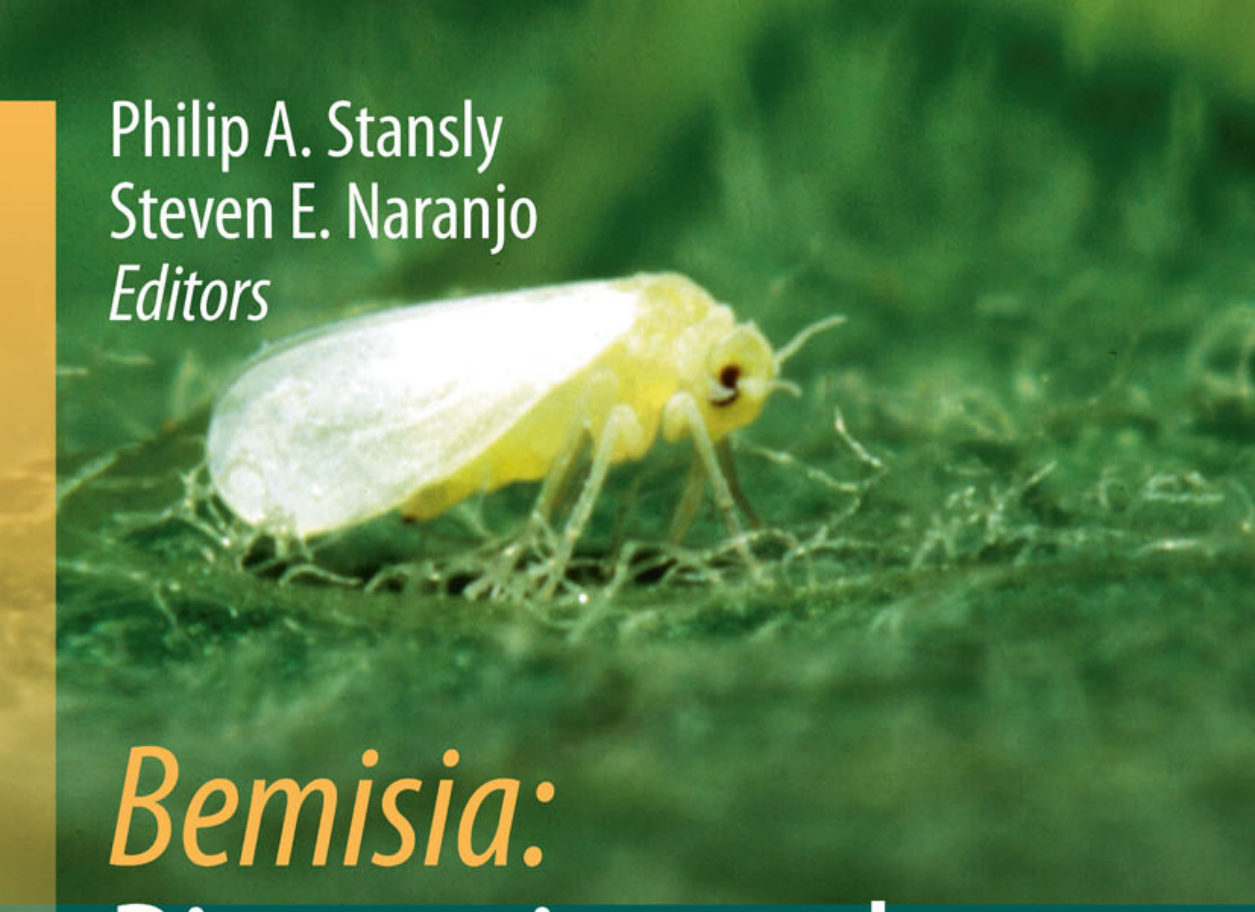
Philip A. Stansly
Steven E. Naranjo
*Editors*



# *Bemisia:* Bionomics and Management of a Global Pest

Springer

# *Bemisia*: Bionomics and Management of a Global Pest

Philip A. Stansly · Steven E. Naranjo
Editors

# *Bemisia*: Bionomics and Management of a Global Pest

Springer

*Editors*
Prof. Philip A. Stansly
University of Florida
Inst. Food & Agricultural
Sciences (IFAS)
Southwest Florida Research &
Education Center
2686 SR 29 North
Immokalee FL 34142-9515
USA
pstansly@ufl.edu

Dr. Steven E. Naranjo
USDA-ARS
Arid-Land Agricultural
Research Center
21881 N. Cardon Lane
Maricopa AZ 85138
USA
steve.naranjo@usda.ars.gov

*Section Editors*
Judith K. Brown
University of Arizona
USA

Dan Gerling
Tel Aviv University
Israel

A. Rami Horowitz
Agricultural Research Organization
Israel

Moshe Lapidot
Agricultural Research Organization
Israel

James P. Legg
International Institute of Tropical
Agriculture
Tanzania

Steven E. Naranjo
USDA-ARS
USA

Jane E. Polston
University of Florida
USA

ISBN 978-90-481-2459-6 e-ISBN 978-90-481-2460-2
DOI 10.1007/978-90-481-2460-2
Springer Dordrecht Heidelberg London New York

Library of Congress Control Number: 2009944102



*Cover illustration*: Whitefly – USDA-ARS, Scott Bauer

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

This book is dedicated to Dr. Jacquelyn (Jackie) L. Blackmer (1954–2008), a co-author of Chapter 5 and a renowned entomologist in the field of insect behavior, insect-plant interactions, and insect dispersal and migration. During postdoctoral work at The University of Arizona and her most recent tenure with the United States Department of Agriculture, Agricultural Research Service in Arizona she made numerous advances in understanding the behavior, physiology and ecology of *Bemisia tabaci*. Dr. Blackmer conducted some of the first and still the most comprehensive studies on the migratory and short-range flight behavior of *Bemisia tabaci* including the role of environment, physiology, morphology, life history and host quality. She advanced our understanding of host quality factors in whitefly life history, developed an artificial diet system, and examined the flight behavior of whitefly parasitoids. She was a tireless, innovative and inspirational researcher as well as being a generous collaborator, colleague and friend to all with whom she worked. Her significant contributions to entomology will be long remembered and will continue to inspire advances in whitefly biology, ecology and management.

# Contents

# Contributors

**Gian Paolo Accotto** Istituto di Virologia Vegetale, Consiglio Nazionale delle Ricerche, Strada delle Cacce 73, Torino 10135, Italy, g.accotto@ivv.cnr.it

**Diann Achor** Citrus Research and Education Center, University of Florida, 700 Experiment Station Road, Lake Alfred, FL 33850, USA, dsar@ufl.edu

**Scott Adkins** USDA-ARS, US Horticultural Research Laboratory, 2001 South Rock Road, Fort Pierce, FL 34945, scott.adkins@ars.usda.gov

**Yehezkel Antignus** Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, Israel, antignus@volcani.agri.gov.il

**Judit Arnó** IRTA Entomology, Ctra. Cabrils km 2, E-08348, Cabrils (Barcelona) Spain, judit.arno@irta.es

**Carlye A. Baker** Division of Plant Industry, Florida Department of Agriculture and Consumer Services, 1911 SW 34th Street, Gainesville, FL 32608, USA, bakerca@doacs.state.fl.edu

**Jacquelyn L. Blackmer** USDA-ARS, Arid-Land Agricultural Research Center, 21881 N. Cardon Ln., Maricopa, AZ 85138, USA (deceased)

**Judith K. Brown** School of Plant Sciences, The University of Arizona, Tucson, AZ 85721, USA, jbrown@ag.arizona.edu

**Benny D. Bruton** USDA-ARS, 911 East Highway 3, Lane, OK 74555-0159, USA, benny.bruton@ars.usda.gov

**Steven J. Castle** USDA-ARS, Arid-Land Agricultural Research Center, 21881 N. Cardon Ln., Maricopa, AZ 85138, USA, steven.castle@ars.usda.gov

**Henryk Czosnek** Faculty of Agriculture, The Robert H. Smith Institute for Plant Sciences and Genetics in Agriculture, The Hebrew University of Jerusalem, Rehovot 76100, Israel, czosnek@agri.huji.ac.il

**Paul J. De Barro** CSIRO Entomology, 120 Meiers Rd., Indooroopilly, Queensland 4068, Australia, paul.debarro@csiro.au

**Ian Denholm** Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK, ian.denholm@bbsrc.ac.uk

**Thomas P. Freeman** Electron Microscopy Center, North Dakota State University, Fargo, ND 58105-5677, USA, thomas.freeman@ndsu.edu

**Rosa Gabarra** IRTA Entomology, Ctra. Cabrils km 2, E-08348, Cabrils (Barcelona) Spain, rosa.gabarra@irta.es

**Dan Gerling** Department of Zoology, Tel Aviv University, Tel Aviv 69970, Israel, DanGE@tauex.tau.ac.il

**Raymond J. Gill** California Department of Food & Agriculture, Plant Pest Diagnostics Center, 3294 Meadowview Road, Sacramento, CA 95832-1448, USA, rgill@cdfa.ca.gov

**Margarita Hadjistylli** Department of Environmental Science, Policy, and Management, University of California, Berkeley, CA 94720-3114, USA, margarita@berkeley.edu

**A. Rami Horowitz** Department of Entomology, Agricultural Research Organization, Negev 85280, Israel, hrami@volcani.agri.gov.il

**Moshe Inbar** Department of Evolutionary and Environmental Biology, University of Haifa, Haifa 31905, Israel, minbar@research.haifa.ac.il

**Chandrasekar S. Kousik** USDA-ARS, US Vegetable Laboratory, 2700 Savannah Highway, Charleston, SC 29414, USA, shaker.kousik@ars.usda.gov

**Moshe Lapidot** Department of Vegetable Research, Institute of Plant Sciences, Agricultural Research Organization ARO, Bet Dagan 50250, Israel, lapidotm@volcani.agri.gov.il

**James P. Legg** International Institute of Tropical Agriculture (IITA), PO Box 34441, Dar es Salaam, Tanzania, j.legg@cgair.org

**Tong-Xian Liu** Laboratory of Applied Entomology, Northwest A&F University, Yangling, Shaanxi 712100, China, txliu@nwsuaf.edu.cn

**Shu-Sheng Liu** Institute of Insect Science, Zhejiang University, 268 Kaixuan Road, Hangzhou 310029, China, shshliu@zju.edu.cn

**Francisco J. Morales** Virology Research Unit, International Center for Tropical Agriculture, Km 17, Recta Cali-Palmira, Valle Cali, Colombia, f.morales@cgiar.org

**Enrique Moriones** Plant Virus Laboratory, Estación Experimental "La Mayora", Consejo Superior de Investigaciones Científicas, 29750 Algarrobo-Costa, Málaga, Spain, moriones@eelm.csic.es

**Rosa M. Muchovej** United States Sugar Corporation, III Ponce de Leon Avenue, Clewiston, FL 33440, USA, rmuchovej@ussuggar.com

**Mariano Muñiz** Department of Plant Protection, Institute for Agricultural Sciences, Spanish National Research Council (CSIC), Serrano 115 Dpdo., Madrid 28006, Spain, mmuniz@ccma.csic.es

**Steven E. Naranjo** USDA-ARS, Arid-Land Agricultural Research Center, 21881 N. Cardon Ln., Maricopa, AZ 85138, USA, steve.naranjo@ars.usda.gov

**Eric T. Natwick** University of California Cooperative Extension, Imperial County, Holtville, CA 92250, USA, etnatwick@ucdavis.edu

**Jesús Navas-Castillo** Plant Virus Laboratory, Estación Experimental "La Mayora", Consejo Superior de Investigaciones Científicas, 29750 Algarrobo-Costa, Málaga, Spain, jnavas@eelm.csic.es

**Gloria Nombela** Department of Plant Protection, Institute for Agricultural Sciences, Spanish National Research Council (CSIC), Serrano 115 Dpdo., Madrid 28006, Spain, gnombela@ccma.csic.es

**John C. Palumbo** Department of Entomology, Yuma Agricultural Center, 6425 W. 8th St. University of Arizona, Yuma, AZ 85364, USA, jpalumbo@ag.arizona.edu

**Thomas M. Perring** Department of Entomology, University of California, Riverside, CA 92521, USA, thomas.perring@ucr.edu

**Jane E. Polston** Department of Plant Pathology, University of Florida, Gainesville, FL 32611, USA, jep@ufl.edu

**Nilima Prabhaker** Department of Entomology, University of California, Riverside, CA 92521, USA, nilima.castle@ucr.edu

**Pamela D. Roberts** Department of Plant Pathology, Southwest Florida Research and Education Center, University of Florida, 2685 SR 29 N., Immokalee, FL 34142, USA, pdr@ufl.edu

**George K. Roderick** Department of Environmental Science, Policy, and Management, University of California, Berkeley, CA 94720-3114, USA, roderick@berkeley.edu

**Rosemarie C. Rosell** Department of Biology, University of St. Thomas, Houston, TX 77006, USA, rrosell@stthom.edu

**Luca Sardo** Istituto di Virologia Vegetale, Consiglio Nazionale delle Ricerche, Strada delle Cacce 73, Torino 10135, Italy, l.sardo@ivv.cnr.it

**Alvin M. Simmons** USDA-ARS, US Vegetable Laboratory, 2700 Savannah Highway, Charleston, SC 29414, USA, alvin.simmons@ars.usda.gov

**Philip A. Stansly** Department of Entomology and Nematology, Southwest Florida Research and Education Center, University of Florida, 2685 SR 29 N., Immokalee FL 34142, USA, pstansly@ufl.edu

**Gregory P. Walker** Department of Entomology, University of California, Riverside, CA 92521, USA, gregory.walker@ucr.edu

**Susan E. Webb** Department of Entomology and Nematology, University of Florida, 2213 Building 970, Natural Area Drive, Gainesville, FL 32611, USA, sewe@ufl.edu

**William M. Wintermantel** USDA-ARS, 1636 East Alisal Street, Salinas, CA 93905-3018, USA, bill.wintermantel@ars.usda.gov

# Introduction

**Philip A. Stansly and Steven E. Naranjo**

*Bemisia tabaci* (Gennadius) has distinguished itself from the more than 1,000 whitefly species in the world by its adaptability, persistence and potential to damage crops of all types. Its only rival for this distinction is perhaps the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood, which fills the niche of polyphagous whitefly pest in temperate regions, while *B. tabaci* reigns supreme in the tropics and subtropics. Nevertheless, proliferation of protected agriculture and mass movement of plants and produce have extended the range of *B. tabaci* into most of the temperate regions of the world as well. *B. tabaci* stands out against any whitefly rival in a number of other aspects: host range, virus vectoring ability, damage potential that includes physiological disorders and honeydew associated quality issues, an ability to adapt morphologically to its host, and the existence of multiple sibling species, just to name a few. These and many other characteristics of *B. tabaci* are examined here in depth.

First named *Aleyrodes tabaci* and described from tobacco in Greece (Gennadius 1889), the insect now known as *Bemisia tabaci* was soon afterward reported from Florida as *A. inconspicua* (Quaintance 1900). The "tobacco whitefly" later solidified its pest status in that crop as the vector of tobacco leaf curl disease in East Africa (Storey 1931) and Indonesia (Thung 1932). At the same time, the self same "cotton whitefly" was recognized in West Africa and Sudan as the vector of cotton leaf curl disease (Golding 1930; Kirkpatrick 1931), which had been reported from Nigeria two decades earlier (Farquharson 1912). The most effective control for both these epidemics was found to be timely removal of the principal inoculum source, the previous year's crop residue (Hopkins 1932; Tarr 1951).

The cotton/tobacco whitefly soon began to be reported as a pest in its own right, first in India (1929), and subsequently in the Sudan (1950s), El Salvador (1961), Mexico (1962), Brazil (1968), Turkey (1974), Israel (1976), Thailand (1978) and Arizona and California (1981), mostly on cotton (Horowitz 1986). In many cases, whitefly infestations reached outbreak proportions, a situation attributed to various causes including improper use of insecticides, climate, and increased agricultural intensification.

The number of plant species known to host *B. tabaci* also proliferated, especially after Takahashi (1936) and Russell (1958) synonymized it with some 18 other described species. The large number of synonyms was in part due to host induced

morphological variation (Mound 1963; Neal and Bentz 1999). Mound and Halsey (1978) listed 302 species in 74 families to which Greathead (1986) added 204 more to make the widely quoted total of 506. The list continues to expand as the insect invades new areas and more records are collected in regions where it has established. The largest numbers in Greathead's list are in Leguminosae (56) and Compositae (33), but these are also the two largest plant families. The greatest percentage of species within plant families known to serve as hosts for *B. tabaci* is attributed to the Malvaceae (3.5%) followed by the Curcubitaceae (2.7%), Euphoriaceae (1.7%), and Convolvulaceae (1.7%). With the addition of the Cruciferae, this list includes most crops on which *B. tabaci* is a serious pest.

It was not until 1986 that Florida experienced outbreaks of what is now known as *Bemisia tabaci* biotype "B", first in greenhouse poinsettia, then in a wide diversity of vegetable, ornamental and agronomic crops throughout the state. The number of reported outbreaks soon accelerated around the globe. The old biotype "A" of more limited host range, totally disappeared in the USA and damage attributable to the new biotype was estimated at 500 million $US in 1993. The subsequent worldwide outbreak of biotype B stimulated greater attention to whiteflies in general, and *B. tabaci* in particular, from the agricultural, research and extension communities.

Our goal with this volume is to follow up on two previous books edited by Dan Gerling: Whiteflies: their Bionomics, Pest Status and Management (1990, ISBN 0-946707-16-2) and *Bemisia*: 1995 – Taxonomy, Biology, Damage, Control and Management (1996, ISBN 1 898298 33.5). The latter, co-edited by Richard Mayer, consisted of proceedings from the first International *Bemisia* Workshop that took place in Israel in 1994. The present volume is a somewhat belated follow-up of the 4th International *Bemisia* Workshop that took place in Florida, December 2006.

It was perhaps fitting that we met in Florida, 20 years after the onset of the latest and perhaps most dramatic chapter in the history of encounters between whiteflies and humans. Much has been learned about *Bemisia* in the interim, but as is usually the case, we only find that there is much more yet to discover. The arrival to the USA, Australia and China of biotype "Q", originally from in the Middle East and southern Europe and thought to be especially resistant to insecticides, sets the stage for another epic struggle among biotypes, the outcome of which may again be mitigated by effective management. Only a deepening knowledge of genetics, biology, interactions with hosts, natural enemies, vectored viruses, and responses to chemicals and environmental conditions (bionomics) will provide the tools needed for satisfactory management. Thus, the broad focus of this book, from basic to applied.

Our specific objective is to provide a review of *Bemisia* taxonomy, genetics, biology, ecology and management, focusing mostly on progress during the last 10–15 years, and directed at workers in the field as well as the informed professional who may not necessarily specialize in whitefly research. We have divided this review into 5 sections: (1) Taxonomy and Genetics, (2) Biology and Ecology, (3) Epidemiology of Whitefly Transmitted Viruses, (4) Management, and (5) Genomics, in a total of 18 chapters. This structure is in keeping with our goal of providing a broad but thorough review of the subject matter. Each section has its own section editor(s), all leaders in their fields. Section editors were responsible for writing an introduction

to each section, and working with the chapter authors on content and style. It is our pleasure to help bring this work to fruition and also provide this short introduction to what has been truly a group effort.

Following an introduction by Brown, Section One opens with an overview by Gill and Brown of whitefly systematics at the generic level, including an evolutionary history and description of the *B. tabaci,* and *B. afer* complexes as well as *Lipaleyrodes* and other *Bemisia*-like species, posing the question: Can molecular techniques solve the *Bemisia tabaci* complex conundrum? Chapter 2 by Brown examines the phylogenetic biology of the *B. tabaci* sibling species group that has so occupied evolutionary biologists as well as management practitioners since the first biotype B outbreaks were recorded in the 1980s. Chapter 3 by Hadjistylli et al. describes the toolbox of molecular methods that are being used to unravel the genetic complexities and relationships within the *B. tabaci* species group.

Section Two provides an overview of the biology and ecology of this fascinating and complex insect. After an introduction by editors Naranjo and Legg, we find a thorough discussion of life history, functional anatomy, feeding and mating behavior by Walker et al (Chapter 4) which includes many outstanding illustrations, but still comes to the unsettling conclusion (as in many of the chapters) that although much is known, much remains to be discovered. Rosell et al. (Chapter 5) take us into the bewildering world of whitefly symbionts and their myriad effects on whitefly life history, behavior and virus transmission. They also review our current understanding of the interaction of *Bemisia* with other herbivores, mediated by the host plants they share. Chapter 6 by Naranjo et al. places our whitefly into its environment, describing demographics as revealed by laboratory and field-based life tables, spatial and temporal dynamics, sampling methods, population models and invasion and outbreak mechanisms.

The worst damage from *B. tabaci* infestation is usually as a consequence of its role as virus vector. Editors Lapidot and Polston open Section Three with an introduction to set the stage for a discussion of these relationships with emphasis on biology and epidemiology of different whitefly transmitted viruses. The *Geminiviridae* are covered by Legg (Chapter 7) with a review of the cassava mosaic viruses, Moriones and Navas-Castillo (Chapter 8) who discuss *Tomato yellow leaf curl virus* (TYLCV), and Morales (Chapter 9) who provides a general review of begomoviruses in Latin America. Wintermantel (Chapter 10) reviews the *Criniviridae*, and Adkins and colleagues (Chapter 11) briefly discuss the *Ipomoviridae*. The section concludes with a review of evidence for transovarial transmission of TYLCV by Accotto and Sardo (Chapter 12), a truly daunting prospect.

Following an introduction to Section Four by editors Gerling and Horowitz, the authors examine progress made during recent years in perfecting the components of *Bemisia* IPM: optical manipulation (Anignus, Chapter 13), host plant resistance (Nombela and Muñiz, Chapter 14), biological control (Arnó et al., Chapter 15) and insecticides examined through the lens of resistance in ecological terms (Castle et al., Chapter 16). Stansly and Natwick (Chapter 17) wrap up the section by

reviewing the integration of these components into biologically based management systems for protected and open field crops.

The final Section Five consists of a single chapter by Czosnek and Brown, which in some sense projects the previous 17 chapters into the future by describing the potential of genomic methods to further illuminate many of these same areas of research. Overall, we hope our efforts may serve to advance basic and applied research on *Bemisia* for some time to come, leading ultimately to solutions for one of the most significant agricultural pests, worldwide.

## Literature Cited

Farquharson CO. 1912. Report of the Mycologist, Annual Report, Agricultural Department, Nigeria.

Gennadius P. 1889. Disease of the tobacco plantations in the Trikonia. The aleurodid of tobacco. *Ellenike Georgia* 5:1–3.

Golding FD. 1930. A vector of leaf curl of cotton in southern Nigeria. *Empire Cotton Growers Rev.* 7:120–126.

Greathead AH. 1986. Host plants. In *Bemisia tabaci – A Literature Survey on the Cotton Whitefly with an Annotated Bibliography*, ed. MJW Cock, pp. 17–25. Ascot, UK: CAB International Institute of Biological Control.

Hopkins JCF. 1932. Further notes on leaf curl of tobacco in Southern Rhodesia. *Rhodesian Agric. J.* 29:680–686.

Horowitz AR. 1986. Population dynamics of *Bemisia tabaci* (Gennadius): with special emphasis on cotton fields. *Agric. Ecosyst. Environ.* 17:37–47.

Kirkpatrick TW. 1931. Leaf curl disease in Sudan. *Bull. Entomol. Res.* 21:127–137.

Mound LA. 1963. Host-correlated variation in *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae). *Proc. R. Entomol. Soc. Lond. Ser. A Gen. Entomol.* 38:171–180.

Mound LA, Halsey SH. 1978. *Bemisia tabaci* (Gennadius). In *Whitefly of the World, A Systematic Catalog of the Aleyrodidae (Homoptera) with Host Plant and Natural Enemy Data*, pp. 118–124. Chichester, New York, Brisbane, Toronto: British Museum (Natural History) and John Wiley & Sons.

Neal JA, Bentz JA. 1999. Evidence for the stage inducing phenotypic plasticity in pupae of the polyphagous whiteflies *Trialeurodes vaporariorum* and *Bemisia argentifolii* (Homoptera: Aleyrodidae) and the *raison d'être*. *Ann. Entomol. Soc. Am.* 92:774–787.

Quaintance AL. 1900. Contribution towards a monograph of the American Aleurodidae. *Tech. Ser., U.S. Dept. Agric. Bureau Entomol.* 8:9–64.

Russell LM. 1958. Synonyms of *Bemisia tabaci* (Gennadius) (Homoptera, Aleyrodidae). *Bull. Brooklyn Entomol. Soc.* 52:122–123.

Storey HH. 1931. A new virus of the tobacco plant. *Nature* 128:187.

Takahashi R. 1936. Some Aleyrodidae, Aphidae, Coccidae, (Homoptera) and Thysanoptera from Micronesia. *Tenthredo* 1:109–120.

Tarr SAJ. 1951. *Leaf Curl Disease of Cotton*. Kew: Commonwealth Mycological Institute.

Thung TH. 1932. De krul- en kroepoekziekten van tabak en de oorzaken van hare verbreiding. *Proejsta. Vorstenland. Tabak Mededeel.* 72:1–54.

# Section I
# Taxonomy, Molecular Systematics, and Gene Flow in the *Bemisia tabaci* Complex and *Bemisia* Relatives

**Judith K. Brown**

## Introduction

The *Bemisia tabaci* (Gennadius) complex is one of the most agriculturally important insect pests worldwide, predominately as a vector of the plant virus genus *Begomovirus* (Family, *Geminiviridae*). The increased importance of begomoviruses as emerging viral pathogens is directly related to the adaptability of certain *B. tabaci* haplotypes – and biotypes – to agricultural cropping systems, which are increasingly expanding into whitefly habitats.

This whitefly complex has attracted attention because of its unusually plastic or variable phenotypic traits, including host range, environmental adaptation, fecundity, and variable dispersal behaviors. Also, the *B. tabaci* complex has long confounded systematists owing to lack of morphological characters that can be linked to diverse phenotypes. Members of the *B. tabaci* complex further vary with respect to composition of secondary endosymbionts that are thought to contribute to certain aspects of fitness. The suite of phenotypic characters of certain biological types closely aligns with recognized invasive behaviors. Particularly invasive *B. tabaci* often exhibit resistance to certain insecticides used in agricultural production systems, which similarly, seems related to the inherent plasticity of the species.

Although distinct biotypes and haplotypes may be equally plastic and adaptive in different environments, some have a greater capacity to become invasive; others are moderately problematic, or even benign. Likewise, certain biotypes/haplotypes have an extensive or only a moderate range of host species, while others are host-specific, or nearly so. Monophagous and polyphagous biological variants are potentially equally damaging as virus vectors, depending on vector phenotypes and crop (or non-crop) hosts involved. The *B. tabaci* complex is poorly studied in genetic and genomics terms; whether behavioral traits are solely expressed in a gene for gene manner, and/or if the different phenotypes are the result of differential gene expression and probably epigenetic regulation is not known, mainly because tools are lacking with which to conduct functional genomics analyses.

---

Judith K. Brown (Section Editor)

A comparison of the sequences for the mitochondria cytochrome oxidase I gene (mtCO1; 780 bases) and a nuclear intron from the whitefly nuclear voltage-gated sodium channel gene (850 bases) for the Sida (moderately fecund, polyphagous) and Jatropha (monophagous, low fecundity) biotypes sympatric in the Caribbean region, diverge by less than 1% (mtCO1), and are otherwise indistinguishable (unpublished data). Although not entirely conclusive, these haplotypes (biotypes) appear to be very closely related but are known to be differentially host-adapted, although these differences are not correlated with notable genetic variation.

Unexpectedly, phylogenetic variation (mtCO1) for worldwide *B. tabaci* was found to range between 0 and 26%, making the gene somewhat useful for haplotype taxonomic classification despite its saturation, which precludes assignment of a basal clade for the *B. tabaci* complex. Phylogenetic inferences based on the mtCO1 sequence delineate ~6 major clades (depending on nodes used for demarcation) of *B. tabaci* that group phylogeographically, each with sister clades.

Although much emphasis has been leveraged on the invasiveness of the *B. tabaci* complex, only a few haplotypes have been truly invasive following their transport to exotic locales by people. As expected, they are not phylogenetically aligned with the haplotypes endemic to the region. These invasive haplotypes are the B (introduced worldwide, 1980s) and Q (introduced, 2005-onward) biotypes, and the (holotype) *B. tabaci* from Greece (first recorded outbreak, 1889), the latter, responsible for the first documented outbreak in a cultivated crop – e.g. tobacco – and the impetus for naming the species. Unexpectedly, the Greek mtCO1 haplotype of adults recovered from leaves archived in the British Museum may have originated in Asia, perhaps arriving as a stow-away aboard a cargo ship bringing goods from Asia to Europe via the Mediterranean Sea (Gill and Brown Chapter 1; Brown Chapter 2).

From the turn of the century to the 1960s, outbreaks of *B. tabaci* became increasingly more frequent in vegetable and fiber crops in the tropics and subtropics. Virus-like diseases became problematic in cotton in Sudan, in vegetable crops in India, and in cassava throughout Sub-saharan Africa. In Brazil, whitefly-transmitted viruses caused damage to soybean and tomato crops, and throughout Latin America and the Caribbean Basin bean crop yields were reduced by *Bean golden yellow mosaic virus* infection. In the Southwestern USA, *Cotton leaf crumple virus* negatively affected lint quality and reduced cotton yields. The literature reveals that much attention was centered on pesticide solutions to reduce whitefly and virus-induced crop damage, and reports of the ineffectiveness of certain pesticides emerged as a common theme. Since then, the economic importance of *B. tabaci* as a pest and vector has continued to increase, and research efforts began to provide new knowledge about this phloem-feeding insect.

In subsequent years, both endemic and introduced *B. tabaci* have continued to increase in importance in agricultural systems as their magnitude and prevalence in irrigated crop production systems have become commonplace, particularly in tropical and subtropical regions and in temperate greenhouse production areas. Spill-over from the field into ornamental crops resulted in the infestation of additional plant species and their addition to the host list for *B. tabaci*, presently topped off at over 500 species. In addition, at least two haplotypes have reached invasive status,

after having been transported to exotic locales on ornamental plants. The worldwide introduction and rapid establishment of the pesticide resistant biotype B beginning in ~1980 (B. Kumashiro and R. Gill personal communication) further underscored the potential significance of *B. tabaci*. Most recently, whitefly-transmitted viruses have been introduced into non-native locations where they have established, and in most instances, have been found to cause greater damage than their endemic counterparts.

Consequently, *B. tabaci* and the viral pathogens it transmits are no longer restricted to native habitats or contained by natural geographic boundaries. Increased monoculture cropping, reduced genetic variation in cultivated species, and widespread use of insecticides in agriculture, together with international transport of infested plants are substantial contributing factors to the global significance of both indigenous and exotic *B. tabaci* and the plant viruses they transmit.

The three chapters in Section I address the current state of knowledge about the systematic of *B. tabaci* with biological-genetic, and classical and molecular taxonomy perspectives (Gill and Brown Chapter 1). Also addressed is the status of population genetics research, which is essential for revealing important insights in population structure, clarifying evolutionary patterns, and providing new insights into the extent of gene flow within the complex (Brown Chapter 2; Hadjistylli et al. Chapter 3). In all arenas, greater research and understanding is needed.

The invasiveness of several main exotic and/or indigenous biotypes of *B. tabaci* – e.g. A and B, and most recently Q biotype – has contributed widely to raised awareness of the economic importance of this whitefly and viruses it transmits to food, fiber, and ornamental crops. The unique biology of the *B. tabaci* complex, and a quest to elucidate the basis for conserved virus-vector interactions have become of increasing interest to the scientific community. Whether *B. tabaci* will continue to be thought of as a "complex of genetic and phenotypic variants", or as a number of separate species, is still not clear. Even so, comparative biological investigations, together with molecular and population biology approaches, are now crucial to addressing a number of central questions. Recent studies have engaged multiple disciplines with diverse perspectives, each contributing new and exciting knowledge. The authors hope that these writings will inspire continued transdisciplinary collaborations and creative dialog.

The last Section (V; Chapter 18) in this book is particularly relevant to Section I in that it underscores the crucial need for a *The Whitefly Genome Project* and the associated functional genomics tools to facilitate answers to many unresolved questions. It is anticipated that the collective knowledge and dedication expressed in this book will garner enthusiasm, provide clearer rationale, and forge in-roads to acquire the support needed to develop a genomics toolbox, now essential for devising new technologies and advancing old paradigms. That the functional genomics (and other "omics") studies are hindered because the *B. tabaci* genome sequence has not been determined seems unfathomable. At a time of world food shortage and with the impending effects of global change on food production, it seems unlikely that a more relevant biological and genetic indicator could be identified. We envision an undertaking analgous to "The *Drosophila* species" sequencing project, with *B. tabaci*

serving as the first "agricultural model study system", a tractable and exciting undertaking given the abundant phenotypic and genetic attributes already known and the threat of prevalent pest outbreaks. Collectively, we look forward to solution-driven research directed by such a full blown genome project for the *B. tabaci* complex, and to highlighting those significant inroads in future communications.

# Chapter 1
# Systematics of *Bemisia* and *Bemisia* Relatives: Can Molecular Techniques Solve the *Bemisia tabaci* Complex Conundrum – A Taxonomist's Viewpoint

**Raymond J. Gill and Judith K. Brown**

## Introduction

The whitefly, *Bemisia tabaci* (Gennadius), has a long history as a serious pest of agriculture worldwide. One of the most serious attacks began in 1985–1986 in Florida where very heavy populations infested ornamental and crop plants. By 1990, it had reached the fertile desert croplands of southern Arizona and California, where clouds of whiteflies could be seen flying over the desert valleys. Crops were destroyed before they could produce fruit, and the resultant infestation totally changed the cropping procedures and methods that had been used in these desert valleys for years. However, sweetpotato whitefly had previously caused economic losses to many crops in these valleys in the years 1980–1985, largely due to increased population levels and the transmission of closteroviruses to the plants. But in 1990, the situation changed. Much heavier populations of the whitefly developed, and although closteroviruses disappeared from the scene, conditions in the valleys became extremely serious with losses to growers of many millions of dollars, in addition to loss of jobs and livelihoods for farm workers and others associated with agriculture in these areas. For further information on these events see Gill (1992).

### *History of the Problem*

The different responses of these two separate population explosions in the southwestern desert suggested that possibly two species were involved under the name *B. tabaci*. By 1991 at least, molecular research and differing biologies had begun to suggest that there were in fact major differences between these two populations that eventually became known as biotype A for those that had occurred in the southwestern desert areas of the United States prior to 1990, and the B biotype for populations

R.J. Gill (✉)
California Department of Food & Agriculture, Plant Pest Diagnostics Center, 3294 Meadowview Road, Sacramento, CA 95832-1448, USA
e-mail: rgill@cdfa.ca.gov

P.A. Stansly, S.E. Naranjo (eds.), *Bemisia*: Bionomics and Management of a Global Pest,
DOI 10.1007/978-90-481-2460-2_1, 

that occurred in Florida starting in 1985–1986, and found on poinsettia plants in Arizona in 1986–87, and after 1990 in the desert valleys. This eventually led to the species description of biotype B as the silverleaf whitefly, *Bemisia argentifolii*, by Bellows et al. (1994). At the same time, several laboratories were already demonstrating the existence of many different populations around the world, referred to as biotypes in addition to the A and B populations. Therefore the naming of this new species has been soundly refuted, with eventually the species being suggested by Brown et al. (1995) as a member of a sibling species complex, and by DeBarro et al. (2005) as a race of *tabac*i and thus a synonym of it. Part of the reasoning behind the synonymy is that there are basically no morphological characteristics of *tabaci* whitefly populations that can distinguish them as distinct species. Part of the basis for the original description of *B. argentifolii* was a slight difference in the occurrence of a single minute seta on the cephalothoracic submargin of the pupal usually being present in A populations and usually not in B populations. This seta is also usually lacking in most other biotypes of *tabaci*, as will be explained later.

## The Problem

Similar issues with setal placement and population separation exist within another species, *Bemisia afer* Priesner and Hosny, as well as many other details regarding the species and generic placement of other whiteflies. Since morphology is considered the foremost basis for species separation by all systematists, one would assume that the case for synonymy within the *B. tabaci* group of populations should be an easy decision. However, this is not the case, as several important taxonomic problems exist because, as a result of morphological studies of *Bemisia* species and others, it appears that morphological characteristics of many whitefly species are very poorly understood (Martin 2003).

Much has been discussed over the years concerning plasticity in the morphology of whitefly pupae. Several authors have mentioned this observation, including Husain and Trehan (1933) and Deshpande (1933). Russell (1948) showed morphological variation within species of the genus *Trialeurodes* based on the host plant, and Mound (1963) conducted rearing experiments with *B. tabaci* showing that pupal morphology varied with the character of the leaf surface. David (1987) summarized these findings and added other examples, particularly in species occurring in India, and Sundararaj and David (1992) discussed host-correlated variation in *Dialeurodes kirkaldyi* (Kotinsky). In the same light, Mohanty and Basu (1986) suggested that pupal morphological variations could be affected by temperature and humidity as well. There is some evidence, based on *B. afer*-like forms from the Macaronesian Islands, that host plants may affect species morphology in ways other than through leaf hair topology.

With the advent of molecular based investigations into whitefly species relationships and phylogeny, it has come time to analyze some of the problems with the current genus/species relationships within the Aleyrodidae. Due in part to this

pupal plasticity, it appears that there is a lot of confusion, not only regarding species limits, but generic levels of classification as well. Mound (1984) suggested that several larger generic groups represent arbitrary units consisting of similar appearing species rather than evolutionarily related forms, and further adds that many species are ill defined and probably represent host-induced forms or localized strains. While this problem occurs throughout the family, much work is being done currently on the genus *Bemisia* and its apparent relatives and on other species and genera that have at least a close similarity in pupal case morphology.

In research stemming from the introduction of the B biotype of *B. tabaci* into the United States and the subsequence astronomical losses in agriculture in that country, it was shown by Neil and Bentz (1999) that morphological changes in the pupal development of *B. tabaci* and *Trialeurodes vaporariorum* are influenced by the environment surrounding the hatching first instar nymphs. If the surrounding leaf surface of the host is smooth and clean, *tabaci* nymphs show little or no development of long dorsal setae. However, if the leaf surface is hairy, if there are a lot of dirt particles, or there are other instars or pupae and the as yet unsettled nymph bumps into these objects, long dorsal setae of varying numbers probably will be produced. For *T. vaporariorum*, long wax spine-like extrusions are produced by papilla-like dorsal pores if the host has many leaf hairs, but the extrusions and papillae do not necessarily form if the host leaf is smooth. The resulting differences in morphological appearance of *B. tabaci* are considered to be the cause of the large amount of synonymy of *tabaci* over the years. Russell (1957) published a list of these synonyms, and this list was followed by a list in Mound and Halsey (1978) in their catalog work.

With the introduction of the B biotype (Costa and Brown 1990, 1991; unpublished data) into the United States, and its appearance in economically damaging populations in Florida in 1986, taxonomists were aware that this whitefly was acting differently from populations of *B. tabaci* that had occurred in that state prior to the arrival of B, but that there appeared to be no discernable morphological differences. It was not until later that work by Brown et al. (1992, 1995a, b, 2000), Coats et al. (1994), Costa and Brown (1990, 1991), Costa et al. (1993), Perring et al. (1992, 1993), and others, showed molecular differences (Gawel and Bartlett 1993), with the designation then of the A and B biotypes for those populations in the USA (Brown et al. 1995a, b; based on Costa and Brown 1991, Costa et al. 1993). Later, numerous other biotypes were added to the list, with the Q biotype also causing considerable economic problems to agriculture (Dennhey et al. 2006).

After a major meeting of scientists in Dallas, Texas in 1990 to discuss control methods for this new problem with *tabaci*, it was determined that a study should be made into the morphology of *tabaci* and its apparent relatives and simultaneously look at molecular aspects of *tabaci* and other closely related species. This study would hopefully shed some light on the possible origins of this particular biotype, and could determine if in fact more than one species was present as a complex. Partial results in this study of the molecular nature of *tabaci* and other whiteflies were published by Campbell et al. (1994, 1995), and will be discussed later. The

morphological studies were also aimed at finding significant and reliable characters for separating genera and species, and which characters might be a product of convergence and not related to phylogenetic sequences, and thus not particularly useful in determining whitefly hierarchy and generic placements.

Specimens of various species of *Bemisia* have been assembled and illustrated. Collections of specimens with *tabaci* characteristics have been studied from all over the globe, and many have been illustrated, although most of these illustrations have yet to be published, as the study is still ongoing. Illustrations from published descriptions of most known species of whiteflies around the world were studied to determine whether other similar appearing genera might contain species that are actually closely related to *Bemisia* or might even belong in the genus. Most of these illustrations will soon be published in a refereed systematics journal.

Representative species of whiteflies that were considered to belong to the genus *Bemisia*, as well as species similar in appearance to *Bemisia*, were studied morphologically. This included the careful mapping of dorsal setae and pore groups as well as other morphological structures that occur on pupal stages. These structures were then illustrated for later comparison purposes. Specimens were gathered from various collections, and included syntype specimens of *B. tabaci* and paratypes of some other species. Recent collections of specimens for molecular testing were also checked for the proper identification and illustrated if necessary. Adults were also studied when available.

## Problems in Whitefly Systematics

Early work by Sampson (1943, 1947) and Sampson and Drews (1956) included the first family-wide keys to the genera of world whiteflies. Basically, these keys break apart some major groupings of genera based on the important characters found in the vasiform orifice or associated with it in the pupal stage. Important considerations include whether or not the operculum nearly or completely covers the orifice, whether the posterior end of the orifice is closed or open, and whether or not the lingula extends posteriorly beyond the operculum and the end of the orifice (Fig. 1.1). Also important is whether or not there is a dorsal furrow extending from the rear of the orifice to the posterior body margin. Length of the orifice in relation to the distance to the posterior margin might also have importance, as could the relative lengths of the lingulae. In the genus *Bemisia*, the operculum does not fill the orifice completely, the orifice is open posteriorly and the lingula is long enough to extend beyond the operculum and sometimes beyond the posterior end of the orifice. Some other species and genera outside of *Bemisia* also have these characteristics to a degree, so separation of these can rely on other characters, or they can be open to question as being part of the *Bemisia* generic group or not. Many of these species have been studied morphologically and hopefully most will be studied molecularly in the future.

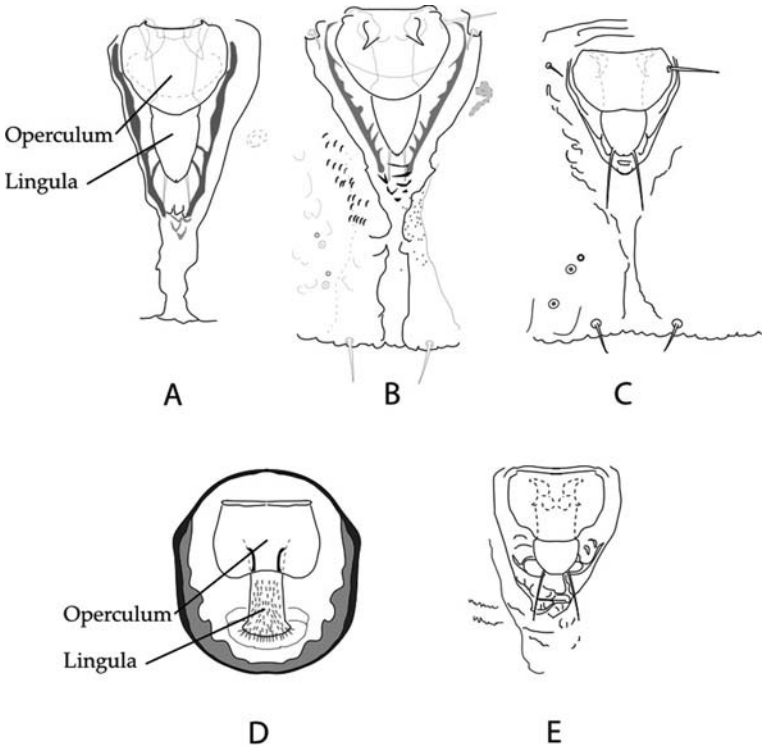



**Fig. 1.1** Comparison of shapes of the vasiform orifice and lingula of: **a**. syntype specimen of *Bemisia tabaci*, **b**. *Bemisia tuberculata* (Brazil), **c**. *Aleyrodes spiraeoides*, **d**. *Pealius maskelli*, **e**. *Pealius azaleae*

It has become even more apparent that the systematics of whiteflies is in a bit of disarray. Whitefly taxonomists are aware of problems in the placement of species in a number of the world genera (Mound 1984) and this study and others are beginning to emphasize this very strongly. A prime example of this is the species *Pealius azaleae* (Baker and Moles). It has a vasiform orifice that is open posteriorly, with the lingula having two apical setae and a spatulate shape. These characters are in complete opposition to the type species of the genus, *Pealius maskelli* (Bemis), which has the posterior margin of the orifice closed like the lip of a bowl, with the lingula blunt and covered with microsetae instead of having one set of long setae (Fig. 1.1). The overall vasiform orifice characteristics of *azaleae* would then suggest that it might possibly be a *Bemisia*, but definitely it belongs in some other genus than *Pealius*. Hopefully more molecular work will help solve this placement dilemma.

While samples of *maskelli* have not been available for molecular testing, note the position in Fig. 1.2 of another North American species, *Pealius kelloggi* (Bemis), morphologically similar and thus probably congeneric with *P. maskelli*. The results of cytochrome oxidase 1 analysis shown in the tree (Fig. 1.3) indicated that

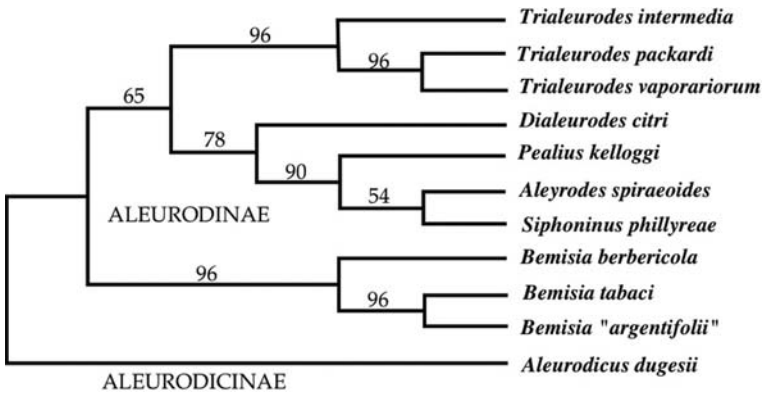



**Fig. 1.2** Phylogenetical tree based on 18S ribosomal RNA gene (rDNA). Data simplified from Campbell et al. (1994) and showing possible relationships of *Bemisia tabaci* to some other common species of whiteflies

*P. azaleae* is basal to other *Bemisia* species tested at that time. However, see also Fig. 1.4 where in another analysis, it is basal to both the *B. tabaci* and *B. afer-hancockii* complexes.

That the molecular versus morphological approach to systematics of whiteflies has been partially successful at least, is illustrated by the fact that species in the genus *Aleyrodes* have very similar pupal morphology to the *B. afer* complex of species (discussed below), but as seen in the Campbell tree (Fig. 1.2) is distinct from *Bemisia* and not particularly related to it based on 18S ribosomal DNA (rDNA) sequences. Species in the genus *Aleyrodes*, for example, meet the requirements of the genus *Bemisia* and are very similar in appearance to the *B. afer* group of species in respect to the shape and structure of the vasiform orifice and lingula, setal placements, variable development of long dorsal setae, overall body shape and adult structures. *Aleyrodes* species differ in pupal morphology from the *afer* complex only in having relatively shorter lingulae in the vasiform orifice, and the 7th and 8th abdominal segments subequal in width on the midline ahead of the orifice. In *afer* species, the lingulae are long and narrow, and the width of the 7th abdominal segment on the midline is much shorter than that of the 8th. The adults of most *Aleyrodes* species are very similar to some *afer* complex adults in having completely divided upper and lower compound eyes, but differ in being more sclerotized, having some diffuse dark markings along the wing veins, and most importantly, having the lengths of antennal segments 4 and 5 subequal. In the known adults of *Bemisia* species, and for most other Aleyrodine species, antennal segment 4 is always much shorter than segment 5. Apparently then, the only reliable morphological characters that tend to separate the genus *Aleyrodes* from *Bemisia* are the widths of abdominal segments 7 and 8 in the pupae, and the lengths of the 4th and 5th antennal segments in the adults. Therefore, these particular characters represent valid generic level separations between the two genera. Without molecular data, this genus level concept could not be proven either way.

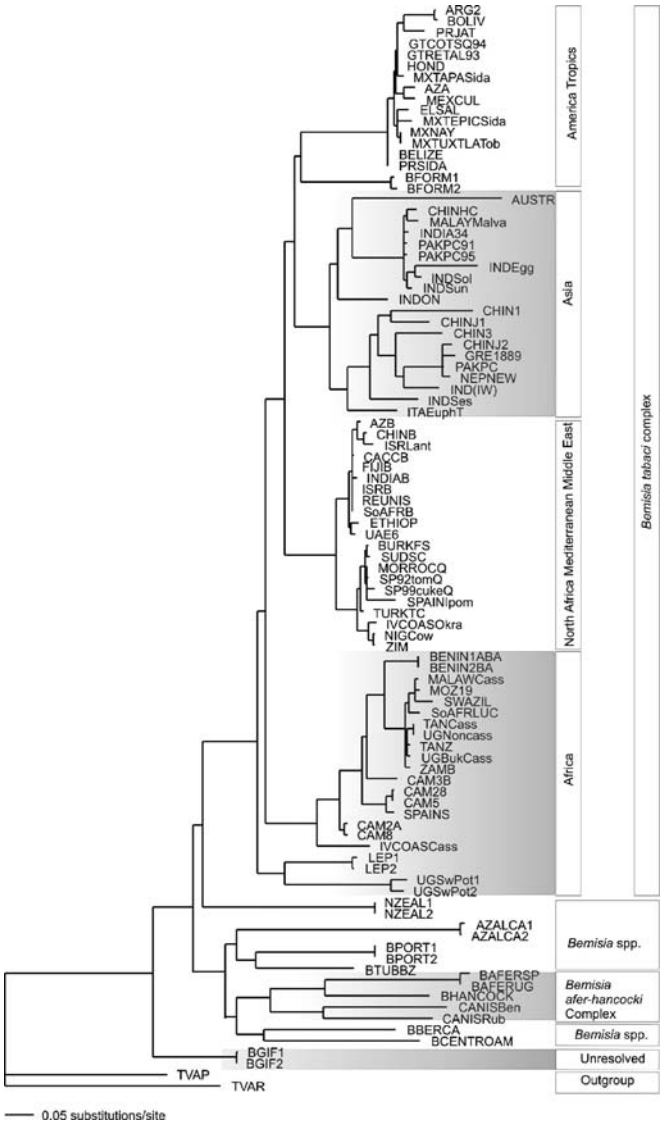



**Fig. 1.3** A condensed and simplified tree based on the mitochondria cytochrome oxidase I sequence (780 bases) molecular data showing inferred (ML, PAUP) phylogenetic relationships of *Bemisia tabaci* populations with other related species [selected acronymns referred to are: *Bemisia centroamericana* from Belize (sample courtesy J. Martin, UK); for *B. tabaci* ABA from Benin on *Asystasia* (Bedford et al. 1994) and Sweet potato Uganda (UgSwPot) (courtesy J. Legg), GRE-1889 pupae from the paratype collection at the British Museum or USDA-collection, Beltsville, MD]; the other *B. tabaci* haplotypes/biotypes are well-known reference sequences, available in the Genbank database; *Bemisia* spp undescribed from New Zealand (NZEAL); *Lipaleyrodes emiliae* Taiwan (LEP); *Pealius azaleae (AZALCA)* from Azalea, USA (orig Asia); Outgroups are: *Trialeurodes vaporariorum* (TVAP) and *T. variabilis* (TVAR)

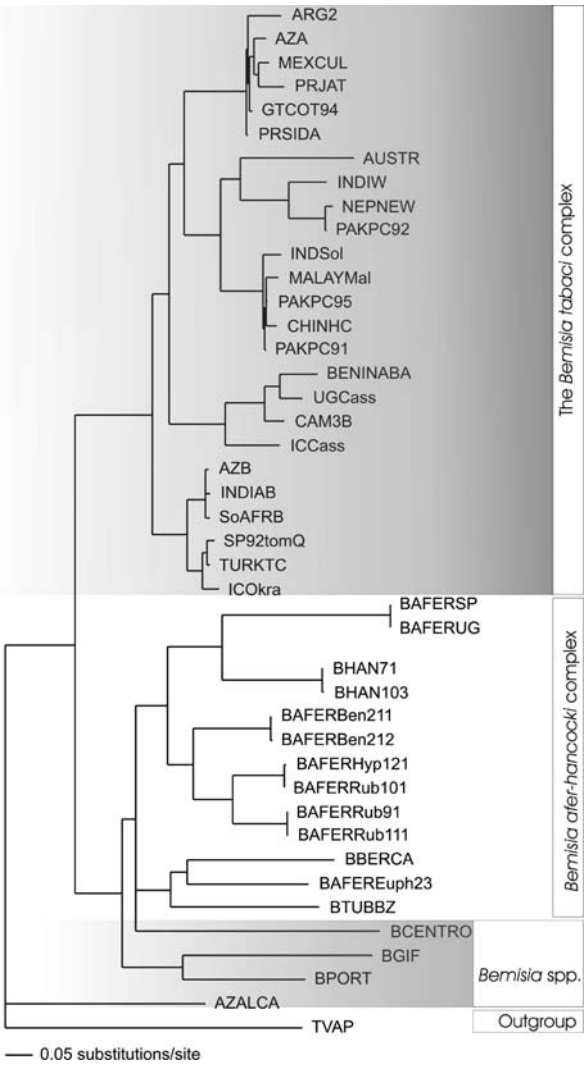



**Fig. 1.4** Phylogenetic tree based on mtCO1 molecular data showing possible relationships of various *Bemisia after* complex populations collected in the Macaronesian Islands. In addition to above, abbreviations are for *B. afer* (BAFER from different hosts (*Euphorbia, Hypericum,* or *Rubus* spp., from the Canary Islands, courtesy, E. Hernandez) or locations Spain, courtesy I. Bedford, UK or Uganda, courtesy J. Legg, IITA, Tanzania); *B. berbericola* (BBER); *B. giffordi* (BGIF) courtesy C-C Ko; *B. hancocki* (BHAN) courtesy, P. De Barro; *B. portiera* (BPORT), courtesy C-C Ko; *B. tuberculata (*BTUB from Brazil)

Separation of *Bemisia* species from other genera based on morphology alone is difficult. Adults, when known, are less useful even than pupae in most cases, nor are they much use in separating species within *Bemisia* itself (although see comments on the *afer* complex of species discussed below). Similar issues occur with many other species of whiteflies, not only in *Bemisia*- and *Pealiu*s-like species, but many other generic groups as well. In addition, many original descriptions are woefully inadequate by modern standards, adults are known for only a very small percentage of described species, and in many cases type material is not available or adequate for further study.

## Morphological Studies: A Versus B Biotype

The first phase of *Bemisia* morphological studies involved the search for differences between the A and B biotypes. Careful study of the pupal cases of both biotypes revealed no major differences, although it was noted that a submarginal seta (ASMS4) occurred on almost all specimens collected from the New World prior to 1980 (Fig. 1.5). However, this seta is very difficult to find on specimens with long setal development and margins that are indented from proximity to leaf hairs [see comments on the significance of this setal pair in Roselle et al. (1997)]. This

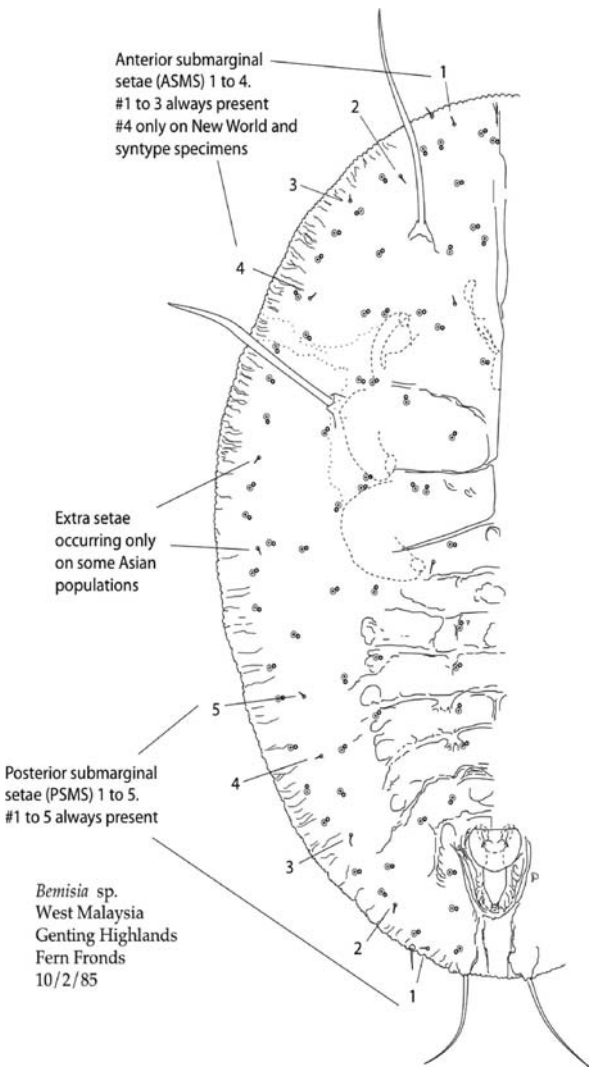



**Fig. 1.5** Outline of left side of a *Bemisia tabaci* complex specimen showing positions of normal anterior and posterior submarginal setae (ASMS, PSMS) as well as the supernumerary setae

ASMS4 setal arrangement included specimens from the United States and Central and South America, including types of the possible synonym *B. poinsettiae* Hemple from Brazil and the Jatropha biotype of *B. tabaci* from Puerto Rico (Bird 1957, Bird and Maramorosch 1978). This setal position is absent in the B biotype except that a setal base would occasionally be present on some specimens although a seta would almost never appear to be present. The ASMS4 setal position is essentially absent from specimens of all biotypes known from Europe and Africa except for its presence in the type specimens of *tabaci* from Greece. Among other things, this could suggest that the original type population of *tabaci* may have been introduced into Greece from the New World. However, work by Brown et al. (2004) using Bayesian analysis and maximum likelihood analyses of mtCO1 sequences (see the position of the haplotype "GRE" in Fig. 1.3) suggests that the original type specimens of *tabaci* have affinities with populations from China, India, and Nepal and therefore may have an originated in Asia, or that this locale represents a cross-roads (hybrid zone?) for the Asian and Mediterranean haplotypes.

Johnson and Bowden (1973) and Mound (1983) suggested that both *B. tabaci* and *B. hancocki* Corbett may have been transported across the Atlantic by man during the seventeenth or eighteenth centuries, so there is no reason why New World populations couldn't move the other direction, considering the amount of traffic between Europe and Central and South America during those periods as well as from the Orient. Multiple different scenarios can obviously be suggested here. Similarly, the South American species *B. tuberculata* may be populations of *hancocki* (Mound 1983), a possible synonym of *B. afer*. In contrast, Anderson et al. (2001) suggest that *afer* has only recently been identified from Peru; perhaps the recently discovered specimens resulted from a recent introduction from Europe or Africa.

The significance of placement of this tiny ASMS4 seta in some *tabaci* populations that are otherwise indistinguishable from other populations around the World is open to question. Because the setal positions in *tabaci* sensu strictu are variable anyway, the presence of the ASMS4 seta may not have any significance at all, and further study of *Bemisia* morphology has shown some interesting and often confusing morphological plasticity. What this all means is that pupal morphological plasticity is making it very difficult to determine just how many species of whiteflies there are in the genus *Bemisia* itself, in addition to whether there may or may not be more than one species within the *tabaci* assemblage of populations.

Aside from the extra ASMS4 submarginal seta found in New World populations and the *tabaci* syntypes, European and African populations of *tabaci* always have three pairs of submarginal setae on the cephalothorax (ASMS 1 to 3) and five pairs on the abdomen, for a total of eight pairs. Several species of *Bemisia* have been described from Africa including *afer* (Preisner and Hosny), *combreticula* Bink-Moenen, *hirta* Bink-Moenen, and *guieriae* Bink-Moenen that have 14 pairs of submarginal setae, as does *Bemisia berbericola* (Cockerell), known from the western United States. This number of submarginal setae is expressed in a number of other genera as well. In *afer* at least, these setae may be elongate or short, and are in addition to long or short dorsal setae. The 14 submarginal setae in these particular species suggest a primitive condition, and this is shown to be correct in part in

molecular-based trees developed by Campbell et al. (1995) (see Fig. 1.2) and from data adapted from Brown (Figs. 1.3 and 1.4). This then suggests that the loss of some of the marginal setae in *tabaci* could be a more recent event in evolutionary time. However, it appears that the genes necessary to control the missing setae are still present, but may be lying dormant. Such is the case with the ASMS4 setae of the A biotype, which apparently has the gene for setal expression at that location turned on. It is interesting at this point that populations of *tabaci* have been collected in Hong Kong and in Malaysia that have at least 11 pairs of submarginal setae instead of eight, three more than any other Euro-African population studied so far (Fig. 1.5). Unfortunately, these populations have not been molecularly characterized, but it may be part of the more primitive, less derived Asian forms that will be discussed here later. It is therefore apparent that, while the plasticity of the morphology of *tabaci* pupal cases is affected by host plant substrates and other environmental factors, there are also several subtle differences in morphology between populations of *tabaci* that are likely gene-linked rather than environmentally driven.

Another confusing characteristic found among *tabaci* relatives is the species *Bemisia formosana* Takahashi. The species *Bemisia graminus* David and Winstone was synonymized under *tabaci* by David and David (2001), but has been further synonymized under *formosana* by Martin and Mound (2007). In specimens identified as either *formosana* or *graminus* there appear to be no differences in chaetotaxy among pupae from other Euro-African *tabaci* biotypes including the B biotype. In addition, an intact, un-emerged adult specimen inside one of the *formosana* pupae studied here has the upper and lower compound eye connected by one ommatidium, a character that is rarely found in other whitefly adults that is frequently used to separate adult *tabaci* individuals from adults of other commonly encountered economic species. Possibly these are the reasons for the synonymy under *tabaci* by David and David (2001). However, specimens of both *formosana* and *graminus* are so far found only on grasses, and in all cases the shape of the pupal cases differ from those of *tabaci* only in that they are elongated, rather than having the typical oval shape of *tabaci* pupae. This pupal shape suggests a long and monophagus association with grasses, and suggests further that either of these entities, synonyms or not, are closely related to *tabaci* but are not the same species. It is common among species within the Sternorrhyncha including aphids, scale insects, and other whitefly species that are associated primarily with grasses, to have an elongated shape. Some whitefly examples of the grass-feeding habit are members of the genera *Aleurocybotu*s and *Vasdavidius*, the species *Aleurolobus barodensis* (Maskell) and the species *Aleurocyperus humus* Ko and Dubey, feeding on grass-like Cyperaceae. Occasional access to grasses does not illicit the elongated shape, which is borne out by specimens of the B biotype with pupae of the typical oval shape taken from sorghum leaves in the Imperial Valley of California during a very heavy valley-wide population explosion. It should be noted, however, that other species of whiteflies may have elongated puparia that are not associated with grasses, such as *Bemisia giffardi*, known from *Citrus* and many other plants, as well as *Peraccius durantae* Lima and Racca-Filho on Verbenaceae and *Dialeurolonga hoyti* Mound on *Coffea*,

to name a few. A tree adapted from unpublished work by Brown using mtCO1 molecular studies has shown that *formosana* at least is part of a clade of populations near *tabaci* from Southern Asia and is less derived than all non-Asian populations (Fig. 1.3).

Another close relative of *tabaci*, *Bemisia capitata* Regu and David, is also very similar in pupal morphology. The oval pupal case is nearly identical to *tabaci* in general outline, setation, and pore placements, but it differs in having elongated dorsal setae that are capitate, or clubbed, on the ends. The pupae also tend to be more tuberculate or rugose dorsally than does *tabaci*, especially on the dorsal midline and in a subdorsal row in line with the dorsal setae. So far, nothing has been published on the molecular aspects of this species, so its relationships to *tabaci* are not yet understood, although it should prove to be very closely related.

The assemblages of the *tabaci* biotypes, plus the extremely similar *Bemisia* species *formosana*, *graminus* and *capitata*, make up a natural grouping here called the "*tabaci* complex." But there is another assemblage of *Bemisia* species that forms a logical group as well, here called the "*afer* complex."

*Bemisia afer* was described from Egypt in 1934. *Bemisia hancocki*, which may be a synonym of *afer*, was described from Uganda in 1936. These two species, and others in this "*afer* complex," differ from species in the *tabaci* complex in several ways. The pupae of the *afe*r complex species are generally bigger in overall size than those in the *tabaci* complex, and they are more rounded in shape and do not taper gradually posteriorly. *Afer* complex species have more submarginal setae than those of the *tabaci* complex; the pore/porette (geminate pore) combinations are mostly non-adjacent, usually with the porette part of the pair closer to the margins (pore/porette combinations are always adjacent in tabaci related species, with no particular alignment with the margins); there are two pore/porette pairs on the first abdominal segment in the *afer* complex, but only one pair in the *tabaci* complex, and the caudal setae are very short compared with the longer setae of the *tabaci* complex. Like *tabaci*, *afer* complex species exhibit variable length setae based on environmental stimuli, but also show a very great development of variously shaped and environmentally induced dorsal tubercules and rugosities that do not develop much in *tabaci* related forms (Figs. 1.6, 1.7, 1.8, and 1.9). Species thought to belong to the *afer* complex have the basic morphology, chaetotaxy, and vasiform orifice shape as those illustrated for *afer* by Bink-Moenen (1983, pp. 96, 157–159). These species include at least the following: *alni* Takahashi, *antennata* Gameel, *berbericola* (Cockerell), *citricola* Gomez-Menor, *combreticula* Bink-Moenen, *guieriae* Bink-Moenen, *lauracea* Martin, Aguiar and Pita, *leakii* (Peal), *medinae* Gomez-Menor, *moringae* David and Subramaniam, *ovata* (Goux), *spiraeoides* Mound and Halsey and *tuberculat*a Bondar. Species in the genus *Asterobemisia* probably belong in this complex as well.

As in the *tabaci* complex, species within the *afer* complex are a puzzle to the whitefly taxonomist. Determining whether or not multiple species exist in this group, and how many, will require further very careful study of the morphology of both the pupae and adults, and will require intensive molecular studies as well. For example, the two New World species, *berbericola* and *tuberculata*, appear to

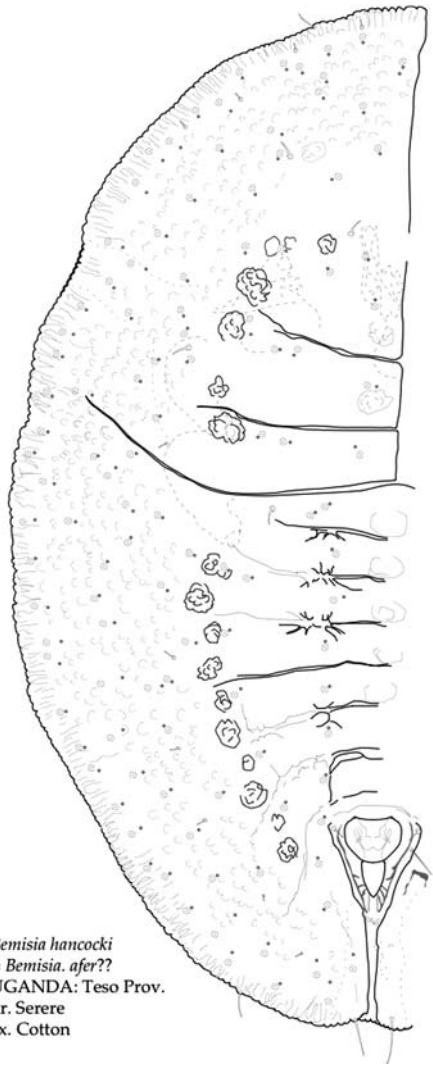



**Fig. 1.6** Illustration of left side of the puparium of *Bemisia hancocki* showing general appearance and development of rugosities

have no significant morphological differences, at least in the pupae. Nor does there appear to be any differences between these two and *afer* itself. And significantly now, *afer* is considered established in South America (Anderson et al. 2001), the former stronghold of *tuberculata*. Since *afer* has been recorded as an agricultural problem in Peru, it will probably require molecular techniques to distinguish the two if they are in fact different species.

Another example of the problem is the species *guieriae*. Bink-Moenen (1983) described this species on the basis of a pair of submedian setae on each of the thoracic segments. A careful study of other species in the complex, including *afer*,

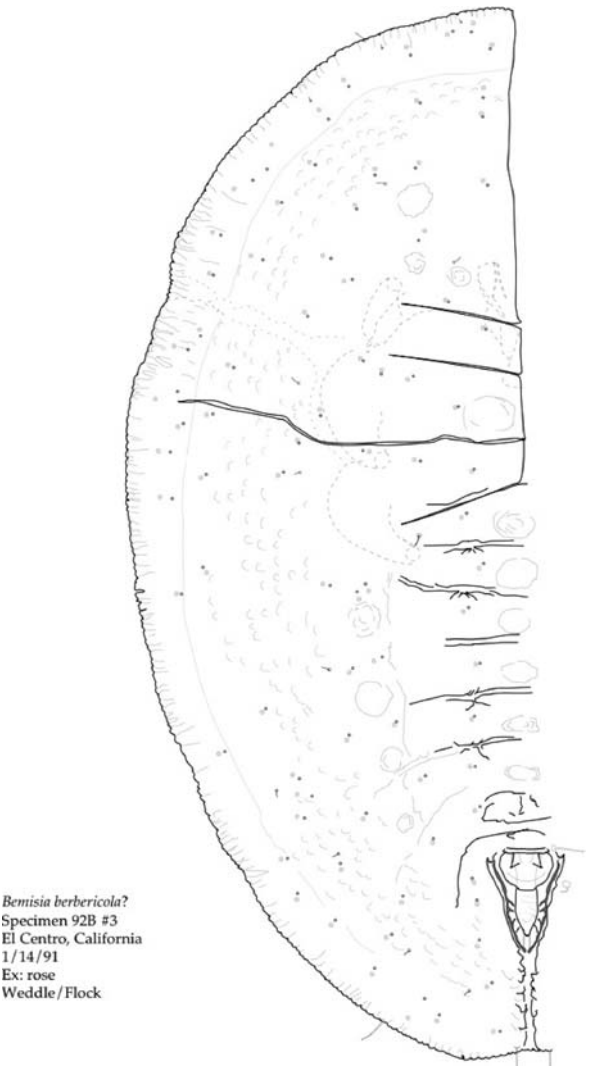



**Fig. 1.7** Illustration of the left side of the puparium of *Bemisia berbericola*, showing general form and the lack of any elongated setae or rugosities

shows that there are also setal pairs on the thorax, although usually smaller and in more lateral positions, and some but usually not all may be elongated. It has been shown that in *tabaci*, submarginal and sometimes dorsal setae will be in different locations depending on whether the seta remains small or is elongated.

We have discussed the plasticity of whitefly pupal morphology that is affected by host leaf surfaces, and possibly gene based population differences. There also may be other host plant induced variables in addition to than those causal agents already discussed. These occur in the *afer* complex of populations, and seem to involve non-setal variations including rugosities and tuberculations on the dorsal

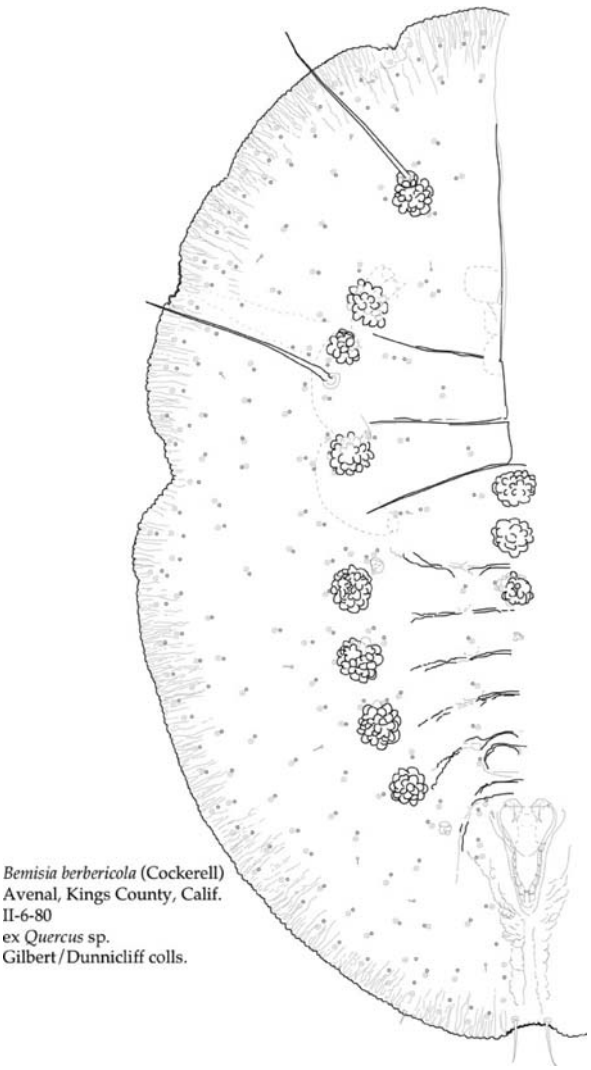



**Fig. 1.8** Illustration of the left side of puparium of *Bemisia berbericola* showing development of elongated setae and rugosities

surfaces of the pupae that appear to be host plant specific. This variation is especially apparent in populations of the *afer* complex occurring on the Macaronesian Islands, particularly on the Canary and Madeira Islands. One of these populations, on endemic Macaronesian lauraceous plant hosts including *Ocotea foetens*, *Laurus azorica* and *Persea indica*, has been described by Martin et al. (1996) as *Bemisia lauracea*. A unique feature of this whitefly is the raised, delimited submedian dorsal area, which is associated with a glassy wax secretion. However, some specimens from the same leaves do not show these raised submedian areas and are thought to be another species, possibly *B. medinae*. That may be, but the discovery that the

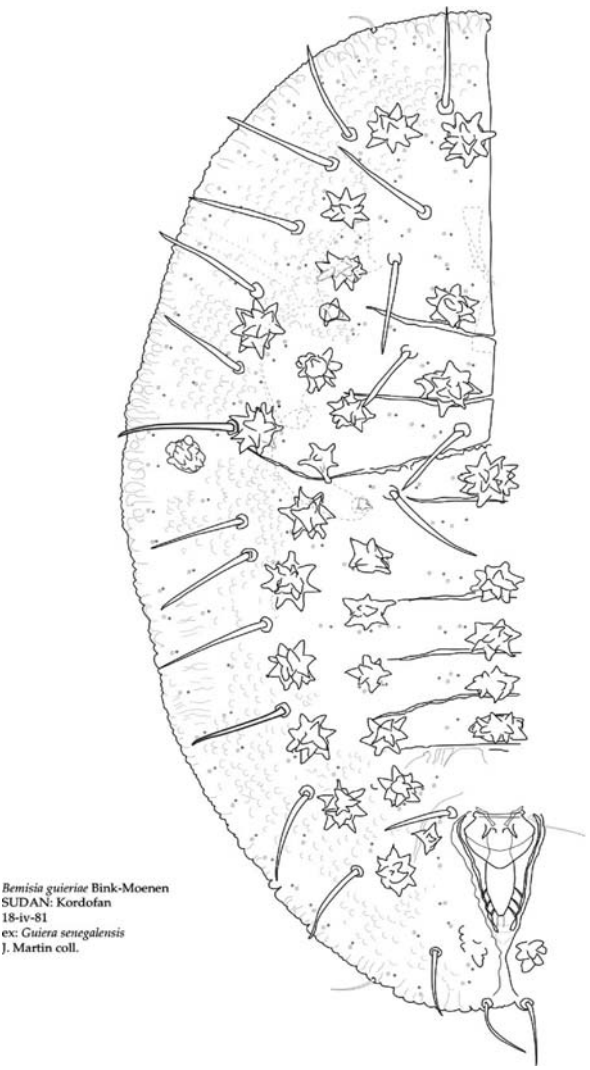



**Fig. 1.9** Illustration of the left side of puparium of *Bemisia guieriae* showing extensive enlargment of dorsal setae and star-like rugosities

division/non-division of the adult compound eyes in males and females of some *afer*-like populations on those islands raises some questions, and adults of both of these two populations on lauraceous hosts need to be studied in detail on the chance that there are differentiating eye characteristics (see below). Careful collection of adults and molecular study needs to be done in this case to resolve the species issue. Some Macaronesian adult males associated with *afer*-like pupae have single ommatidium eye connections on at least the following hosts: *Gesnouinia arborea*, *Clethra arborea*, *Chamaespartium* sp., and *Hypericum grandifolium*, although the female eyes are divided.

As with most whitefly species, adult whiteflies in the *afer* complex are not known for all species or populations, or they may not be positively associated with the pupae with which they were collected. Unlike *tabaci* populations, however, there are differences among adults in some cases. A good example is that the adults of the New World species *Bemisia berbericola* have the upper and lower eye completely divided. Two *afer*-like (*Bemisia tuberculata*?) specimens from Guyana on *Manihot* have unhatched females inside the pupae which have the upper and lower eye connected by two ommatidia. In a similar case, an unhatched male from an *afer*-like puparium from Hong Kong on *Phyllanthus cochinchinensis* has the upper and lower eye connected by one ommatidium. On various hosts in the Macaronesian Islands listed above, most of the populations there have the female upper and lower eye separate, but in the males, the eyes are connected by one ommatidium, as in both sexes of *tabaci*. But one population collected from *Euphorbia* has the upper and lower eye completely divided in both sexes, which should indicate immediately that two species occur there, even though there are no significant differences in pupal morphology between the *Euphorbia* specimens and those from other hosts. This is further borne out by molecular research carried out by Brown (Fig. 1.3). The *Euphorbia* population appears to be ancestral to the other Macaronesian *Bemisia* populations. Whether or not this population is indigenous to the islands or is an introduction is unknown. Interestingly, the *Euphorbia* specimens align with New World *afer* complex specimens (Fig. 1.4), indicating, as for several other cases, that these taxa are still confounded and that additional molecular studies are needed.

A Central American species, *Bemisia centroamericana* Martin, seems to bridge the gap between the *tabaci* and *afer* types of pupal shape. As in *tabaci*, the pupa is more oval in shape, the enlarged dorsal setae are in the same relative positions, the caudal setae are as long or longer than the vasiform orifice and the submarginal setae are missing from the central areas of the body, but present on five abdominal segments and with three submarginal setae on the cephalothorax, and the pore/porette combinations adjacent. However, unlike *tabaci*, there are two pairs of pore/porettes on the first abdominal segment, the pore/porette combinations are aligned so that the porettes are closest to the margin, not randomly aligned, and the large dorsal setae are set atop long tubercles. Also, the adult upper and lower eyes are completely divided, not connected by one ommatidium as with tabaci.

Molecularly *B. centroamericana* aligns with the *afer* complex in spite of the similarities to *tabaci* (Fig. 1.3). Were it not for intermediate morphologies of this species and of several other undescribed Southern Hemisphere species, there are actually enough morphological differences between *tabaci* and *afer* complexes to put them in separate genera. Molecular data would also seem to suggest that as a possibility. There are a number of described species, either in the genus *Bemisia*, or in the genera *Asterobemisia* and *Neobemisia*, that have the general shape and appearance of *afer* pupae. The major difference between these three genera seems to be that in some, the transverse molting suture of the pupa does not just end laterally near the margin, but continues anteriorly and usually connects with the suture from the opposite side at the midline near the front margin. This characteristic appears to be variable also, however, just like the variability in dorsal rugosities and

tuberculations that occurs so commonly in *afer*-like populations, and in fact some species with this character are still included in *Bemisia* such as *Bemisia shinanoensis* Kuwana. In recent work by Martin and Mound (2007), *Neobemisia* has been synonymized under *Asterobemisia*, and the *Bemisia* species *curvata* Qureshi, *salicaria* Danzig, *silvatica* Danzig, *yanagicola* Takahashi and the *Neobemisia* species *obenbergeri* Zahradnik, *paveli* Zahradnik and *trifolii* Danzig have now been placed in *Asterobemisia*. Wherever they belong, there is still need for molecular data to better determine the exact relationships between the species and the genera. The curved molting suture character that separates *Asterobemisia* from *Bemisia* is not well defined in some of the species such as *silvatica* and *eoa* (now equals *shinanoensis*), and this character also occurs weakly in the species *Bemisiella lespedezae* Danzig, which may belong in the genus *Bemisia* as well. Could the variable development of this molting suture be affected by environment, since many of the *Asterobemisia* species occur in colder Eurasian localities?

Besides species having typical *tabaci* or *afer* pupal characteristics as already discussed, there are several other groups of species that are included in the genus *Bemisia*, or if in other genera, probably should be included. Recent molecular work has suggested that at least some species in the Asian genus *Lipaleyrodes* should belong in the genus *Bemisia*. This genus contains nine species. *Lipaleyrodes* is unique in that the pupae have small marginal clusters of round pore-like structures grouped segmentally along the margins that apparently produce fluffy white wax. One, the species *emiliae* Chen and Ko, (Fig. 1.10) has been tested for mtCO1 enzyme and in a phylogenetic tree produced by Brown (adapted from published and unpublished data) indicates that it may be a recent derivation of the older Asian races of *B. tabaci* due to its central position in the tree between Asian forms and those of Europe, Africa and the New World. An apparently undescribed species recently introduced into New Zealand has many *tabaci*-like characteristics, but produces fluffy white wax on the dorsal disk, rather than around the submargins as is the case with *Lipaleyrodes* species. While it has the *tabaci*-like characters of adjacent pore/porette groups, a single pore/porette pair on the first abdominal segment and elongated caudal setae, it apparently has *afer*-like molecular affinities. Yet another undescribed form is known from a single specimen from Gambiers Island, in the eastern Samoan Island chain, collected in 1931. It also has *tabaci* pupal characteristics, except for having two pairs of pore/porettes on the first abdominal segment.

Two species of *Bemisia*, *hirta* Bink-Moenen and *combreticula* Bink-Moenen, exhibit some *tabaci*-like pupal morphology, except that the submarginal setae are clearly evident, much enlarged, and number from 11 to 14 pairs. Enlarged dorsal discal setae are also present in *combreticula*. They also have *afer*-like characteristics, except that most of the pore/porette combinations are adjacent and not particularly aligned with the margin (less so in *hirta*). It is not beyond reason to suspect that these two species may be synonyms, the slight differences in the pupae possibly being affected by environmental conditions. These two species are more interesting, however, because of the enlarged submarginal setae and the fact that the lingula in both species has a slight protuberance or bulge on either side just posterior to the trailing edge of the operculum. These two characteristics are very similar to those of

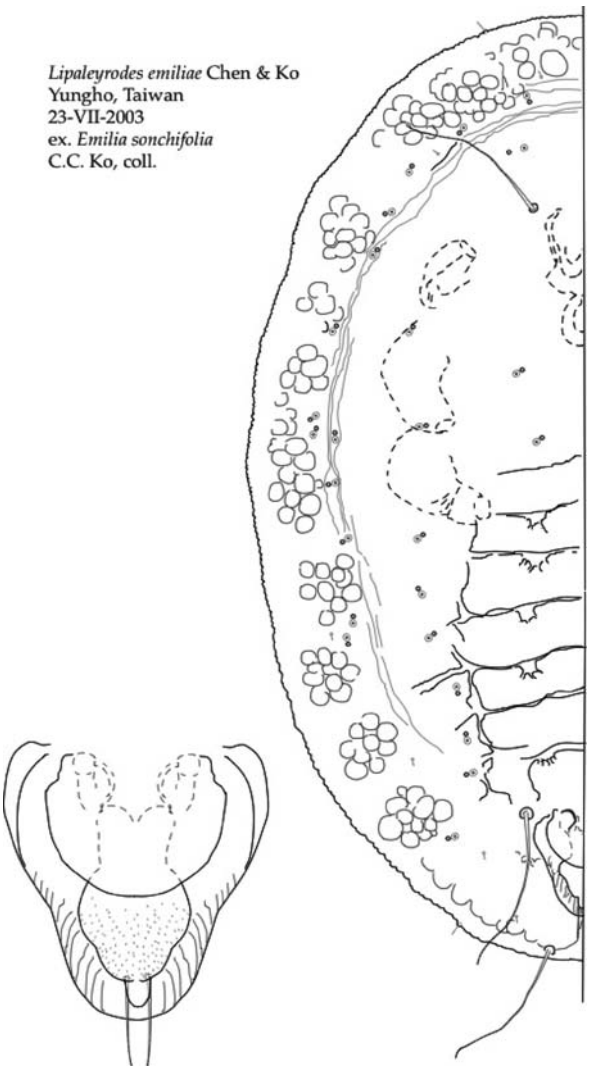



**Fig. 1.10** Illustration of the left side of puparium of *Lipaleyrodes emiliae* showing morphology similar to that of *Bemisia* species

*Parabemisia myricae* (Kuwana), which has 14 pairs of submarginal setae and triangular protuberance on either side of the lingula. Molecular data may help determine the exact nature of the morphological similarity between species in the two genera, and whether or not these three species belong in *Bemisia*.

## What All This Means

It is unfortunate that the work of Campbell et al. (1994, 1995) was not continued due to project re-assignments. Fortunately similar work is currently being carried out in Australia. Based on Campbell's 18S rDNA sequences, however, a rough,

molecular-based whitefly phylogeny is now available for some of the more common or economic whitefly genera. Even though adding new genera to this phylogenetic tree or using other gene sequences in other trees may alter the eventual position of genera in the phylogeny, this data is beginning to shed light on some of the basic relationships. As an example, follow the positions of *Pealius azaleae* and *Bemisia porteri* in the trees in Figs. 1.3 and 1.4. The Aleurodicinae, for instance, were considered by whitefly systematists to be more primitive than other whiteflies, and this is verified in the Campbell tree. Further, the genus *Trialeurodes* is of moderate size and has been placed along with two others (*Aleuroparadoxus* and *Aleurotithius*) in a separate tribe from other Aleurodine genera by Russell (1948). The DNA sequence was determined for three species of *Trialeurodes* (Campbell 1994), although, unfortunately, none of the other genera in the tribe were tested, nor were other Trialeurodines from Central and South America. However the phylogenetic tree indicates that the ancestral path for this group is closely-knit and quite old, much more so than that of the other Aleyrodines tested except possibly *Bemisia*. Most species in the genera *Trialeurodes* and *Aleurotithius* share the palisade wax layer characteristic of the pupal case with the Aleurodicines such as *Aleurodicus*. It would be interesting to know if this character was passed down from the Aleurodicines as an ancestral trait or whether it is a matter of convergence.

The world distribution of *Trialeurodes* species is of interest here. The genus has developed well in North America and the New World in general, with more than 35 species in the USA especially in California, and there are several as yet undescribed species in the western USA However, there are a few *Trialeurodes* species in Africa, Europe, Southeast Asia and more interestingly, in Australia. The occurrence of many endemic whitefly species in Australia (Martin 1999) including *Trialeurodes*, and whitefly distributions across the globe for that matter, indicates that whitefly morphology was largely established, in place, and widespread prior to the beginnings of the formation of the present day continents, and that probably species moved with the breakup of the Laurasian and Gondwonan landmasses. For a review of continental drift and how the landmasses of South America, Africa, Australia, Antarctica, New Guinea, Borneo, New Zealand and Madagascar are all interrelated, see Schlinger (1974) and Gressitt (1974). Speciation has obviously occurred within the genus *Trialeurodes* and the Trialeurodini after the present continent formation, but the presence of papillae and/or lobed lingula occurs in all species across the globe, and in *Trialeurodes* pupae at least, species morphological differences are very slight.

In this light then, the Campbell phylogenetic tree indicates that the genus *Bemisia* may be nearly as old as that of *Trialeurodes* and older than the other Aleurodine genera studied so far. So basically, the morphological characteristics of *B. tabaci* would then appear to have been in place prior to the movement of the continents, and *tabaci* itself may well have been extant at that time. This scenario would then explain why there are the various population differences between specimens collected from Africa, South America, Asia and Australia. They have essentially been isolated, except for more recent cross-introductions by man, basically by millions of years. So in *tabaci* at least, the presence or absence of certain submarginal setae seem to

suggest that these continental populations have taken on their own morphological characteristics, however slight, as well as the molecular differences indicated in the already cited work by Brown, DeBarro and others.

Are some or all of the various populations of *tabaci* around the world separate species? That question may never be answered to everyone's satisfaction. But consider some of the scenarios. First, there are numerous accounts of *tabaci* populations (or biotypes) with crossbreeding incompatibilities of one level or another (see De Barro et al. 2005, but also Caballero and Brown 2008). This is not unexpected for instance, if in fact the New World A populations have been separated from the African B populations for millions of years. Secondly, virus transmissions by these two populations seem to be different since the B biotype apparently does not transmit closteroviruses as well as the A biotype, probably for the same reasons (see Gill 1992). Thirdly, Kanmiya (2006) has found significant differences in the acoustic male mating sounds between local populations of *tabaci* and the introduced B populations in Japan. Whatever the situation is, the mtCO1 work by Brown (Fig. 1.3) clearly indicates major groupings within the *tabaci* complex that align with current continental placements, recent man-facilitated distributional changes of the B and Q populations aside.

Following the description of the species *Bemisia argentifolii* by Bellows et al. (1994), much discussion has taken place on the validity of this entity as a true species. Publications by Rosell et al. (1997), Brown et al. (1995b) and De Barro et al. (2005) have discussed the issue at length, with Brown and predecessors referring to *argentifolii* and other populations as biotypes, and De Barro calling *argentifolii* a race of *tabaci*, and further suggesting that the name *argentifolii* be dropped as a synonym of *tabaci*. While that move for synonymy may be premature, it is none the less probably necessary until a better understanding of whitefly morphology, systematics and phylogeny can be accomplished, especially in light of all the other biotypes (species?) of the *tabaci* complex that are now known, as well as the very confusing *afer* complex. As mentioned, most of the subfamily Aleurodinae is in serious need of in-depth study in order to properly characterize generic and species placements.

Comments by authors mentioned above aside, other aspects of the interrelationships of these entities, be they biotypes, races or species, should be mentioned. These aspects are not meant to resolve the issues either way, but to point out that there are still many things to be learned, not only with the *tabaci* group as a whole, but with other *Bemisia* species and other Aleyrodines. That *tabaci* is not alone in causing confusion within the field of whitefly systematics is the main focus of these discussions. One seemingly very essential aspect in all of this that until now has not been discussed is the role of evolution and biogeography, and how it seems to have affected the biology and morphology of these various entities.

As for the discussion of what the biotypes actually are, and if more than one species is involved, consider first the generally accepted definition of a species. The definition has been repeated in several similar wordings in many books and publications over the years. But take for example a recent rendition of the meaning of "species" from Gordh and Headrick (2001): (1) The primary biological unit,

debatably an actual thing or a purely subjective concept; or (2) A static moment in the continuum of life; an aggregation of individuals similar in appearance and structure, mating freely and producing young that themselves mate freely and bear fertile offspring resembling each other and their parents, including all varieties and races. Definition 1 above rather strongly sets the groundwork for all of this, i.e. "a purely subjective concept." Basically what is a species?

Based on the second definition, there are obvious mating incompatibilities between the various geographically oriented populations. Perring et al. (1993) first noted this problem between the A and B biotypes, although Caballero (2007) and Caballero and Brown (2008) have managed to cross-breed three biotypes in the laboratory by varying environmental conditions, and Duffus (presented at a UC Extension symposium on *Bemisia*, (ca. 1991) and published on-line by Hsing-Yeh et al. 1992) showed electrophoretic evidence for hybrids obtained by putting large numbers of both biotypes together in the same cages. De Barro et al. (2005) have, however, mentioned that there are examples of crossbreeding difficulties among representatives of four major groupings (clades) examined by others thus far. Whatever the reasons for some of the interbreeding barriers (see Brown Chapter 2), and in spite of the fact that they may be overcome in the laboratory, there still are some deterrents that exist, and whether they exist in the field is still yet to be determined. The point is that similar morphologies aside, there are barriers to free mating within *tabaci* populations, and molecular data seem to coincide with where these barriers were actually found. The issues are further complicated by the discovery in some populations of *tabaci* that have inclusions of *Wohlbachia* and Cardinium spp. (Brown et al. 1998a, b; Caballero and Brown, 2008) and other symbionts that are known to affect insect fertility through cytoplasmic incompatibility.

## Conclusions

The question has been whether or not *tabaci*, *argentifolii* and any other of these biotypes or haplotypes (variants) are actually separate species. They have been placed in all sorts of categories including types, strains, races, populations, biotypes and so on. And while biotypes have not been suggested so far, the same can likewise be said for the many described species within the *B. afer* complex. The answer is probably in the anthropomorphic concept of what a species really is. Mating incompatibilities alone would suggest, apart from molecular evidence, that this is a complex of cryptic species that have evolved over time in isolation without the need to change morphologically. They are now being introduced into other areas of the world where they are exerting economic influence while away from natural controls, and in some cases, apparently displacing other populations of endemic forms, as witnessed by the disappearance of the A biotype from most areas of North and Central America. In light of some of these studies, the apparent lack of differentiating morphological characteristics should not necessarily be a deterrent to species separation in whiteflies.

From a taxonomist's viewpoint, the *B. tabaci* complex conundrum merely points out what we have already known about whitefly systematics as a whole but so far cannot solve. It also has clearly defined, however, some of the problems for other scientists to see and understand. Whether *tabaci* populations are separate species or not, economic control of the various populations have required separate and individual research into control measures for each, as if they were in fact separate. Molecular research has aided in clarifying where to direct the search, and to suggest where various controls need to be applied. Molecular research has also indicated the probable site of origin of several of the "biotypes" which may eventually aid in better implementation of natural controls. Traditional taxonomists have not been able to provide these answers, so we need this help. Molecular techniques appear to be solving some problems in whitefly phylogenies and most likely will solve more if they are further studied in synchrony with more thorough morphological studies of at least pupae and adults. This is indeed a fertile field for further research.

## Literature Cited

Anderson PK, Martin JH, Hernandez P, Lagnaoui A. 2001. *Bemisia afer* sens. lat (Homoptera: Aleyrodidae) outbreak in the Americas. *Fla. Entomol.* 84:316–317.

Bedford ID, Briddon RW, Brown JK, Rosell RC, Markham PG. 1994. Geminivirus transmission and biological characterization of *Bemisia tabaci* (Gennadius) biotypes from different geographic regions. *Ann. Appl. Biol*. 125:311–325.

Bellows TS, Perring TM, Gill RJ, Headrick DH. 1994. Description of a species of *Bemisia* (Homoptera: Aleyrodidae). *Ann. Entomol. Soc. Am.* 87:195–206.

Bink-Moenen RM. 1983. Revision of the African whiteflies (Aleyrodidae). *Monografieën van de Nederlandse Entomologishe Vereniging, Amsterdam* 10:1–211.

Bird J. 1957. A whitefly-transmitted mosaic of *Jatropha gossypifolia*. *Tech. Paper. P. R. Agric. Exp. Stn.* 22:1–35.

Bird J, Maramorosch K. 1978. Viruses and virus diseases associated with whiteflies. *Adv. Virus Res.* 22:55–110.

Brown JK, Caballero R, Torres-Jerez I. 1998a. First report of *Wolbachia* infection in *Bemisia tabaci* (Genn.) and a possible case of cytoplasmic incompatibility as evidenced by unidirectional mating between uninfected and infected whiteflies. In *Proceedings of The Whitefly-Geminivirus Workshop*, Managua, Nicaragua, October 24–30, 1998. p. 217.

Brown JK, Coats S, Bedford ID, Markham PG, Bird J. 1992. Biotypic characterization of *Bemisia tabaci* populations based on esterase profiles, DNA fingerprinting, virus transmission, and bioassay to key host plant species. *Phytopathology* 82:1104.

Brown JK, Coats S, Bedford ID, Markham PG, Bird J, Frohlich DR. 1995a. Characterization and distribution of esterase electromorphs in the whitefly, *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae). *Biochem. Genet.* 33:205–214.

Brown JK, French R, Rogan D, Caballero R, Bird J. 2004. Genetic variability and phylogeographical relationships of the *Bemisia tabaci* complex based on the mt cytochrome oxidase I gene. In *Proceedings 2nd European Whitefly Symposium*, Cavtat, Croatia, October 5–9, 2004.

Brown JK, Frolich DR, Rosell RC. 1995b. The sweetpotato or silverleaf whiteflies: biotypes of *Bemisia tabaci* or a species complex? *Annu. Rev. Entomol.* 40:511–534.

Brown JK, Perring TM, Cooper AD, Bedford ID, Markham PG. 2000. Genetic analysis of *Bemisia* (Homoptera: Aleyrodidae) populations by isoelectric focusing electrophoresis. *Biochem. Genet.* 38:13–25.

Brown JK, Torres-Jerez I, Caballero R, Brentt D. 1998b. First report of *Wolbachia* spp. in the whitefly, *Bemisia tabaci*, and unidirectional production of female offspring due to apparent

cytoplasmic incompatibility. In *Third International Symposium on Molecular Insect Science,* Snowbird, Utah, June 5–10, 1998.

Caballero R. 2007. Systematics of the *Bemisia tabaci* complex and the role of endosymbionts in reproductive compatibility. Ph.D. dissertation, The University of Arizona, 107 pp.

Caballero R, Brown JK. 2008. Positive evidence for interbreeding and differential gene flow between three well characterized biotypes of the *Bemisia tabaci* complex (Gennadius) (Hemiptera: Aleyrodidae) excludes geographic and host barriers as isolating factors. *J. Insect Sci.* 8:10–11.

Campbell BC, Steffen-Campbell JD, Gill RJ. 1994. Evolutionary origin of whiteflies (Hemiptera: Sternorhyncha: Aleyrodidae) inferred from 18S rDNA sequences. *Insect Mol. Biol.* 3:73–88.

Campbell BC, Steffen-Campbell JD, Gill RJ. 1995. Origin and radiation of whiteflies: an initial molecular phylogenetic assessment. In *Bemisia 1995: Taxonomy, Biology, Damage, Control and Management*, ed. D Gerling, RT Mayer. Andover, Hants: Intercept Ltd.

Coats SA, Brown JK, Hendrix DL. 1994. Biochemical characterization of biotype-specific esterases in the whitefly *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae). *Insect Biochem. Mol. Biol.* 24:723–728.

Costa HS, Brown JK. 1990. Variability in biological characteristics, isozyme patterns and virus transmission among populations of *Bemisia tabaci* (Genn.) in Arizona. *Phytopathology* 80:888.

Costa HS, Brown JK. 1991. Variation in biological characteristics and in esterase patterns among populations of *Bemisia tabaci* (Genn.) and the association of one population with silverleaf symptom development. *Entomol. Exp. Appl.* 61:211–219.

Costa HS, Brown JK, Sivasupramaniam S, Bird J. 1993. Regional distribution, insecticide resistance, and reciprocal crosses between the 'A' and 'B' biotypes of *Bemisia tabaci*. *Insect Sci. Appl.* 14:127–138.

David BV. 1987. Biosystematics of Aleyrodidae (Homoptera: Insects). *Proc. Indian Acad. Sci (Anim. Sci.)* 96:583–586.

David PMM, David BV. 2001. Revision of whiteflies (Aleyrodidae: Homoptera) infesting rice in India. *Entomon. Kariavattom* 26(Special Issue):353–356.

De Barro PJ, Trueman JWH, Frolich DR. 2005. *Bemisia argentifolii* is a race of *B. tabaci* (Hemiptera: Aleyrodidae): the molecular differentiation of *B. tabaci* populations around the world. *Bull. Entomol. Res.* 95:193–203.

Dennehy TJ, DeGain BA, Harpold VS, Brown JK, Morin S, Fabrick JA, Byrne FJ, Nichols RL. 2006. New whitefly biotype with high levels of resistance to insecticides. The University of Arizona – Cooperative Extension Report, Tucson, Arizona. 32pp.

Deshpande VG. 1933. On the anatomy of some British Aleurodidae. *Trans. R. Entomol. Soc. London* 81:117–132.

Gawel NJ, Bartlett AC. 1993. Characterization of differences between whiteflies using RAPD-PCR. *Insect Mol. Biol.* 2:33–38.

Gill RJ. 1992. A review of the sweetpotato whitefly in southern California. *Pan-Pac. Entomol.* 68:144–152.

Gordh G, Headrick D. 2001. *A Dictionary of Entomology*. Wallingford, Oxon: CABI Publishing, 1032pp.

Gressitt JL. 1974. Insect biogeography. *Annu. Rev. Entomol.* 19:293–321.

Hsing-Yeh L, Duffus JE, Cohen S. 1992. Work Group on *Bemisia tabaci* Newsletter No. 6. November, Tropical Whitefly IPM Project. www.tropicalwhiteflyipmproject.cgiar.org/bemisianewsletter06.jsp

Husain MA, Trehan KN. 1933. Observations on the life-history, bionomics and control of the whitefly of cotton (*Bemisia gossypiperda* M and L). *Indian J. Agric. Sci.* 3:701–753.

Johnson CG, Bowden J. 1973. Problems related to the transoceanic transport of insects, especially between the Amazon and Congo areas. In *Tropical Forest Ecosystems in Africa and South America*, ed. BJ Meggers, ES Ayensu, WD Duckworth, pp. 207–222. Washington, DC: Smithsonian Institution.

Kanmiya K. 2006. Mating behaviour and vibratory signals in whiteflies (Hemiptera: Aleyrodidae). In *Contemporary Topics in Entomology Series: Insect Sounds and Communication*, ed. S Drosopoulous, M Claridge, pp. 365–379. London: Taylor & Francis.

Martin JH. 1999. The whitefly fauna of Australia (Sterorrhyncha, Aleyrodidae), a taxonomic account and identification guide. Technical Paper, CSIRO Entomology. *Canberra* 38:1–197.

Martin JH. 2003. Whiteflies (Hemiptera: Aleyrodidae) – their systematic history and the resulting problems of conventional taxonomy, with special reference to descriptions of *Aleyrodes proletella* (Linnaeus, 1758) and *Bemisia tabaci* (Gennadius, 1889). *Entomol. Gaz.* 54:125–136.

Martin JH, Aguiar AMF, Pita MT. 1996. Aleyrodidae of Madeira – descriptions of three new species and notes on a pan-Mediterranean species of Aleurotrachelus. *J. Nat. Hist.* 30:113–125.

Martin JH, Mound LA. 2007. An annotated check list of the world's whiteflies (Insecta: Hemiptera: Aleyrodidae). *Zootaxa* 1492:1–84.

Mohanty AK, Basu AN. 1986. Effects of host plants and seasonal factors on intraspecific variations in pupal morphology of the whitefly vector *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae). *J. Entomol. Res.* 10:19–26.

Mound LA. 1963. Host correlated variation in *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae). *Proc. R. Entomol. Soc. Lond. A* 38:171–180.

Mound LA. 1983. Biology and identity of whitefly vectors of plant pathogens. In *Plant Virus Epidemiology*, ed. RT Plumb, JM Thresh, pp. 305–313. Oxford: Blackwell Scientific Publications.

Mound LA. 1984. Zoogeographical distribution of whiteflies. *Curr. Topics Vector Res.* 2:185–197.

Mound LA, Halsey SH. 1978. *Whitefly of the World*. Chichester: British Museum (Natural History)/John Wiley & Sons, 340pp.

Neil JW, Bentz J. 1999. Evidence for the stage inducing phenotypic plasticity in pupae of the polyphagus whiteflies *Trialeurodes vaporariorum* and *Bemisia argentifolii* (Homoptera: Aleyrodidae) and the raison d' être. *Ann. Entomol. Soc. Am.* 92:744.

Perring TM, Cooper A, Kazmer DJ. 1992. Identification of the poinsettia strain of *Bemisia tabaci* (Homoptera: Aleyrodidae) on broccoli by electrophoresis. *J. Econ. Entomol.* 85:1278–1284.

Perring TM, Cooper A, Rodriguez RJ, Farrar CA, Bellows TS, Jr. 1993. Identification of whitefly species by genomic and behavioral studies. *Science* 259:74–77.

Rosell RC, Bedford ID, Frolich DR, Gill RJ, Brown JK, Markham PG. 1997. Analysis of morphological variation in distinct populations of *Bemisia tabaci* (Homoptera: Aleyrodidae). *Ann. Entomol. Soc. Am.* 90:575–589.

Russell LM. 1948. The North American species of whiteflies of the genus *Trialeurodes*. *Misc. Publ. U.S. Dept. Agric.* 635:1–85.

Russell LM. 1957. Synonyms of *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae). *Bull. Brooklyn Entomol. Soc.* 52:122–123.

Sampson WW. 1943. A generic synopsis of the Hemipterous superfamily Aleyrodoidea. *Entomol. Americana* 23:173–223.

Sampson WW. 1947. Additions and corrections to "a generic synopsis of the Aleyrodoidea". *Bull. Brooklyn Entomol. Soc.* 42:45–50.

Sampson WW, Drews EA. 1956. Keys to the genera of the Aleyrodinae and notes on certain genera. (Homoptera: Aleyrodinae). *Ann. Mag. Nat. Hist.* 9:689–697.

Schlinger EI. 1974. Continental drift, Nothophagus, and some ecologically associated insects. *Annu. Rev. Entomol.* 19:323–343.

Sundararaj R, David BV. 1992. Host correlated variation in *Dialeurodes kirkaldyi* (Kotinsky) (Aleyrodidae: Homoptera: Insecta). *Hexapoda* 4:33–38.

# Chapter 2
# Phylogenetic Biology of the *Bemisia tabaci* Sibling Species Group

**Judith K. Brown**

## Introduction

Whiteflies are classified in the family Aleyrodidae (Sternorrhyncha: Hemiptera [suborder Homoptera]) (Mound 1984; Mound and Halsey 1978). The closest relatives to whiteflies are aphids, mealybugs, psyllids, and scales, which all feed using piercing and sucking mouthparts (Martin 1987, 2003; Martin and Mound 2007). Reproductive modulation is one of the many examples of plasticity in the Aleyrodidae, an ancient insect family. They are unique among most of their close relatives in employing a haplodiploid sex determination system, in which fertilized eggs yield females and males are produced from unfertilized eggs (Blackman and Cahill 1998; Schrader 1920). Thus, all male offspring inherit only the maternal genome, whereas female offspring inherit genes from both parents. The sex ratio is modulated by increased production of females when males are abundant (see Byrne et al. 1990).

Haplodiploid sex determination, combined with infection by bacteria such as *Wolbachia* and *Cardinium* spp. that cause cytoplasmic incompatibility (CI), could result in the demise of the germline, or at the least, a severely bottlenecked population due to increased inbreeding. CI in *B. tabaci* is expressed as a reduction in the number of female offspring resulting from crosses between infected males and uninfected females (or females infected with different bacterial strains) (Caballero 2007; Stouthamer et al. 1999). This process could decrease genetic diversity while also reducing fitness and altering other traits which *B. tabaci* is widely known to employ, presumably for adaptive advantage. How the interactions between the host and its prokaryotic passengers influences genetic diversity and evolution in *B. tabaci* is not well understood.

Whiteflies, like aphids and many other plant-feeding hemipterans, feed on plant sap, which is deficient in certain essential amino acids. A hallmark of this group is that they harbor species-specific mutualistic endosymbionts (referred to

J.K. Brown (✉)
School of Plant Sciences, The University of Arizona, Tucson, AZ 85721, USA
e-mail: jbrown@ag.arizona.edu

P.A. Stansly, S.E. Naranjo (eds.), *Bemisia*: Bionomics and Management of a Global Pest,
DOI 10.1007/978-90-481-2460-2_2, 

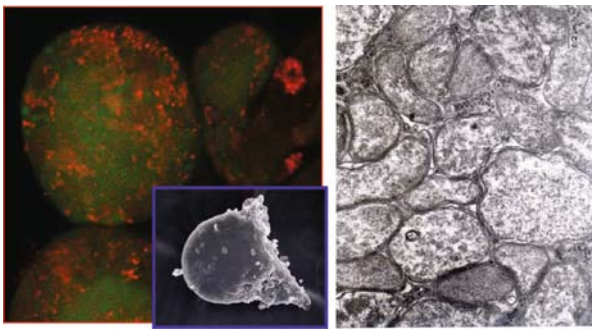


**Fig. 2.1** Bacteriomes by SEM (*left*, *color* and *black* and *white inset*) containing the primary endosymbionts (*right*) of the whitefly *B. tabaci* B biotype (Photo courtesy E Zchori-Fein and JK Brown)

as "primary") having a genome reduced in size compared to free-living counterparts that is housed in a specialized structure called a bacteriome. The bacteriome (Fig. 2.1) is tightly associated with whitefly ovaries from which founder bacteria migrate to the immature egg and are passed on to the offspring (Costa et al. 1996). Based on studies of other homopterans (hemipterans), primary symbionts are less complex in comparison to free-living bacteria (Douglas 1998; Thao and Baumann 2004a, b; Zchori-Fein and Brown 2002). These insect host-bacterial complexes have evolved a mutualistic relationship in which each contributes to the survival of the other. Tightly co-evolved associations have been corroborated for certain whitefly species based on evidence for congruent evolution of both host and bacterial genes (Campbell 1993; Clark et al. 1992; Zchori-Fein and Brown 2002). See Rosell et al. (Chapter 5) for further discussion on endosymbionts.

The *B. tabaci* complex is a "cryptic species" in that its members exhibit a range of genetic variation and are collectively considered a sibling species group, although morphological characters in the pupal case (Fig. 2.2) useful for identification to species lack variation sufficient for finer scale taxonomic purposes. This external morphology for the species complex is thought to have remained static since ancient times (Gill 1990; Martin 2003; Rosell et al. 1997). Variants of *B. tabaci* for which biological (phenotypic) differences are recognized have most recently been referred to as "biotypes", and previously, as races (Bird 1957; Bird and Sanchez 1971; Bird and Maramorosch 1975, 1978). More than fifteen biotypes have been characterized to varying degrees in biological and genetic terms, and a number of additional variants are recognized but are incompletely studied. In fact, the majority of biological variants that occur throughout the world probably remain unstudied. The best studied phenotypic differences among *B. tabaci* biotypes include host-specialization, host range of polyphagous haplotypes, dispersal behavior, mating behavior (Fig. 2.3), reproductive compatibility, differential resistance to distinct classes of insecticides, variable efficiency in the transmission of plant viruses, and secondary endosymbiont composition.


**Fig. 2.2** Pupal case containing pre-eclosed adult and key morphological characters used for morphological identification of whiteflies to genus and species. (Photo courtesy R Rosell, I Bedford, and JK Brown; see Rosell et al. 1997)

**Fig. 2.3** Adult male and female *B. tabaci* on a leaf with one pair mating (Courtesy M Hadjistylli)

Aside from certain basic knowledge about whitefly species included in higher-level taxonomic studies, there have been far fewer studies of whiteflies at the species level compared to homopterans that predominantly inhabit temperate zones (Campbell et al. 1994; 1996; Gill 1990,1992; Neil and Bentz 1999). As a result, the evolutionary origin or basal and derived taxa have not been ascertained, and so the evolutionary history of *B. tabaci* is not yet understood. Relatively few molecular markers are available for inferring the evolutionary history of *B. tabaci*. At present only the 16S rRNA, the cytochrome oxidase I genes in the mitochondrial genome, and the nuclear ribosomal intergenic spacer 1 (ITS1), a non-coding sequence, have been explored.

Microsatellite markers have been developed to study population structure (Hadjistylli et al., Chapter 3), revealing broad geographic affiliations and levels of substructure not yet revealed for the sibling group. A study of the Asian-Pacific region revealed robust geographic structure accompanied by reduced or negligible gene flow, suggesting that as many as 10 major groupings (sibling species?) could occur there (DeBarro et al. 2005). Mound (1993) postulated that *B. tabaci* could follow one of two scenarios based on the duration that these whiteflies have inhabited earth, i.e. at least 1.2 MY (Schlee 1970). Depending on extent of isolation, *B. tabaci* would either be limited in its ecological and therefore geographical distribution and would have low intraspecific genetic variability, or conversely, it would have a broad distribution across variable ecological geographic boundaries, and wide genetic variation. As predicted by Mound, the latter scenario seems to best explain the evolutionary history of *B.tabaci* and is supported by population and molecular phyologenetic studies (see Hadjistylli et al. Chapter 3). Even so, more extensive population studies and new and more informative molecular markers are needed to facilitate deeper phylogenetic predictions. Exciting times lie ahead!

This chapter will review the present status of the phylogenetic biology of the *B. tabaci* sibling species group (see Gill and Brown, Chapter 1), and highlight well known examples of phenotypic variation and genetic diversity revealed by a limited number of molecular markers. Although insufficient in the larger sense, molecular studies have revealed the existence of many *B. tabaci* haplotypes that group phylogeographically with their extant origin. Furthermore, a bar coding system employing the cytochrome oxidase I gene to identify haplotypes, coupled with definitive biological characteristics, can provide the necessary criteria to define a biotype. Thus, at least some commonly occurring variants are distinguishable for the first time.

## The *Bemisia tabaci* Sibling Species Group or Assemblage

### *Historical Underpinnings*

The terms biotype, strain, or race have been used to differentiate morphologically identical organisms that exhibit distinct phenotypic behaviors. For *B. tabaci*, variants have been distinguished based on host range (Abdullah et al. 2006; Attique et al. 2003; Bayhan et al. 2006; Bedford et al. 1994; Burban et al. 1992; Bird 1957; Bird and Brown 1998; Butler and Brown 1985; Carabali et al. 2005; Costa and Brown 1990, 1991; Costa et al. 1991a, b; Costa and Russell 1975; Legg 1994, 1996; Maruthi et al. 2001, 2002; Osmondi et al. 2005; Sseruwagi et al. 2006; Servin-Villegas et al. 2006; Simon et al. 2003; Thompson 2003) life history traits (Bethke et al. 1991; Costa et al. 1991b; Demichelis et al. 2005; Viscarret et al. 2003), and other evidence for phenotypic variation, including differential virus transmission, which influences the epidemiology of viral outbreaks and their control (Brown et al. 1995, 2004a; Brown and Bird 1996; Brown 2007; Legg et al. 2002, 2008).

The term "host race" was first applied to distinguish between two variants of *B. tabaci* by Bird (1957) who demonstrated monophagous feeding behavior in *B. tabaci* associated with a polyphagous habit of another endemic population, the "Sida race" (SIDA-PR) (after *Sida cordifolia*) (Bird and Brown 1998). The two races also differed in having distinct virus-vector relationships owing primarily to host range preference. This seminal study provided the first evidence for *B. tabaci* as a key host range determinant of begomoviruses (see Bird and Maramorosch 1978).

The first major outbreak in the Americas of a then uncharacterized race or biotype of *B. tabaci* occurred in the summer of 1981 in the southwestern USA (Brown 1990; Brown and Nelson 1984, 1986; Butler et al. 1986; Gill 1992; Rosemeyer et al. 1986), later becoming known as the Arizona "A" biotype (AZ-A) (Costa and Brown 1990, 1991), which represented the local or endemic haplotype. The A biotype reached unprecedented regional levels, resulting in widespread infection by whitefly-transmitted viruses in cotton and vegetable crops (Brown 1990, 1994; Brown and Bird 1992; Gill 1992; Gill and Brown Chapter 1). This outbreak has been correlated with the possible development of insecticide resistance, based on known exposure of endemic populations to insecticides used for primary cotton pest control. Mild winter temperatures together with residual insecticide resistance in the local *B. tabaci* population may possibly have contributed to the population explosion in southwestern USA desert cropping systems (Anthony et al. 1995; Coats et al. 1994; Costa et al. 1993; Morin et al. 2002).

## The Invasion of the B Biotype

A second outbreak of *B. tabaci* in the USA occurred first in Florida during 1985–1986 and was characterized by the unprecedented ability of *B. tabaci* to colonize poinsettia plants, *Euphorbia pulcherrima* (Willd), a previously unreported species for *B. tabaci* in Florida (Hamon and Salguero 1987). In 1988, *B. tabaci* was identified in Arizona on poinsettia (Byrne and Miller 1990), also a non-host of *B. tabaci* endemic there (unpublished data). General esterase patterns were used to investigate genetic polymorphism between the two populations (Figs. 2.4 and 2.5) making it possible for the first time to differentiate the two haplotypes, referred to as the "A" and "B" biotypes (Costa and Brown 1990, 1991). Thereafter, the use of "biological type" instead of "race" became the accepted way to refer to phenotypically distinguishable *B. tabaci*. However, the intent of the term "biotype" did not fundamentally change the evolving paradigm that phenotypic differences were widespread in *B. tabaci*.

Follow on studies explored differences with respect to host range, fecundity, and the induction of "silvering" symptoms in pumpkin (*Cucurbita* spp.) plants, a phenotype that had not been associated with New World *B. tabaci* and so was a useful indicator for the B biotype (in the Tropical Americas) (Costa and Brown 1990; 1991). In Florida, a link had been made between *B. tabaci* infestations and silvering of squash leaves (Yokomi et al. 1990), and similarly, with irregular ripening of tomato fruit (Schuster et al. 1990), and elsewhere with stem whitening or blanching of cole crops (Brown et al. 1991) among others. Genetic analysis (Costa et al. 1993)

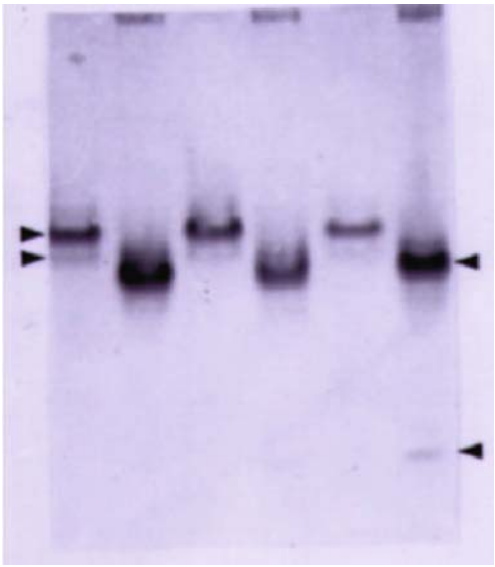


**Fig. 2.4** General esterase banding patterns for the A and B biotypes that allowed for the first genetic differentiation. Major (*upper*) and minor (*lower*) bands are denoted by the *arrows* (Courtesy JK Brown and HS Costa)

confirmed that *B. tabaci* in Florida and Arizona were identical, but that the "newcomer" was not likely a native haplotype. The B biotype next invaded the Americas south of the USA including the Caribbean region, and by 1990–1991 had displaced the A biotype in the southwestern USA (Brown and Bird 1998; Brown et al. 1995a; Costa et al. 1993). It is assumed that the endemic Florida *B. tabaci* was similarly displaced, however, unfortunately no samples of *B. tabaci* from Florida have been found from which DNA can be isolated, and so the original Florida haplotype has not been compared to others genetically.

The novel silvering phenotype (Fig. 2.6) was used in the tropical Americas as a reliable field "diagnostic" for the B biotype as it rapidly invaded the region. Since the first report of silverleaf associated with the B biotype, two additional polyphagous populations have been discovered that also induce silvering in *Cucurbita* species, one from Uganda, referred to as non-B (Sseruwagi et al. 2005, 2006) and the other from the Reunion Islands, designated the "MS" biotype (Delatte et al. 2005). Phylogenetic analysis (COI) of the three population indicates that the B biotype and the non-B/MS haplotypes occupy different sister clades within the major North Africa-Mediterranean-Middle East clade (Delatte et al. 2005; Sseruwagi et al. 2005).

## Taxonomic Conundrums

Genetic and behavioral studies led one group to propose that the B biotype differed sufficiently from the SW-USA endemic A biotype to warrant its recognition as the distinct species, *B. argentifolii* (Bellows and Perring) (Bellows et al. 1994). This was based on lack of evidence for gene flow between A and B biotypes, and higher than expected (but possibly borderline) genetic differentiation (RAPDs and

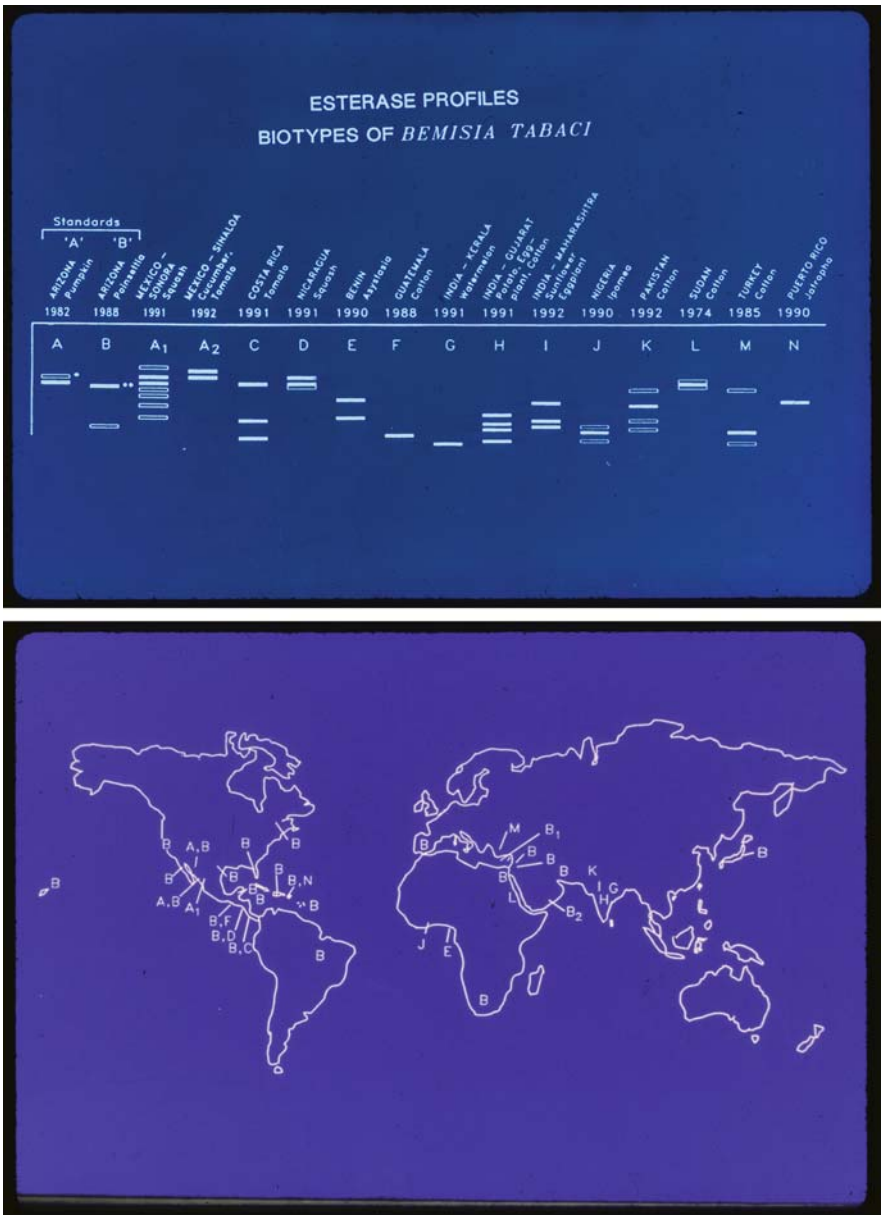



**Fig. 2.5** Esterase profiles (*upper panel*) and a map showing the origin of each haplotype colony (*lower panel*), revealing the first greater-than-expected genetic polymorphisms in *B. tabaci* (Courtesy, JK Brown, S Coats, HS Costa, and RC Rosell)

**Fig. 2.6** Silvering induced by B biotype feeding on the majority of *Cucurbita* species leaves, including pumpkin, develop subsequent to the colonization of lower leaves by whitefly immatures (and adults) (Costa and Brown 1990). Recent studies have shown that silvering-inducing haplotypes also occur among Uganda non-B (Sseruwagi et al. 2005) and Reunion Island (Ms biotype) (Delatte et al. 2005)

isozymes) (Bellows et al. 1994; Perring et al. 1993). The presence of a fourth instar (Fig. 2.2) seta in position ASMS4 on the A biotype and its absence on B biotype specimens was also cited. The differentiation values could possibly have been less significant than initially interpreted, given non-equilibrium conditions. However, further studies have provided convincing evidence for higher than expected genetic diversity within this sibling species group. Presently a large (and expanding) number of morphologically indistinguishable biotypes are recognized. However, separation of a single variant from the complex does not seem useful or informative, unless many more follow. One seemingly more manageable approach has been to consider *B. tabaci* as a sibling species group (see Gill and Brown Chapter 1). Indeed a number of species recognized in the genus *Bemisia* are difficult to separate from one another or from *B. tabaci* using morphological differences, and so may eventually be grouped into a *B. tabaci* complex that contains the *B. tabaci* sibling species assemblage of variants (see Gill and Brown Chapter 1). Examples that fall outside of the major *B. tabaci* sibling species group and form a tight complex basal to *B. tabaci* have been studied using molecular markers. This concept is supported by Mound's (1993) scenario of evolution in *Bemisia* characterized by high genetic diversity, and population expansion with substantial barriers to gene flow.

## From Concept to Working Definition

The whitefly *B. tabaci* (Gennadius 1889) is probably best understood as a sibling species group, or an assemblage of biologically and genetically *diverse* variants (biotypes or races) (Abdullahi et al. 2003; Berry et al. 2004; Bird and Maramorosch 1978; Brown 2000; Brown et al. 1995; Costa et al. 1991a, b; De Barro et al. 2005;

Frohlich et al. 1999; Gawel and Bartlett 1993; Gill and Brown Chapter 1; Guirao et al. 1997; Mound 1963; Perring 2001; Russell 1957; Zhang et al. 2005). This system is generally adhered to, and interestingly, is underscored by the historical taxonomic synonymization by Russell (1957) of numerous species and several genera into *B. tabaci*. This synonymy was later challenged by Bellows et al. (1994) and Perring et al. (1993) who proposed establishing separate species for the A and B biotypes, although it is now clear that they represented only two of the many variants that exist worldwide. Their proposal did not clarify the taxonomy for *B. tabaci* as a whole, and so has not met with consensus among the scientific community.

The working strategy for classifying *B. tabaci* is based on morphological and molecular characters that group *Bemisia* into a sibling species group, or as close relatives in the *B. tabaci* species complex (Gill and Brown Chapter 1). Currently, the COI phylogeny supports at least seven major phylogeographic clades (Fig. 2.7a, b), while the phylogenies based on the mitochondria16S and the ribosomal intergenic spacer 1 (ITS1), although not as well resolved, do reflect a phylogeography similar to the COI tree (Berry et al. 2004; Boykin et al. 2007; De Barro et al. 2005; Frohlich et al. 1999; Kirk et al. 2000; Sseruwagi et al. 2005, 2006; Ueda et al. 2009). The latter two markers differ primarily with respect to extent of genetic variation, with the COI being more divergent than the others (Boykin et al. 2007; De Barro et al. 2000; 2005; Frohlich and Brown 1994; Frohlich et al. 1996, 1999). As may be expected, different kinds of analyses are revealing different demarcations for sub- and sister-clades (Boykin et al. 2007; De Barro et al. 2005; De la Rua et al. 2006), although this could also be due to the sets of sequences employed.

Presently it is not possible to predict a single origin for the *B. tabaci* sibling species because the markers explored to date are saturated (to much variation) and do not allow the designation of a basal clade. For example the COI phylogeny often predicts that New World *B. tabaci* are basal to the Asian clades, whereas, one ITS phylogeny places the New World clade as sister to the North Africa-Mediterranean-Middle East group (De Barro et al. 2000). However, another study did not support this conclusion (Xingxia et al. 2003).

Abundant evidence underscores the existence of extensive differentiation for the group as a whole (Brown and Idris 2005; Brown et al. 2000; Gawel and Barlett 1993; Perring et al. 1993; Figs. 2.7a, b). A number of divergence estimates point to Africa and/or a portion of Asia as likely geographical centers of population expansion and diversification (Berry et al. 2004; Brown 2000; Brown and Idris 2005; Brown et al. 2004a; Boykin et al. 2007; De la Rua et al. 2006; Moya et al. 2001; Qui et al. 2007; Sseruwagi et al. 2005).

At the present a haplotype "working cutoff" has not been established. Such a cutoff to demarcate biotypes might feasibly range from ~1 to 3% based on comparisons with a set of reference biotypes. However, it may be difficult to arrive at a consensus in this sibling species group because within each major clade, a wide range of inter-haplotype variation is evident. For example, divergence is relatively narrow among the Western Hemisphere haplotypes at about 5–6%, a case in point being the Jatropha and Sida biotypes that diverge at about 1.6% (COI). Consequently,

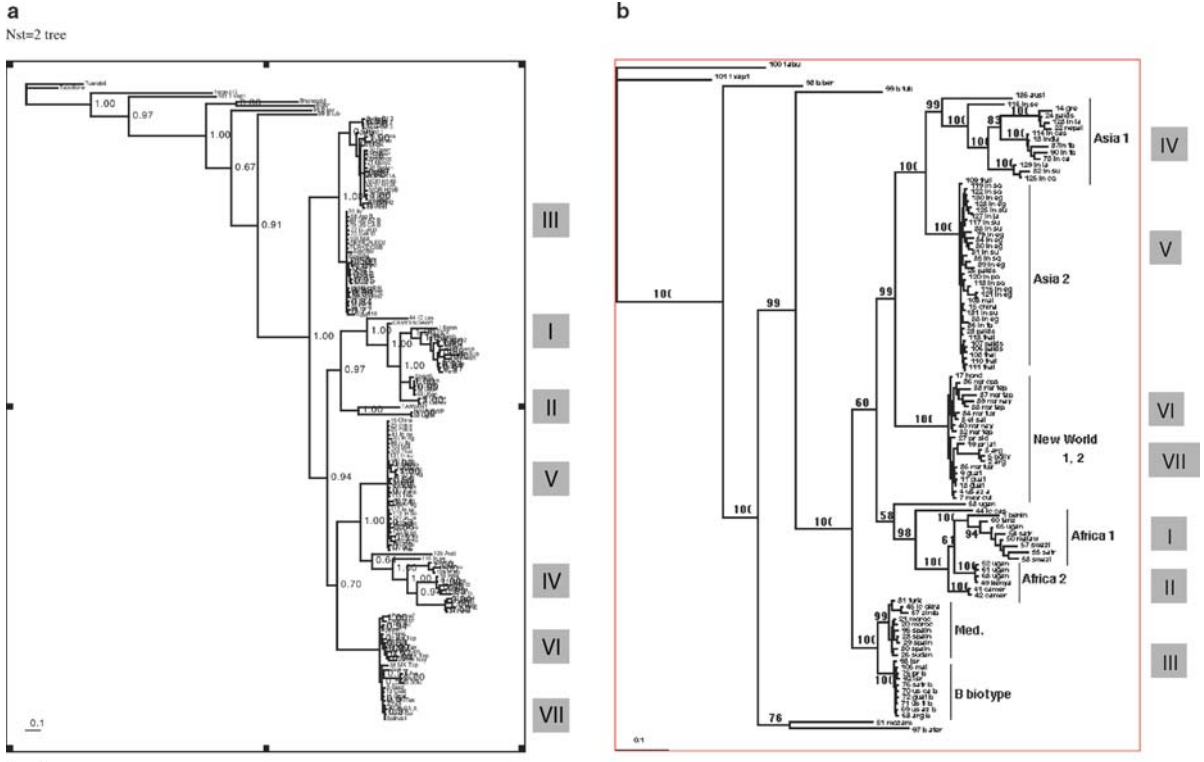



**Fig. 2.7** Mr. Bays tree (Huelsenbeck and Ronquist 2001) (Nst=2 tree) in collaboration with R. French, USDA-ARS and the University of Nebraska; presented in Brown et al. (2004a). (**a**, *top figure*): seven major phylogeographic clades are resolved with robust support 99–100% (except for one at 60%) based on the cytochrome oxidase I gene fragment; (**b**, *bottom figure*) A large subset of COI sequences from each clade was separately analyzed to illustrate the seven major clades and numerous sister clades within each. The greatest nucleotide variation occurs first in the sub-Saharan Africa clade, and second in the Asia 1 group, indicating the greatest extant variation in this sibling group is probably in sub-Saharan Africa

establishing such a set criterion could lead to erroneous designations for closely related haplotypes, which would not be supported by the criteria used to distinguish biotypes.

## Biological Criteria for *B. tabaci* Biotypes

A suite of phenotypic and genotypic criteria have been employed for biotype characterization, and include: (1) esterase or isozyme profiling (Figs. 2.4 and 2.5; Brown et al. 1995a, 2000; Costa and Brown 1990, 1991; Costa et al. 1993; Perring et al. 1993; Wool et al. 1989, 1993), (2) bar-coding (COI) (Figs. 2.7a, b), (3) life history traits (Abdullah et al. 2006; Bethke et al. 1991; Bonato et al. 2007; Butler et al. 1983; Costa and Brown 1991; Hussain and Trehan 1933; Viscarret et al. 2003; De Latte et al. 2005), (4) host range and/or host preference (Abdullahi et al. 2003; Attique et al. 2003; Brown and Butler 1985; Bayhan et al. 2006; Bedford et al. 1994; Bird 1957; Bird and Maramorosch 1978; Burban et al. 1992; Cock 1986; 1993; Costa and Brown 1991; Costa and Russell 1975; Delatte et al. 2006; Demichelis et al. 2005; Gennadius 1889; Legg 1994; 1996; Lopez-Avila 1986; Mound and Halsey 1978; Osmondi et al. 2005; Russell 1957; Thompson 2003), (5) co-evolved virus-vector interactions that contribute to different transmission competency (Brown 2007), (6) composition of endosymbionts (Baumann et al. 2000; Brown et al. 1998; Caballero and Brown 2008; Caballero et al. 2001; Campbell 1993; Chiel et al. 2007; Costa et al. 1995; Zchori-Fein and Brown 2002); (7) dispersal behavior (long distance, or not), (8) ability or inability to induce silverleaf symptoms in *Cucurbita* spp. (Fig. 2.6) (Costa and Brown 1991; Delatte et al. 2005; Sserwuwagi et al. 2005), (9) insecticide resistance (Abdullah et al. 2006; Anthony et al. 1995; Dittrich et al. 1990; Morin et al. 2002; see Castle et al. Chapter 16), and (10) discontinuous gene flow (Bedford et al. 1994; Byrne et al. 1995; Caballero 2007; Caballero and Brown 2008; Caballero et al. 2001; Costa et al. 1993; De Barro and Hart 2000; Hadjistylli 2003; Kanmiya 2006; Liu et al. 2007; Perring et al. 1993; Perring and Symmes 2006; Pascual and Callèjas 2004; Ronda et al. 2000).

### Biotype Classification and Nomenclature

Multiple studies have revealed genetic and behavioral polymorphisms for *B. tabaci* and despite recent improvements in standardization of nomenclature, some confusion still persists. Some relief has been provided by the use of reference strains that now serve as the basis for establishing the major clade system (Figs. 2.7a, b; 2.8). Herein, it is proposed that the anomalous alphabetical esterase-based designations be abandoned except for those already well established haplotypes (A-T). In its place, a revised nomenclature system would group new haplotypes within their respective major phylogeographic clade based on a reference suite of COI sequences (Fig. 2.7a, b and Fig. 2.8). The system would become the basis of a code that conveys geographic affiliation as well as host species and other biological characteristics. This relevant information would thus become the signature for a distinct haplotype,

or when characterized with respect to a number of key biological characteristics, a biotype signature.

The next level of demarcation would be based on affiliation of the COI haplotype with the closest sister clade, typically of local or nearby origin. For example, the major North Africa-Mediterranean-Middle East clade contains three recognized sister clades of haplotypes, most closely related to the B biotype, the B, Q, and MS sister clades. The sub-Saharan haplotypes are many and incompletely accounted for, but diversity is possibly greatest within these (at least) two major clades. Within the Australasian/Pacific clade, there are at least two and possibly three major subgroups, each containing sister clades that include haplotypes unique to Asia, Australia and the Pacific Islands. In the Western Hemisphere there are at least three sister clades, and perhaps more (Figs. 2.7a, b and 2.8). However, these haplotypes diverge minimally as indicated by the shortest branch lengths among all clades, suggesting relatively recent expansion (Fig. 2.9). Recent studies suggest that haplotypes that are closely related may or may not be reproductively isolated by geographical or host restrictions. For example, reproductive isolation has been demonstrated among several closely related members of the Q sister clade, even though nucleotide divergence is rather minimal (<2%) (Hadjistylli 2003; Pascual 2006; Pascual and Callèjas 2004; Ronda et al. 2000).

Associating a haplotype with an endemic geographic locale would make origins and possible introduced status clear, thereby reducing confusion about what is meant by Q biotype, for example. In this example, the endemic Israel-Q (ISR-Q)

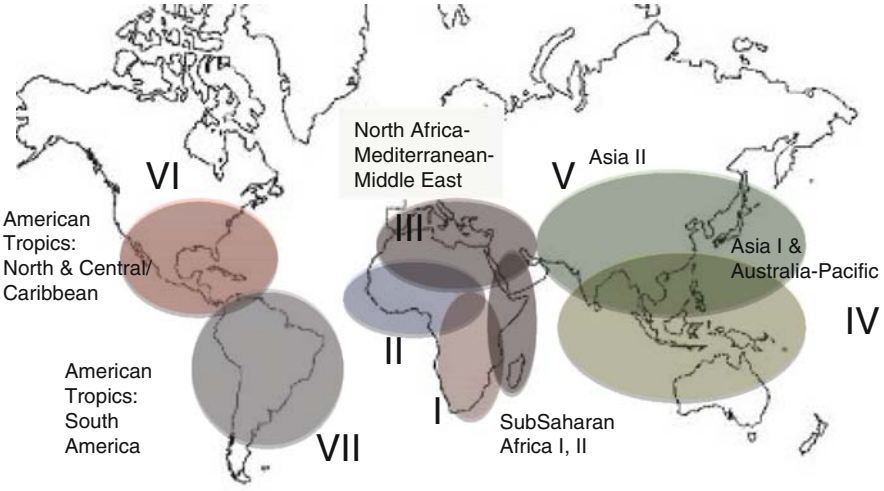



**Fig. 2.8** World map showing the seven major phylogeographic clades resolved in the mitochondria cytochrome oxidase I phylogeny. This scheme defines at least two in sub-Saharan Africa: I and II, one in the North Africa-Mediterranean-Middle East region: III, two in Austral-Asia: IV and V, and two in the Tropical Americas, VI and VII. With the exploration of additional markers more and/or possibly different haplotypes as well as new clades and/or sister clades may be recognized

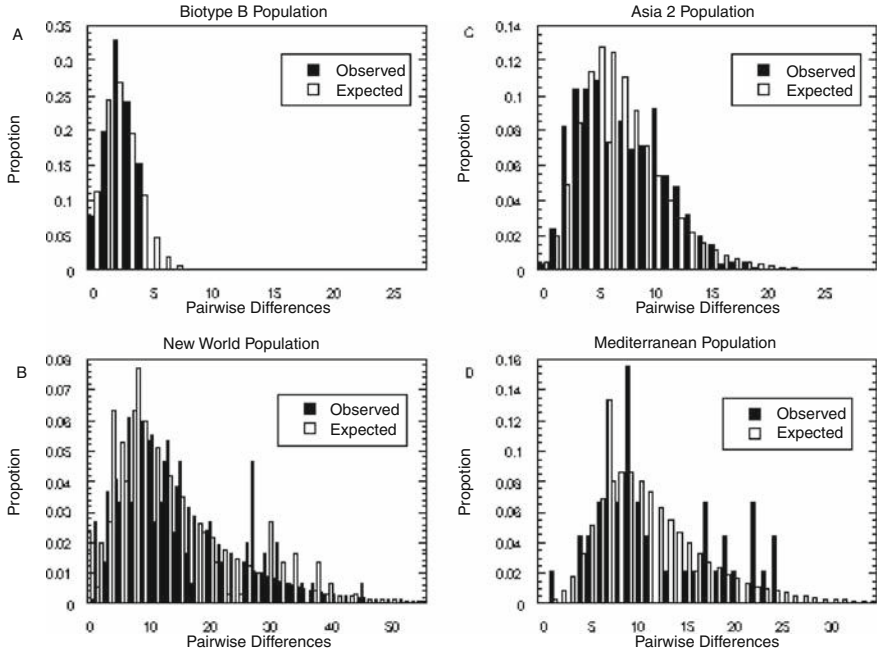



**Fig. 2.9** Mismatch analysis (Rogers and Harpending 1992, using DnaSP) illustrating population growth curves and the extent of population expansion for representative biotypes. The observed data was compared to a step-wise model of growth, estimating three model parameters: Tau, time of population expansion, expressed in units of mutational time, and the population sizes before (Thetai), and following (Thetaf) expansion. Confidence intervals for those parameters were obtained by comparing observed values with those resulting from coalescent simulations of 1,000 random samples. The distribution of expected pairwise differences between two sequences drawn at random, given the derived values of Tau, Thetai, and Thetaf, was calculated and plotted along with the observed values. Other population test statistics calculated were the raggedness index, Tajima's D, and Fs. Statistical significance estimates were also obtained by coalescent simulations (Courtesy R French, University of Nebraska)

(Horowitz et al. 2003) would be differentiated from the Spanish-Q (SPAN-Q) (Rosell et al. 1997) or the Turkish-Q [TURK-Q (analogous to the TC population from cotton) (Bedford et al. 1994)]. A similar scenario holds for New World haplotypes that are closely related to the AZ-A biotype, but are not precisely the A biotype, which by historical definition it is endemic to the southwestern USA. The A biotype does not exist in South America for example, unless it has been introduced there, but its close relatives do, and they can thereby be demarcated to reveal a sister group affiliation (A biotype clade) followed by the (local) endemism, such as COLUM-A for Colombia. Other examples would be the Culiacan, Mexico A-like relative (>1% nt identity) that would become Culiacan-A (CUL-A), and the endemic *B. tabaci* from Panama would become A-Panama, etc. If more than one haplotype exists in the same location, a number can be attached, such as PanamaA1,

PanamaA2, and a reference barcode would be deposited for each in the public database to provide ready comparison.

At the next higher level of classification, in line with the suggestion of Gill and Brown (Chapter 1), all *B. tabaci*-like *Bemisia* haplotypes or members of the *B. tabaci* sibling species group would become a subclade of the *B. tabaci* species complex that contains other *B. tabaci*-like whiteflies but are sufficiently divergent to fall outside of the sibling species group. A higher order classification would involve another species complex designation such as the *B. afer-hancockii* haplotypes, or the *B. afer* complex. This or a similar system would groups the designated *Bemisia* species and closest relatives into two major species complexes based on morphological and molecular evidence. Additional studies are needed to completely validate the proposed classification and take into account both biological phenotypes, gene flow potential, and molecular and population genetics data sets.

## Molecular Markers and Phylogeography

### *Mitochondrial Molecular Markers*

The mitochondrial genome is maternally inherited in most organisms, and its coding regions exhibit higher rates of evolution (mutation) compared to the nuclear genome (Brown 1983). Two mitochondrial markers, the 16S and COI, have been examined for their utility in inferring phylogenetic relationships within the *B. tabaci* sibling species group. Of the two, the COI sequence (780 bases) has proven the most informative and so is now widely used in "barcoding" to facilitate identification (Brown 2000). The use of barcoding has facilitated phylogenetic analysis, and the rapid identification of field specimens to the level of haplotype, and in one instance made possible the immediate recognition the introduction of the Q biotype (Brown and Dennehy 2006; Dennehy et al. 2006; see position of the Spanish Q in Fig. 2.7).

Two other molecular markers – the mt16S ribosomal DNA and, the nuclear noncoding ITSI sequence – predict a similar but not identical phylogeography for *B. tabaci* haplotypes (De Barro et al. 2000, 2005; Frohlich and Brown 1994; Frohlich et al. 1999). The ITS region has been less satisfactory for bar coding because the sequence is less variable. Nonetheless, these other two markers generally reveal similar phylogeographic groupings, with respect to those demarcated in the COI phylogeny, irrespective of the algorithm, e.g. neighbor-joining, maximum likelihood, or Bayesian (Berry et al. 2004; Brown et al. 2004a; Boykin et al. 2007; De Barro et al. 2000; 2005; Frohlich et al. 1996, 1999; Sseruwagi et al. 2005, 2006; Viscarret et al. 2003). Only extant haplotypes known to be recent exotic introductions, namely the B and Q biotypes, group outside the phylogeographic boundaries demarcated by the mtCO1 and other molecular markers as would be expected (Brown et al. 1995a, b; Costa et al. 1993; Dennhey et al. 2006; Perring et al. 1993).

### Cytochrome Oxidase I

Although the functional basis for the unexpected degree of divergence within this sibling species group is not understood, this gene sequence has proven highly valuable for classifying haplotypes of *B. tabaci* and close relatives worldwide. The greater-than expected variation in this gene could reflect some aspects of direct or indirect adaptability. Unfortunately, gene expression studies have not yet determined if certain relevant genes are differentially regulated and/or are determinants of the behavioral plasticity observed within the complex. Likewise, over-estimation of genetic variability owing to abundance of "numts" (nuclear mitochondrial DNA) (Zhang and Hewitt 1996) is yet another possibility.

## Phlogenetic Relationships Are Generally Congruent with Extant Geography

With the exception of recently introduced biotypes, members of the *B. tabaci* sibling species group into seven major geographic clades based on COI sequence analysis: five in the Eastern Hemisphere and two in the Western Hemisphere (Figs. 2.7a, b and 2.8).

The major Eastern Hemisphere groups sort by region of extant origin and include at least five major clades. In Africa, these are represented by the (i) Mediterranean-North Africa-Middle Eastern group (MED-NA-ME) and at least two sub-Saharan Africa (SSAF) groups that contains a large number of divergent haplotypes (Berry et al. 2004; Brown 2000; Legg et al. 2002; Sseruwagi et al. 2005, 2006; Figs. 2.7a, b and 2.8). The former group contains the well-known B, Q, and MS biotypes and closest relatives, whereas the latter group contains both polyphagous and cassava-associated haplotypes. Asia and adjacent land masses are represented by the large Southeast Asian-Australian group (SA-AUS), comprising two obvious sister groups (I, II) extant in China, Pakistan, India and adjacent countries, a small Asian subgroup found only in China, and a subpopulation known only from Australia. In contrast, *B. tabaci* in the American Tropics form two clades comprising the Americas-Caribbean and South America sister clades, albeit divergence (and genetic differentiation) among these groups is less than for Eastern Hemisphere haplotypes (Figs. 2.7a, b, 2.8 and 2.9).

In the New World groups, certain haplotypes overlap with respect to regional distribution, suggesting that there is some potential for gene flow between haplotypes in certain regions. The 'American Pacific haplotypes' prevail throughout North and Central America, whereas, the Caribbean haplotypes and close relatives are mostly distributed in Central America, southern Mexico, and the Caribbean islands. The haplotypes from Argentina and Bolivia in South America form a distinct group from the northerly haplotypes and diverge by ~4–6% from their neighboring sister clades (Brown 2000; Viscarret et al. 2003). However, this estimate is based on a small sample size, and so is not necessarily indicative of reproductive isolation.

## Global Genetic Divergence

Among the major geographic clades, the Asia-Australia and sub-Saharan Africa clades exhibit the greatest within-clade divergence, up to 20%, and 26% or more, respectively (Fig. 2.8). Intermediate levels of divergence, up to ~14%, exists within the major Mediterranean-North Africa-Middle East clade, whereas, strikingly less than ~8% within-clade divergence is seen in the major Tropical Americas clade (see Fig. 2.7a, b and 2.8 for examples). Nevertheless, within-population divergence is less than between-population differences for all comparisons. Similar results were obtained in a comparison of over 500 COI sequences representing haplotypes from the major geographic regions, and are depicted for certain sister clades (Figs. 2.7a, b and 2.8; Brown et al. 2004a).

An alternative means of illustrating pair-wise variation is through mismatch analysis (Fig. 2.9). From this analysis, it is possible to discern real differences in population size and histories, for example, founder events and genetic bottlenecks. It is possible to deduce that the greatest variability exists in Sub-Sahara Africa, with the second most in the Asia I subclade. Much less population expansion is evident in the New World and also in Asia II, whereas, with the North Africa-Mediterranean-Middle East populations expansion is moderate. Additional studies may reveal the patterns of ancient migrations and founder events, as well as the relative time frames of population expansions (and contractions) and extinctions. It is also interesting to speculate whether additional *B. tabaci* have migrated out of Africa along the same corridors as humans in conjunction with the movement of germplasm and its cultivation. An intimate association with humans could feasibly have begun with the advent of agriculture 10–14,000 years ago. Molecular clock analysis is now needed to establish the basal taxa and to arrive at sound estimates of evolution for this sibling species assemblage. The complete genome sequence will reveal a multitude of molecular marker candidates that can then be analyzed using new, more powerful phylogenetic and population genetics approaches (see Czosnek and Brown Chapter 18).

## Selection and Differentiation

Evaluation of conserved and variable sites in the 780 base COI sequence reveals unusually high divergence at both the nucleotide and protein levels (Brown and Idris 2005). For example, alignment of the consensus amino acid sequence identified 26 polymorphic sites clustered at the C-terminus end of the protein. Whether there is a functional explanation for this observation is not known.

The inter-population coefficient of genetic differentiation, $G_{ST}$, was used to estimate the extent of differentiation between haplotypes, irrespective of evolutionary history. Results indicated an overall differentiation of 0.599 (SE = 0.015), or a relatively high degree of genetic differentiation within the complex. Positive Darwinian selection was demonstrated for 99 of 141 COI sequences, each representing a single individual from worldwide collections (Brown and Idris 2005). The greatest number of haplotypes exhibiting positive selection were those in the major clade demarcating Asia I-Australia at 79%, compared to the second greatest for the major

clade comprising Sub-Saharan Africa haplotypes, at 69%. This is not necessarily in contrast to percent nucleotide comparisons that have predicted more extensive divergence in Sub-Saharan Africa populations (Berry et al. 2004; Boykin et al. 2007; Sseruwagi et al. 2005; 2006), a discrepancy that might be explained by the specific data sets used in the various analyses. The third greatest differentiation occurred for the Mediterranean-North Africa-Middle East clade at 60%, while the lowest frequency is evident among haplotypes in the two American Tropics major clades in the Western Hemisphere.

In summary, the greatest diversification for the *B. tabaci* sibling species group based on a rather extensive, though incomplete, representation of haplotypes appears to be either sub-Saharan Africa (Berry et al. 2004; Boykin et al. 2007; De la Rúa et al. 2006; Hsieh et al. 2006; Sseruwagi et al. 2005, 2006) or Asia (Brown and Idris 2005; Qiu et al. 2007; Ueda et al. 2009). There is good evidence that genetic variation is low in the Western Hemisphere, where both inter- and intra-population divergence and differentiation estimates are very low (Boykin et al. 2007; Brown and Idris 2005; Viscarret et al. 2003; Zhang et al. 2005; Hadjistylli et al. Chapter 3). In some instances, mitochondria bar coding has been found to possibly overestimate the number of species or extent of divergence if based on the inclusion of nuclear pseudogenes (numts) that can be PCR-amplified with mitochondrial primers (Zhang and Hewitt 1996). Therefore, validation of the gene source has become essential (http://www.zen36595.zen.co.uk/avoidance.html).

## *Nuclear Markers*

### 18S rRNA Gene

The A and B biotype vary by a single nucleotide at the level of the highly conserved 18S rRNA gene (Campbell 1993). The higher order taxonomic tree inferred from the analysis did not separate the A and B biotypes and so is clearly not informative at the level of the sibling species group. However, it does support the identification of *B. tabaci* in relation to other taxa examined.

### Non-coding Nuclear Sequences – Ribosomal ITS1

As mentioned above, non-coding, or intergenic "spacers", in this case the ITS1 intron that links the ribosomal 18S–23S coding regions (exons), are often found to be useful for reconstructing phylogenetic trees. The ITS1 has been employed as a molecular marker (DeBarro et al. 2000) and major phylogeographic groupings do not vary discernibly with respect to the COI phylogeny, although branch lengths differ. Nonetheless, an overall congruence between the two trees is clear. A combined analysis of the ITS1 and COI nucleotide sequences demarcated the same major groups, referred to as: (i) Asia + Bali +Australia, Sub-Saharan Africa, Mediterranean-Asia Minor-Africa, and New World (Boykin et al. 2007; De Barro et al. 2000, 2005), with Sub-Saharan Africa showing the greatest overall divergence, as also is observed for the faster evolving mtCO1.

### Sodium Voltage Gated Channel Gene Intron

An intron (domain II, 4–6) (Khasdan et al. 2005; Morin et al. 2002) for the voltage-gated sodium channel gene intron, also a nuclear non-coding sequence, has been employed to study a relatively small set of haplotypes. Even so it has proven useful for discerning certain geographical differences, namely in the demarcation of eastern and western haplotypes from cassava in Africa, which diverge by ~7–8% (Brown et al. 2004b; Legg et al. 2008). It has also been used to a small degree for differentiation of haplotypes in Africa and India (Legg et al. 2008). This marker also was used to discriminate the B biotype from the ISR-Q biotype in insecticide resistance studies among laboratory populations (Alon et al. 2006). The "knock down resistance" (KDR) or sodium voltage gated channel intron sequence currently under exploration in several laboratories appears only somewhat less divergent than the ITS-1 sequence when tested with representative haplotypes (mtCO1). It is possible that this non-coding region could advance the understanding of the overall phylogeography, or at least provide another nuclear marker, albeit, non-coding, for comparative purposes.

## Some Biological Characteristics Used to Differentiate Biotypes

The hallmark of the *B. tabaci* sibling species group is extensive variation in phenotypic characteristics, including host range, and other phenotypes that were responsible for the earliest recognition of *B. tabaci* as a polymorphic and cryptic complex (Bird 1957; Bird and Maramorosch 1978; Costa and Brown 1990, 1991; Bedford et al. 1994; Berry et al. 2004; Burban et al. 1992; Costa and Russell 1975; De Barro and Hart 2000; De Latte et al. 2006; Legg 1996; Maruthi et al. 2001). Several of these criteria will be discussed in detail.

### *Host Range and Preference*

#### Monophagous Biotypes

In Puerto Rico, the monophagous JAT-PR biotype was sympatric with the polyphagous Sida race (Bird and Maramorosch 1978; Bird and Brown 1998; Brown and Bird 1992, 1996). Establishment of the exotic B biotype with its broad host range there during the late 1990s lead to rapid displacement of both endemic biotypes (Bird and Brown 1998).

In contrast, the apparently monophagous cassava-restricted biotype associated with the severe cassava mosaic disease outbreak caused by endemic viruses and a new recombinant virus erupted in Uganda in ca. 1990 (Gibson et al. 1996; Harrison et al. 1997; Legg 1996), and subsequently spread to over eight countries in east-central Africa (Legg et al. 2002; Legg Chapter 7). In this instance, an exotic West African haplotype is thought to have invaded east African cassava plantings, transmitting one to three cassava-infecting viruses. The primary phenotype was more

severe than previously encountered. Research uncovered a new, recombinant begomovirus, containing sequences from eastern and western cassava mosaic viruses. The invader biotype as it became referred to, apparently is more fecund than east African cassava populations, and also dispersed longer distances, resulting in the rapid spread of the virus and vector southward in sub-Saharan Africa. The *B. tabaci* involved in the post-epidemic was a hybrid between the two vector haplotypes, providing the first example for hybridization and viable offspring production in nature (Brown et al. 2004b; Legg et al. 2008). This study also underscores the potential for a host-restricted begomovirus-*B. tabaci* sibling species to emerge under optimal conditions and with profound consequences.

Several biotypes are known from the Eastern and Western Hemispheres that exhibit limitations in host range. The most common plant family with which they are associated is the Euphorbiaceae, and many of these are thought to be nearly monophagous. To rule out the importance of these specialists of euphorbiaceous hosts as sources of begomoviruses that may emerge in crop species would be a mistake, because many *B. tabaci* colonize *Euphorbia* species and at least six begomoviral species have been identified in such hosts that are of economic importance (Brown and Bird 1996; Legg 1996; Maruthi et al. 2001, 2002).

Monophagy has been most often associated with colonizers of Euphorbiaceae, including the JAT-PR, the vector of *Jatropha mosaic virus* (JMV) in Puerto Rico and the Caribbean region, cassava in Africa and also in India (different haplotype groups), among others. Although the JMV host range was thought possibly limited because of its host-adapted monophagous vector, experimental transmission studies with polyphagous B biotype revealed instead that the JMV host range was limited by its monophagous vector. Consequently, upon establishment of the B biotype in Puerto Rico, JMV was found infecting passion vine plants *Passiflora* spp. (Bird and Brown 1996), confirming that the vector was responsible for JMV host bottlenecking. Based on sampling over time and molecular identification, the JAT-PR biotype appears to have been displaced by the B biotype since it has not been found on *J. gossypifolia* since 1994 (J Bird and JK Brown, unpublished data). In 2002, a rare population of the Sida biotype was identified near Rio Piedras Puerto Rico by Bird and Brown (see the SIDA-PR COI sequence), however more recent collections have not produced additional Sida haplotypes but have instead been of the B biotype.

## Polyphagous Biotypes

The best known Old World polyphagous biotypes are the B (B sister clade in the North African-Mediterranean-Middle East clade) and other B relatives (MS/non-UG sister clade), and the Spanish-Q (endemic in Spain) and Q-like (Q sister clade) haplotypes, including SUD-Q, ISR-Q, among others. New World polyphgous *B. tabaci* biotypes include AZ-A (USA-AZ and CA lowland deserts) and closely related A-like haplotypes from Mexico and Central America (Tropical Americas clade).

## Habitat

Most New World haplotypes that have been studied are found to be adapted to a range of eudicots and occur in varied habitats spanning desert, Mediterranean, and dry-subtropical and tropical niches. They are moderately fecund and disperse short distances in search of food. In contrast, the B biotype prevails in dry, irrigated monoculture habitats and exhibits high fecundity, extreme polyphagy, and long-distance dispersal, and in hot, dry habitats can outcompete at least some New World haplotypes. The Q-like haplotypes appear to favor higher humidity than the B biotype, but probably their adaptation to moderate temperature fluctuations is more important. Analysis of the mtCO1 has revealed a possible origin for the B biotype in the eastern African Sahel region that probably extends into the Middle East, including in Egypt, Israel, Jordan, and Iran (Brown, unpublished data), underscoring their favored habitat. These studies also reveal an overlap in geographic and host ranges for Q-like and B-like haplotypes, there, although the natural range of the Q sister clade also extends into Europe and Asia, where the B biotype is now invasive, not endemic (Berry et al. 2004; Brown 2000; Brown et al. 2004a; Frohlich et al. 1999; Sseruwagi et al. 2005). Also Q-like haplotypes are abundant in northern Africa particularly in the Sahel region.

## Host Range

The host range of the B biotype comprises a large number of eudicot genera and species: totally 500–600 species for the sibling species group, collectively (Attique et al. 2003; Bayhan et al. 2006; Cock 1986, 1993.). It seems likely that host range plasticity accounts for an important adaptive trait, allowing for survival when preferred hosts are in short supply. This adaptive feature could favor upsurge of new, more aggressive haplotypes, leading to emergence of previously unidentified begomovirus species in crops, given the close dependency of begomoviruses on their whitefly vector for exposure to new hosts (Brown 2001).

The polyphagous A biotype and relatives have their origin in the southwestern USA and northern Mexico where irrigated agriculture is practiced nearly year round. In terms of crop species, it exhibits a preference for bean, cotton, cucurbits, and composites. It also colonizes a large number of endemic and naturalized eudicots (Butler and Brown 1985). In this environment, the introduced B biotype showed a preference for *Brassica* spp., Solanaceae, composites, Cucurbitacae, Malvaceae and the Euphorbiaceae, with a marked propensity for cotton, eggplant, squash, tomato, and lantana.

In Puerto Rico, the Sida biotype host range included legumes, composites, and species in the Malvaceae, but not cucurbits (Bird and Sanchez 1971; J Bird, personal communication), as is known for A-like biotypes from the western USA Mexico, and the Caribbean region, and generally did not prefer solanaceous or *Brassica* species. However, pre-biotype B records of *B. tabaci* in the Florida Department of Agriculture Division of Plant Industries Museum in Gainesville do include

cruciferous hosts (P Stansly, personal communication), so perhaps its origin was distinct from western USA haplotypes.

### Dispersal Behavior

Overall, little is known about differences in dispersal behavior, with the exception of the long distance flight reported for the B biotype noted immediately upon its introduction into the Americas, where the endemic A biotype was known to move only short distances between crops and native plant hosts. Also, only short distance flights were observed between understory plants in Puerto Rico where the Sida and JAT-PR biotypes predominated until they were displaced by the B biotype in ~1987–1988 (J Bird, personal communication). These polymorphic behaviors could perhaps be explained as an adaptation to combat unreliable and seasonally unavailable food sources brought about by desert weather and subsequently, agricultural practices. Long distance flight behavior (and high fecundity) would seem to lend itself to greater survivability in desert habitats where seasonal rainfall results in sudden and prolific plant growth conducive to increased reproductive rates followed by migration to locate additional food sources (Brown et al. 2004a; Brown and Bird 1992). Such conditions are particularly documented in the Sudan, Uganda, and other locales in the Sahel region of Africa.

Prior to the near-global invasion of the B biotype and reports of long-distance flight behavior consistent for this biotype, little other information was widely available regarding this trait. However, such flight behavior has been reported, including in cotton crops in Sudan where DDT was widely employed to control cotton pests from the 1950s onward (Dittrich et al. 1990; V Dittrich, personal communication). Based on what is now known about haplotype distribution in that part of Africa, the population was probably a Q or B biotype relative. Indeed, insecticide resistance in the SUD-Q R and S populations has been the subject of studies in the Rothamsted laboratory in the UK, and these haplotypes originate from the same area afflicted by aggressive *B. tabaci* populations described over 50 years ago.

A second example is more recently known from Israel, where silverleaf symptoms were first reported in squash plants (Be'eri and Kapuler 1963). This observation may refer to the introduction of the B biotype, possibly from Africa, having dispersed across the Red Sea (haplotype relatives are known in Uganda, Ethiopia, and Eritrea) (unpublished data) into Israel and Jordan at about this time (AR Horowitz and A Alabdallat, respectively, personal communication). Subsequently, increased fecundity and long distance dispersal were noted in agricultural areas, including heavy infestations in cotton, cucurbits, and tomato crops 1978–1980 (AR Horowitz, personal communication).

An inventory of flight behavior for the *B. tabaci* complex has not been carried out on a worldwide level but it would seem likely that this trait could go hand in hand with other invasive traits, including extreme polyphagy and fecundity, and perhaps insecticide resistance if detoxification of allelochemicals and insecticides is found to be tightly linked. Indeed, one study described elevated free amino acids (concomitant with decreased sugar concentration) and a modified oviposition in response

to insecticides, with positive fitness affects in the *B. tabaci* observed in this study (Abdullah et al. 2006).

Clearly, multiple factors could alter dispersal behavior although these need not all be "hard-wired". Perhaps it will be found that certain of these behaviors and other adaptive responses are modulated by whitefly gene expression in response to the changing environments in which *B. tabaci* must adapt and survive in the long term.

### Silvering Phenotype in *Cucurbita* spp. (and Other Hosts that Exhibit Phytotoxic-Like Symptoms)

The phytotoxic-like disorder referred to as squash "silverleaf" associated with *B. tabaci* was first described in Israel (Be'eri and Kapuler 1963), and later with *B. tabaci* in Florida (Yokomi et al. 1990) and specifically with the non-native, invasive B biotype by Costa and Brown (1990, 1991). As mentioned above, the silverleaf-inducing MS-like haplotypes from Uganda (Sseruwagi et al. 2005) and La Reunion Island (Delatte et al. 2005) are close relatives of B biotype in a third distinct sister clade. The MS biotype from La Reunion is very closely related to the "Uganda non-B haplotype", that together form a unique sister clade to the B and Q biotype sister clades, respectively. These three groups comprise the major North African-Mediterranean-Middle East clade of the *B. tabaci* sibling species group (Fig. 2.7a, b). Interestingly, the two most invasive haplotypes to have emerged in recent years, the B and Q biotypes, are members of this major geographical clade.

## *Gene Flow and Reproductive Isolation*

### Evidence from Reciprocal Crosses Under Laboratory Conditions

The population genetics surrounding gene flow and mating in the *B. tabaci* sibling species group are poorly understood. It has been suggested that haplotype-specific behaviors influence not only gene flow within and between haplotypes, but also fitness and the success of the resultant haplotype. Several studies have focused on invasive traits in haplotypes that are particularly adapted to contemporary agricultural environments (Perring et al. 1993; De Barro et al. 2006; Liu et al. 2007; see also Naranjo et al. Chapter 6), but in many ways, these examples generalize and over-state the potential for members of the *B. tabaci* to become invasive species. Abundant evidence suggests the vast majority of *B. tabaci* haplotypes are relatively unimportant or not important at all in agriculture, but instead inhabit primarily uncultivated, endemic and naturalized species (Attique et al. 2003; Bayhan et al. 2006; Butler and Brown 1985; Bird and Maramorosch 1978; Delatte et al. 2006; Mound 1963; Mound and Halsey 1978). However, an argument could be made for convergent evolution of the common traits needed to adapt to both kinds of non-static environments.

Reproductive behaviors and factors that confer mating compatibility, and therefore patterns of gene flow, remain unsatisfactorily elucidated. This is due, in part, to the relatively few studies that have been carried out, particularly in comparison to the vast number of haplotypes that prevail. Even so, an overriding caveat to the mating studies that have been carried out is that experimental conditions have been highly variable. Improved experimental design and representative haplotypes for such studies would go a long way to reduce experimental error and clarify discrepancies presently apparent between different laboratories. Also new behavioral and molecular tools that enable pedigree testing of parents and offspring (COI, KDR, others) are available to facilitate quantitative assessment of key mating behaviors and their outcomes (Walker and Perring 1994; Liu et al. 2007; Perring and Symmes 2006).

Logic would suggest that reproductive isolation would be expected to some extent between haplotypes in a sibling species group. The worldwide distribution in tropical and subtropical latitudes imposes geographical barriers to gene flow. Host plant isolation could also result in behavioral or post-zygotic isolation in sympatric haplotypes. Taken together with the extensive plasticity conjectured to promote rapid adaptation to variable and even changing conditions, it seems likely that a number of as yet unknown factors influence gene flow directly and indirectly. Gene flow patterns may run the gamut from local to long distance. Further host preferences/range barriers are expected to come into play in a sibling species group exhibiting host range extremes from monophagous to polyphagous. Thus, gene flow is likely to be straightforward and direct, or highly complex and indirect. Factors involved in human-mediated movement of plants harboring *B. tabaci* further increase the complexity. Thus, it is not possible to unequivocally state precise cut-offs or to delineate or define the extent of gene flow, nor can the associated behaviors that delimit gene flow be inferred by extrapolating solely from phylogeographic inferences. Likewise, phylogeography can provide a guide, but does not provide a substitute for carrying out sound biologically experiments (environmental control, backcrosses) that implement reliable technologies to assess and unravel mating behaviors (monitoring, quantitative analysis) in relation to estimates of gene flow in parents, offspring, and back cross populations (microsatellites, SNPs, others).

## Specific Results and Trends

Several studies have been carried out for isolates of the B biotype and Q/Q-like relatives, owing primarily to their status as agricultural pests that have developed insecticide resistance or as an invasive haplotype whose movement was mediated by human activity. However, haplotypes that attain pest or invasive status are minor representatives of the group as a whole, so these results may or may not be relevant to more benign haplotypes. A survey of the literature reveals that, in general, two types of studies have been carried out: those involving allopatric or sympatric haplotypes. In a few cases, monophagy and polyphagy have also been taken into account.

### Sympatry and Sister Clades

Studies involving sympatric haplotypes in different sister clades of the major North Africa-Mediterranean-Middle East have reported similar and contradictory results: A single female offspring was produced from a cross between the invasive B biotype and the SUD-Q, native to Sudan (Byrne et al. 1995; Bedford et al. 1994). SUD-Q is a close relative to the Spanish Q, members of the same Q sister clade, whereas, the B biotype is placed in a separate sister clade. Both are classified in the same major clade (North Africa-Mediterranean-Middle East), which spans both sides of the Mediterranean Sea to Europe, including Turkey, the Middle East, and the North Africa-Sahel region.

- Ronda et al. (2000) reported minimal or no gene flow between biotype B and the Spanish-Q biotype under laboratory conditions.
- In mixed B and Q field populations, hybridization was not detected based on genetic analysis (Moya et al. 2001).
- In a study involving Spanish-Q × B crosses, a few female offspring were produced in reciprocal crosses between the Spanish-Q × B (Hadjstylli 2003; Fig. 2.3). Experiments were conducted to investigate the potential of the two biotypes to interbreed and whether behavioral aspects of mating and courtship were barriers to successful copulation, and thus, of interbreeding between biotypes. One experimental result provided evidence for successful mating in reciprocal inter-biotype crosses. The progeny were viable and fertile, although the hybridization rate was significantly lower than intra-biotype crosses ($P<0.001$). A second observation was that inter-biotype pairs spent more time courting than intra-biotype pairs, and copulation in inter-biotype pairs only occurred in rare instances. In addition, the behavioral patterns were thought to account for unsuccessful copulation in the inter-biotype pairs, leading to asymmetric mating, based on observations that Q biotype males were more active and courted females of either biotype more readily than did B biotype males. This suggested that in sympatry, Q males could have a competitive advantage over the B biotype males.
- Similarly, female offspring were not produced in ISR-Q × B biotype crosses. It should be noted that the ISR-Q is a distinct and geographically isolated haplotype compared to the Spanish Q, and both group within the Q sister clade (AR Horowitz and JK Brown unpublished data). Field interactions between mixed biotypes that are members of distinct sister clades within the same major clade, were examined using population genetics methods. The native MS and the introduced B biotype exist sympatrically in La Reunion since the recent introduction of the B there. Using microsatellite markers to study field population-host interactions, the two biotypes were found to colonize a suite of unique plant species, some of which they shared in common. No evidence of interbreeding between MS and B biotypes (Delatte et al. 2006) was observed. The two biotypes are not naturally sympatric, but they are about as closely related to one another as are the B and Q-like biotypes.

## Allopatry

There are several studies of potential gene flow studies under allopatry. Pascual and Callèjas (2004) studied intraspecific and interspecific mating in mixed colonies of the B biotype (Tenerife) and Q (Majorca). Whether the Majorca Q has been introduced from mainland Spain or is indigenous to the island is not known. Based on fecundity (number and density of eggs, instars; developmental rate; mortality) and sex ratio, they predicted that B should displace Q biotype. They observed an increase in population size of B biotype compared to Q in a single generation, from 50 to 79%, and a higher ratio of B to Q female offspring. Reproductive interference, based on lower reproductive rate of the Q, or differential resource acquisition was cited as a possible mechanism (Pascual 2006).

Female offspring were produced bi-directionally in reciprocal crosses of two New World endemic populations, naturally isolated by host preference and also by geography. These same biotypes when mated to the introduced, non-endemic B biotype yielded viable offspring unidirectionally. For example, AZ-A × JAT-PR were bidirectional, whereas AZ-A or JAT-PR crosses were unidirectional.

In Australia the WAN and EAN haplotypes belong to the native Australian (Asian-Australian clade) and occur in proximity to one another but are host isolated. The B biotype groups with the North Africa-Mediterranean-Middle East clade and are not naturally sympatric with either of them. The cross between the invasive B biotype and WAN was marginally compatible, whereas the cross between B with EAN produced no female offspring (De Barro and Hart 2000). Thus, at least some gene flow may be possible between some members of these two major clades.

Host-restricted and geographically isolated cassava-haplotypes extant in India and Africa respectively did not produce viable female offspring. Similarly, when the Indian cassava type was mated to a sympatric, polyphagous (non-cassava specialist) (Maruthi et al. 2001) haplotype no female offspring were produced (Maruthi et al. 2002). In this case it appeared that no gene flow occurred.

Qiu et al. (2007) studying phylogeography in relation to the host associations for haplotypes from China and India showed that haplotypes associated with cassava or other euphorbiaceous species were also found on other hosts. Thus, it is not entirely certain which, if any haplotypes might be cassava-adapted in India. Some may be strictly monophaous while others are polyphagous, as also is suspected for African haplotypes (Sseruwagi et al. 2006). This strategy could ensure adaptability under conditions when cassava is scarce.

Liu et al. (2007) reported studies with two allopatric haplotypes endemic to China (ZHJ1, ZHJ2) and the B biotype, mated in all possible combinations. The Chinese haplotypes, each from a different locale, exhibited mating compatibility. However, in reciprocal crosses, ZHJ1, and ZHJ2 females copulated with B males, whereas, B females rejected ZHJ1 and ZHJ2 males and copulated only with B males. Because the two heterologous females mated frequently and longer with B males, homologous males are thought to have had fewer opportunities to approach their respective females, which significantly reduced the number of native female offspring produced. These results were reported to reveal a new mechanism by which the

B biotype imposes displacement through mating competition and corroborated a similar pattern reported by Hadjistylli (2003) mentioned above, which predicted that the Q biotype would displace the B biotype. In this scenario, it was the Q biotype that out-competed the B for attention to homologous and heterologous females.

An example of allopatric populations that have produced hybrid offspring comes from molecular evidence for hybridization between the Uganda 1 (east Africa native) × putative west African Uganda 2. The latter is thought to be a recent introduction that catalyzed the initiation of the severe cassava mosaic disease, which involves several virulent begomoviruses (Legg et al. 2002, 2008). If so, this new haplotype would be the first naturally occurring hybrid documented. The extreme fitness (high fecundity) of this east-west hybrid is hypothesized to have been the driving force behind the severe cassava mosaic virus pandemic in eastern and Central Africa, beginning in Uganda in 1990 and spreading to at least 8 countries as of 2008 (Legg et al. 2002, 2008; Legg Chapter 7; JK Brown and JP Legg, unpublished data).

Among New World haplotypes examined together and/or in crosses with the B biotype, Costa et al. (1993) reported that several female offspring were produced in AZ-A × Arizona B (AZ-B) crosses, but were unable to corroborate this result. Caballero (2007) carried out all possible reciprocal crosses of single pairs and groups of 20 males and females of polyphagous AZ-A (Tropical Americas, native to southwestern USA), the polyphagous AZ-B (North Africa-Mediterranean Middle East clade), and the monophagous JAT-PR (Tropical America clade-Caribbean). Maternal hosts were used for all crosses, either cotton or *J. gossypifoli*. Female offspring were produced in all but two combinations, AZ-B♀ × AZ-A♂, and AZ-B♀ × JAT-PR♂. In this case at least, the Old World haplotype (AZ-B) was reproductively isolated from both New World biotypes, one polyphagous (AZ-A) and the other monophagous (JAD-PR). The AZ-A and JAT-PR are infected with divergent CI (cytoplasmic incompatibility)-causing bacteria *Cardinium* (Weeks et al. 2001) and *Wolbachia* (Stouthammer et al. 1999), respectively. In contrast, no CI bacteria could be detected in AZ-B, either by polymerase chain reaction or fluorescent hybridization. This difference could feasibly explain the observed unidirectional production of offspring from AZ-B crosses with either AZ-A or JAT-PR, given the expected restricted gene flow due to post zygotic interference from CI bacteria (Brown et al. 1998; Caballero 2007; Caballero and Brown 2008; Caballero et al. 2001).

## *Endosymbionts of the B. tabaci Complex*

Endosymbionts of *B. tabaci* are reviewed by Rosell et al. (Chapter 5) and briefly summarized here because of their relevance to speciation. The earliest studies on endosymbionts for *B. tabaci* were conducted prior to molecular tool development. Costa et al. (1995) described three symbiont morphotypes associated with the *B. tabaci* colonies maintained in the Arizona laboratory, AZ-A, AZ-B, and JAT-PR. A pleomorphic morphotype and another referred to as coccoid type I were observed in all three biotypes, but a third type, coccoid type II, was present in only AZ-A and JAT-PR biotypes. The pleomorphic morphotype, later named *Candidatus* Porteira aleyrodidarum (GenBank AY268082), has no visible cell wall and has been

identified as the primary symbiont of *B. tabaci*, (Thao and Baumann 2004a, b). It is most closely related to pseudomonads and not to *E. coli*, as are the primary symbionts of aphids.

### Secondary Symbionts

The two coccoid types were both characterized by Gram-negative cell walls and inner and outer cell membranes. Both types of have been identified in 13 whitefly species based initially on electron transmission studies (Costa et al. 1995) and later on 16S rRNA or 16S–23S rRNA sequence analysis, respectively. These are considered to be secondary and important, but non-essential (Thao and Baumann 2004a, b). Secondary symbionts are associated with all well-studied homopterans, and their specific biological role is not known. They may contribute to adaptation and ecological fitness, and their diversity is greater than primary symbionts (Chiel et al. 2007; Costa et al. 1995; Zchori-Fein and Brown 2002).

Molecular analysis of endosymbionts indicated that the AZ-A and AZ-B harbored a T-type symbiont, whereas, the "A-type symbiont" (*Arsenophonus* spp; Enterobacteriaceae, Proteobacteria) was associated with the remaining 11 species examined. The whitefly "T-type symbiont" ("Type I" according to Costa et al. 1995) is most closely related to T-type symbionts of aphids and the wasp, *Nasonia vitripennis* (Walker) (Thao and Baumann 2004a, b).

Coccoid type II morphologies (0.66–0.78 × 0.70–2.5 μm; cell wall ~30 nm) were associated with the AZ-A and JAT-PR biotypes (Costa et al. 1995). The morphotype observed in the AZ-A biotype had a distinctive filament-like structure in the cytoplasm that has recently has been identified in mites and dipteran parasitoids as *Cardinium*, which causes cytoplasmic incompatibility (Weeks et al. 2001, 2003). The coccoid type II bacteria was grouped in clusters in AZ-A ovarian tissues in greater numbers than the coccoid type I, a feature that is consistent with transovarial transmission typical of other sex-ratio distortion bacteria (Weeks and Breeuwer 2003; Stouthammer et al. 1999). In contrast, a coccoid type II symbiont was found in JAT-PR whiteflies scattered in groups similar to what was observed for coccoid type I is suspected to be *Wolbachia* (Stouthammer et al. 1999). This is consistent with the unidirectional pattern of reproduction observed in JAT-PR × AZ-B crosses.

In an independent study, a *Chlamydia* species was associated with field collection of *B. tabaci* and also with the laboratory colonies: AZ-A, AZ-B, and JAT-PR, based on PCR amplification of the 16S rRNA gene (Zchori-Fein and Brown 2002). These results support the hypothesis that secondary symbionts have infected *B. tabaci* multiple times, some being horizontally transmitted (Douglas 1998; Thao and Baumann 2004a, b).

### *Wolbachia* and *Cardinium* Infection

*Wolbachia* and *Cardinium* can cause reproductive abnormalities, including sex-linked mortality in arthropods. Both of these kinds of organisms have been identified in *B. tabaci* using molecular approaches (Brown et al. 1998; Caballero 2007;

Caballero and Brown 2008; Weeks and Breeuwer 2003; Weeks et al. 2001, 2003; Zchori-Fein et al. 2001), which support conclusions of Costa et al. (1995) based on electron microscopy examination of the identical colonies used for mating studies (Caballero 2007).

In another example, *Wolbachia* influenced sex ratio by re-directing developmental programs, sometimes causing female mortality (i.e. CI) (Stouthamer et al. 1999). CI is thought to manifest as unidirectional incompatibility by creating a post-zygotic barrier in which uninfected females do not produce female offspring. However, when a mixed infection occurs, infected females may produce female progeny, as observed in *B. tabaci* doubly infected with *Cardinium* and *Wolbachia* (Caballero 2007).

In summary a diverse suite of prokaryotes is associated with the *B. tabaci* sibling species group. It is well known in other arthropods that endosymbionts influence adaptation, fitness, survivability, sex ratio, and even reproductive isolation. A metagenomic analysis of diverse biotype-endosymbiont complexes in *B. tabaci* and other species would address this interesting hypothesis.

## Conclusions

The crossing studies reported here represent experiments conducted under different conditions with different haplotypes that are of agricultural importance, a small subset of haplotypes that may or may not be representative of the larger overall species. Nevertheless, the available results suggest a trend in which sympatric haplotypes were more likely than allopatric haplotypes to exhibit reproductive isolation. In other sibling species scenarios, gene flow can occur by direct transfer between compatible haplotypes, or indirectly between selectively compatible genotypes. Taken together, these results suggest that, as with other sibling species, gene flow within the *B. tabaci* group occurs between some but not all members and thus may be incomplete. Although speculative, this hypothesis is testable using strategic field collections and informative populations markers.

Other haplotypes that could be studied to test an allopatric-sympatric barrier hypotheses, are: (1) members of the B-like and Q-like sister clades, e.g. sympatric in North Africa and the Middle East; (2) the Q clade including the Spanish-Q and related haplotypes: TC (Turkey), SUD-Q, ISR-Q, and Tenerife-Q, all polyphagous haplotypes native to the Mediterranean region but not sympatric; (3) the polyphagous MS and non-B haplotypes comprising the same sister clade but not sympatric; (4) B- and Q-like haplotypes in Egypt, Israel, Jordan, Uganda, and Sudan that are sympatric (Berry et al. 2004; Brown, unpublished data; Delatte et al. 2005, 2006; Sserwuwagi et al. 2005); (5) Q-like haplotypes sympatric or allopatric with the S bioype (Spain and sub-Saharan Africa), (6) T haplotypes (Demichelis et al. 2005) that occur in the Mediterranean islands and Italy that are not sympatric (Berry et al. 2004; Demichelis et al. 2005; Simon et al. 2003); (7) two New World haplotypes that are geographically and host isolated, one being a desert dweller while the other is tropical (A, Jat); (6) EAN and WAN that are native to Australia and

isolated from all other haplotypes; and (7) ZHJ1 and ZHJ2, sympatric haplotypes endemic to China (Liu et al. 2007).

Widespread discontinuous gene flow in *B. tabaci* could explain why barriers exist between some, but not all, populations. Gill and Brown (Chapter 1) suggest that *B. tabaci* probably inhabited the earth before the most recent continental rearrangements occurred. If so, continuous gene flow may have once been common and possible. Following continental drift that achieved our extant configuration, climate change, and habitat adaptation, gene flow has been interrupted between certain haplotypes. Even so, catastrophic geologic and weather/climate events and human activities have fostered whitefly movement in various ways sufficient to bring together divergent haplotypes that have enabled periodic gene flow between haplotypes in the absence of reproductive barriers that either did not develop during isolation were subsequently shed. There is evidence that supports different mechanisms of reproductive isolation that could result in discontinuous gene flow within this sibling species assemblage.

Well-known biotypes of *B. tabaci* are now readily distinguished using bar coding and the extensive COI reference database. The most informative approach utilizes PCR amplification and DNA sequencing of the 780 base pair COI fragment that provides immediate identification upon comparative sequence analysis, or reveals a previously unstudied haplotype. Even so, morphological analysis identification remains the most common method for identifying *B. tabaci* in many laboratories.

A major impediment to advancing the biotype concept is the shortage of biological data available for the growing number of well-defined genetic variants. There is a need for quarantine-level insectaries in which whitefly colonies can be maintained in isolation and used to carry out rigorous life history, host range, mating compatibility, and virus-vector studies, among others. New and expanded population genetics tools are needed to further resolve key questions regarding gene flow and reproduction in the *B. tabaci* sibling species group. Microsatellite analysis of lesser-known haplotypes, and additional sister-, sub-, and major-clades is essential. With expanded microsatellite sets, it will become possible to employ high throughput SNPs and other new approaches to investigate population-level questions at more informative levels.

The *B. tabaci* sibling species group holds enough mysteries to fuel scientific inquiry for many years to come. Determining the whitefly genome and transcriptomes for representative biotypes will soon be possible, and at a fraction of yesterdays costs. The availability of the proteome will make it possible to link genotypic with phenotypic traits and reveal gene expression patterns that may underpin biotype formation and other important processes that contribute to behavioral plasticity in *B. tabaci*. Identifying proteins conserved in arthropods as a group and also in cross-kingdom interactions will pinpoint genes involved in adaptive processes ranging from reproductive isolation to phenotypic plasticity, as well as the basis for morphological recalcitrance, and the cellular basis of virus-vector specificity, collectively under diverse and challenging environments.

## Literature Cited

Abdullah NMM, Singh J, Sohal BS. 2006. Behavioral hormoligosis in oviposition preference of *Bemisia tabaci* on cotton. *Pestic. Biochem. Physiol.* 84:10–16.

Abdullahi I, Winter S, Atiri GI, Thottappilly G. 2003. Molecular characterization of whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) populations infesting cassava. *Bull. Entomol. Res.* 93:97–106.

Alon M, Benting J, Lueke B, Ponge T, Alon F, Morin S. 2006. Multiple origins of pyrethroid resistance in sympatric biotypes of *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Insect Biochem. Mol. Biol.* 36:71–79.

Anthony N, Brown JK, Markham PG, ffrench-Constant RH. 1995. Molecular analysis of cyclodiene resistance-associated mutations among populations of the sweetpotato whitefly *Bemisia tabaci*. *Pestic. Biochem. Physiol.* 51:220–228.

Attique MR, Rafiq M, Ghaffar A, Ahmad Z, Mohyuddin AI. 2003. Hosts of *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae) in cotton areas of Punjab, Pakistan. *Crop Prot.* 22:715–720.

Baumann P, Moran N, Baumann L. 2000. Bacteriocytes-associated endosymbionts of insects. In *The Prokaryotes*, ed. M Dworkin. New York: Springer.

Bayhan E, Ulusoy MR, Brown JK. 2006. Host species, distribution, and natural enemies of *Bemisia tabaci* 'B biotype' (Homoptera: Aleyrodidae) in Turkey. *J. Pest Sci.* 79:233–240.

Bedford ID, Briddon RW, Brown JK, Rosell RC, Markham PG. 1994. Geminivirus transmission and biological characterization of *Bemisia tabaci* (Gennadius) biotypes from different geographic regions. *Ann. Appl. Biol.* 125:311–325.

Be'eri Y, Kapuler B. 1963. Summer squash. In *Growing of Vegetables, Part B*, ed. YM Margalit, pp. 235–240. Tel Aviv (in Hebrew): Sifriyyat Hassadeh Publications.

Bellows TS, Perring TM, Gill RJ, Headrick DH. 1994. Description of a species of *Bemisia* (Homoptera: Aleyrodidae). *Ann. Entomol. Soc. Am.* 87:195–206.

Berry S, Rey MEC, Rogan D, Fondong VN, Fauquet CM, Brown JK. 2004. Molecular evidence for distinct *Bemisia tabaci* geographicaly genotypes from cassava in Africa. *Ann. Entomol. Soc. Am.* 97:852–859.

Bethke JA, Paine TD, Nuessly GS. 1991. Comparative biology, morphometrics and development of two populations of *Bemisia tabaci* (Homoptera: Aleyrodidae) on cotton and poinsettia. *Ann. Entomol. Soc. Am.* 84:407–411.

Bird J. 1957. A whitefly-transmitted mosaic of *Jatropha gossypifolia*. *Tech. Pap. P. R. Agric. Exp. Stn.* 22:1–35.

Bird J, Brown JK. 1998. Displacement of the *Sida* race of *Bemisia tabaci* by the B biotype of the *B. tabaci* complex in Puerto Rico. In *Proceedings of the Second International Whitefly and Geminivirus Workshop*, San Juan, Puerto Rico, June 8–12, 1998.

Bird J, Maramorosch K. 1975. *Tropical Diseases of Legumes*. New York: Academic Press.

Bird J, Maramorosch K. 1978. Viruses and virus diseases associated with whiteflies. *Adv. Virus Res.* 22:55–110.

Bird J, Sanchez J. 1971. Whitefly-transmitted viruses in Puerto Rico. *Tech. Pap. P. R. Agric. Exp. Stn.* 55:461–467.

Blackman RL, Cahill M. 1998. The karyotype of *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Bull. Entomol. Res.* 88:213–215.

Bonato O, Lurette A, Vidal C, Fargues J. 2007. Modelling temperature-dependent bionomics of *Bemisia tabaci* Q biotype. *Physiol. Entomol.* 32:50–55.

Boykin LM, Shatters RGJ, Rosell RC, McKenzie CL, Bagnall RA, De Barro PJ, Frolich DR. 2007. Global relationships of *Bemisia tabaci* (Hemiptera: Aleyrodidae) revealed using Bayesian analysis of mitochondria COI DNA sequences. *Mol. Phylogenet. Evol.* 16:1306–1319.

Brown WM. 1983. Evolution of animal mitochondrial DNA. In *Evolution of Genes and Proteins*, ed. M Nei, RK Koehn, pp. 62–88. Sunderland, MA: Sinauer.

Brown JK. 1990. An update on the whitefly-transmitted geminiviruses in the Americas and the Caribbean Basin. *FAO Plant Prot. Bull.* 39:5–23.

Brown JK. 1994. The status of *Bemisia tabaci* (Genn.) as a pest and vector in world agroecosystems. *FAO Plant Prot. Bull.* 42:3–32.

Brown JK. 2000. Molecular markers for the identification and global tracking of whitefly vector-begomovirus complexes. *Virus Res.* 71:233–260.

Brown JK. 2001. The Molecular Epidemiology of Begomoviruses. In *Trends in Plant Virology*, ed. JA Khan, J Dykstra, pp. 279–316. New York: The Haworth Press, Inc.

Brown JK. 2007. The *Bemisia tabaci* complex: genetic and phenotypic variability drives begomovirus spread and virus diversification. *Plant Dis.* http://www.apsnet.org/online/feature/btabaci/

Brown JK, Bird J. 1992. Whitefly-transmitted geminiviruses in the Americas and the Caribbean Basin: past and present. *Plant Dis.* 76:220–225.

Brown JK, Bird J. 1996. Introduction of an exotic whitefly (*Bemisia*) vector facilitates secondary spread of Jatropha mosaic virus, a geminivirus previously vectored exclusively by the Jatropha biotype. In *Bemisia 1995: Taxonomy, Biology, Damage, Control and Management*, ed. D Gerling, RT Mayer, pp. 351–353. Andover, Hants: Intercept Ltd.

Brown JK, Caballero R, Torres-Jerez I. 1998. First report of *Wolbachia* infection in *Bemisia tabaci* (Genn.) and a possible case of cytoplasmic incompatibility as evidenced by unidirectional mating between uninfected and infected whiteflies. In *Proceedings of the Whitefly-Geminivirus Workshop*. Managua, Nicaragua, October 24–30, 1998. p. 217.

Brown JK, Coats S, Bedford ID, Markham PG, Bird J, Frohlich DR. 1995a. Characterization and distribution of esterase electromorphs in the whitefly, *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae). *Biochem. Genet.* 33:205–214.

Brown JK, Costa HS, Laemmlen F. 1991. First incidence of whitefly-associated squash silverleaf (SSL) of *Cucurbita*, and of white streaking (WSt) disorder of cole crops in Arizona and California. *Plant Dis.* 76:426.

Brown JK, Dennehy TJ. 2006. First report of the Q biotype of *Bemisia tabaci* (Gennadius) in the U.S.A. and resistance to insecticides in an Arizona population. *Eur. Whitefly Stud. Network Newslett.* http://www.whitefly.org/whiteflyforum/forum_posts.asp?TID=32&PN=1

Brown JK, French R, Rogan D, Caballero R, Bird J. 2004a. Genetic variability and phylogeographical relationships of the *Bemisia tabaci* complex based on the mt cytochrome oxidase I gene. In *2nd European Whitefly Symposium*, Cavtat, Croatia, October 5–9, 2004.

Brown JK, Frolich DR, Rosell RC. 1995. The sweetpotato or silverleaf whiteflies: biotypes of *Bemisia tabaci* or a species complex? *Annu. Rev. Entomol.* 40:511–534.

Brown JK, Idris AM. 2005. Genetic differentiation of the whitefly *Bemisia tabaci* (Genn.) mitochondria COI and geographic congruence with the coat protein of the plant virus genus: *Begomovirus*. *Ann. Entomol. Soc. Am.* 98:827–837.

Brown JK, Nelson MR. 1984. Geminate particles associated with cotton leaf crumple disease in Arizona. *Phytopathology* 74:987–990.

Brown JK, Nelson MR. 1986. Whitefly-borne viruses of melons and lettuce in Arizona. *Phytopathology* 76:236–239.

Brown JK, Perring TM, Cooper AD, Bedford ID, Markham PG. 2000. Genetic analysis of *Bemisia* (Homoptera: Aleyrodidae) populations by isoelectric focusing electrophoresis. *Biochem. Genet.* 38:13–25.

Brown JK, Rogan D, Legg J. 2004b. Hybridization between the 'invader' and 'local' *B. tabaci* haplotypes associated with severe mosaic disease of cassava in East Africa. In *2nd European Whitefly Symposium*, Cavtat, Croatia, October 5–9, 2004.

Burban C, Fishpool LDC, Fauquet C, Fargette D, Thouvenel JC. 1992. Host associated biotypes within West African populations of the whitefly *Bemisia tabaci* (Genn.) (Hom., Aleyrodidae). *J. Appl. Entomol.* 113:416–423.

Butler GD, Jr, Brown JK. 1985. Sweetpotato whitefly infection of cotton leaf crumple from weed hosts in 1984. The University of Arizona, Tucson.

Butler GD, Jr, Brown JK, Henneberry TJ. 1986. Effect of cotton seedling infection by cotton leaf crumple virus on subsequent growth and yield. *J. Econ. Entomol.* 79:208–211.

Butler GD, Jr, Henneberry TJ, Clayton TE. 1983. *Bemisia tabaci* (Homoptera: Aleyrodidae): development, oviposition, and longevity in relation to temperature. *Ann. Entomol. Soc. Am.* 76:310–313.

Byrne DN, Bellows TS, Parrella MP. 1990. Whiteflies in agricultural systems. In *Whiteflies: Their Bionomics, Pest Status and Management*, ed. D Gerling, pp. 227–261. Andover, Hants: Intercept Ltd.

Byrne FJ, Cahill M, Denholm I, Devonshire AL. 1995. Biochemical identification of interbreeding between B-type and non-B-type strains of the tobacco whitefly *Bemisia tabaci*. *Biochem. Genet.* 33:13–23.

Byrne DN, Miller WB. 1990. Carbohydrate and amino acid composition of phloem sap and honeydew produced by *Bemisia tabaci*. *J. Insect Physiol.* 36:433–440.

Caballero R. 2007. Systematics of the *Bemisia tabaci* complex and the role of endosymbionts in reproductive compatibility. PhD Dissertation, The University of Arizona, Tucson, AZ. 107pp.

Caballero R, Brown JK. 2008. Positive evidence for interbreeding and differential gene flow between three well characterized biotypes of the *Bemisia tabaci* complex (Gennadius) (Hemiptera: Aleyrodidae) excludes geographic and host barriers as isolating factors. *J. Insect Sci.* 8:10–11.

Caballero R, Torres-Jerez I, Brown JK. 2001. Two distinct *Wolbachia* identified in *Bemisia tabaci* (Genn.) are associated with unidirectional cytoplasmic incompatibility in infected and uninfected biotypes. In *Proceedings of The Sixth International Whitefly and Geminivirus Workshop*, Ragusa, Sicily, February 27–March 3, 2001.

Campbell B. 1993. Congruent evolution between whiteflies and their bacterial endosymbionts based on the respective 18S and 16S rDNAs. *Curr. Microbiol.* 26:129–132.

Campbell BC, Steffen-Campbell JD, Gill RJ. 1994. Evolutionary origin of whiteflies (Hemiptera: Sternorhyncha: Aleyrodidae) inferred from 18S rDNA sequences. *Insect Mol. Biol.* 3:73–88.

Campbell BC, Steffen-Campbell JD, Gill RJ. 1996. Origin and radiation of whiteflies: an initial molecular phylogenetic assessment. In *Bemisia 1995: Taxonomy, Biology, Damage, Control and Management*, ed. D Gerling, RT Mayer, pp. 29–51. Andover, Hants: Intercept Ltd.

Carabali A, Bellotti AC, Montoya-Lerma J, Cuellar ME. 2005. Adaptation of *Bemisia tabaci* biotype B (Gennadius) to cassava, *Manihot esculenta* (Crantz). *Crop Prot.* 24:643–649.

Chiel E, Gottlieb Zchori-Fein E, Mozes-Daube N, Katzir N, Inbar M, Ghanim M. 2007. Biotype-dependent secondary symbiont communities in sympatric populations of *Bemisia tabaci*. *Bull. Entomol. Res.* 97:407–413.

Clark MA, Baumann L, Munson MA, Baumann P, Campbell BC, Duffus JE, Osborne SL, Moran NA. 1992. The eubacterial endosymbionts of whiteflies (Homoptera: Aleyrodidae) constitute a lineage distinct from the endosymbionts of aphids and mealybugs. *Curr. Microbiol.* 25:119–123.

Coats SA, Brown JK, Hendrix DL. 1994. Biochemical characterization of biotype specific esterases in the whitefly *Bemisia tabaci* Genn. (Homoptera: Aleyrodidae). *Insect Biochem. Mol. Biol.* 24:723–728.

Cock MJW. 1986. *Bemisia tabaci – a Literature Survey on the Cotton Whitefly with an Annotated Bibliography*. Ascot: FAO/CAB.

Cock MJW. 1993. *Bemisia tabaci, an Update 1986–1992 on the Cotton Whitefly with an Annotated Bibliography*. Silwood Park, Ascot, Berks: CAB International Institute of Biological Control, 78 pp.

Costa HS, Brown JK. 1990. Variation in biological characteristics, isozyme patterns and virus transmission among populations of *Bemisia tabaci* (Genn.) in Arizona. *Phytopathology* 80:888.

Costa HS, Brown JK. 1991. Variation in biological characteristics and in esterase patterns among populations of *Bemisia tabaci* (Genn.) and the association of one population with silverleaf symptom development. *Entomol. Exp. Appl.* 61:211–219.

Costa HS, Brown JK, Byrne DN. 1991a. Host plant selection by the whitefly, *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae) under greenhouse conditions. *J. Appl. Entomol.* 112:146–152.

Costa HS, Brown JK, Byrne DN. 1991b. Life history traits of the whitefly *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae) on six virus-infected or healthy plant species. *Environ. Entomol.* 20:1102–1107.

Costa HS, Brown JK, Sivasupramaniam S, Bird J. 1993. Regional distribution, insecticide resistance, and reciprocal crosses between the 'A' and 'B' biotypes of *Bemisia tabaci*. *Insect Sci. Appl.* 14:127–138.

Costa AS, Russell LM. 1975. Failure of *Bemisia tabaci* to breed on cassava plants in Brazil (Homoptera: Aleyrodidae). *Cienc. Cult.* 27:388–390.

Costa HS, Toscano NC, Henneberry TJ. 1996. Mycetocyte inclusion in the oocytes of *Bemisia argentifolii* (Homoptera: Aleyrodidae). *Ann. Entomol. Soc. Am.* 89:694–699.

Costa HS, Wescot DM, Ullman DE, Rosell R, Brown JK, Johnson MW. 1995. Morphological variation in *Bemisa* endosymbionts. *Protoplasma* 189:194–202.

De Barro PJ, Bourne A, Khan SA, Brancatini VAL. 2006. Host plant and biotype density interactions-their role in the establishment of the invasive B biotype of *Bemisia tabaci*. *Biol. Invasions* 8:287–294.

De Barro PJ, Driver F, Trueman JWH, Curran J. 2000. Phylogenetic relationships of world populations of *Bemisia tabaci* (Gennadius) using ribosomal ITS1. *Mol. Phylogenet. Evol.* 16:29–36.

De Barro PJ, Hart PJ. 2000. Mating interactions between two biotypes of the whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Australia. *Bull. Entomol. Res.* 90:103–112.

De Barro PJ, Trueman JWH, Frolich DR. 2005. *Bemisia argentifolii* is a race of *B. tabaci* (Hemiptera: Aleyrodidae): the molecular differentiation of *B. tabaci* populations around the world. *Bull. Entomol. Res.* 95:193–203.

De la Rúa P, Simón B, Cifuentes D, Martinez-Mora C, Cenis JL. 2006. New insights into the mitochondrial phylogeny of the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) in the Mediterranean Basin. *J. Zool. Syst. Evol. Res.* 44:25–33.

Delatte H, David P, Granier M, Lett JM, Goldbach R, Peterschmitt M, Reynaud B. 2006. Microsatellites reveal extensive geographical, ecological and genetic contacts between invasive and indigenous whitefly biotypes in an insular environment. *Genet. Res. Camb.* 87:109–124.

Delatte H, Reynaud B, Granier M, Thornary L, Lett JM, Goldbach R, Peterschmitt M. 2005. A new silverleaf-inducing biotype Ms of Bemisia tabaci (Hemiptera: Aleyrodidae) indigenous to the islands of the southwest Indian Ocean. *Bull Entomol. Res.* 95:29–35.

Demichelis S, Arno C, Bosco D, Marian D, Caciagli P. 2005. Characterization of biotype T of *Bemisia tabaci* associated with *Euphorbia characias* in Sicily. *Phytoparasitica* 33:196–208.

Dennehy TJ, DeGain BA, Harpold VS, Brown JK, Morin S, Fabrick JA, Byrne FJ, Nichols RL. 2006. New whitefly biotype with high levels of resistance to insecticides. The University of Arizona – Cooperative Extension Report, 32pp. Series P-144, ed. DN Byrne, P Baciewicz. http://cals.arizona.edu/pubs/crops/az1382/az1382_2.pdf

Dittrich V, Ernst GH, Ruesch O, Uk S. 1990. Resistance mechanisms in sweetpotato whitefly (Homoptera: Aleyrodidae) populations from Sudan, Turkey, Guatemala, and Nicaragua. *J. Econ. Entomol.* 83:1665–1670.

Douglas AE. 1998. Nutritional interactions in insect-microbial symbioses: aphids and their symbiotic bacteria Buchnera. *Annu. Rev. Entomol.* 43:17–37.

Frohlich DR, Brown JK. 1994. Mitochondrial 16S ribosomal subunit as a molecular marker in *Bemisia tabaci* and implications for population variability. In *Abstract of Papers Presented at The International Workshop on Bemisia spp.*, Shoresh, Israel, October 3–7, 1994.

Frohlich DR, Brown JK, Bedford I, Markham PG. 1996. Mitochondrial 16S ribosomal subunit as a molecular marker in *Bemisia*, and implications for population variability. In *Bemisia 1995: Taxonomy, Biology, Damage, Control and Management*, ed. D Gerling, RT Mayer, pp. 143–145. Andover, Hants: Intercept Ltd.

Frohlich D, Torres-Jerez I, Bedford ID, Markham PG, Brown JK. 1999. A phylogeographic analysis of the *Bemisia tabaci* species complex based on mitochondrial DNA markers. *Mol. Ecol.* 8:1593–1602.

Gawel NJ, Bartlett AC. 1993. Characterization of differences between whiteflies using RAPD-PCR. *Insect Mol. Biol.* 2:33–38.

Gennadius P. 1889. Disease of tobacco plantations in the Trikonia. The aleurodid of tobacco. [In Greek]. *Ellenike Georgia* 5:1–3.

Gibson RW, Legg JP, Otim-Nape G. 1996. Unusually severe symptoms are a characteristic of the current epidemic of mosaic virus disease of cassava in Uganda. *Ann. Appl. Biol.* 128:479–490.

Gill RJ. 1990. The morphology of whiteflies. In *Whiteflies: Their Bionomics, Pest Status and Management*, ed. D Gerling, pp. 13–46. Andover, Hants: Intercept Ltd.

Gill RJ. 1992. A review of the sweetpotato whitefly in southern California. *Pan-Pac. Entomol.* 68:144–152.

Guirao PF, Beitia F, Cenis JL. 1997. Biotype determination of Spanish populations of *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Bull. Entomol. Res.* 87:587–593.

Hadjistylli MC. 2003. Mating interactions between two *Bemisia tabaci* biotypes and their implications for pest and resistance management. MS Thesis, Imperial College, London. 80pp.

Hamon A, Salguero V. 1987. *Bemisia tabaci*, sweetpotato whitefly, in Florida (Homoptera: Aleyrodidae: Aleyrodinae). Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville. Entomology Circular No. 292.

Harrison BD, Zhou X, Otim-Nape GW, Liu Y, Robinson DJ. 1997. Role of a novel type of double infection in the geminivirus-induced epidemic of severe cassava mosaic in Uganda. *Ann. Appl. Biol.* 131:437–448.

Horowitz AR, Denholm I, Gorman K, Cenis JL, Kontsedaloy S. 2003. Biotype Q of *Bemisia tabaci* identified in Israel. *Phytoparasitica* 31:94–98.

Hsieh CH, Wang CH, Ko CC. 2006. Analysis of *Bemisia tabaci* (Hemiptera: Aleyrodidae) species complex and distribution in eastern Asia based on mitochondrial DNA markers. *Ann. Entomol. Soc. Am.* 99:968–975.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17:754–755.

Husain MA, Trehan KN. 1933. Observations on the life-history, bionomics and control of the whitefly of cotton (*Bemisia gossypiperda* M and L). *Indian J. Agric. Sci.* 3:701–753.

Kanmiya K. 2006. Mating behaviour and vibratory signals in whiteflies (Hemiptera: Aleyrodidae). In *Contemporary Topics in Entomology Series: Insect Sounds and Communication*, ed. S Drosopoulous, M Claridge, pp. 365–379. London: Taylor & Francis.

Khasdan V, Levin I, Rosner A, Morin S, Konsedalov S, Rippa M, Kleitman S, Mory R, Horowitz AR. 2005. Using DNA markers to identify biotypes B and Q of *Bemisia tabaci* in population dynamics studies in Israel. In *21st Annual Meeting of the Entomology Society of Israel*, Bet Dagan, Israel.

Kirk AA, Lacey LA, Brown JK, Ciomperlik MA, Goolsby JA, Vacek DC, Wendel LE, Napompeth B. 2000. Variation within the *Bemisia tabaci* s.l. species complex (Hemiptera: Aleyrodidae) and its natural enemies leading to successful biological control of *Bemisia* biotype B in the USA. *Bull Entomol. Res.* 90:317–327.

Legg JP. 1994. Genetic polymorphisms amongst Ugandan populations of *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae), vector of African cassava mosaic geminivirus. *Trop. Sci.* 34:73–81.

Legg JP. 1996. Host-associated strains within Ugandan populations of the whitefly *Bemisia tabaci* (Genn.) (Hom, Aleyrodidae). *J. Appl. Entomol.* 120:523–527.

Legg JP, Asiimwe P, Njukeng AP, Sseruwagi P, Brown JK. 2008. Distribution trends of two haplotypes of the *Bemisia tabaci* complex associated with cassava and the severe mosaic disease pandemic in Africa. In *Proceedings of the 4th International Whitefly Meeting*, Duck Key, Florida, December 3–6, 2006. *J. Insect Sci.* 8:27–28.

Legg JP, French R, Rogan D, Okao-Okuja G, Brown JK. 2002. A distinct *Bemisia tabaci* (Gennadius) (Hemiptera: Sternorrhyncha: Aleyrodidae) genotype cluster is associated with the epidemic of severe cassava mosaic virus disease in Uganda. *Mol. Ecol.* 11:1219–1229.

Liu SS, De Barro PJ, Xu J, Luan JB, Zang LS, Ruan YM, Wan FH. 2007. Asymmetric mating interactions drive widespread invasion and displacement in a whitefly. *Science* 318:1769–1772.

Lopez-Avila A. 1986. Taxonomy and biology. In *Bemisia tabaci – A literature Survey on the Cotton Whitefly with an Annotated Bibliography*, ed. MJW Cock, pp. 3–11. Silwood Park, Ascot, Berks: CAB International Institute of Biological Control.

Martin JH. 1987. An identification guide to common whitefly pest species of the world (Homoptera: Alerodidae). *Trop. Pest Manage.* 33:298–322.

Martin JH. 2003. Whiteflies (Hemiptera: Aleyrodidae) – their systematic history and the resulting problems of conventional taxonomy, with special reference to descriptions of *Aleyrodes proletella* (Linnaeus, 1758) and *Bemisia tabaci* (Gennadius, 1889). *Entomol. Gaz.* 54:125–136.

Martin JH, Mound LA. 2007. An annotated check list of the world's whiteflies (Insecta: Hemiptera: Aleyrodidae). *Zootaxa* 149:21–84.

Maruthi MN, Colvin J, Seal S. 2001. Mating compatibility, life-history traits, and RAPD-PCR variation in *Bemisia tabaci* associated with the cassava mosaic disease pandemic in East Africa. *Entomol. Exp. Appl.* 99:13–23.

Maruthi MN, Colvin J, Seal S, Gibson G, Cooper J. 2002. Co-adaptation between cassava mosaic geminiviruses and their local vector populations. *Virus Res.* 86:71–85.

Morin S, Williamson MS, Goodson SJ, Brown JK, Tabashnik BE, Dennehy TJ. 2002. Mutations in the *Bemisia tabaci* sodium channel gene associated with resistance to a pyrethroid plus organophosphate mixture. *Insect Biochem. Mol. Biol.* 32:1781–1791.

Mound LA. 1963. Host correlated variation in *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae). *Proc. R. Entomol. Soc. London A* 38:171–180.

Mound LA. 1984. Zoogeographical distribution of whiteflies. *Curr. Topics Vector Res.* 2:185–197.

Mound LA. 1993. Whitefly evolutionary strategies: host-specificity, polyphagy, or biotypes. In *Memorias, XX Congresso de la Sociedad Colombiana de Entomologia*, July 13–16, 1993, Cali, Colombia, pp. 244–250.

Mound LA, Halsey SH. 1978. *Whitefly of the World: A Systematic Catalogue of the Aleyrodidae (Homoptera) with Host Plant and Natural Enemy Data*. Chichester: British Museum and John Wiley & Sons, 340pp.

Moya A, Guirao P, Cifuentes D, Beitia F, Cenis JL. 2001. Genetic diversity of Iberian populations of *Bemisia tabaci* (Hemiptera: Aleyrodidae) based on random amplified polymorphic DNA-polymerase chain reaction. *Mol. Ecol.* 10:891–897.

Neil JW, Bentz J. 1999. Evidence for the stage inducing phenotypic plasticity in pupae of the polyphagus whiteflies *Trialeurodes vaporariorum* and *Bemisia argentifolii* [(sic) *tabac*i] (Homoptera: Aleyrodidae) and the raison d' être. *Ann. Entomol. Soc. Am.* 92:774–787.

Osmondi AB, Obeng-Otori D, Kyerematen RA, Danquah EY. 2005. Host preferences and suitability of some selected crops for two biotypes of *Bemisia tabaci* in Ghana. *Entomol. Exp. Appl.* 115:393–400.

Pascual S. 2006. Mechanisms in competition under laboratory conditions between Spanish biotypes B and Q of *Bemisia tabaci* (Gennadius). *Span. J. Agric. Res.* 4:351–354.

Pascual S, Callèjas C. 2004. Intra- and interspecific competition between biotypes B and Q Bemisia tabaci (Hemiptera: Aleyrodidae) from Spain. *Bull. Entomol. Res.* 94:369–375.

Perring TM. 2001. The *Bemisia tabaci* species complex. *Crop Prot.* 20:725–737.

Perring TM, Cooper A, Rodriguez RJ, Farrar CA, Bellows TS, Jr. 1993. Identification of whitefly species by genomic and behavioral studies. *Science* 259:74–77.

Perring T, Symmes EJ. 2006. Courtship behavior of *Bemisia argentifolii* (Hemiptera: Aleyrodidae) and whitefly mate recognition. *Ann. Entomol. Soc. Am.* 99:598–606.

Qiu B-L, Coats SA, Ren SX, Idris AM, Caixia XU, Brown JK. 2007. Phylogenetic relationships of native and introduced *Bemisia tabaci* (Homoptera: Aleyrodidae) from China and India based on mtCO1 sequencing and host plant comparisons. *Prog. Nat. Sci.* 17:645–654.

Rogers AR, Harpending H. 1992. Population growth makes waves in the distribution of pairwise genetic differences. *Mol. Biol. Evol.* 9:552–569.

Ronda M, Adan A, Beitia F, Cifuentes D, Cenis JL. 2000. Interbreeding between biotypes of *Bemisia tabaci*. *Eur. Whitefly Stud. Network Newslett.* November (Issue No. 07).

Rosell RC, Bedford ID, Frolich DR, Gill RJ, Brown JK, Markham PG. 1997. Analysis of morphological variation in distinct populations of *Bemisia tabaci* (Homoptera: Aleyrodidae). *Ann. Entomol. Soc. Am.* 90:575–589.

Rosemeyer ME, Brown JK, Nelson MR. 1986. Five viruses isolated from field-grown buffalo gourd, *Cucurbita foetidissima* HBK, a potential crop for semi-arid lands. *Plant Dis.* 70:405–409.

Russell LM. 1957. Synonyms of *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae). *Bull. Brooklyn Entomol. Soc.* 52:122–123.

Schlee D. 1970. Verwandtschaftsforschung an fossilen und rezenten Aleyrodina (Insectas, Hemiptera). *Stuttgarter Beiträge zur Naturkunde* 213:1–74.

Schrader F. 1920. Sex determination in the whitefly (*Trialeurodes vaporariorum*). *J. Morphol.* 34:267–297.

Schuster DJ, Mueller TF, Kring JB, Price JF. 1990. Relationship of sweet potato whitefly to a new tomato fruit disorder in Florida. *Hortic. Sci.* 25:1618–1620.

Servín-Villegas R, Troyo-Dieguez E, Martínez-Carrillo JL. 2006. Wild hosts of *Bemisia argentifolii* Bellows & Perring in semiarid northwest Mexico. *Southwest. Entomol.* 26:239–244.

Simon B, Cenis JL, Demichelis S, Rapisarda C, Caciagli P, Bosco D. 2003. Survey of *Bemisia tabaci* (Hemiptera: Aleyrodidae) biotypes in Italy (T) from *Euphorbia characias*. *Bull. Entomol. Res.* 93:259–264.

Sseruwagi P, Legg J, Maruthi MN, Colvin J, Rey MEC, Brown JK. 2005. Genetic diversity of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) populations and presence of the B biotype and a non-B biotype that can induce silverleaf symptoms in squash, in Uganda. *Ann. Appl. Biol.* 147:253–265.

Sseruwagi P, Maruthi MN, Colvin J, Rey MEC, Brown JK, Legg JP. 2006. Colonization of non-cassava plant species by cassava whiteflies (*Bemisia tabaci*) in Uganda. *Entomol. Exp. Appl.* 119:145–153.

Stouthamer R, Breeuwer JAJ, Hurst GDD. 1999. *Wolbachia pipientis*: microbial manipulator of arthropod reproduction. *Annu. Rev. Microbiol.* 53:71–102.

Thao ML, Baumann P. 2004a. Evolutionary relationships of primary prokaryotic endosymbionts of whiteflies and their hosts. *Appl. Environ. Microbiol.* 70:3401–3406.

Thao ML, Baumann P. 2004b. Evidence for multiple acquisition of *Arsenophonus* by whitefly species (Sternorrhyncha: Aleyrodidae). *Curr. Microbiol.* 48:140–144.

Thompson WMO. 2003. A new host plant species for the cassava biotype of Bemisia tabaci (Gennadius) (Hom., Aleyrodidae). *J. Appl. Entomol.* 127:374–376.

Ueda S, Kitamura S, Kijima K, Honda KI, Kanmiya K. 2009. Distribution and molecular characterization of distinct Asian populations of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Japan. *J. Appl. Entomol.* 133:355–366.

Viscarret MM, Torres-Jerez I, Agostini de Manero E, López SN, Botto EE, Brown JK. 2003. Mitochondrial DNA evidence for a distinct clade of New World *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae) from Argentina and Bolivia, and presence of the Old World B biotype in Argentina. *Ann. Entomol. Soc. Am.* 96:65–72.

Walker GP, Perring TM. 1994. Feeding and oviposition behavior of whiteflies (Homoptera: Aleyrodidae) interpreted from AC electronic feeding monitor waveforms. *Ann. Entomol. Soc. Am.* 87:363–374.

Weeks AR, Breeuwer JAJ. 2003. A new bacterium from the *Cytophaga-Flavobacterium-Bacteroides* phylum that causes sex-ratio distortion. In *Insect Symbiosis*, ed. K Bourtzis, TA Miller, pp. 165–176. Boca Raton, FL: CRC Press.

Weeks AR, Marec F, Breeuwer JAJ. 2001. A mite species that consists entirely of haploid females. *Science* 292:2479–2482.

Weeks AR, Velten R, Stouthamer R. 2003. Incidence of a new sex-ratio-distorting endosymbiotic bacterium among arthropods. *Proc. R. Soc. Lond. B Biol. Sci.* 270:1857–1865.

Wool D, Gerling D, Belloti AC, Morales FJ. 1993. Esterase electrophoretic variation in *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae) among host plants and localities in Israel. *J. Appl. Entomol.* 115:185–196.

Wool D, Gerling D, Nolt BL, Constantino LM, Bellotti AC, Morales FJ. 1989. The use of electrophoresis for identification of adult whiteflies (Homoptera: Aleyrodidae) in Israel and Colombia. *J. Appl. Entomol.* 107:344–350.

Xingxia WU, Zhengxi LI, Dunxiao HU, Zuorui S. 2003. Identification of Chinese populations of *Bemisia tabaci* (Gennadius) by analyzing ribosomal ITS1 sequence. *Prog. Nat. Sci.* 13:276–281.

Yokomi RK, Hoelmer KA, Osborne LS. 1990. Relationship between the sweetpotato whitefly and the squash silverleaf disorder. *Phytopathology* 80:895–900.

Zchori-Fein E, Brown JK. 2002. Diversity of prokaryotes associated with *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae). *Ann. Entomol. Soc. Am.* 95:711–718.

Zchori-Fein E, Gottlieb EY, Kelly SE, Brown JK, Wilson JM, Karr TL, Hunter MS. 2001. A newly discovered bacterium is associated with parthenogenesis and a change in host selection behavior in parasitoid wasps. *Proc. Natl. Acad. Sci.* 98:12555–12560.

Zhang DX, Hewitt GM. 1996. Nuclear integrations: challenges for mitochondrial markers. *Trends Ecol. Evol.* 11:247–251.

Zhang LP, Zhang YJ, Zhang WJ, Wu QJ, Xu BY, Chu D. 2005. Analysis of genetic diversity among different geographical populations and determination of biotypes of *Bemisia tabaci* in China. *J. Appl. Entomol.* 129:121–128.

# Chapter 3
# Tools and Recent Progress in Studying Gene Flow and Population Genetics of the *Bemisia tabaci* Sibling Species Group

**Margarita Hadjistylli, Judith K. Brown, and George K. Roderick**

## Introduction

The use of molecular markers in the *Bemisia tabaci* complex has been a definitive step in identifying the enormous genetic diversity hidden behind the morphological likeness among its members (see Gill and Brown, Chapter 1), and in determining interrelationships. The presence of biologically-based biotypes in *B. tabaci* was first realized in the 1950s by Bird (Bird 1957; Bird and Maramorosch 1978), who found that morphologically indistinguishable populations of the whitefly differed substantially in biological and ecological traits, including host range, adaptability to different hosts, and plant virus-transmission efficiencies. Later studies used ecological and biological experiments to examine mating compatibilities as well as differences among distinct populations in phytotoxic induction, insecticide resistance, and behavior (Brown et al. 1995b). The use of molecular markers, starting in the late 1980s with allozymes, was an attempt to assess variability at the molecular level and provide a tool to distinguish among biological and ecological variants. Allozymes were used for more than 10 years in *B. tabaci* studies for identification of certain variants in a region, and also served as the basis for biotype characterization and nomenclature which is still largely in use today. Interest in the *B. tabaci* system grew following the first major outbreak of the invasive B biotype in the southwestern United States in 1991, and the subsequent displacement of the indigenous A biotype (Brown et al. 1995b). The worldwide expansion in range of biotype B in the years that followed, accompanied by significant economic consequences in the agricultural sector, prompted an international effort involving intensive studies aimed at understanding genetic variability and possible correlations with host use and/or geographic origins.

While the use of allozymes revealed significant polymorphism within *B. tabaci*, rapid technological advancements in the field of molecular genetics allowed the

M. Hadjistylli (✉)
Department of Environmental Science, Policy, and Management, University of California, Berkeley, CA 94720-3114, USA
e-mail: margarita@berkeley.edu

P.A. Stansly, S.E. Naranjo (eds.), *Bemisia*: Bionomics and Management of a Global Pest,
DOI 10.1007/978-90-481-2460-2_3, 

exploitation of new, neutral multilocus molecular markers. A wealth of literature continues to be produced by research laboratories worldwide using molecular tools to examine differences among biotypes in biological and ecological traits including host use, virus transmission efficiency, insecticide resistance, breeding incompatibility, endosymbiont composition, and finally, geographical distribution. Most of these studies have only been able to make associations between these characteristics and genetic variants leading to general conclusions about the genetic and geographic structure of biotypes.

The use of molecular markers in *B. tabaci* has not only revealed important biological information, but also has practical applications. In pest management for example, it is useful to identify specific biotypes that are associated with economically important traits, including invasiveness, ability to disperse, insecticide resistance, higher potential for plant damage and transmission of plant viruses. For example, specific molecular markers were developed to identify the invasive B biotype and track its spread into new regions. This ability to determine early the presence of a biotype possibly carrying resistance alleles in a new region has allowed timely adjustments in management practices in the USA and elsewhere. Molecular markers have also been used to identify and track natural enemies of *B. tabaci* in its native range, making use of correlations between the biogeographic lineages, and pinpointing the most likely geographic locale to scour for natural enemies that co-evolved with their whitefly host (Kirk et al. 2000).

More complex issues can also be addressed with the use of genetic markers coupled with appropriate analyses. For example, one can ask questions concerning the colonization history of a biotype in an area, e.g., when was a biotype introduced in an area, where did it come from, and what route did it follow? One can also address the demographic history, including the founding effective population size, the extent of a genetic bottleneck associated with the founding event, and the pattern of subsequent population growth. A final, and often neglected point is that different genetic markers may originate from different parts of the genome, and as such, may have distinct properties that will affect suitability for certain studies. The choice of markers should therefore depend on the question one is seeking to answer, the objectives of the study, the extent of genetic polymorphism required to address the specific questions, and the statistical approaches available for the application of each technique (Parker et al. 1998).

This review aims to provide a historical perspective on use of the types of multilocus molecular markers that are available to study population genetics of organisms in general. At the same time, we highlight studies of the *B. tabaci* sibling species group, which therein illustrates the paucity of available population genetics data from which profound or definitive conclusions can be drawn. The use of allozymes to categorize biotypes based on esterase bands is summarized, as well as the use of markers and microsatellite allele frequencies to detect fine scale population differentiation. We distinguish between markers based on protein variation, DNA sequence variation and frequency variation through the polymerase chain reaction (PCR). For each, we provide an overview of the marker system, outline advantages and limitations, and provide a review of the applications (Table 3.1). We conclude by noting

**Table 3.1** Multilocus molecular markers used in studies of *Bemisia tabaci*

| | Allozymes | RFLPs[1] | RAPDs[2] | AFLPs[3] | Microsatellites |
|---|---|---|---|---|---|
| *A. Markers and properties, reproduced from Lowe et al. (2004) by permission of the authors and publisher* | | | | | |
| Polymorphism | Charged amino acid substitutions | Nucleotide substitutions, indels, inversions | Nucleotide substitutions, indels, inversions | Nucleotide substitutions, indels, inversions | Repeat number changes |
| Level of polymorphism | Low | Medium | Medium | Medium | High |
| Abundance in genome | Low | High | Very high | High | Medium |
| Dominance | Usually codominant | Codominant | Dominant | Codominant/dominant | Codominant |
| Development costs | Low | Medium | Low | Medium | High |
| Start up costs | Low | Medium/high | Low | Medium | High |
| Automation | No | Limited | Yes | Yes | Yes |
| Reproducibility | Medium/high | High | Low | Medium | High |
| *B. Applications in* Bemisia tabaci | | | | | |
| Host associations | Brown et al. (1995a); Brown and Bird (1996); Burban et al. (1992); Costa and Brown (1991); Wool et al. (1991, 1993) | Abdullahi et al. (2004) | Abdullahi et al. (2003) | – | De Barro (2005) |
| Insecticide resistance | Anthony et al. (1995); Byrne et al. (2000); Coats et al. (1994) | – | – | – | – |
| Sex determination, haplodiploidy | Byrne and Devonshire (1996) | – | – | – | – |
| Interbreeding | Bedford et al. (1994); Byrne et al. (1995); Caballero (2007); Costa et al. (1993); Gunning et al. (1997) | – | – | – | Delatte et al. (2006) |

**Table 3.1** (continued)

| | Allozymes | RFLPs[1] | RAPDs[2] | AFLPs[3] | Microsatellites |
|---|---|---|---|---|---|
| Virus epidemic associations | Bedford et al. (1994); Brown and Bird (1992); Brown (2007); Brown and Bird (1996); Brown et al. (1993); Legg et al. (2002) | – | Maruthi et al. (2002) | – | – |
| Biotype/Haplotype/ Species identification | Costa and Brown (1990, 1991); Costa et al. (1993); Perring et al. (1992); Brown et al. (1995a); Brown et al., (2000) | Bosco et al. (2006) | Gawel and Bartlett (1993); Guirao et al. (1997); Delatte et al. (2005); Horowitz et al. (2003); Simon et al. (2003); McKenzie et al. (2004); Khasdan et al. (2005) | Cervera et al. (2000) | Tsagkarakou et al. (2007) |
| Geographic structure | – | – | De Barro and Driver (1997); De Barro et al. (1998); Rekha et al. (2005) | Cervera et al. (2000); Zhang et al. (2005) | De Barro (2005) |
| Population differentiation | Costa et al (1993); Brown et al. (1995a); Brown et al. (2000); Wool et al. (1989) | Abdullahi et al. (2004) | Perring et al. (1993); De Barro and Driver (1997); De Barro et al. (1998); Guirao et al. (1997); Moya et al. (2001); Lima et al. (2000) | Cervera et al. (2000); Zhang et al. (2005) | De Barro (2005); Delatte et al. (2006); Tsagkarakou et al. (2007); Simon et al. (2007); Dalmon et al. (2008) |

[1]Restriction fragment length polymorphism
[2]Random amplification of polymorphic DNA
[3]Amplified fragment length polymorphisms

considerations for the selection of molecular markers and prospects for future studies of the *B. tabaci* sibling species group, or complex.

## Protein Markers

### *Enzyme Electrophoresis*

Allozymes are biochemical variants of an enzyme encoded by different alleles at the same locus, whereas the term isozyme refers to variants of an enzyme encoded by different loci (Lowe et al. 2004). Thus, the use of allozymes as markers relies on obtaining different allelic variants for the same enzyme when screening individuals from one or more populations. Detection of allozyme variation was introduced in the 1960s and represents the first use of true molecular markers (Schlötterer 2004).

Enzyme electrophoresis is based on the fact that non-denatured proteins with different net charges have differential mobility when moving through a gel to which an electrical current is applied (Avise 2004). If an enzyme marker is polymorphic, meaning it has different variants in the same or different individuals, then the data produced are visible in the gel as different bands, representing different alleles. Thus, differences in the overall banding pattern obtained for different populations indicate that there is some degree of genetic differentiation between the populations, at least for that particular locus. In most cases, heterozygous individuals will be distinguished from homozygotes as having two bands (alleles) for a specific locus.

#### Uses, Assumptions, and Limitations

The first studies that utilized allozyme markers were undertaken by Harris (1966) to quantify variation in populations of humans and by Johnson and colleagues (1966) and Hubby and Lewontin (1966) to assess variation in natural *Drosophila* populations. Since then, allozymes have been used in many systems to examine and analyze the degree of genetic differentiation, inbreeding, genetic drift, and gene flow between populations (migration/hybridization) (Hoy 2003), as well as to provide evidence for polyploidy and introgression between species (Lowe et al. 2004).

Development of an array of methods of data analysis and interpretation for allozymes, together with the large availability of protocols (Lowe et al. 2004), facilitated their use in studies of insects and other organisms. Also, the ease and low cost of application for large numbers of samples allowed their extensive use across laboratories and for different study systems. Although a large number of allozymes proved not to be polymorphic for some species, their initial use provided much more information about intraspecific variation than earlier genetic markers which relied on phenotypic Mendelian traits such as flower or fruit color (Parker et al. 1998).

The amount of polymorphism that can be revealed using allozymes is limited, as they only allow detection of variation at the protein level, i.e., only what DNA encodes for, not variation in DNA itself (Schlötterer 2004). For example, mutations occurring in introns or synonymous substitutions in DNA sequences that code for

the same amino acid and thus not reflected at the protein level cannot be scored with allozymes (Behura 2006). As a result, much of the polymorphism observed at the DNA level goes undetected with allozymes, leading to underestimation of true genetic variation. Furthermore, because allozymes are proteins encoded by genomic DNA, some of the observed polymorphism may not be neutral and thus could have been exposed to selective processes, potentially obscuring analysis and data interpretation. For example Karl and Avise (1992) showed that balancing selection acting upon enzyme loci rendered them poor markers for estimation of genetic variation in oysters and provided misleading evidence for gene flow whereas mitochondrial DNA and nuclear RFLPs showed high degrees of genetic structure and population subdivision.

Limitations during practical application may also be an issue with allozyme analysis, as it requires high quality samples that must be kept alive or deeply frozen until use. In addition some bands generated with allozyme analysis may not follow Mendelian inheritance (Roderick 1996), confounding data scoring and interpretation. Finally, enzyme markers provide little genealogical information, and as such they have limited applications for systematics and phylogenetic studies (Buth 1984).

## Applications of Allozyme Analysis to *Bemisia tabaci*

The use of allozymes was the first attempt towards resolving genetic diversity among whitefly species. The first study was conducted by Prabhaker et al. (1987), who exploited variation in nonspecific esterases (enzymes that hydrolyze esters) to differentiate three whitefly species. Subsequent studies were carried out in the late 1980s – early 1990s to look for polymorphism within *B. tabaci*. Wool et al. (1989) used general esterases and α-Glycerophosphate dehydrogenase isozymes to assess polymorphism in populations from Colombia and Israel and showed that esterases were variable between the two *B. tabaci* populations.

The earliest detailed study of *B. tabaci* variants using general esterases was undertaken by Costa and Brown (1991), who identified the distinct "B" band and proposed the A and B biotype nomenclature. In this study, esterase variation was assessed in native polyacrylamide gels (PAGE) using α- and β-naphthyl acetate as substrates. The two B esterase bands, unique to a population derived from imported poinsettia plants (named the B type for the unique pattern in lane B), migrated faster than two major bands associated with a colony of *B. tabaci* from Arizona in the USA reared on cotton or pumpkin (later named the A type for the unique pattern in lane A). Importantly, the B type electromorph was associated with the squash silverleaf (SSL) phenotype in *Curcubita* spp. (Costa and Brown 1991), thus providing a second distinguishing trait used to separate B type from non-B type whiteflies. The SSL induction was later shown also to be associated with a variant of *B. tabaci* from Yemen (YC), which was characterized as having the two distinctive B type bands, plus one additional faster migrating band (Bedford et al. 1994). In further pursuit of the origin of the B biotype, additional possible correlations were sought. The SSL phenotype was found associated with a population of *B. tabaci* in Uganda (Sseruwagi et al. 2005) and another from the Indian Ocean islands

(Byrne et al. 1995); both haplotypes are members of sister clades to that housing the B biotype but all are members of the major N. Africa-Mediterranean-Middle East major clade (Berry et al. 2004; Delatte et al. 2005; Sseruwagi et al. 2005, 2006). Although esterase profiles were not examined for either population, these results have revealed that the SSL phenotype is not strictly linked with the B biotype, but most likely, is a trait associated with it and some of its closest relatives (Brown 2007; Delatte et al. 2005; Sseruwagi et al. 2005).

Allozyme markers were also employed to test for host specificity of populations with different allozyme patterns. Burban et al. (1992) used isozyme electrophoresis in an attempt to identify host-associated differences among sympatric *B. tabaci* populations in the Ivory Coast of West Africa. The study revealed two different esterase profiles that corresponded to the host range of two *B. tabaci* biotypes, the polyphagous okra and monophagous cassava biotypes. In another study in Colombia, however, Wool et al. (1991) showed that esterase profiles were not correlated with specific host-plant species, but differed between geographic locations. Furthermore, in a study from Israel, esterase profiles failed to show correlation with host plant or geographical location (Wool et al. 1993), suggesting that, in general, esterases were useful markers for detecting variants based on banding patterns but not for determining host plant associations.

The presence of the poinsettia strain or "B" biotype in California, USA, which was suspected since 1990 (Costa and Brown 1990), was confirmed by Perring et al. (1992) with the use of isoelectric focusing (IEF), a technique that is based on the separation of molecules by their differing electric charges moving through a changing pH gradient. In this study, allelic variation in 18 loci encompassing 14 enzymes was assessed in whitefly populations from poinsettia, cotton, and broccoli. Perring et al. (1992) identified 6 loci that were polymorphic, with the cotton strain (A biotype, Western Hemisphere) expressing unique alleles, in contrast to the poinsettia and broccoli isolates of the B biotype (Eastern Hemisphere) that were identical in presence/absence of alleles and in induction of SSL symptoms in squash. This degree of divergence, together with evidence for reproductive isolation, was considered sufficient to warrant the description of the B biotype as a separate *Bemisia* species (see section below). However, subsequent examination of additional haplotypes representing different extant geographical origins revealed a far more complex scenario, with evidence for a range of differentiation across the complex (Brown et al. 2000). Using the above criteria, together with evidence for reproductive isolation, many species would require recognition even though biological data are unavailable for most taxa investigated using molecular and population biology approaches.

## Protein Polymorphisms

General esterases were the first *B. tabaci* proteins explored in the search for genetic (biochemical) polymorphisms, providing corroborative support for observed phenotypic variation. The use of general esterases (allozymes), combined with biological and morphological studies facilitated the discovery that the A and B biotypes were

genetically and phenotypically different. These results established the A and B biotype nomenclature as noted above. Additional polymorphic patterns were assigned alphabetical designations. Protein polymorphisms were supported by differences in host range and fecundity, and evidence that the poinsettia type induced phytotoxic silvering symptoms in the leaves of pumpkin, whereas, the Arizona cotton colony did not. The esterase polymorphisms were useful for revealing genetic differences between the two biologically distinct populations, thereafter, referred to as the A (endemic isolate) and B (poinsettia isolate) biotypes.

Allozyme frequencies were used by Perring et al. (1993) along with data from behavioral and crossing experiments to characterize the recently introduced (to North America) B biotype (common name, silverleaf whitefly) as a new species. Bellows et al. (1994) described the species as *Bemisia argentifolii* Bellows & Perring, distinct from *B. tabaci* based on mating incompatibilities, morphological, and behavioral differences, along with evidence for differential migration distances of allozymes for three enzymatic systems. Although now it is generally accepted that *B. tabaci* represents a sibling species complex (Brown et al. 1995a, b), with the silverleaf whitefly or biotype B (Costa and Brown 1990, 1991) falling somewhere within a continuum, rather than as a separate species, these latter data together with distinctive esterase profiles (Brown et al. 1995a; Costa et al. 1993) provided the first insights into the unexpected extent of genetic diversity present within *B. tabaci*.

Studies that followed confirmed the extensive variation at the protein level among distinct *B. tabaci* populations, and esterase electromorphs were used to categorize variants into biotypes given alphabetical designations following Costa and Brown (1991). In addition, the use of the variable esterase patterns was expanded to include studies that investigated the biogeographic distribution of *B. tabaci* variants. For example, in a survey over the Americas and the Caribbean basin, Costa et al (1993) found the A biotype in Northern Mexico and Southwestern USA, and the B biotype predominating in distribution and present throughout most of the Caribbean basin, the USA, and Brazil. They also found, however, that Costa Rican and Nicaraguan populations of *B. tabaci* exhibited distinct esterase patterns, namely C and D type, respectively. In another study, Brown et al. (1995a) examined esterase profiles for over 40 populations of *B. tabaci* and found 12 non-A or non-B electromorphs, representing unique populations from Central America, Africa, Middle East, and India. The same study confirmed the widespread distribution and abundance of the B-type, and concluded that given the extreme amount of genetic diversity, *B. tabaci* was likely a species complex.

Extensive monitoring studies made it possible to identify and characterize more polymorphic variants based on distinctive esterase banding patters, and also to document the extensive genetic differentiation within *B. tabaci*. Brown et al. (2000) analyzed 21 populations in the *Bemisia* genus using isoelectric focusing electrophoresis, and found that 9 of these populations exhibited polymorphism in allozyme loci. They further analyzed these populations using 10 enzymes and were able to cluster these into 3 main groups: Western Hemisphere A- type variants, B-type variants and a single population from Benin, West Africa. The genetic distances (measures of the degree of dissimilarity between groups of individuals) among these

variants ranged between 0.03 and 0.52. Based on Nei's (1976) genetic distance, the values calculated in this study were suggestive of species level boundaries, again suggesting that *B. tabaci* possibly represents a complex with more than one species (Brown et al. 2000). The limited availability of multiple informative markers has hindered progress greatly in advancing our thinking about the status of *B. tabaci* as a sibling species complex or a suite of separate species, or some of both. For example, a single informative nuclear marker (ITS-23S) and two mitochondrial markers (16SRNA, COI) have been explored in an attempt to advance one hypothesis or the other (see Brown (Chapter 2) for coverage of these molecular markers). Hopefully the necessary studies will be carried out in the next 5 or more years.

### Esterases as Markers

With the discovery of the invasive and highly damaging B biotype, and its associated esterase pattern (Costa and Brown 1991), the use of esterase electrophoresis proved to be a very useful and powerful tool in detecting and tracking the worldwide spread of the B type *via* trade of plant material, particularly poinsettias. Hence, the first surveys of this kind documented the presence of the B biotype in the United States (Costa and Brown 1991; Perring et al. 1992), in the Caribbean Basin and Brazil (Costa et al. 1993) in the Middle East, Central America, Northern Europe (Byrne and Devonshire 1993), in Australia (Gunning et al. 1995), in Africa, India, and many other locations throughout the world (Brown et al. 1995a).

Beyond their use as markers for characterizing and identifying biotype variants, esterases were also used to detect the presence of hybrids in the field or in the lab from crossing experiments, and provide evidence for interbreeding between some biotypes (Byrne et al. 1995; Caballero 2007; Gunning et al. 1997). This use of esterases relies on the fact that hybrids between biotypes will likely be heterozygotes and express allozyme bands from both parental biotypes, assuming of course that the enzymes under consideration follow Mendelian inheritance. Polymorphic esterases were also used to confirm that *B. tabaci* has a haplodiploid sex-determination system; that is, males are haploid, produced by unmated females and females are diploid, produced by mated females (Byrne and Devonshire 1996).

Insecticide resistant *B. tabaci* populations/biotypes have been shown to exhibit characteristically high esterase activity. However, the use of such markers may not be appropriate in monitoring or surveying populations with a recent history of exposure to certain insecticides and even more in studying genetic structure of populations because local insecticide regimes may affect selection at these allozyme loci.

Despite the limitations of esterases as markers, the ease and low cost of application of the esterase electrophoresis technique as well as its reliability in biotype determination has prompted continued use of the approach by some laboratories in subsequent years (Horowitz et al. 2003), especially for the purposes of simply detecting the presence of specific biotypes in an area (e.g., B and Q). However, as the interest in researching further the genetic variation within *B. tabaci* increased, several groups have focused their efforts on the use of DNA based markers that allow for finer resolution of genetic polymorphisms compared to allozymes.

# DNA-Level Markers

## *RFLP Analysis*

Restriction fragment length polymorphisms (RFLPs) reveal polymorphisms in DNA fragments of different size due to the absence or presence of restriction enzyme sites (Mitton 1994). Changes in the patterns of restriction fragments can occur from base pair substitutions, or insertions or deletions (indels) in the restriction site, allowing assessment of DNA variation (Schlötterer 2004). The technique works by using restriction endonucleases, enzymes that cut the DNA strand in vitro at a specific nucleotide sequence 4–5 nucleotides in length. Restriction fragments can be separated by electrophoresis in agarose or acrylamide gels and detected by Southern blot hybridization (Botstein et al. 1980). Detecting the target sequences with Southern blotting requires binding to probes, which may be available from studies of related species or developed after cloning and sequencing species specific-DNA (Hoy 2003). Alternatively, if the target is small DNA fragments, RFLP variation can be visualized directly in the agarose gel by staining with ethidium bromide after electrophoresis (Parker et al. 1998).

### Uses, Assumptions, and Limitations

RFLPs were initially used for constructing a genetic linkage map of the human genome (Botstein et al. 1980). They were the first molecular markers that investigated within species variability at the DNA level and their advantages over allozymes, such as the ability to detect silent changes in protein sequences, or selectively neutral, non-coding polymorphisms (Karl and Avise 1992; Schlötterer 2004) were quickly appreciated. Thus, their use was subsequently extended in studies of population differentiation, hybridization, introgression, gene flow, autopolypoloidy and allopolyploidy, and for phylogeographic and phylogenetic inferences using animal mitochondrial and ribosomal DNA (Avise 2004; Lowe et al. 2004).

One of the major advantages of RFLP markers is that they are codominant (Lowe et al. 2004) meaning that homologous alleles can be detected in an electrophoretic gel, allowing the identification of both heterozygous and homozygous individuals. Codominant markers are preferred over dominant markers for population genetics, not only because they allow estimation of allele frequencies but also because they increase the analytical power of the study with more alleles available for analysis in a given sample size (Lynch and Milligan 1994). Another advantage of RFLPs is that they can generate ordered data, i.e., the ancestral states mutate into derived states and the evolutionary direction can be identified (Lowe et al. 2004). Thus, they have a phylogenetic signal, allowing inferences on genealogies to be made. In addition, RFLP analysis allows a large amount of variation to be assessed in polymorphic loci and also provides highly repeatable results.

Since the RFLP technique was first developed before the invention of polymerase chain reaction (PCR), the first applications of RFLP markers relied on Southern

blot hybridization, which is a time consuming, laborious, and expensive technique (Parker et al. 1998). Using a PCR step in RFLP analysis however eliminates some of its disadvantages (Karl and Avise 1992). Arbitrary PCR primers can be used to amplify random DNA sequences; from these, allele-specific PCR primers can be designed and used for amplification of the particular locus in other individuals. Subsequently, the product can be digested with restriction enzymes, and the fragments can then be separated on an electrophoretic gel and stained to visualize and identify the RFLPs (Hoy 2003). A modification of the RFLP technique, for example, is cleaved amplified polymorphic sequences (CAPS), which relies on restriction digestion of PCR-amplified DNA fragments (Lowe et al. 2004; Schlötterer 2004). In RFLP-PCR, no labeled probes are required, and it is faster, easier and less expensive than non PCR-amplified RFLP. However, it can only analyze DNA of very small specimens and is still relatively laborious as it requires constructing a genomic DNA library and DNA sequencing.

### Applications of RFLP Analysis to *Bemisia tabaci*

Despite their wide application for population genetics and phylogenetics in other organisms, RFLPs were not adequately explored as markers in studies of *B. tabaci*. This may be due to the extended use of allozymes and subsequently RAPDs by researchers, especially among collaborating laboratories that used similar protocols, which made their application more approachable. In addition, the need for designing allele-specific PCR primers from already studied sequences limits the feasibility of this approach.

Abdullahi et al. (2004) analyzed RFLPs of the ribosomal DNA internal transcribed spacer regions of *B. tabaci* in order to assess the genetic differentiation between cassava and non-cassava populations in Africa. They found that monophagous cassava-associated populations were genetically distinct from the polyphagous non-cassava populations as they clearly clustered in separate groups. Even though there was a significant subdivision of groups within the cassava cluster, these did not show any geographically associated structure. The results of this study are in agreement with previous studies that used other molecular techniques, e.g., allozymes (Burban et al. 1992), RAPDs and ribosomal DNA sequences (Abdullahi et al. 2003) to discriminate cassava from non-cassava populations. They authors suggest however that PCR-RFLP is a more reliable and cost-effective technique, especially when compared to RAPDs which failed to provide consistent patterns and tended to overestimate genetic differences between populations. Thus, for the purposes of routine identification and monitoring the spread of biotypes, particularly the B biotype, the use of the internal transcribed spacer RFLP markers is probably a much more practical approach than RAPDs or DNA sequencing (Abdullahi et al. 2004).

The utility of RFLP markers for quick identification of *B. tabaci* biotypes was also demonstrated recently by Bosco et al. (2006) in a survey of populations in the Mediterranean basin. The authors used a restriction enzyme to digest the PCR amplified cytochrome oxidase I mitochondrial gene and were able to successfully

identify five biotypes from samples in the Mediterranean region. From these, biotypes B and Q were already known to be widespread in the area, whereas M, S, and T biotypes were considered to have a more restricted geographical and host-plant range. This approach provided clearly distinct and reproducible RFLP patterns for each biotype, demonstrating its potential for precise identification of the studied biotypes (Bosco et al. 2006).

Although novel and more informative molecular markers are being introduced that may be more appropriate for population genetics and phylogenetics studies in *B. tabaci*, RFLPs still have promise as a rapid and inexpensive means of monitoring for known biotypes at a regional scale.

## DNA-Level, PCR-Based Markers

The invention of the polymerase chain reaction (PCR) in 1983 (Mullis et al. 1986; Saiki et al. 1988) revolutionized the field of molecular biology and amongst other advances it allowed the exploration of DNA-based markers for studies of molecular population genetics and systematics. PCR allows the selective amplification of a very small DNA fragment using specific primers that anneal to the regions flanking the locus of interest. The production of multiple copies of the genomic region under study in vitro allows analysis of numerous individuals without the need for cloning or isolating large amounts of pure genomic DNA (Schlötterer 2004). Thus, PCR enables studies with genetic markers to be undertaken quickly and at relatively low costs by almost any molecular laboratory. As a result, after the arrival of PCR molecular markers used for population studies largely shifted from protein based (allozymes) to DNA based (e.g., randomly amplified polymorphic DNAs, microsatellites, amplified fragment length polymorphisms, single nucleotide polymorphisms, and DNA sequencing) (Schlötterer 2004).

### *RAPD Analysis*

RAPD (Random amplification of polymorphic DNA) markers were initially used by Williams et al. (1990) and Welsh and McClelland (1990) for the construction of genetic maps for different species, plant and animal breeding studies, intra-specific level identification (e.g., strains, varieties), epidemiology studies, as well as DNA fingerprinting and population genetics. The acronym RAPD stands for randomly amplified polymorphic DNA and the approach involves the use of short PCR arbitrary primers to amplify random DNA sequences in the genome (Avise 2004). The resulting PCR products represent polymorphic DNA segments that can be separated and visualized in an appropriate electrophoretic gel. Polymorphisms in RAPDs arise from single base substitutions, insertions, or deletions in primer recognition sites that result in a change in the pattern of amplified DNA segments (Williams et al. 1990).

### Uses, Assumptions, and Limitations

Beyond their initial uses, RAPDs found applications in many organisms for studies of genetic diversity, systematics, and to investigate hybridization and introgression (Harris 1999). In a review paper, Hadrys et al. (1992) discuss the applications of RAPDs in molecular ecology and emphasize their usefulness for the determination of taxonomic identity, analysis of interspecific gene flow and hybrid speciation, paternity and kinship studies and analysis of mixed genome samples (e.g., sperm competition). In insect systems in particular, RAPDs have been used to study gene flow and genetic differentiation among insect biotypes or races. For example, Edwards and Hoy (1993) used RAPDs to analyze genetic variation in two Hymenopteran parasitoids, and were able to detect greater polymorphism than when using allozyme analysis. In another study, Black et al. (1992) were able to detect high genetic variation in RAPDs among biotypes, populations and color morphs of four aphid species that exhibited very little allozyme variability. The authors suggested that the method would be particularly successful for rapid species diagnostics, especially in groups where adult or larval characters are poor for species identification. Clearly, the ability to amplify small, potentially polymorphic DNA segments through PCR, made RAPD analysis a much more powerful tool for studying intraspecific variation compared to allozyme analysis.

The RAPD method was initially widely adopted because of technical advantages, such as low cost of application and technical simplicity. In addition, it is a fast method that allows multiple variable loci to be analyzed at once. The main advantage, however, is that it does not require any prior knowledge of the sequence of the target organism and a universal set of primers can be used for genomic analysis in a wide variety of species (Welsh and McClelland 1990; Williams et al. 1990).

RAPDs, however, have also been severely criticized and their drawbacks limited their extended application as new markers were explored. Their main limitation is that, like amplified fragment length polymorphisms (AFLPs), they segregate as dominant markers so they are scored as present or absent in a gel, thus making it impossible to distinguish between homozygote and heterozygote individuals (Black et al. 1992; Harris 1999). Thus the estimation of genetic diversity and the way it is partitioned can only be determined indirectly (Harris 1999). Another severe drawback, not shared by AFLPs, is that RAPDs can be unreliable and very difficult to reproduce or even provide comparable results among different laboratories that use different reaction conditions, different types of *Taq* polymerase or even different thermal cycling machines (Black 1993; Jones et al. 1997; Schierwater and Ender 1993). Hadrys et al. (1992) also discuss the limitation of co-migration of bands with similar size or the formation of fragments from non-specific priming. In addition, like RFLPs and AFLPs, RAPDs provide much lower resolution than microsatellites which target short tandem repeats in the DNA sequence, so they cannot give as much insight into within-population genetic diversity. Finally, their interpretation relies on the assumption that DNA fragments (alleles) from different individuals that have the same position on a gel are identical due to common descent (homologous) and not the result of size homoplasy (similarity in size because of convergent

mutations) (Black 1993; Harris 1999). Beyond the issues regarding the interpretation of data produced by RAPDs, there may be difficulties in publishing results obtained from these markers. For example, due to the problems of reproducibility, dominance, and homology, the journal *Molecular Ecology* rarely accepts for peer review papers based on RAPDs for population genetic studies, and encourages the use of other markers, that do not suffer from these limitations.

As a result of the issues discussed above, despite the early promise of RAPDs as a quick, easy and reliable technique, the problems of reproducibility limited their extended use in systematics, phylogeographic and phylogenetics studies (Harris 1999), especially in molecular systematics above the intraspecific level (Black 1993). Their popularity in later years was surpassed by the extensive application of microsatellites which are also highly polymorphic but are codominant, allowing the identification of homozygotes and heterozygotes, and therefore more genetically informative (Avise 2004).

## Applications of RAPD Analysis to *Bemisia tabaci*

RAPD markers were used extensively for the identification and analysis of genetic diversity in biotypes of *B. tabaci.* Their application was particularly focused in studies of biotype B whose spread over the continents in the past two decades required extensive monitoring for the application of more efficient control measures. The first study in this direction was undertaken by Gawel and Bartlett (1993), who were able to distinguish between the A and B biotype based on genetic differences revealed by 20 RAPD markers. The authors concluded that, based on their RAPD data, the degree of similarity between the two biotypes is similar to that between other whitefly species, even genera. However, they suggested that RAPDs are probably of limited value for taxonomic determination of biotypes without the use of additional genetic, morphological, and physiological data. Since RAPDs were the first DNA-based markers used in *B. tabaci*, this study was also the first to demonstrate the advantages of DNA-based markers over allozymes – the ability to analyze dead specimens preserved for years in alcohol and the flexibility of using a small amount of material of any whitefly stage.

Perring et al. (1993) used RAPDs to detect variation in A and B biotypes, and showed that populations of either biotype shared 80–100% genetic similarity in bands whereas between them similarity was only 10%. The authors used these data along with differences in allozyme frequencies, and behavioral and mating experiments to propose that the B biotype should be re-classified as a new species, which was later named *Bemisia argentifolii* (Bellows et al. 1994). This extreme genetic differentiation among worldwide populations as shown in RAPD and other molecular markers was the basis for suggesting that *B. tabaci* is a group or complex of species, (instead of a sibling species group) (Brown et al. 1995a; Perring 2001) of which biotype B is a member rather than a different species.

Most studies that followed used RAPDs similarly to allozymes, that is, as a means of detecting the presence of biotypes in an area, or the distribution of biotypes within and between populations in different geographic regions, host plants,

and in indoor versus outdoor cultivations. De Barro and Driver (1997), for example, used RAPDs to compare B biotype populations from Australia, the Cook Islands, Israel, the Netherlands, New Caledonia, and the USA, and showed that they had similar banding patterns but differed from other native biotypes in Australia. They also confirmed the flexibility of the technique to utilize alcohol preserved material and analyze all stages of the whitefly. In a subsequent survey of populations from Australia and 18 Pacific island countries, De Barro et al. (1998) showed that biotypes of *B. tabaci* were present and widespread in each country surveyed. Analysis of RAPD profiles of these samples revealed the presence of three distinct biotypes in these regions. The first was the widespread B biotype and was found in islands with strong links with France or the USA, suggesting introduction through the plant trade. The authors also detected a type with unique RAPD bands which was widely distributed in 13 countries and was termed the Nauru biotype, and a third biotype, termed Australasian, which is most probably native to the region as it was reported previously from Australia (De Barro and Driver 1997).

In a study in the Iberian Peninsula, Guirao et al. (1997) used both esterases and RAPDs, along with physiological examinations of silverleaf induction to characterize Spanish populations of *B. tabaci*. This study demonstrated that two genetically distinct populations existed in Spain and the Canary Islands, the already known B biotype and a new variant with unique RAPD and esterase patterns, referred to by others as the Q biotype (Rosell et al. 1997). This was the first use of the Q biotype designation in the Mediterranean region, corresponding to endemic haplotypes from Spain and Sudan populations that were previously detected (referred to in Kirk et al. 2000). A cluster analysis based on RAPD band sharing sorted the sampled populations into four groups. The first included only the A biotype from Arizona, the second included the Spanish Q type, the third included populations of the B biotype, similar by 90% from eight different countries, and the fourth included populations from Pakistan, India, and Turkey. Genetic similarity estimates (% of RAPD bands shared) used in this study showed that the new genetic type found in Spain was closer to B biotype than to any other non-B type, but only shared 55% of bands observed. An interesting conclusion in this study was that the new biotype had not been displaced as a result of competitive interaction with the B biotype as was the case in the USA with the A biotype (Guirao et al. 1997). Some have speculated that the Q biotype and its closest relatives are endemic in the North African-Mediterranean-Middle East, and that the Q biotype in Spain competitively displaced the exotic B biotype there with the intervention of neonicotinoid insecticides. This was the inverse to the permanent displacement of the endemic A biotype in the Southwestern USA by the introduced B biotype, which was susceptible to locally applied insecticides including pyrethroids, to which the B biotype harbored inherent resistance (Coats et al. 1994; Costa et al. 1993). The widespread displacement of indigenous biotypes by the invasive B has been documented worldwide. For example a recent study showed competitive displacement of indigenous biotypes in Australia and China by biotype B, both in the field and in laboratory settings (Liu et al. 2007).

In a more detailed analysis of *B. tabaci* populations from the Iberian Peninsula, Moya et al. (2001) were able to identify six genetically distinct clusters consisting of either biotype Q, or B, or a mixture of the two. In contrast to previous studies that used RAPDs to estimate genetic relationships among haplotypes, Moya et al. (2001) used analysis of molecular variance (AMOVA) of RAPD data to investigate the genetic differentiation among populations. They found that genetic variance is partitioned more between biotypes than among populations within the same biotype, suggesting that gene flow is restricted between the two biotypes even in mixed field populations. This means that the two biotypes are genetically isolated under the existing ecological conditions in the area, which confirms previous studies of the B and Q biotypes. In addition, populations of the Q biotype were shown to be more genetically polymorphic than populations of B which suggested that the Q is more ancestral, existing long before the introduction of B in the region (Moya et al. 2001). The hypothesis that biotype Q and its variants are possibly the indigenous Mediterranean genetic type is also supported by studies showing its predominance in fields in Italy, in contrast to B biotype which is widespread in greenhouses, possibly introduced through the plant trade (Demichelis et al. 2000).

Similar to the Mediterranean studies, Lima et al. (2000) used RAPD markers to survey *B. tabaci* populations and identify the distribution of biotypes in Brazil. They showed that B biotype was well established and distributed across 20 Brazilian states in the northeast, east and midwest of the country. In contrast to the Spanish surveys, Lima et al. found that B was the dominant genetic type in Brazil, but also coexisted in some areas with BR, a native Brazilian, non-B biotype with distinctive RAPD patterns. In a subsequent study of Brazilian populations, Lima et al. (2002) used analysis of molecular variance of RAPD data and demonstrated that there is a significant genetic differentiation between B and the Brazilian biotype (BR). The B biotype was found to predominate over the A or the BR biotype throughout Brazil, even in regions or hosts where BR was dominant before. It also exhibited considerable genetic variability despite its relatively recent introduction into Brazil in the early 1990s, perhaps due to multiple founder events or because of the differential selection regimes caused by variable insecticide applications across different crops (Lima et al. 2002).

In a study of *B. tabaci* in association with the cassava mosaic disease (CMVD) in East Africa, Maruthi et al. (2002) used 10 RAPD markers along with mating and life history studies to test whether a new biotype was responsible for the spreading of the severe pandemic across East Africa. In a comparison of populations between pandemic and non-pandemic zones, the authors found no difference in RAPD patterns or life history traits, and no mating incompatibilities, suggesting that the CMVD pandemic in East Africa may not be associated with a new genetically distinct *B. tabaci* biotype. In this study, the RAPD variability data that grouped pandemic and non-pandemic colonies in a single cluster were confirmed by mating and life history tests. However, the results of this study contradicted those of Legg et al. (2002) who collected samples across several years and in transects across the spreading epidemic zone. This revealed the presence of both a western and eastern type African haplotype, suggesting that a mixture of two distinct *B. tabaci* haplotypes was

associated with the epidemic of CMVD in Uganda. The subsequent reversion to the east African haplotype resulted in the hypothesis that the invasive population may represent a unidirectional hybrid between the eastern and western haplotypes, based on mitochondrial DNA phylogenetic data (Sseruwagi et al. 2004). The contradictory findings may be due to the limited power of RAPDs to discriminate phylogenetic lineages compared to mtDNA. Even so, the lack of association of a distinct genotype with the CMVD epidemic was subsequently supported by assays that showed no difference in transmission efficiencies among sampled populations (Maruthi et al. 2002). Conversely, assays performed with field populations collected early in the outbreak and maintained in colonies used in the transmission experiments may not necessarily have represented collections from the field assessed by mtCO1 analysis as the dynamic epidemic continued to unfold. Indeed, the mtCO1 was not assessed for the lab colonies (Maruthi et al. 2001), nor were transmission studies performed with the field haplotypes identified later in the epidemic zone, and so it has not been possible to explain the differing results. Further studies employing typed colonies and possibly new markers, together with transmission experiments will be necessary to elucidate the genetic status of cassava-associated populations in relation to the spreading pandemic (see Legg Chapter 7).

Similarly to studies initiated by Burban et al. (1992), who looked for associations between distinct biotypes (based on allozyme patterns) and host range, Abdullahi et al. (2003) analyzed RAPD data using AMOVA and showed that samples collected from cassava and non-cassava populations represent two genetically isolated groups, supporting the hypothesis of a distinct cassava specific lineage in Africa. This finding was also in agreement with ribosomal DNA data, although RAPDs exhibited a greater ability to differentiate geographically distinct populations. More importantly, these results were supported by physiological experiments showing that cassava populations were monophagous and restricted to cassava, whereas populations from other hosts were polyphagous but could not colonize cassava. These results suggest that host specialization in this case may drive isolation, leading to genetically differentiated populations. More extensive studies of cassava populations based on mitochondrial cytochrome oxidase I (mtCO1) sequence analyses supported the hypothesis of host-specificity but also revealed a high variation within cassava populations in Sub-Saharan Africa, with a strong phylogeographic basis (Berry et al. 2004). However, it is clear that more studies are needed, especially focusing on comparisons with non-cassava populations in the continent in order to provide an overall picture of host association within *B. tabaci*. Furthermore, to support the hypothesis of host-specificity, it will be critical to ensure that individuals collected from the host actually colonize and breed on that host. Indeed, one recent study in Uganda addressed this issue by sampling pupae from leaves of cassava and non-cassava hosts (Sseruwagi et al. 2006). In this study, phylogenetic analysis of mtCO1 indicated that, while cassava is only colonized by the cassava *B. tabaci* (Ug1 genotype), this type is also capable of colonizing other non-cassava plant species suggesting that it is not monophagous but can possibly survive on other hosts when cassava is not available. This work highlights the importance of the sampling procedures used for such studies, but also illustrates that more molecular markers

and new analyses could be explored to identify host-associated genotypes on the continent (see also Chapter 7).

In another study from south India, Rekha et al. (2005) analyzed RAPD diversity in *B. tabaci* populations and identified two indigenous genotypic clusters, a southern and a northern cluster, as well as a third group represented by the B biotype. These results point to a geographic, rather than host-associated, structuring of populations, perhaps related to distinct cropping regimes and climatic conditions prevailing in the two areas. The unique RAPD banding patterns of the B biotype made it possible to identify its distribution in the region, which has been rapidly expanding since it was first reported in India in 1999 (Banks et al. 2001; Rekha et al. 2005). Additional phylogenetic analysis using mtCO1 sequences revealed the presence of three distinct genotypes that are possibly indigenous to India but were also found in other Asian countries from which reference samples were used. These indigenous types were found in higher frequencies in areas where the B type is considered to have invaded recently, but were probably excluded in regions where B has been present for at least 2 years and where it now represents the dominant type (Rekha et al. 2005).

Along the same lines, Delatte et al. (2005) identified a biotype indigenous to the South-West Indian Ocean islands based on distinct RAPD bands and sequencing of the mt(CO1) gene. This new biotype, Ms, occurs along with B on the islands and, although considered to have lower fecundity and restricted host range, is also capable of inducing silverleaf symptoms in *Cucurbita sp.* and acquiring and transmitting TYLCV (Delatte et al. 2005). Subsequent studies with microsatellites (Delatte et al. 2006) showed that, although Ms is the common biotype in weeds across the island, the invasive B biotype has spread in vegetable plantations, possibly interbreeding at some parts with the Ms biotype.

Besides their use in population genetics, RAPDs found wide application as markers for quick detection of a biotype in a region. For example, in Israel, the Q biotype was first identified by RAPD and esterase profiles and was found in sympatry with the B biotype in three crops, although the Q biotype predominated in numbers (Horowitz et al. 2003). The authors suggested that the dynamic distribution of biotypes could have been a result of the differential inherent levels of resistance exhibited by the two biotypes. In a survey of *B. tabaci* biotypes across Italy, Simon et al. (2003) detected biotypes B and Q (which was almost identical to the Spanish Q) using RAPDs, squash silverleaf assays, and esterases. More importantly, they were able to identify a genetically distinct population, namely biotype T, which unlike B and Q is geographically isolated, monophagous, restricted to *Euphorbia characias* at high altitudes and possibly endemic to the island of Sicily. In a survey of *B. tabaci* from crop fields and weeds in Florida, RAPD patterns showed only the presence of biotype B, suggesting that native biotypes have been displaced from the agricultural ecosystems in the area (McKenzie et al. 2004). Khasdan et al. (2005) further demonstrated the usefulness of RAPDs as diagnostic markers by developing a new protocol for distinguishing between B and Q biotypes based on two techniques: SCAR (sequence characterized amplified regions), which requires sequencing of RAPD fragments and designing sequence specific

PCR primers, and CAPS (cleaved amplified polymorphic sequences), which uses restriction endonucleases to further cleave the RAPD amplified DNA fragments and reveal polymorphisms.

Despite the limitations of RAPD markers, they were used extensively for almost 15 years in population genetics studies and molecular identification in *B. tabaci*. The use of RAPDs provided insights into the genetic structure of biotypes across the world. More importantly, RAPDs revealed aspects of the dynamic distribution and rapid spreading of the invasive B biotype in several regions in relation to indigenous biotypes which were generally less competitive and less damaging than the B biotype. The use of RAPDs in the future will no doubt decline as they are replaced by new, more informative markers, such as microsatellites, DNA sequencing, single nucleotide polymorphisms (SNPs) and expressed sequence tags (ESTs) that allow for high throughput genotyping in large scale population genetics studies (Behura 2006). Due to the ease and low cost of application however, it is possible that RAPDs will continue to be used in studies of detection and monitoring for the presence of a biotype in a region.

## *AFLP Analysis*

The analysis of amplified fragment length polymorphisms (AFLPs) was developed as a technique for DNA fingerprinting in 1995 (Vos et al. 1995). The AFLP protocol involves three steps. First, genomic DNA is digested with restriction enzymes and oligonucleotide double stranded adapters are ligated to the ends of the restriction fragments. Subsequently, the fragments are selectively amplified with PCR primers that are complementary to the adaptor and the restriction site fragments. Finally, the amplified fragments are separated through gel electrophoresis and visualized or scored in an automated sequencing machine. Polymorphisms in AFLPs are scored as differences in the lengths of the amplified sequences which may be caused by base substitutions within or near the restriction sites or by insertions or deletions of sequences (Avise 2004). The AFLP technique has elements of both RFLP and RAPD analysis, and as such it is regarded as a clever combination of steps from the two older marker systems (Bensch and Akesson 2005).

### Uses, Assumptions, and Limitations

AFLP markers have been used for molecular characterization of closely related species or strains, for population and conservation genetics, fingerprinting, and parentage analysis (Mueller and Wolfenbarger 1999). These authors also found application in the construction of genetic linkage maps in insects and other organisms with unexplored genomes (e.g., Parsons and Shaw 2002). The AFLP technique combines the ease of RAPDs and the reliability of RFLPs (Behura 2006). One major advantage is that they do not require previous knowledge of primer sequences in the target species (Schlötterer 2004), thus there is no need to obtain or design species-specific primers for the analysis. In addition, with AFLPs, a large number of marker

loci drawn from the entire genome (typically 50–100 fragments per reaction), are selectively amplified and can be analyzed at once (Vos et al. 1995). Although some of these characteristics are shared by other markers, AFLPs are more time and cost-efficient, and provide higher resolution of genetic variability when compared to allozymes, RFLPs and RAPDs (Mueller and Wolfenbarger 1999). The higher reliability and reproducibility of AFLPs compared to RAPDs can be attributed to the fact that they are amplified by primers of longer sequence with precise annealing to their target sequence, rather than by short, arbitrary primers (Lowe et al. 2004).

The application of the AFLP protocol may be restricted by the need for high technical laboratory skills and thus may not be approachable to less experienced researchers. Another issue that emerges from studies with AFLPs is the need for large amounts of high quality DNA (Lowe et al. 2004), which may restrict the number and variety of samples that can be analyzed in a study. AFLPs also suffer from data interpretation problems, such as size homoplasy, an issue also discussed with RAPDs above, or uncertain locus/allele designations (Harris 1999).

The major drawback of AFLPs for population studies is that, like RAPDs, AFLPs are dominant markers and so PCR amplification reveals only the presence or absence of bands, making the technique less suitable for studies that require analysis of allelic diversity or heterozygosity (Mueller and Wolfenbarger 1999). There have been cases, however, where codominant AFLPs were detected, possibly due to insertions/deletions of repeats in microsatellites often present in AFLP fragments, producing multiple variable alleles for the same locus (Wong et al. 2001). If this is the case, with the vast number of fragments generated it would be impossible to determine whether alleles in different individuals belong to the same AFLP locus. Because dominance is assumed in AFLP analysis, the production of codominant AFLPs may compromise their use in population genetics studies as the estimation of parameters such as allele frequencies and heterozygosity will be biased (Wong et al. 2001). For example, Yan et al. (1999) showed that average heterozygosity and population differentiation ($F_{ST}$) in populations of the yellow fever mosquito in Trinidad were underestimated when AFLP markers were used compared to data obtained from RFLP markers. Nevertheless, for large data sets with the vast number of markers typically scored in ALFP analysis, the frequency of codominant bands may be minimal, thus reducing the resulting bias (Parsons and Shaw 2002). In addition, it is possible to detect codominance in AFLP data following several procedures that have been developed for this purpose (Jansen et al. 2001).

Despite their high promise as molecular markers for population genetics, phylogenetics and quantitative trait loci (QTL) mapping (Mueller and Wolfenbarger 1999), AFLPs are largely underrepresented in animal studies in these fields. On the contrary, the method has been widely adopted and applied in studies of plants, bacteria, and fungi (Bensch and Akesson 2005). In the limited number of animal studies, AFLPs were shown to be powerful in delineating genetic relationships among cryptic sympatric species (Parsons and Shaw 2002). Their use in phylogenetic and phylogeographic studies on the other hand, may be limited by the fact that they are not ordered markers, i.e., they do not carry genealogical information, and thus their use for such purposes is controversial (Robinson and Harris 1999).

Bensch and Akesson (2005) strongly advocated for the advantages of ALFPs and suggested that the drawback of dominance can be overcome by employing more AFLP loci; they also urged their further exploitation in studies of genetic diversity and population structure in animals.

### Applications of AFLP Analysis to in *Bemisia tabaci*

Similar to other animal systems, AFLPs are also poorly represented in the *B. tabaci* literature. Although from a theoretical perspective it seems that these markers could provide further insights into the genetic diversity within the *B. tabaci* sibling species group, there are only a few published studies in the literature on AFLP analysis of *B. tabaci* populations. In one such study, Cervera et al. (2000) analyzed the genetic diversity of nine biotypes, two field populations of *B. tabaci* (from cowpea and cassava in Africa), and two field populations of two other *Bemisia* species, *B. medinae* and *B. afer*. Based on the AFLP profiles, the nine esterase types (Brown et al. 1995a; Costa et al. 1993) (A, B, H, K, M, Q, S, Pakistan I, and Pakistan II) were grouped together and separately from the other two *Bemisia* species. This suggests that all genetic types (erroneously referred to as biotypes) studied are more closely related to each other than to any of the two other species, which is consistent with morphological differentiation and in agreement with the existing literature. A further grouping within *B. tabaci* separated the nine esterase types into four clusters: a Near East and Indian subcontinent cluster (H, K, M biotypes), a cluster with biotypes B, Q, and a Nigerian cowpea population, a third group consisting only of New World A biotype, and finally a cluster with the S biotype and a Nigerian cassava population. The results from the AFLP analysis were in agreement with previous studies based on RAPDs and provided similar resolution of genetic differentiation, although; AFLPs offered better quality and reproducibility (Cervera et al. 2000). These findings have been further supported by mitochondrial sequence analysis (Berry et al. 2004; Qiu et al. 2007).

In a recent study of *B. tabaci* in China, Zhang et al. (2005) used both AFLP data and mtCO1 sequences to assess genetic diversity in geographically discrete populations. The AFLP analysis indicated that at least four genetically distinct groups of *B. tabaci* are present in China, and their genetic isolation is most likely being maintained by geographic barriers. The use of mtDNA sequences allowed the assignment of the two groups to known mtCO1 clades – the first represented by the widespread B biotype and the second by Q biotype populations from Spain and Israel. The third and fourth groups were assigned to a non-B/Q clade as they were significantly differentiated from either biotype. The populations belonging to B biotype were the most widespread throughout the country, while the Q type was reported for the first time from China and was found to be much more restricted in range (Zhang et al. 2005).

With the growing use in the literature of new molecular markers for population level genetic studies, we expect that research on *B. tabaci* will also shift towards new approaches, especially as more information on the whitefly genome becomes available. However, traditional markers that are reliable and cost-effective, like AFLPs,

still will have a place in small scale studies and may reveal new and useful information on population differentiation from previously unexplored parts of the *B. tabaci* genome.

## *Microsatellite Analysis*

Microsatellites are tandem repeats of usually 1–6 nucleotide bases that are randomly distributed throughout the eukaryotic nuclear genome, and are also known as simple sequence repeats (SSR), variable number tandem repeats (VNTR), and short tandem repeats (STR) (Selkoe and Toonen 2006). The high degree of polymorphism of microsatellites in natural populations was first appreciated in the late 1980s (Tautz 1989) and they have since become particularly attractive as markers for population genetics studies (Ellegren 2004). Their high popularity relies partly on their Mendelian inheritance and codominance, meaning that, in contrast to RAPD's and AFLP's, heterozygotes can be distinguished from homozygotes in the electrophoretic gel (Lowe et al. 2004). In addition, their high degree of polymorphism allows for fine resolution for intra-population level studies in contrast to other markers discussed here.

The high degree of polymorphism among microsatellites reflects the great variability in repeat length, commonly between 5 and 40, a result of high rates of mutation. The most common types of microsatellites used in molecular genetics studies are dinucleotide, trinucleotide, and tetranucleotide repeats (Selkoe and Toonen 2006). Microsatellites are thought to gain and lose repeat units typically through polymerase slippage, an error during DNA replication which results in an increase or decrease in the number of repeats (Levinson and Gutman 1987; Schlötterer and Tautz 1992), leading to the formation of different alleles. The mutation rate of microsatellites has been estimated at between $10^{-6}$ and $10^{-2}$ per generation (Schlötterer 2000), although it may vary among different loci, especially between di- and tetra-nucleotides (Weber and Wong 1993). The flanking regions, DNA sequences adjacent to either side of the microsatellite locus, are generally conserved across individuals of the same species or between closely related species, and thus can serve as primers for polymerase chain reaction (PCR) amplification of the microsatellite locus in con-generic species (Selkoe and Toonen 2006). The assumption is that any mutations will likely occur at the microsatellite locus as insertions or deletions of a repeat and not at the more conserved flanking region (Schlötterer 2001; Schlötterer and Pemberton 1994; Weber and Wong 1993).

Microsatellite data are obtained following amplification in a PCR with species-specific primers, and subsequent genotyping and allele scoring in an automated DNA sequencer. Microsatellite analysis is based on a locus of known size, and subsequent alleles are characterized by the number of repeats that differ from the known locus. Thus, depending on the questions being asked, the type of data generated can

be raw alleles for parentage analysis or allele frequencies per locus and estimates of heterozygosity for population studies. Statistical analysis of microsatellite data can be performed using maximum likelihood, Bayesian, or coalescent methods in a variety of software freely available on the internet (Excoffier and Heckel 2006; Luikart and England 1999).

### Uses, Assumptions, and Limitations

Microsatellites have been used extensively in the past decade to test for inbreeding, parentage and relatedness, to examine genetic diversity and demographic patterns, as well as to investigate population differentiation in many insect systems (e.g., Endersby et al. 2005; Kim and Sappington 2006). Contrary to allozymes, several thousand potentially polymorphic markers are available with microsatellites (Schlötterer 2000) and their fast rate of evolution compared to mitochondrial genes or other nuclear loci makes them more suitable for resolving the genetic diversity among closely related, recently founded populations (Davies et al. 1999). The relatively large number of microsatellites usually available allows multi-locus analyses, providing much more information and statistical power at a fine scale. However, the use of microsatellite DNA in population genetics requires knowledge and understanding of the mutation processes they undergo and their rate of evolution (Schlötterer 2000). Additionally, there are other issues and limitations that need to be considered before using microsatellite markers, some of which are discussed here:

(a) *Size homoplasy*. Microsatellite analysis methods assume that alleles identical in size have identical genealogical history, or common descent, although this may not hold true because of size convergence as discussed earlier for RAPDs and AFLPs. When mutation rates are high, homoplasy can inflate estimates of gene flow and give misleading inferences of population structure (Viard et al. 1998). However, Estoup et al. (2002) suggest that high polymorphism often compensates for the homoplasious evolution of microsatellites, although it may still be an issue in studies of large populations and at loci with high mutation rates.
(b) *Complex pattern of mutation*. Mutation rates may be complex and may vary considerably among loci, complicating the use of models (e.g., infinite allele model, stepwise mutational model) to infer statistics of population differentiation based on estimates of allele frequencies such as $F_{ST}$ and $R_{ST}$ (Selkoe and Toonen 2006).
(c) *Neutrality and selection.* Most methods used to analyze microsatellite data assume a neutral rate of evolution, i.e., that the locus used as a marker is not under selection and evolves at a more or less constant rate. Although microsatellites are generally considered selectively neutral markers, this assumption is sometimes questionable (Zhang and Hewitt 2003) as microsatellites have been detected in loci under selection or linked to such loci (Schlötterer and

Pemberton 1994). Thus, before using microsatellites in population studies, it is recommended that researchers use tests of neutrality, for example the Ewens-Watterson test that compares the distribution of allele frequencies at each locus for deviations from an expected distribution under neutrality (Selkoe and Toonen 2006).

(d) *Scoring errors*. Genotyping errors or mistyping during allele scoring in microsatellite analysis may obstruct data interpretation and bias the conclusions of the study. DeWoody et al. (2006) review three common scoring errors that may occur during microsatellite analysis: stuttering, large allele dropout, and presence of null alleles, and discuss implications and potential ways to circumvent these problems. Null alleles represent the most problematic scoring error and also the best studied (Callen et al. 1993; Dakin and Avise 2004; Pemberton et al. 1995). These are alleles that fail to amplify in a PCR reaction due to a mutation in the primer-binding region of a microsatellite locus, leading to either no amplification at all in homozygous individuals or amplification of only one allele in heterozygotes (Dakin and Avise 2004). Presence of null alleles could result in the mistyping of heterozygotes as homozygotes in the analysis, and consequently in heterozygote deficiency, leading to underestimation of genetic diversity and population structure. Several approaches are available to test for null alleles (Dakin and Avise 2004). For example, deviations from Hardy-Weinberg equilibrium in some loci but not in others might suggest the presence of null alleles. Additionally, testing for suspected null alleles could be done by directly sequencing PCR products. New analytical methods are better at dealing with null alleles (e.g., Geneland, Guillot et al. 2008).

(e) *Limited use for genealogical studies*. Microsatellites, like most of the other markers discussed here, produce mostly unordered data which means that they do not contain much ancestral information because of the multiple allele states often present. Thus, unlike nuclear or mitochondrial DNA sequence data, microsatellite data will not be very useful for phylogenetic inferences (Zhang and Hewitt 2003), although it has been demonstrated that phylogenetic inference is possible in some cases (Schlötterer 2001 for a review; Wilson and Balding 1998).

(f) *Mendelian inheritance*. Although microsatellites have generally been considered to be markers with Mendelian inheritance (Jarne and Lagoda 1996), some researchers have questioned this assumption (e.g., Dobrowolski et al. 2002; Smith et al. 2000) and suggest that evaluation of microsatellite loci for this property in parent-offspring pairs should be done before using microsatellites in analyses (Smith et al. 2000).

## Applications of Microsatellite Analysis in *Bemisia tabaci*

Microsatellites have been used as molecular markers for almost 20 years (Schlötterer 2004), although their application to studies of *B. tabaci* populations was only recently explored. Microsatellite loci were first isolated in *B. tabaci* in

2003 by two independent groups (De Barro et al. 2003; Tsagkarakou and Roditakis 2003), and more recently by Dalmon et al. (2008), Delatte et al. (2006), Gauthier et al. (2008), Schwartz et al. (unpubl. data), and Tsagkarakou et al. (2007). Studies undertaken so far using microsatellites have examined the structure of *B. tabaci* populations at a regional scale.

With the use of 15 microsatellite loci, De Barro (2005) showed that *B. tabaci* in the Asia-Pacific region consists of six genetically distinct populations – more than previously revealed by mitochondrial (De Barro et al. 2005) and ribosomal (De Barro et al. 2000) DNA markers – with little or no gene flow between them. This study also demonstrated the presence of strong geographic structure possibly maintained by physical barriers and/or competitive interactions, leading to allopatric divergence in most populations. However, De Barro (2005) did not find evidence for strong host-plant associated structure, suggesting that the polyphagous nature of *B. tabaci* is common among most populations, preventing host-based structuring of genotypes.

Delatte et al. (2006) used a set of eight microsatellite loci to examine the distribution of the invasive biotype B in the island of La Réunion and its interactions with the indigenous Ms biotype. They demonstrated that the invasive biotype has spread throughout the island, overlapping in range with the indigenous biotype. However, segregation into different hosts, with the invasive B occupying mostly vegetable crops and the indigenous Ms colonizing weeds, determines relative distribution in distinct parts of the island. The examination of shared alleles among populations indicated the presence of a cluster that could represent a hybrid population, a result of asymmetrical and locus specific introgression of Ms alleles into B genotypes, possibly from a unidirectional cross between B females and Ms males. In addition, despite the estimated recent invasion of the B biotype, the putative hybrid population exhibited considerable genetic variation and levels of geographic structure similar to the local biotype, suggesting that multiple introductions may have contributed to invasion of the island.

More recently, Tsagkarakou et al. (2007) used six microsatellite loci and mtCO1 DNA sequences to study the genetic polymorphism of *B. tabaci* populations from Greece. Although analysis of mtCO1 sequences failed to differentiate the populations into discrete clusters, the use of microsatellite loci provided better resolution of the genetic structure, even at this small geographic scale, and differentiated the samples into at least two distinct genetic populations. It was also possible to use two polymorphic microsatellite loci to discriminate Q from B biotype on the basis of shared and unique alleles detected in reference populations and accordingly to characterize the Greek samples as belonging only to the Q biotype.

A more recent study examined the microsatellite variation of biotypes B and Q in the Mediterranean region by examining a number of populations from different areas (Simon et al. 2007). This study looked specifically for signatures of recent bottleneck events – sudden reductions in population size – and was able to detect such genetic signatures in Iberian Q, Canarian Q and Egyptian B populations. In addition, they tested for genetic differentiation between pairs of populations and inferred close relationships among Q biotype populations from Israel, the Iberian

Peninsula and Italy, and likewise between B biotype populations from Egypt and Israel. However, the origins of the Mediterranean populations were not resolved.

Finally, another study looked at the genetic structure of 22 *B. tabaci* populations sampled from vegetable and ornamental glasshouses in southern France (Dalmon et al. 2008). This study tested the hypothesis that the enclosed environment and patchy distribution of glasshouses in combination with the limited outdoor survival of whiteflies in the winter would promote genetic differentiation among populations. Using seven microsatellite loci and mtCO1 sequences, two genetic groups were detected, corresponding to B and Q-like biotypes. While the Q group was predominant across the sampled areas, the B type group showed limited distribution. The authors suggested that extreme temperature tolerance and insecticide resistance might have favored Q over B in glasshouses in southern France. Within the Q group, the microsatellite analysis indicated limited genetic differentiation among glasshouse populations, suggesting a recent colonization event and dispersal over large distances likely facilitated by movement of plant material between glasshouses. As the authors discuss, high genetic similarity among localities resulting from human trade is also evident at a worldwide scale, with individuals on different continents exhibiting 100% nucleotide identity of mtCO1 sequences.

Although not fully explored in *B. tabaci*, the high degree of polymorphism displayed by microsatellites makes them promising for studies measuring gene flow, identifying genetic structure and inferring past demographic histories of populations. Future studies employing microsatellites likely will focus on associating populations with disease epidemics, investigating host-associated structure, and untangling complex population histories.

## Additional Considerations and Future Directions

To date, research using multilocus molecular markers in the *B. tabaci* complex system has targeted identifying the current distribution of biotypes, patterns of gene flow and population structure, host-associations, and surveys for the presence or evidence of introductions of specific biotypes in a region. Information about genetic differentiation among populations/biotypes can be obtained by calculating "summary statistics" such as the fixation index $F_{ST}$. Although such analyses are informative for presenting a current perspective, they do not distinguish among histories that could have produced that level of genetic differentiation, i.e., between contemporary and historical gene flow (Lowe et al. 2004). Genetic data obtained from molecular markers, particularly DNA sequences (see Brown, Chapter 2) and microsatellites, can reveal genealogical information and answer questions pertaining to the evolutionary history of biotypes. One can ask for example: how long ago did biotypes in the *B. tabaci* complex diverge from one another? How large was the population that diverged to give rise to a new biotype? Was the population constant in size throughout its history or did it fluctuate as a result of bottlenecks, founder

events, or expansions? Have some biotypes continued to exchange migrant whiteflies at some times in their history or did they become completely geographically isolated following the classical model of allopatric diversification? All these questions are important to understanding how biotypes and species diverge and evolve, especially while maintaining cryptic and often overlapping morphologies. In the case of *B. tabaci*, these questions can also help us understand the long-term histories of invasive "pest" biotypes and how past demographic processes (e.g., selection, gene flow, fluctuations in population size) have shaped their current high genetic diversity, ability to adapt to extreme conditions and range expansions.

Employing methods and programs that are based on the theory of the coalescent can help approach questions in population biology (Marjoram and Tavaré 2006). The coalescent refers to the process by which, looking backward in time, the genealogies of two alleles merge at a common ancestor. Thus, according to this approach, the descent of any given sample of alleles can be traced back in history, with a coalescent event occurring at every node of common ancestry. By developing a model for the time intervals between each coalescent event for a given sample of genes, the ancestral history can be inferred. Any historical changes in population size affects the probability distribution of the coalescent times, with stable, exponentially expanding or declining population sizes leaving distinct signatures on the pattern of coalescence (Hartle and Clark 2007). Demographic histories of populations can be inferred using this approach from samples of gene sequences or alleles obtained from extant populations (Emerson et al. 2001), and interesting scenarios about the evolution of the species/organism under consideration can be hypothesized. The *B. tabaci* complex of biotic and genetic variants – the most well studied of which have been named as biotypes – are quite likely at the point of divergence into distinct species, representing an excellent model for answering such theoretical questions that can nevertheless serve as a basis for addressing more applied issues, such as understanding the biology and spread of invasive organisms.

The use of molecular markers in ecological genetics has other, often neglected, considerations, the most important being the natural history of a chosen molecular marker. Different markers have different rates of mutation, with nuclear DNA typically evolving slower than mitochondrial or ribosomal DNA. In addition, the mtDNA does not undergo recombination and is only inherited through the mother, therefore only the maternal lineage is traced in mtDNA analysis. As a consequence, the effective population size of mtDNA is smaller than nuclear DNA because fewer copies of mtDNA are passed to each generation. These issues may have implications for phylogenetic studies, as the inferred genealogical histories will only reflect the history of the mtDNA locus without necessarily corresponding to the species histories (Ballard and Whitlock 2004). To provide an overall and non-biased perspective of population and species histories, a set of multiple non-linked markers from both the nuclear and mitochondrial genome must be investigated. Complementing markers from these two different genomes having different natural histories can also help detect past hybridization events between biotypes by testing for discrepancies between the generated nuclear and mitochondrial phylogenies (Lowe et al. 2004). Moreover, use of microsatellite loci can add significant information because they

are examined as multilocus markers and a good number of polymorphic loci are already available in the literature for *B. tabaci* (Dalmon et al. 2008; De Barro et al. 2003; Delatte et al. 2006; Gauthier et al. 2008; Schwartz et al. unpubl. data; Tsagkarakou and Roditakis 2003; Tsagkarakou et al. 2007). Even though microsatellites are nuclear DNA, they evolve faster than mtDNA owing to the frequent error of polymerase slippage during replication. Although not as appropriate for phylogenetic inference as other DNA markers, microsatellites have been used successfully to infer population demographic histories, and their high polymorphism allows for analysis at a finer scale. These observations illustrate that selection of a marker must be evaluated upon the natural history of the marker and the type of question being asked, whether the focus is short-term or long-term estimations of population parameters.

As more of the *B. tabaci* genome becomes available, more recently developed markers can also be employed for future studies in this system. Single nucleotide polymorphisms (SNPs) for example have been widely used in human population genetics (Hartle and Clark 2007) because of their high abundance in the genome and the ability to use them in high-throughput analysis. Recent technological advances in the sequencing methods, e.g., pyrosequencing, 454-sequencing technology (Ellegren 2008), can also enhance future molecular genetic studies in the *B. tabaci* system and help our understanding of biotype diversification.

## Conclusions

Since the first appreciation of the high genetic polymorphism within the *B. tabaci* sibling species group in the mid-1980s, a wealth of literature has been produced, with studies focusing on a range of molecular markers, from allozymes, to RFLP, RAPD, AFLP, and more recently to microsatellites. These studies addressed questions ranging from host associations and geographic structure of biotypes, to mating compatibilities between biotypes, providing a worldwide perspective of these topics. Our knowledge of biotype biology and ecology has been complemented with genetic information through the use of molecular markers that allowed us to appreciate the unique nature of the *B. tabaci* sibling species group – a complex of cryptic variants that exhibit genetic variation comparative to species level in other organisms, while having maintaining indistinguishable morphologies.

In this chapter, our aim was to provide a historical overview of studies that employed multilocus markers to answer these questions, by focusing on the main findings of each study, while also providing some background information about the markers used. We also consider possible future studies that can help enhance our knowledge, not only of the current status and distribution of biotypes, but also of the long-term history that contributed to the evolution of the present biotype variants. With these thoughts, we hope that future research will focus on the *B. tabaci* sibling species group as a model system with which to address broad evolutionary questions.

## Literature Cited


Abdullahi I, Atiri GI, Thottappilly G, Winter S. 2004. Discrimination of cassava-associated *Bemisia tabaci* in Africa from polyphagous populations, by PCR-RFLP of the internal transcribed spacer regions of ribosomal DNA. *J. Appl. Entomol.* 128:81–87.

Abdullahi I, Winter S, Atiri GI, Thottappilly G. 2003. Molecular characterization of whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) populations infesting cassava. *Bull. Entomol. Res.* 93:97–106.

Anthony N, Brown JK, Markham PG, ffrench-Constant RH. 1995. Molecular analysis of cyclodiene resistance-associated mutations among populations of the sweetpotato whitefly *Bemisia tabaci*. *Pestic. Biochem. Physiol.* 51:220–228.

Avise JC. 2004. *Molecular Markers, Natural History, and Evolution*. Sunderland, MA: Sinauer Associates.

Ballard JWO, Whitlock MC. 2004. The incomplete natural history of mitochondria. *Mol. Ecol.* 13:729–744.

Banks GK, Colvin J, Chowda Reddy RV, Maruthi MN, Muniyappa V. 2001. First report of the *Bemisia tabaci* B biotype in India and an associated tomato leaf curl virus disease epidemic. *Plant Dis.* 85:231.

Bedford ID, Briddon RW, Brown JK, Rosell RC, Markham PG. 1994. Geminivirus transmission and biological characterization of *Bemisia tabaci* (Gennadius) biotypes from different geographic regions. *Ann. Appl. Biol.* 125:311–325.

Behura SK. 2006. Molecular marker systems in insects: current trends and future avenues. *Mol. Ecol.* 15:3087–3113.

Bellows TS, Perring TM, Gill RJ, Headrick DH. 1994. Description of a species of *Bemisia* (Homoptera: Aleyrodidae). *Ann. Entomol. Soc. Am.* 87:195–206.

Bensch S, Akesson M. 2005. Ten years of AFLP in ecology and evolution: why so few animals? *Mol. Ecol.* 14:2899–2914.

Berry SD, Fondong VN, Rey C, Rogan D, Fauquet CM, Brown JK. 2004. Molecular evidence for five distinct *Bemisia tabaci* (Homoptera: Aleyrodidae) geographic haplotypes associated with cassava plants in sub-Saharan Africa. *Ann. Entomol. Soc. Am.* 97:852–859.

Bird J. 1957. A whitefly-transmitted mosaic of *Jatropha gossypifolia*. *Tech. Pap. P. R. Agric. Exp. Stn.* 22:1–35.

Bird J, Maramorosch K. 1978. Viruses and virus diseases associated with whiteflies. *Adv. Virus Res.* 22:55–110.

Black WC. 1993. PCR with arbitrary primers: approach with care. *Insect Mol. Biol.* 2:1–6.

Black WC, Duteau NM, Puterka GJ, Nechols JR, Pettorini JM. 1992. Use of the random amplified polymorphic DNA – polymerase chain reaction (RAPD-PCR) to detect DNA polymorphisms in aphids (Homoptera: Aphididae). *Bull. Entomol. Res.* 82:151–159.

Bosco D, Loria A, Sartor C, Cenis JL. 2006. PCR-RFLP identification of *Bemisia tabaci* biotypes in the Mediterranean basin. *Phytoparasitica* 34:243–251.

Botstein D, White RL, Skolnick M, Davis RW. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* 32:314–331.

Brown JK. 2007. The *Bemisia tabaci* complex: genetic and phenotypic variability drives begomovirus spread and virus diversification. *Plant Dis. APSNet Feature Article*. January 2008. http://www.apsnet.org/online/feature/btabaci

Brown JK, Bird J. 1992. Whitefly-transmitted geminiviruses in the Americas and the Caribbean Basin: past and present. *Plant Dis.* 76:220–225.

Brown JK, Bird J. 1996. Introduction of an exotic whitefly (*Bemisia*) vector facilitates secondary spread of Jatropha mosaic virus, a geminivirus previously vectored exclusively by the Jatropha biotype. In *Bemisia 1995: Taxonomy, Biology, Damage, Control and Management*, ed. D Gerling, RT Mayer, pp. 351–353. Wimborne: Intercept Publications.

Brown JK, Coats SA, Bedford ID, Markham PG, Bird J, Frohlich DR. 1995a. Characterization and distribution of esterase electromorphs in the whitefly, *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae). *Biochem. Genet.* 33:205–214.

Brown JK, Fletcher D, Bird J. 1993. First report of *Passiflora* leaf mottle caused by a whitefly-transmitted geminivirus in Puerto Rico. *Plant Dis.* 77:1264.

Brown JK, Frohlich DR, Rosell RC. 1995b. The sweetpotato or silverleaf whiteflies: biotypes of *Bemisia tabaci* or a species complex? *Annu. Rev. Entomol.* 40:511–534.

Brown JK, Perring TM, Cooper AD, Bedford ID, Markham PG. 2000. Genetic analysis of *Bemisia* (Hemiptera: Aleyrodidae) populations by isoelectric focusing electrophoresis. *Biochem. Genet.* 38:13–25.

Burban C, Fishpool LDC, Fauquet C, Fargette D, Thouvenel JC. 1992. Host-associated biotypes within West African populations of the whitefly *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae). *J. Appl. Entomol.* 113:416–423.

Buth DG. 1984. The application of electrophoretic data in systematic studies. *Annu. Rev. Ecol. Syst.* 15:501–522.

Byrne FJ, Cahill M, Denholm I, Devonshire AL. 1995. Biochemical identification of interbreeding between B type and non B-type strains of the tobacco whitefly *Bemisia tabaci*. *Biochem. Genet.* 33:13–23.

Byrne FJ, Devonshire AL. 1993. Insensitive acetylcholinesterase and esterase polymorphism in susceptible and resistant populations of the tobacco whitefly *Bemisia tabaci* (Genn.). *Pestic. Biochem. Physiol.* 45:34–42.

Byrne FJ, Devonshire AL. 1996. Biochemical evidence of haplodiploidy in the whitefly *Bemisia tabaci*. *Biochem. Genet.* 34:93–107.

Byrne FJ, Gorman KJ, Cahill M, Denholm I, Devonshire AL. 2000. The role of B-type esterases in conferring insecticide resistance in the tobacco whitefly, *Bemisia tabaci* (Genn.). *Pest Manage. Sci.* 56:867–874.

Caballero R. 2007. Systematics of the *Bemisia tabaci* complex and the role of endosymbionts in reproductive compatibility. Ph.D. dissertation, The University of Arizona, 107pp.

Callen DF, Thompson AD, Shen Y, Phillips HA, Richards RI, Mulley JC, Sutherland GR. 1993. Incidence and origin of null alleles in the (AC)n microsatellite markers. *Am. J. Hum. Genet.* 52:922–927.

Cervera MT, Cabezas JA, Simon B, Martinez-Zapater JM, Beitia F, Cenis JL. 2000. Genetic relationships among biotypes of *Bemisia tabaci* (Hemiptera: Aleyrodidae) based on AFLP analysis. *Bull. Entomol. Res.* 90:391–396.

Coats SA, Brown JK, Hendrix DL. 1994. Biochemical characterization of biotype specific esterases in the whitefly, *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae). *Insect Biochem. Mol. Biol.* 24:723–728.

Costa HS, Brown JK. 1990. Variability in biological characteristics, isozyme patterns and virus transmission among populations of *Bemisia tabaci* (Genn.) in Arizona. *Phytopathology* 80:888.

Costa HS, Brown JK. 1991. Variation in biological characteristics and esterase patterns among populations of *Bemisia tabaci*, and the association of one population with silverleaf symptom induction. *Entomol. Exp. Appl.* 61:211–219.

Costa HS, Brown JK, Sivasupramaniam S, Bird J. 1993. Regional distribution insecticide resistance, and reciprocal crosses between the A-biotype and B-biotype of *Bemisia tabaci*. *Insect Sci. Appl.* 14:255–266.

Dakin EE, Avise JC. 2004. Microsatellite null alleles in parentage analysis. *Heredity* 93:504–509.

Dalmon A, Halkett F, Granier M, Delatte H, Peterschmitt M. 2008. Genetic structure of the invasive pest *Bemisia tabaci*: evidence of limited but persistent genetic differentiation in glasshouse populations. *Heredity* 100:316–325.

Davies N, Villablanca FX, Roderick GK. 1999. Determining the source of individuals: multilocus genotyping in nonequilibrium population genetics. *Trends Ecol. Evol.* 14:17–21.

De Barro PJ. 2005. Genetic structure of the whitefly *Bemisia tabaci* in the Asia-Pacific region revealed using microsatellite markers. *Mol. Ecol.* 14:3695–3718.

De Barro PJ, Driver F. 1997. Use of RAPD PCR to distinguish the B biotype from other biotypes of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). *Aust. J. Entomol.* 36:149–152.

De Barro PJ, Driver F, Trueman JWH, Curran J. 2000. Phylogenetic relationships of world populations of *Bemisia tabaci* (Gennadius) using ribosomal ITS1. *Mol. Phylogenet. Evol.* 16:29–36.

De Barro PJ, Liebregts W, Carver M. 1998. Distribution and identity of biotypes of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) in member countries of the Secretariat of the Pacific Community. *Aust. J. Entomol.* 37:214–218.

De Barro PJ, Scott KD, Graham GC, Lange CL, Schutze MK. 2003. Isolation and characterization of microsatellite loci in *Bemisia tabaci*. *Mol. Ecol. Notes* 3:40–43.

De Barro PJ, Trueman JWH, Frohlich DR. 2005. *Bemisia argentifolii* is a race of *B. tabaci* (Hemiptera: Aleyrodidae): the molecular genetic differentiation of *B. tabaci* populations around the world. *Bull. Entomol. Res.* 95:193–203.

Delatte H, David P, Granier M, Lett JM, Goldbach R, Peterschmitt M, Reynaud B. 2006. Microsatellites reveal extensive geographical, ecological and genetic contacts between invasive and indigenous whitefly biotypes in an insular environment. *Genet. Res.* 87: 109–124.

Delatte H, Reynaud B, Granier M, Thornary L, Lett JM, Goldbach R, Peterschmitt M. 2005. A new silverleaf-inducing biotype Ms of *Bemisia tabaci* (Hemiptera: Aleyrodidae) indigenous to the islands of the south-west Indian Ocean. *Bull. Entomol. Res.* 95:29–35.

Demichelis S, Bosco D, Manino A, Marian D, Caciagli P. 2000. Distribution of *Bemisia tabaci* (Hemiptera: Aleyrodidae) biotypes in Italy. *Can. Entomol.* 132:519–527.

DeWoody J, Nason JD, Hipkins VD. 2006. Mitigating scoring errors in microsatellite data from wild populations. *Mol. Ecol. Notes* 6:951–957.

Dobrowolski MP, Tommerup IC, Blakeman HD, O'Brien PA. 2002. Non-mendelian inheritance revealed in a genetic analysis of sexual progeny of *Phytophthora cinnamomi* with microsatellite markers. *Fungal Genet. Biol.* 35:197–212.

Edwards OR, Hoy MA. 1993. Polymorphism in two parasitoids detected using random amplified polymorphic DNA polymerase chain reaction. *Biol. Control* 3:243–257.

Ellegren H. 2004. Microsatellites: simple sequences with complex evolution. *Nat. Rev. Genet.* 5:435–445.

Ellegren H. 2008. News and views: sequencing goes 454 and takes large-scale genomics into the wild. *Mol. Ecol.* 17:1629–1631.

Emerson BC, Paradis E, Thebaud C. 2001. Revealing the demographic histories of species using DNA sequences. *Trends Ecol. Evol.* 16:707–716.

Endersby NM, McKechnie SW, Vogel H, Gahan LJ, Baxter SW, Ridland PM, Weeks AR. 2005. Microsatellites isolated from diamondback moth, *Plutella xylostella* (L.), for studies of dispersal in Australian populations. *Mol. Ecol. Notes* 5:51–53.

Estoup A, Jarne P, Cornuet JM. 2002. Homoplasy and mutation model at microsatellite loci and their consequences for population genetics analysis. *Mol. Ecol.* 11:1591–1604.

Excoffier L, Heckel G. 2006. Computer programs for population genetics data analysis: a survival guide. *Nat. Rev. Genet.* 7:745–758.

Gauthier N, Dalleau-Clouet C, Bouvret M-E. 2008. Twelve new polymorphic microsatellite loci and PCR multiplexing in the whitefly, *Bemisia tabaci*. *Mol. Ecol. Resour.* 8:1004–1007.

Gawel NJ, Bartlett AC. 1993. Characterization of differences between whiteflies using RAPD-PCR. *Insect Mol. Biol.* 2:33–38.

Guillot G, Santos F, Estoup A. 2008. Analysing georeferenced population genetics data with Geneland: a new algorithm to deal with null alleles and a friendly graphical user interface. *Bioinformatics* 24:1406–1407.

Guirao P, Beitia F, Cenis JL. 1997. Biotype determination of Spanish populations of *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Bull. Entomol. Res.* 87:587–593.

Gunning RV, Byrne FJ, Conde BD, Connelly MI, Hergstrom K, Devonshire AL. 1995. First report of B-biotype *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) in Australia. *J. Aust. Entomol. Soc.* 34:116–120.

Gunning RV, Byrne FJ, Devonshire AL. 1997. Electrophoretic analysis of non-B and B-biotype *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) in Australia. *Aust. J. Entomol.* 36:245–249.

Hadrys H, Balick M, Schierwater B. 1992. Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. *Mol. Ecol.* 1:55–63.

Harris H. 1966. Enzyme polymorphisms in man. *Proc. R. Soc. Ser. B Biol. Sci.* 164:298–310.
Harris SA. 1999. RAPDs in systematics – a useful methodology? In *Molecular Systematics and Plant Evolution*, ed. PM Hollingsworth, RM Bateman, RJ Gornall, pp. 211–228. London: Taylor & Francis.
Hartle D, Clark A. 2007. *Principles of Population Genetics*. Sunderland, MA: Sinauer Associates.
Horowitz AR, Denholm I, Gorman K, Cenis JL, Kontsedalov S, Ishaaya I. 2003. Biotype Q of *Bemisia tabaci* identified in Israel. *Phytoparasitica* 31:94–98.
Hoy M. 2003. *Insect Molecular Genetics: An Introduction to Principles and Applications*. San Diego: Academic Press.
Hubby JL, Lewontin RC. 1966. A molecular approach to study of genic heterozygosity in natural populations.I. Number of alleles at different loci in *Drosophila pseudoobscura*. *Genetics* 54:595–609.
Jansen RC, Geerlings H, Van Oeveren AJ, Van Schaik RC. 2001. A comment on codominant scoring of AFLP markers. *Genetics* 158:925–926.
Jarne P, Lagoda PJL. 1996. Microsatellites, from molecules to populations and back. *Trends Ecol. Evol.* 11:424–429.
Johnson FM, Kanapi CG, Richardson RH, Wheeler MR, Stone WS. 1966. An analysis of polymorphisms among isozyme loci in dark and light *Drosophila ananassae* strains from American and Western Samoa. *Proc. Natl. Acad. Sci. USA* 56:119–125.
Jones CJ, Edwards KJ, Castaglione S, Winfield MO, Sala F. 1997. Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. *Mol. Breed.* 3:381–390.
Karl SA, Avise JC. 1992. Balancing selection at allozyme loci in oysters – implications from nuclear RFLPs. *Science* 256:100–102.
Khasdan V, Levin I, Rosner A, Morin S, Kontsedalov S, Maslenin L, Horowitz AR. 2005. DNA markers for identifying biotypes B and Q of *Bemisia tabaci* (Hemiptera: Aleyrodidae) and studying population dynamics. *Bull. Entomol. Res.* 95:605–613.
Kim KS, Sappington TW. 2006. Molecular genetic variation of boll weevil populations in North America estimated with microsatellites: implications for patterns of dispersal. *Genetica* 127:143–161.
Kirk AA, Lacey LA, Brown JK, Ciomperlik MA, Goolsby JA, Vacek DC, Wendel LE, Napompeth B. 2000. Variation in the *Bemisia tabaci* s. l. species complex (Hemiptera: Aleyrodidae) and its natural enemies leading to successful biological control of *Bemisia biotype* B in the USA. *Bull. Entomol. Res.* 90:317–327.
Legg JP, French R, Rogan D, Okao-Okuja G, Brown JK. 2002. A distinct *Bemisia tabaci* (Gennadius) (Hemiptera: Sternorrhyncha: Aleyrodidae) genotype cluster is associated with the epidemic of severe cassava mosaic virus disease in Uganda. *Mol. Ecol.* 11:1219–1229.
Levinson G, Gutman GA. 1987. Slipped-strand mispairing – a major mechanism for DNA-sequence evolution. *Mol. Biol. Evol.* 4:203–221.
Lima LHC, Campos L, Moretzsohn MC, Navia D, de Oliveira MRV. 2002. Genetic diversity of *Bemisia tabaci* (Genn.) populations in Brazil revealed by RAPD markers. *Genet. Mol. Biol.* 25:217–223.
Lima LHC, Navia D, Inglis PW, de Oliveira MRV. 2000. Survey of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) biotypes in Brazil using RAPD markers. *Genet. Mol. Biol.* 23:781–785.
Liu SS, De Barro PJ, Xu J, Luan JB, Zang LS, Ruan YM, Wan FH. 2007. Asymmetric mating interactions drive widespread invasion and displacement in a whitefly. *Science* 318:1769–1772.
Lowe A, Harris S, Ashton P. 2004. *Ecological Genetics: Design, Analysis, and Application*. Oxford: Blackwell Publishing Ltd.
Luikart G, England PR. 1999. Statistical analysis of microsatellite DNA data. *Trends Ecol. Evol.* 14:253–256.
Lynch M, Milligan BG. 1994. Analysis of population genetic structure with RAPD markers. *Mol. Ecol.* 3:91–99.

Marjoram P, Tavaré S. 2006. Modern computational approaches for analyzing molecular genetic variation data. *Nat. Rev. Genet.* 7:759–770.

Maruthi MN, Colvin J, Seal S. 2001. Mating compatibility, life-history traits, and RAPD-PCR variation in *Bemisia tabaci* associated with the cassava mosaic disease pandemic in East Africa. *Entomol. Exp. Appl.* 99:13–23.

Maruthi MN, Colvin J, Seal S, Gibson G, Cooper J. 2002. Co-adaptation between cassava mosaic geminiviruses and their local vector populations. *Virus Res.* 86:71–85.

McKenzie CL, Anderson PK, Villarreal N. 2004. An extensive survey of *Bemisia tabaci* (Homoptera: Aleyrodidae) in agricultural ecosystems in Florida. *Fla. Entomol.* 87:403–407.

Mitton JB. 1994. Molecular approaches to population biology. *Annu. Rev. Ecol. Syst.* 25:45–69.

Moya A, Guirao P, Cifuentes D, Beitia F, Cenis JL. 2001. Genetic diversity of Iberian populations of *Bemisia tabaci* (Hemiptera: Aleyrodidae) based on random amplified polymorphic DNA-polymerase chain reaction. *Mol. Ecol.* 10:891–897.

Mueller UG, Wolfenbarger LL. 1999. AFLP genotyping and fingerprinting. *Trends Ecol. Evol.* 14:389–394.

Mullis K, Faloona F, Scharf S, Saiki R, Horn G, Erlich H. 1986. Specific enzymatic amplification of DNA in vitro – the Polymerase Chain Reaction. *Cold Spring Harb. Symp. Quant. Biol.* 51:263–273.

Nei M. 1976. Mathematical models of speciation and genetic distance. In *Population Genetics and Ecology*, ed. S Karlin, E Neve, pp. 723–765. New York: Academic Press.

Parker PG, Snow AA, Schug MD, Booton GC, Fuerst PA. 1998. What molecules can tell us about populations: choosing and using a molecular marker. *Ecology* 79:361–382.

Parsons YM, Shaw KL. 2002. Mapping unexplored genomes: a genetic linkage map of the Hawaiian cricket *Laupala*. *Genetics* 162:1275–1282.

Pemberton JM, Slate J, Bancroft DR, Barrett JA. 1995. Nonamplifying alleles at microsatellite loci – a caution for parentage and population studies. *Mol. Ecol.* 4:249–252.

Perring TM. 2001. The *Bemisia tabaci* species complex. *Crop Prot.* 20:725–737.

Perring TM, Cooper A, Kazmer DJ. 1992. Identification of the poinsettia strain of *Bemisia tabaci* (Homoptera: Aleyrodidae) on broccoli by electrophoresis. *J. Econ. Entomol.* 85:1278–1284.

Perring TM, Cooper AD, Rodriguez RJ, Farrar CA, Bellows TS, Jr. 1993. Identification of a whitefly species by genomic and behavioral studies. *Science* 259:74–77.

Prabhaker N, Coudriet DL, Meyerdirk DE. 1987. Discrimination of 3 whitefly species (Homoptera: Aleyrodidae) by electrophoresis of nonspecific esterases. *J. Appl. Entomol.* 103:447–451.

Qiu BL, Coats SA, Ren SX, Dris AM, Xu CX, Brown JK. 2007. Phylogenetic relationships of native and introduced *Bemisia tabaci* (Homoptera: Aleyrodidae) from China and India based on mtCO1 DNA sequencing and host plant comparisons. *Prog. Nat. Sci.* 17:645–654.

Rekha AR, Maruthi MN, Muniyappa V, Colvin J. 2005. Occurrence of three genotypic clusters of *Bemisia tabaci* and the rapid spread of the B biotype in south India. *Entomol. Exp. Appl.* 117:221–233.

Robinson JP, Harris SA. 1999. Amplified fragment length polymorphisms and microsatellites: a phylogenetic perspective. In *Which DNA Marker for Which Purpose?* ed. EM Gillet, pp. 1–27. Göttingen, Germany: Institut für Forstgenetik und Forstpflanzenzüchtung, Universität Göttingen.

Roderick GK. 1996. Geographic structure of insect populations: gene flow, phylogeography, and their uses. *Annu. Rev. Entomol.* 41:325–352.

Rosell RC, Bedford ID, Frohlich DR, Gill RJ, Markham PG, Brown JK. 1997. Analyses of morphological variation in distinct populations of *Bemisia tabaci*. *Ann. Entomol. Soc. Am.* 90:575–589.

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA-polymerase. *Science* 239:487–491.

Schierwater B, Ender A. 1993. Different thermostable DNA polymerases may amplify different RAPD products. *Nucleic Acids Res.* 21:4647–4648.

Schlötterer C. 2000. Evolutionary dynamics of microsatellite DNA. *Chromosoma* 109:365–371.

Schlötterer C. 2001. Genealogical inference of closely related species based on microsatellites. *Genet. Res.* 78:209–212.

Schlötterer C. 2004. The evolution of molecular markers – just a matter of fashion? *Nat. Rev. Genet.* 5:63–69.

Schlötterer C, Pemberton J. 1994. The use of microsatellites for genetic analysis of natural populations. In *Molecular Ecology and Evolution: Approaches and Applications*, ed. B Schierwater, B Streit, GP Wagner, R DeSalle, pp. 203–214. Basel, Switzerland: Birkhauser Verlag.

Schlötterer C, Tautz D. 1992. Slippage synthesis of simple sequence DNA. *Nucleic Acids Res.* 20:211–215.

Selkoe KA, Toonen RJ. 2006. Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. *Ecol. Lett.* 9:615–629.

Simon B, Cenis JL, De La Rua P. 2007. Distribution patterns of the Q and B biotypes of *Bemisia tabaci* in the Mediterranean Basin based on microsatellite variation. *Entomol. Exp. Appl.* 124:327–336.

Simon B, Cenis JL, Demichelis S, Rapisarda C, Caciagli P, Bosco D. 2003. Survey of *Bemisia tabaci* (Hemiptera: Aleyrodidae) biotypes in Italy with the description of a new biotype (T) from Euphorbia characias. *Bull. Entomol. Res.* 93:259–264.

Smith KL, Alberts SC, Bayes MK, Bruford MW, Altmann J, Ober C. 2000. Cross-species amplification, non-invasive genotyping, and non-Mendelian inheritance of human STRPs in Savannah baboons. *Am. J. Primatol.* 51:219–227.

Sseruwagi P, Legg JP, Maruthi MN, Colvin J, Rey MEC, Brown JK. 2005. Genetic diversity of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) populations and presence of the B biotype and a non-B biotype that can induce silverleaf symptoms in squash, in Uganda. *Ann. Appl. Biol.* 147:253–265.

Sseruwagi P, Maruthi MN, Colvin J, Rey MEC, Brown JK, Legg JP. 2006. Colonization of non-cassava plant species by cassava whiteflies (*Bemisia tabaci*) in Uganda. *Entomol. Exp. Appl.* 119:145–153.

Sseruwagi P, Rey MEC, Brown JK, Legg J. 2004. The cassava mosaic geminiviruses occuring in Uganda following the 1990s epidemic of severe mosaic disease. *Ann. Appl. Biol.* 145:113–121.

Tautz D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Res.* 17:6463–6471.

Tsagkarakou A, Roditakis N. 2003. Isolation and characterization of microsatellite loci in *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Mol. Ecol. Notes* 3:196–198.

Tsagkarakou A, Tsigenopoulos CS, Gorman K, Lagnel J, Bedford ID. 2007. Biotype status and genetic polymorphism of the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Greece: mitochondrial DNA and microsatellites. *Bull. Entomol. Res.* 97:29–40.

Viard F, Franck P, Dubois MP, Estoup A, Jarne P. 1998. Variation of microsatellite size homoplasy across electromorphs, loci, and populations in three invertebrate species. *J. Mol. Evol.* 47:42–51.

Vos P, Hogers R, Bleeker M, Reijans M, Vandelee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M. 1995. AFLP – a new technique for DNA fingerprinting. *Nucleic Acids Res.* 23:4407–4414.

Weber JL, Wong C. 1993. Mutation of human short tandem repeats. *Hum. Mol. Genet.* 2:1123–1128.

Welsh J, McClelland M. 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Res.* 18:7213–7218.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* 18:6531–6535.

Wilson IJ, Balding DJ. 1998. Genealogical inference from microsatellite data. *Genetics* 150:499–510.

Wong A, Forbes MR, Smith ML. 2001. Characterization of AFLP markers in damselflies: prevalence of codominant markers and implications for population genetic applications. *Genome* 44:677–684.

Wool D, Gerling D, Bellotti AC, Morales FJ. 1993. Esterase electrophoretic variation in *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae) among host plants and localities in Israel. *J. Appl. Entomol.* 115:185–196.

Wool D, Gerling D, Bellotti A, Morales F, Nolt B. 1991. Spatial and temporal genetic variation in populations of the whitefly *Bemisia tabaci* (Genn.) in Israel and Colombia – an interim report. *Insect Sci. Appl.* 12:225–230.

Wool D, Gerling D, Nolt BL, Constantino LM, Bellotti AC, Morales FJ. 1989. The use of electrophoresis for identification of adult whiteflies (Homoptera: Aleyrodidae) in Israel and Colombia. *J. Appl. Entomol.* 107:344–350.

Yan G, Romero-Severson J, Walton M, Chadee DD, Severson DW. 1999. Population genetics of the yellow fever mosquito in Trinidad: comparisons of amplified fragment length polymorphism (AFLP) and restriction fragment length polymorphism (RFLP) markers. *Mol. Ecol.* 8:951–963.

Zhang DX, Hewitt GM. 2003. Nuclear DNA analyses in genetic studies of populations: practice, problems and prospects. *Mol. Ecol.* 12:563–584.

Zhang LP, Zhang YJ, Zhang WJ, Wu QJ, Xu BY, Chu D. 2005. Analysis of genetic diversity among different geographical populations and determination of biotypes of *Bemisia tabaci* in China. *J. Appl. Entomol.* 129:121–128.

# Section II
# Biology and Ecology of *Bemisia tabaci*

**Steven E. Naranjo and James P. Legg**

## Introduction

*Bemisia tabaci* (Gennadius) was first described as a pest of tobacco nearly 120 years ago in Greece, and since that time has become one of the most important pests of world agriculture. The insect is capable of inflicting damage to plants in multiple ways. High populations may remove sufficient phloem sap to reduce plant vigor, and secretion of honeydew and resulting sooty mold can reduce the quality and marketability of harvested products. Feeding by even a few insects may induce debilitating plant disorders or the transmission of numerous plant viruses. *B. tabaci* exhibits considerable genetic and biological variability and is consequently considered by many to be a species complex. The *B. tabaci* species complex is cosmopolitan in distribution, with natural populations occurring within tropical and subtropical latitudes on every continent but Antarctica. Commercial plant trade has brought the pest to temperate zones where it has found a home in greenhouses throughout Asia, Europe and North America.

The most recent, large-scale review on the biology, ecology, epidemiology and pest management of *B. tabaci* was based on a workshop held in 1994 and published in 1996 (Gerling and Mayer 1996). Basu (1995) also published a general text on multiple aspects of *B. tabaci* biology and management. Most recently, Naranjo and Ellsworth (2001) published a special journal issue that updated efforts in pest management tactics and their integration into working systems. By early 2009, over 9,000 citations – from the late 1800s on – had been reported on *B. tabaci* with over 300 new papers published each year since 1996 (Naranjo et al. 2009). Advances are being made in many areas, from basic biology and population dynamics, to complex interactions with other organisms, to development and implementation of pest management tactics and systems.

This section focuses on broad areas of the biology and ecology of *B. tabaci*; fundamental and instrumental areas of investigation that underlie and facilitate research in systematics and phylogeny, virus-vector interactions and epidemiology, and integrated pest management. Given the breadth of this topic, we cannot

---

Steven E. Naranjo and James P. Legg (Section Editors)

hope to provide a complete review of all activity in this area. Our approach here was to offer three comprehensive chapters that focus on particular aspects of the biology and ecology of *B. tabaci*, with primary emphasis on research advances made in the past 10–15 years. Some historical literature is also included to set the stage for current advances and to provide the means for re-evaluating and re-interpreting our understanding of this important and complex pest insect.

Walker, Perring and Freeman (Chapter 4) update and synthesize current thinking on the life history and functional anatomy of *B. tabaci* including the mechanics and behavior of feeding and mating. For such a small and seemingly non-descript insect that spends much of its life on leaf surfaces, high power microscopy has highlighted the incredible and intricate details in all life stages and their intimate connection to the host plant. The text is supplemented with a series of scanning electron microscope images and line drawings that detail morphological elements of each developmental stage, and that also highlight the insect's interaction with both the plant surface and the phloem tissue upon which they feed and through which they transmit viruses. Modern tools have lead to new descriptions of anatomy and a better understanding of their functional biology and behavior. Despite advances in this area, there remain differences of interpretation in the organization and function of certain portions of the alimentary canal and these views are discussed. This chapter further reviews our current understanding about courtship and mating behavior of *B. tabaci* as well as competitive interactions among biotypes that may have implications for biotype spread and displacement.

One of the hallmarks of *B. tabaci* biology is its close association with symbiotic organisms that permit the insect to thrive on the dilute nutrient stream provided by plant phloem. In the second chapter of this section, Rosell, Blackmer, Czonek, and Inbar (Chapter 5) review and synthesize recent studies of the complex interactions of *B. tabaci* with primary and secondary endosymbiotic bacteria, the critical role and interaction of these symbionts in begomovirus transmission, as well as the intra-and interspecific relationships among *B. tabaci*, other herbivores and their host plants. Comprehension of these complex interactions is in its infancy, and much is yet to be learned. Fascinating in their own right, these rich and unusual relationships open up important new avenues of research aimed at developing novel tactics and strategies for pest management based on interfering with or manipulating symbiotic and other biological and ecological relationships.

Another hallmark of *B. tabaci* is its amazing ability to reproduce and spread within multiple ecosystems, often leading to population outbreaks that severely impact agricultural and horticultural production. In this section's final chapter, Naranjo, Castle, De Barro and Liu (Chapter 6) focus on advances in our understanding of several broad but interrelated categories within population ecology including sampling (measuring population size), demography (growth and survival of populations), and dispersal (mechanisms for dealing with and exploiting ephemeral environments). The authors also discuss and synthesize our more fundamental understanding of the interaction of population ecology and the environment with regards to the pervasive invasion capacity of certain biotypes, and the ability of

the insect to precipitate population outbreaks. The role of and advances in population modeling are also discussed. Researchers continue to study life history in the laboratory and to develop sampling methods for newly affected crops. Field-based life table studies have recently been completed on a number of host plants, the results of which are helping us to better understand and exploit population processes for better management. The invasion and rapid spread of *B. tabaci* into new regions of the world continues unabated while recent research is helping to explain the mechanisms behind these patterns.

Like any area of investigation, the more we learn the more questions arise and challenge our current understanding. As the scale of the *B. tabaci* research effort continues to grow, it becomes essential that the scientists involved have a broad understanding of the fundamental biology and ecology of this insect pest. We hope that these reviews will contribute to this process, not only by increasing our current knowledge, but also by helping to focus and refine the questions we continue to ask about this pest and its multiple relationships with other organisms and the environment. Ultimately, progress in these activities is essential if we are to provide more efficient and environmentally sound management strategies to deal with this cosmopolitan pest.

## Literature Cited

Basu AN. 1995. Bemisia tabaci (Gennadius): *Crop Pest and Principal Whitefly Vector of Plant Viruses*. Boulder, Colorado: Westview Press.

Gerling D, Mayer RT (eds.). 1996. *Bemisia 1995: Taxonomy, Biology, Damage, Control and Management*. Andover, Hants, UK: Intercept.

Naranjo SE, Butler GD, Jr, Henneberry TJ. 2009. *Bibliography of Bemisia tabaci* and *Bemisia argentifolii*, http://www.ars.usda.gov/Services/docs.htm?docid=10916.

Naranjo SE, Ellsworth PC. 2001. Challenges and opportunities for pest management of *Bemisia tabaci* in the new century. *Crop Prot. (Special Issue)* 20:707–872.

# Chapter 4
# Life History, Functional Anatomy, Feeding and Mating Behavior

**Gregory P. Walker, Thomas M. Perring, and Thomas P. Freeman**

## Introduction

This chapter covers three diverse topics: whitefly anatomy, feeding behavior, and courtship and mating. In previous volumes of the *Bemisia* series (Gerling 1990; Gerling and Mayer 1996), Gill (1990) reviewed whitefly anatomy from a systematics/taxonomic perspective and Harris et al. (1996a) covered the internal anatomy of whiteflies, specifically the alimentary canal, salivary glands, and ovaries. We avoid overlap with these earlier reviews by covering whitefly anatomy from a structure-function perspective and discussing internal anatomy only briefly, although the alimentary canal required more detail to correct a previous misinterpretation and incorporate new information from more recent studies. Feeding and courtship behavior are reviewed here for the first time.

## Morphology of Life Stages

Life history and development stages appear to be virtually uniform throughout the Aleyrodidae. The four nymphal instars are sessile except for the early first instar. Nymphs have a reduced body design while adults retain the well-developed anatomical features characteristic of most hemipteran insects. The transition between immature and adult morphology requires a dramatic metamorphosis in the latter part of the fourth nymphal instar.

### *Egg Stage*

Whitefly eggs have a pedicel that is an extension of the egg chorion. During oviposition, the female inserts the pedicel into the plant, and secures it with a glue-like secretion that keeps the egg anchored in place (Buckner et al. 2002). For *Bemisia,*

G.P. Walker (✉)
Department of Entomology, University of California, Riverside, CA 92521, USA
e-mail: gregory.walker@ucr.edu

P.A. Stansly, S.E. Naranjo (eds.), *Bemisia*: Bionomics and Management of a Global Pest,
DOI 10.1007/978-90-481-2460-2_4, 

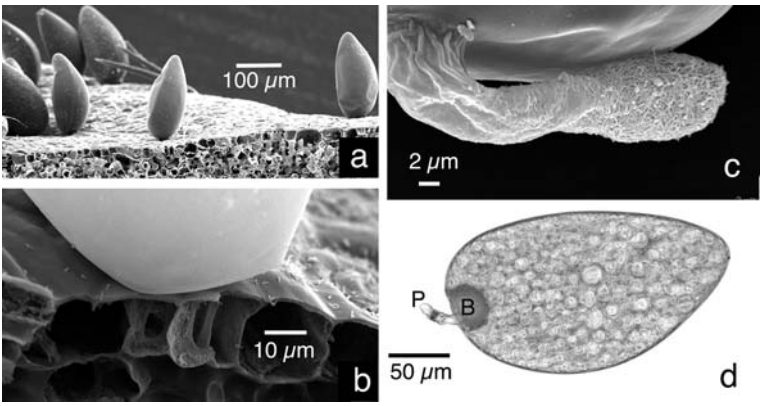



**Fig. 4.1** *Bemisia* egg stage. (**a**) Eggs in situ with pedicels inserted in leaf (SEM) (**b**) egg pedicel in epidermal cell (freeze fracture and SEM) (**c**) close-up of egg pedicel showing fibrous structure (SEM, Fig. 4.7 from Buckner et al. 2002, Archives of Insect Biochemistry and Physiology 49: 22–33; reprinted with permission of John Wiley & Sons, Inc.) (**d**) light micrograph of egg revealing bacteriome (B) at the pedicel (P) end of the egg

the egg pedicel is usually inserted into an epidermal cell (Fig. 4.1b) with the glue securing it in place, whereas most other whiteflies studied insert the egg pedicel into stomata (Paulson and Beardsley 1985). Securing the egg to the plant insures immediate contact of the newly hatched nymph with its host. In addition, the apex of the pedicel, which has a porous fibrous structure (Fig. 4.1c), absorbs water and possibly solutes from the leaf. Water absorption from the leaf is essential for egg development; approximately half of the water content of mature whitefly eggs is obtained from the leaf (Byrne et al. 1990; Castañé and Savé 1993, Buckner et al. 2002).

A large bacteriome (formally mycetome) in each whitefly egg contains symbiotic bacteria received through transovariole transmission from the mother (Weber 1935; Tremblay 1959; Costa et al. 1996). In *Bemisia*, a single bacteriocyte enters the base of each egg (Tremblay 1959; Costa et al. 1996). In the mature egg, the bacteriome is a large, conspicuous, yellow sphere at the base of the egg above the pedicel (Fig. 4.1d). Symbionts are discussed in Chapter 5 by Rosell et al.

Whitefly eggs have a distinct longitudinal eclosion line that splits open during hatching, allowing the newborn nymph to emerge (Fig. 4.2a). The empty egg chorion remains attached to the leaf, providing a convenient record of the starting number of eggs in a cohort for life table studies.

## *Nymphal Stage*

Whitefly nymphs settle permanently in one spot on a leaf and obtain all their nutritional needs for development over four instars from the phloem tissue within reach of their stylets. They have a highly reduced morphology in keeping with this sessile

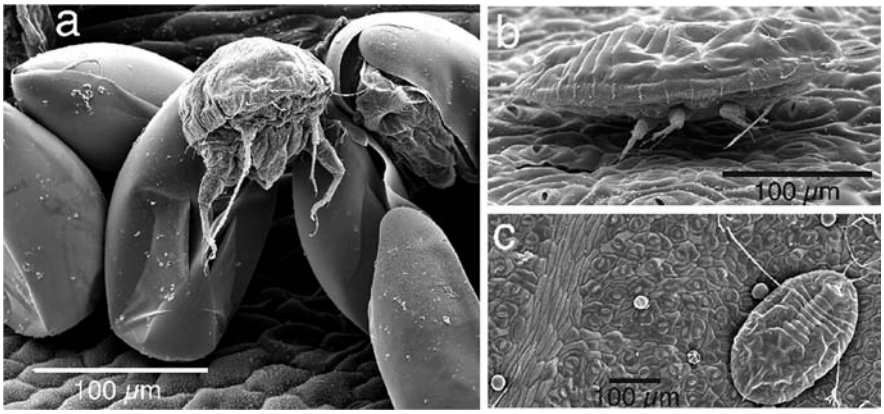



**Fig. 4.2** First instar (SEM). (**a**) Hatching from egg (**b**) first instar crawler on leaf surface (Fig. 4.12 from Freeman et al. 2001; reprinted courtesy of the Entomological Society of America) (**c**) first instar on surface of cotton leaf showing differences in epidermal topography over small veins and in intervenal areas

feeding habit. All instars are oval in shape and flattened dorso-ventrally (Figs. 4.2b, c and 4.3a, b). Height increases within each nymphal instar, while length and width remaining constant until the molt (Gelman et al. 2002). Thus, length and width provide reliable criteria to distinguish among instars, although the size of a given instar can vary among host plants (Bethke et al. 1991; Gelman and Gerling 2003).

The early first instar or "crawler" has well-developed legs and, after hatching, it wanders over the leaf surface in search of a suitable settling site (Fig. 4.2b). Once a site is found, the nymph inserts its stylets and usually remains at that site for the rest of its nymphal development. However, sometimes crawlers withdraw their stylets from the initial site, presumably after determining it to be unacceptable, move to a new site, and re-insert their stylets (GPW unpublished data). The remaining instars have reduced legs and are incapable of movement.

Crawler movement on the leaf surface does not appear to be random although the cues used to determine site acceptability are largely unknown. On cotton, first instars spend 80% of their wandering time over minor veins (the preferred feeding site – Cohen et al. 1996b, c; Jiang and Walker 2007) even though the area over minor veins accounts for less than 25% of the leaf surface area (Cohen et al. 1996a). Cohen et al. (1996a) suggested that crawlers need to settle near a minor vein on a cotton leaf to access it with their stylets. However, based on measurements of stylet length, Freeman et al. (2001) concluded that first instar stylets were capable of reaching phloem from virtually any point on the leaf surface of cotton and hibiscus. Walker (unpublished data) also noted the same on alfalfa. Nonetheless, the probability of actually locating a vascular bundle with the stylets may be greater if the probe is initiated closer to a vein. Surface topography could provide the necessary cues because epidermal cells are often elongate over the veins versus more-or-less isodiametric over intervenal areas (Fig. 4.2c, Cohen et al. 1996a).

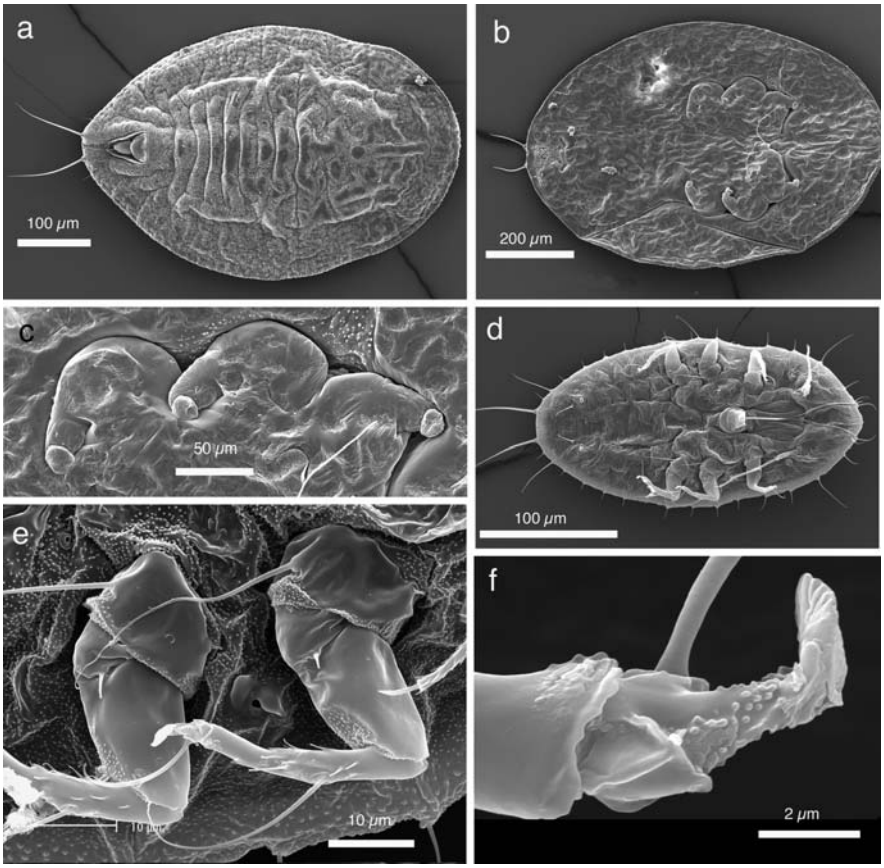



**Fig. 4.3** *Bemisia* nymphs (SEM). (**a**) Third instar, dorsal habitus (**b**) fourth instar, ventral habitus (**c**) fourth instar legs (**d**) first instar, ventral habitus (**e**) first instar legs (**f**) first instar tarsus/pretarsus

The dispersal ability of whitefly crawlers has been considered limited (van Lenteren and Noldus 1990) based on studies showing that crawlers generally settle very close to the egg from which they hatched (e.g., Price and Taborsky 1992). However, if that leaf is unsuitable, some crawlers may successfully move to a more suitable leaf on the plant or even move to a different plant whose leaves are touching (Summers et al. 1996). Nevertheless, the great majority of those attempting to relocate perish, even under ideal conditions in the laboratory (Summers et al. 1996). Thus, host and leaf selection is largely controlled by the ovipositing female rather than the crawler.

Estimates of the duration of the crawler stage vary widely. Cohen et al. (1996a) stated that crawlers must locate a settling site in 3–5 h or die (presumably at 25°C). Price and Taborsky (1992) found that most crawlers settled within 3 h of egg hatch

at 24 ± 2°C, although some were still moving after 2 days. Summers et al. (1996) observed survival of unsettled crawlers up to 5 days at 12–18°C. Ambient temperature and humidity undoubtedly affect how long a crawler can survive without settling and feeding.

Gill (1990) and Byrne and Bellows (1991) stated that whitefly crawlers appear to have 3-segmented legs, presumably based on examination of slide-mounted specimens. However, our scanning electron micrographs, and those of Domenichini (1981), clearly show that the legs of the mobile first instar are relatively well-developed with a distinctly articulated coxa, trochanter, femur, fused tibia/tarsus, and pretarsus (Fig. 4.3d, e). The pretarsus consists of an elongate seta and a bladder-shaped structure on a short stalk (Fig. 4.3f). The legs of the sessile second through fourth instars are short, stubby, and fleshy without distinct segmentation, and bear an adhesive disc at the apex (Fig. 4.3c). Although non-functional for walking, they appear to play an important role in gripping the leaf surface during molting. A good grip is probably important to prevent the nymph from falling off the leaf during the molt (*Bemisia* nymphs usually occur on the abaxial (lower) leaf surface), and may also be needed for leverage when the stylets are inserted. Antennae of first instars are 3 segmented and are relatively long, extending beyond the margin of the head. They bear a number of sensory organs presumably to provide needed input for site selection (Fig. 4.4a, c, e–g). Although as yet unstudied, these sensory structures probably include both mechano-and chemosensory sensillae as in the adults. The rudimentary antennae of second through fourth instars are short, 1 segmented, and have few sensory organs (Fig. 4.4b, d).

The lateral margin of the first instar bears many setae that are absent in later instars (Figs. 4.3d and 4.4a). These setae are presumably mechanosensory, and have been hypothesized to detect plant trichomes while the crawler wanders over the leaf surface in search of a settling site. Detection of plant trichomes by the wandering crawler affects development of the dorsal setae which may be longer in later instars on plants with trichomes and shorter or absent on glabrous plants (Azab et al. 1969; Neal and Bentz 1999; Guershon and Gerling 2001). This phenotypic response to the host plant may represent a form of crypsis (Neal and Bentz 1999).

The “eyes” of the nymphal stage consist of two small spots that lack a cuticular cornea and have an underdeveloped retina (Gelman et al. 2002). They later develop into the compound eyes of the adult, but in the nymph they presumably function as simple light detectors.

Nymphal mouthparts are visible externally only as a short rostrum with sensory structures at the apex that, if homologous to the sensillae of the adults, include both mechano- and chemosensors (Fig. 4.5) (Domenichini 1981). Mouthparts of nymphs and adults will be described in more detail later.

The anus is located dorsally and posteriorly in a depression referred to as the “vasiform orifice” (Fig. 4.6). The anus opens between the bases of two structures, an anterior “operculum” and a posterior “lingua.” The latter serves to catapult drops of sticky honeydew away from the body helping the nymph to avoid entrapment in its own sticky excrement.

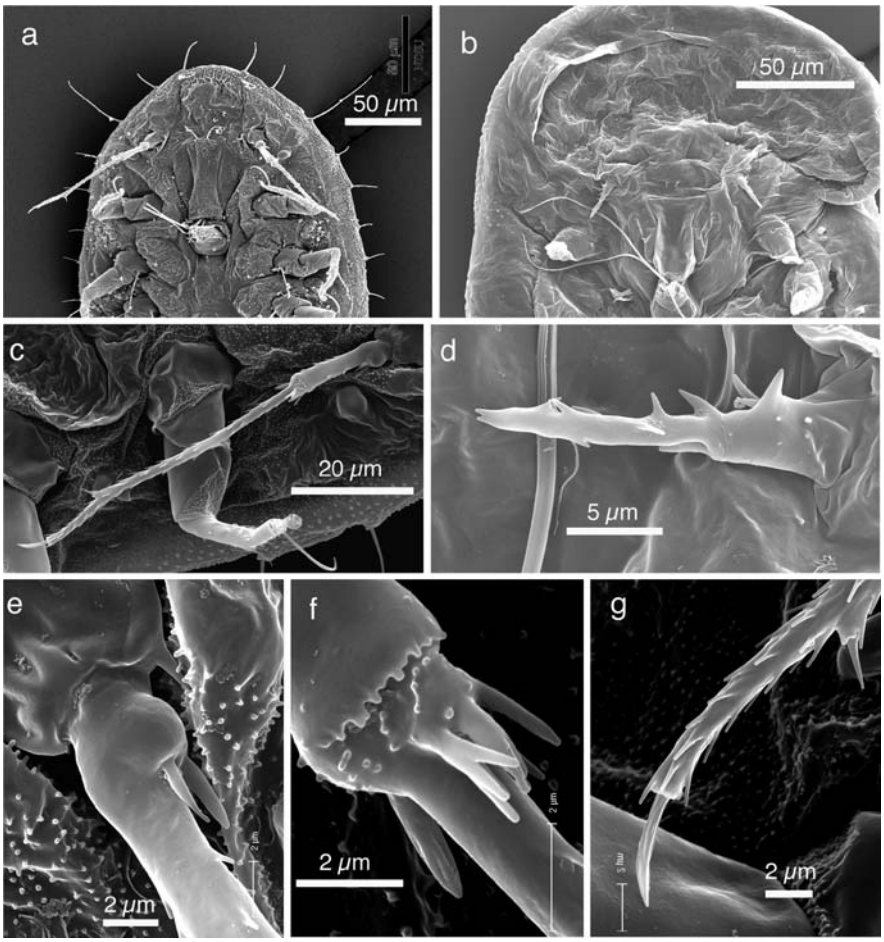



**Fig. 4.4** Antennae of *Bemisia* nymphs (SEM). (**a**) Anterior of first instar showing relative size of antenna (**b**) anterior of later nymphal stage showing relative size of antenna (**c**) overview of first instar antenna (**d**) overview of later nymphal stage antenna (**e**) close-up of sensillae at base of first instar antenna (**f**) close-up of sensillae in mid region of first instar antenna (**g**) close-up of sensillae at apex of first instar antenna

## *"Pupal" Stage*

The dramatic metamorphosis to adult morphology takes place during the "pupal stage" which is the latter part of the fourth nymphal instar (Gelman et al. 2002). Unlike the Holometabola, the pupal stage is not a separate instar; hence, the quotation marks around "pupal" for this developmental stage in whiteflies. The first externally observable feature in the fourth instar that signals this metamorphosis is the enlargement of the eyes from small red pinpoints to larger diffuse red oval spots, and finally to conspicuous red eye spots giving rise to the term "red-eyed nymph"

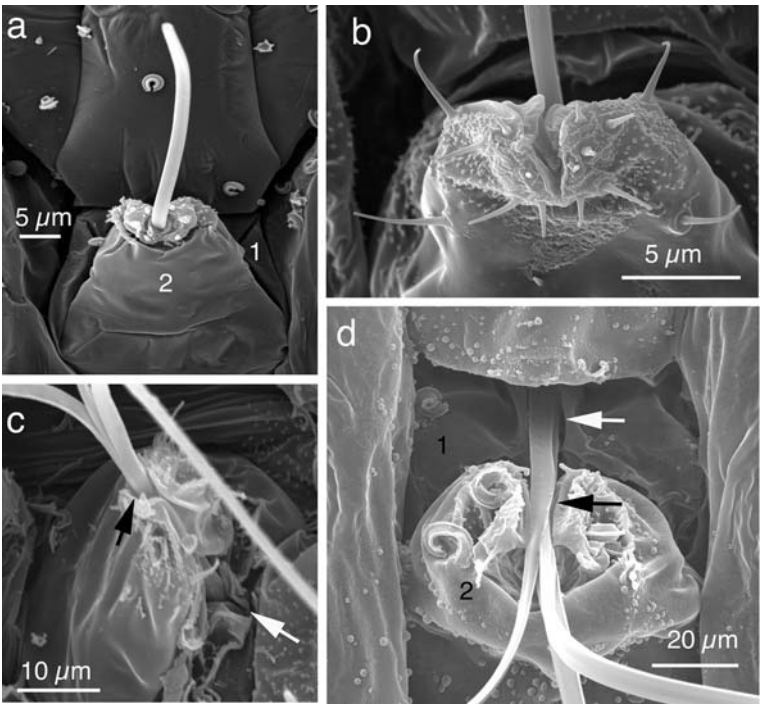



**Fig. 4.5** Nymphal rostrum (SEM). (**a**) Rostrum with stylets protruding; large sclerite behind the stylets is the clypeus (**b**) close up of sensory structures on rostrum (**c** and **d**) views of the rostrum showing where the stylet bundle can be gripped at the apex of the rostrum (*black arrow*) and at the base of the rostrum (*white arrow*). Numbers 1 and 2 indicate the first and second labial segments

(Gelman et al. 2002). At 26°C, in little more than 24 h after the eye spots start to enlarge, the simple nymphal eye transforms to a complex bipartite adult compound eye. At the same time, wings develop from simple bilayers of tissue to highly folded and invaginated wing buds that, if unfolded, would be approximately the size of the adult wings (Gelman et al. 2002). Some time during the red-eyed nymph stage, the fourth instar stops feeding and withdraws its stylets from the plant (Lei et al. 1996a, b; Costa et al. 1999). Close to the time of adult eclosion, the fourth instar cuticle is mostly transparent, and the pharate adult becomes visible underneath.

## *Adult*

In contrast to the highly reduced anatomy of the nymphal stage, adult whiteflies have all the typical anatomical features of adult insects in the Sternorrhyncha.

### Head

The compound eyes have a relatively small number of ommatidia which means that visual acuity is rather poor. Each compound eye is divided into a distinct upper eye

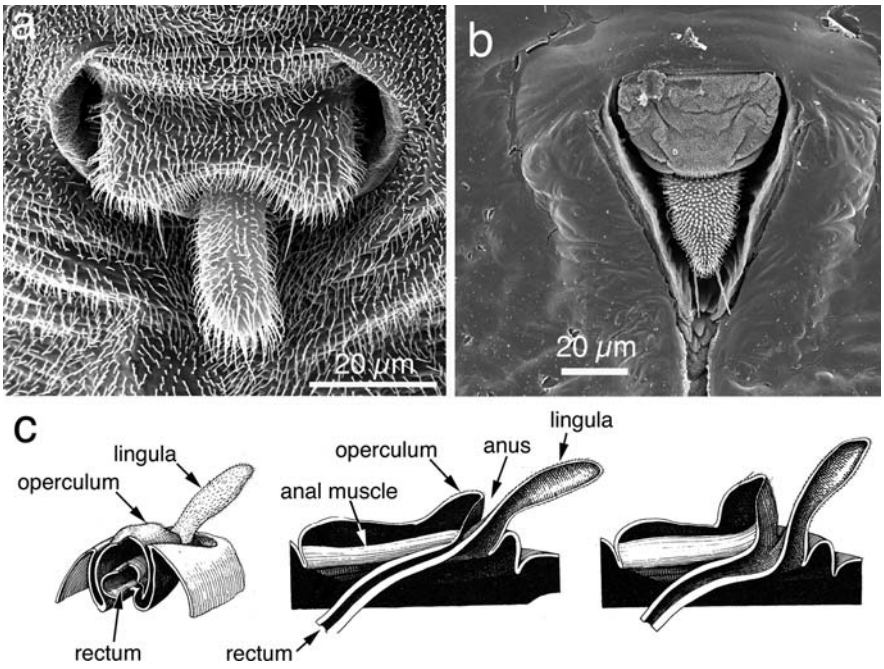



**Fig. 4.6** Vasiform orifice. (**a**) Vasiform orifice of adult (SEM) (**b**) vasiform orifice of nymph (SEM) (**c**) line drawing of adult vasiform orifice of greenhouse whitefly (redrawn from Weber 1935; printed with permission from E. Schweizerbart'sche Verlagsbuchhandlung (http://www.schweizerbart.de))

and lower eye, which in *Bemisia* are connected by a single ommatidium (Fig. 4.7a). When whitefly adults are in a dispersal behavioral mode, they are attracted to UV wavelengths of light, which causes them to fly upward toward the open sky (Mound 1962; Coombe 1982; Blackmer and Byrne 1993a, b). In contrast, when they are in a host-finding behavioral mode, they are attracted to yellow-green wavelengths of light that stimulates them to alight on vegetation (Mound 1962; Moericke et al. 1966; MacDowall 1972; Vaishampayan et al. 1975; Coombe 1982; Blackmer and Byrne 1993a, b). The division of the compound eyes into upper and lower halves may have a functional significance by enabling a differential response to UV and yellow-green wavelengths of light. Indeed, electroretinograms of greenhouse whitefly (*Trialeurodes vaporariorum* [Westwood]) have demonstrated that the relative efficiency of detecting UV versus yellow-green wavelengths is greater for the upper than for the lower compound eye (Mellor et al. 1997). In addition to compound eyes, there is a pair of ocelli just above them (Fig. 4.7).

As in most whiteflies, the antennae of adult *Bemisia* are 7-segmented and possess several different kinds of sensillae (studied in detail by Mellor and Anderson 1995a, b) (Fig. 4.8). The basal antennal segments, scape and pedicel, each bear a single campaniform sensillum that probably serves as a proprioceptor to detect

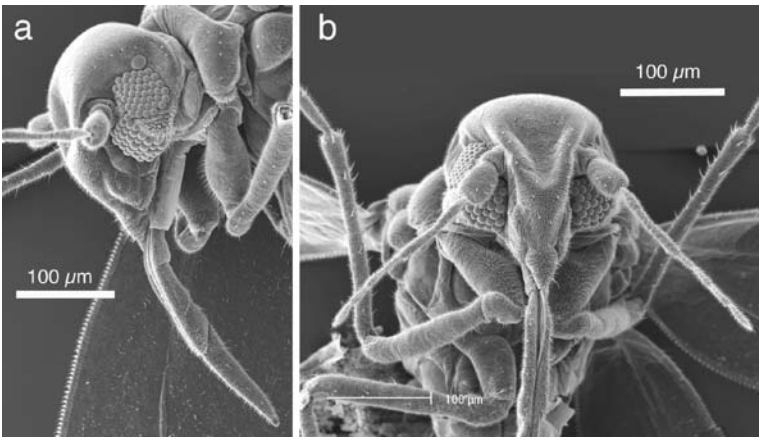



**Fig. 4.7** Adult head (SEM). (**a**) Lateral view (**b**) anterior view

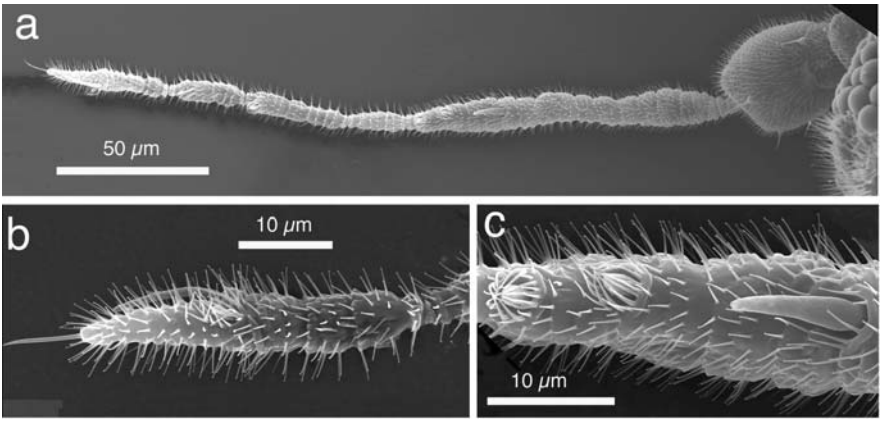



**Fig. 4.8** Adult antennae (SEM). (**a**) Overview. (**b**) Close up of sensillae on apical segment; note apical tactile chaetae sensillum, a coeloconic sensillum and a basiconic sensillum. (**c**) Close-up of sensillae on segment 3; note two a coeloconic sensillae and a basiconic sensillum

antennal movements. The apical 5 segments, the flagellum, lack campaniform sensillae. Internally, scolopidial sensillae, which also function as proprioceptors, occur in the flagellum as well as in the scape and pedicel. Tactile chaetae sensillae (a.k.a. trichoid sensillae) occur on the pedicel and are concentrated on its ventral side. The 7th segment also bears a large tactile chaetae sensillum at the apex (Fig. 4.8b). There are no additional external mechanoreceptors on the flagellum.

In addition to the mechanosensory sensillae, antennae of *Bemisia* bear a single basiconic sensillum and two apical coeloconic sensillae on the third antennal segment, a single coeloconic sensillum at the apex of the fifth antennal segment, a single basiconic sensillum and a peg with digitate tips near the apex of the sixth antennal

segment, and a basiconic and coeloconic sensillum near the apex of the seventh antennal segment (Fig. 4.8). Basiconic sensillae, also referred to as sensory cones in the literature, are relatively large, elongate pegs that lay more-or-less parallel to the longitudinal axis of the antennae and are olfactory in function. Coeloconic sensillae, also referred to as lachneat, rhinaria, and primary sensillae in the literature, consist of a short, longitudinally grooved peg set centrally in the floor of a circular depression and also appear to be olfactory in function. Inwardly oriented non-sensory hairs extend from the rim of the depression and surround the peg. There is a single small peg with digitate tips on the antenna that is on the sixth antennal segment close to the base of the basiconic sensillum. Its function is uncertain, but comparison to sensillae of other insects suggests that it is a thermo-hygroreceptor.

Mouthparts will be discussed below.

## Thorax

Adult whitefly legs are well developed and typical of most insects (Fig. 4.9): coxae and trochanters short, femora and tibiae long, tarsi short and segmented (2-segments in all whiteflies), and pretarsi short. The trochanter and base of the femur each have a field of campaniform sensillae that probably are involved in proprioception and/or detection of stress on the legs (Domenichini 1981) (Fig. 4.9a).

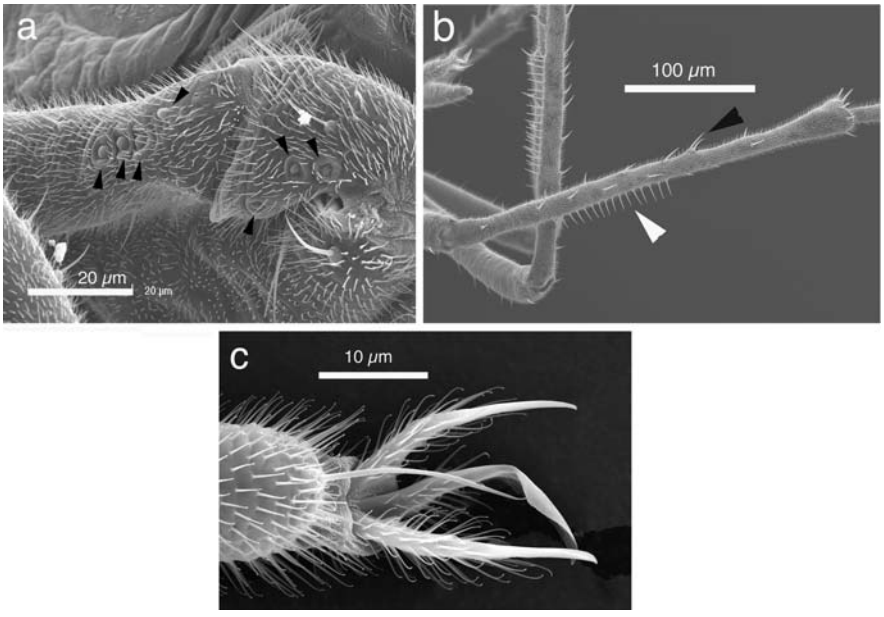



**Fig. 4.9** Adult legs (SEM). (**a**) Campaniform sensillae (*black arrowheads*) on the trochanter (*right*) and base of the femur (*left*) (**b**) metatibial brush (*black arrowhead*) and comb (*white arrowhead*) (**c**) pretarsus and apex of second tarsal segment

Whiteflies contact the substrate only with their pretarsi; the tarsal segments are elevated above the substrate. The pretarsus bears a pair of claws used for gripping the substrate, which is typical of most insects (Fig. 4.9c). The pretarsus also has an arolium, referred to as the paronychium in some literature, which is not as sclerotized as the claws and has microsetae on its ventral surface. It is believed to be an adhesive structure for gripping smooth substrates that the claws cannot penetrate. The apex of the last tarsal segment has a long medial seta that is about the same length as the pretarsus and extends between the two pretarsal claws (Fig. 4.9c). This is probably a mechanosensory sensillum that provides feedback on contact with the substrate.

The front wings are larger than the hind wings and venation is highly reduced. Forewing venation consists of a fused costa-subcosta on the anterior margin of the wing and a radial vein. The hind wing has only a short radial vein.

### Abdomen

The abdomen consists of nine conspicuous segments, and a highly reduced tenth segment. The ninth segment is the posterior-most, and the reduced tenth segment lies dorsally on it (Weber 1935). The tenth segment surrounds the vasiform orifice which is similar in structure and function to the nymphal vasiform orifice described previously (Fig. 4.6).

The female ovipositor consists of three sclerotized appendages (gonapophyses) that fit tightly together and taper to a sharp point at their apex (Fig. 4.10). The three gonapophyses consist of a pair from the eighth abdominal segment and a single gonapophysis, a fused pair, from the ninth segment (Weber 1935). The three gonapophyses are joined together by longitudinal tongue-and-groove articulations and enclose a channel through which the egg travels after leaving the genital opening (Fig. 4.10c, d). The single gonapophysis of the ninth segment encloses a duct from the colleterial gland that produces adhesive that anchors the egg pedicel in the leaf (Fig. 4.10c, d) (Weber 1935).

The ovipositor is folded upward at rest (Fig. 4.10a, d). Egg laying is initiated by extending the ovipositor posteriorly, bending the abdomen ventrally, and forcing the ovipositor into the plant tissue (Fig. 4.10d). The right and left paired gonapophyses then alternately protract and retract, sawing a hole in the leaf epidermis with their distal serrations. The egg then travels, pedicel end first, down the oviduct, guided between the gonapophyses which presumably separate to allow its passage. The pedicel is inserted in the hole and anchored in place by the adhesive that is secreted through the duct in the unpaired gonapophysis. The entire process takes place in less than a minute (Walker and Perring 1994).

The male genitalia extend posteriorly from the ninth abdominal segment and consist of a pair of tong-like parameres, or "claspers," and a single penis (Fig. 4.11). The claspers are used to grasp the female ovipositor during copulation (Weber 1931, 1935) (Fig. 4.11c). The distal ends of their medial surfaces bear "teeth" that grip the apex of the ovipositor.

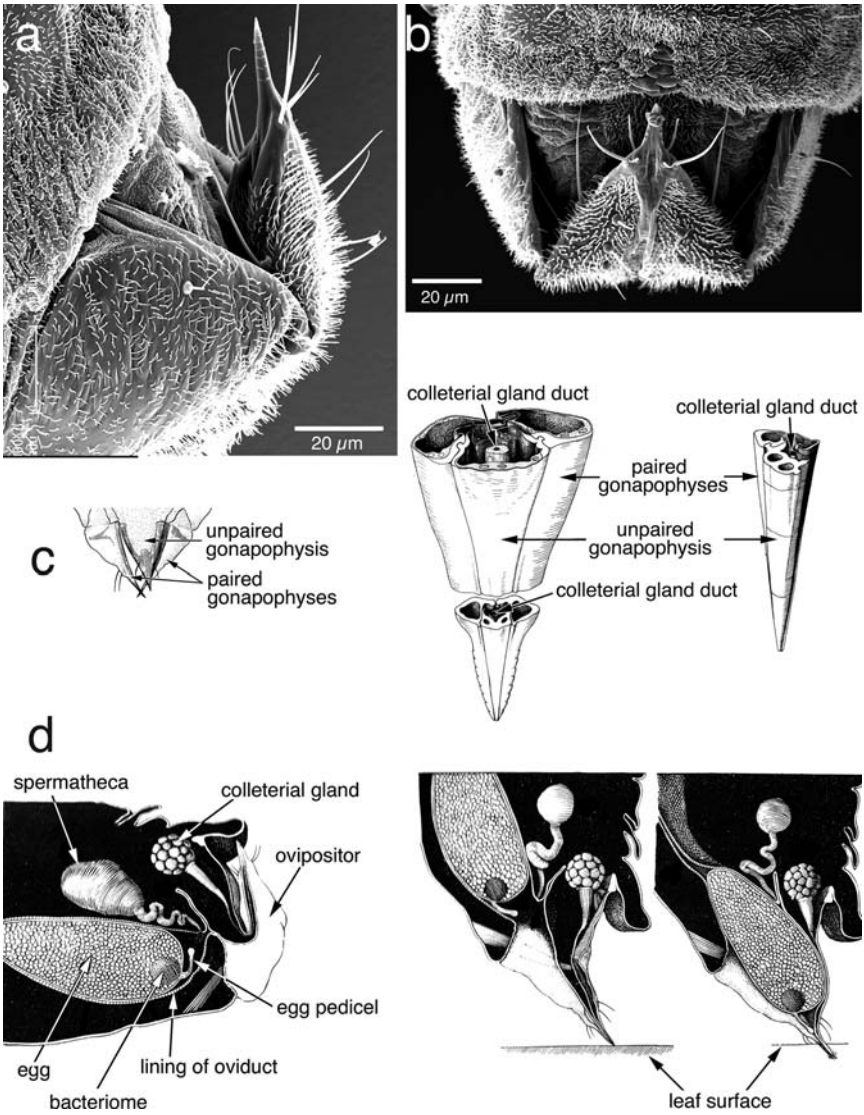



**Fig. 4.10** Oviposition and oviposition (**a**) ovipositor, lateral, almost completely retracted (SEM) (**b**) ovipositor, dorsal, retracted position (SEM) (**c**) line drawings of greenhouse whitefly ovipositor (**d**) internal genital structures and oviposition; *left*, ovipositor retracted; *center* and *right*, ovipositor extended; (**c**) and (**d**) redrawn from Weber (1935); printed with permission from E. Schweizerbart'sche Verlagsbuchhandlung (http://www.schweizerbart.de)

Whiteflies cover their body with tiny wax particles produced by large wax plates located on the ventral side of the abdomen; *Bemisia* and all other members of their subfamily (Aleyrodinae) have two pair of wax plates on females and four pair on males (Fig. 4.12). Hundreds of sclerotized pores in each plate connect to wax glands

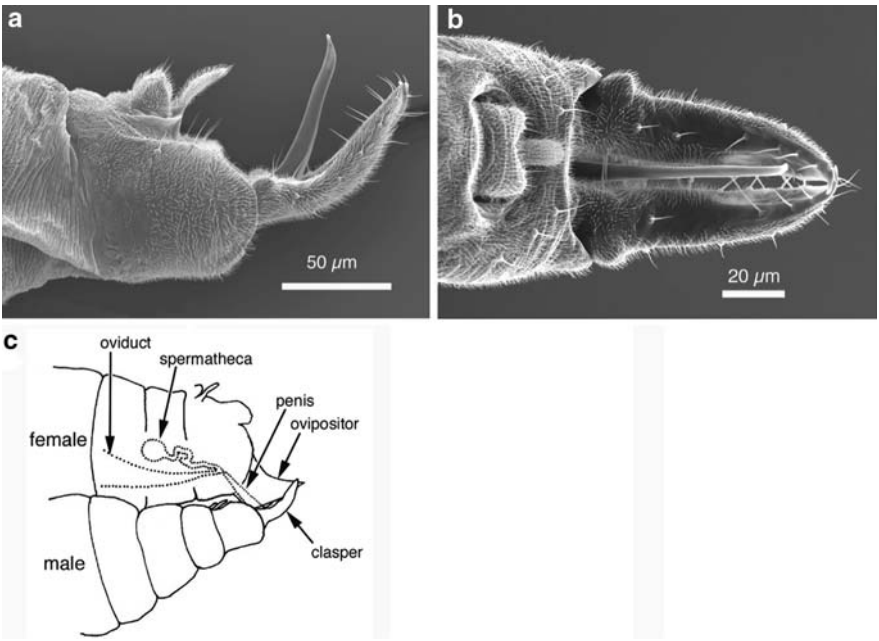



**Fig. 4.11** Male genitalia, (**a**) lateral (SEM) (**b**) dorsal (SEM) (**c**) copulation in greenhouse whitefly; redrawn from Weber (1931)

underneath. Wax extrudes as filaments with a characteristic curled shape determined by the form of the pore (Fig. 4.12c). The hind tibiae rub over the wax plates, breaking off pieces of filament, and then apply the broken particles to the abdomen and wings. Tibial combs on the hind legs (Fig. 4.9b) facilitate this process. The front and middle legs are then used to distribute wax particles from the abdomen and wings to other parts of the body. Most of the body of adult whiteflies is densely covered by very short microtrichia that are slightly bulbous or bent at their apices. The shape and density of the microtrichia make them effective at holding the curled wax particles in place (Fig. 4.12d). Waxing behavior and chemical composition of the wax is described in detail by Byrne and Hadley (1988). The white wax gives whiteflies their common name although the body itself is actually yellow. The wax particles have been hypothesized to slow down water loss and desiccation, repel sticky honeydew, reflect away harmful UV radiation, deter fungal pathogen attack as well as perform other hypothetical functions, none of which have been tested experimentally (Pope 1983; Byrne and Hadley 1988).

## Molting

Molting is triggered by 20-hydroxyecdysone, same as in other insects (Gelman et al. 2005). Prior to molting, the nymph withdraws its stylets from the plant (Lei et al. 1996a, b; Freeman et al. 2001). For molts from one nymphal stadium to the next,

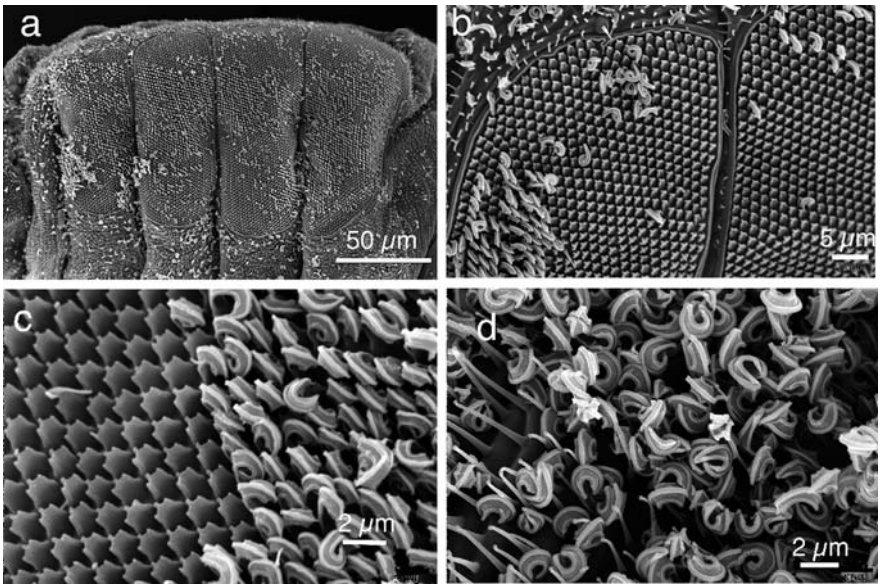



**Fig. 4.12** Wax plates and wax particles (SEM). (**a**) Ventral surface of male abdomen showing four wax plates (**b**) close-up of wax plate (**c**) close-up of wax plate showing a portion where wax particles have been removed and a portion where they are still present (**d**) portion of body covered with dense microtrichia which have knobbed or slightly bent apices and serve to hold the wax particles on the body

the ecdysial suture, which splits open to allow the newly molted insect to escape its old exuvium, is a transverse seam at the anterior end of the body where the dorsum meets the venter (Fig. 4.13a, b). Contractions of the body push the exuvium posteriorly where it eventually falls off. In the fourth instar, the ecdysial suture is dorsal and T-shaped (Fig. 4.13c, d). The transverse portion of the T coincides with the junction of the metanotum and first abdominal tergum and the longitudinal portion of the T extends anteriorly from this along the dorsal midline. During the molt, the T-shaped ecdysial suture splits open and the adult emerges head-first. The fourth instar exuvium generally remains attached to the leaf, especially in the absence of wind and rain (i.e., greenhouse conditions), another convenient feature for life table studies.

## Feeding Apparatus and Feeding

### *Mouthpart Morphology*

#### Adults

Mandibles and maxillae are elongated into stylets. Each stylet is individually muscled at its base in the head; thus the stylets can protract and retract independently of

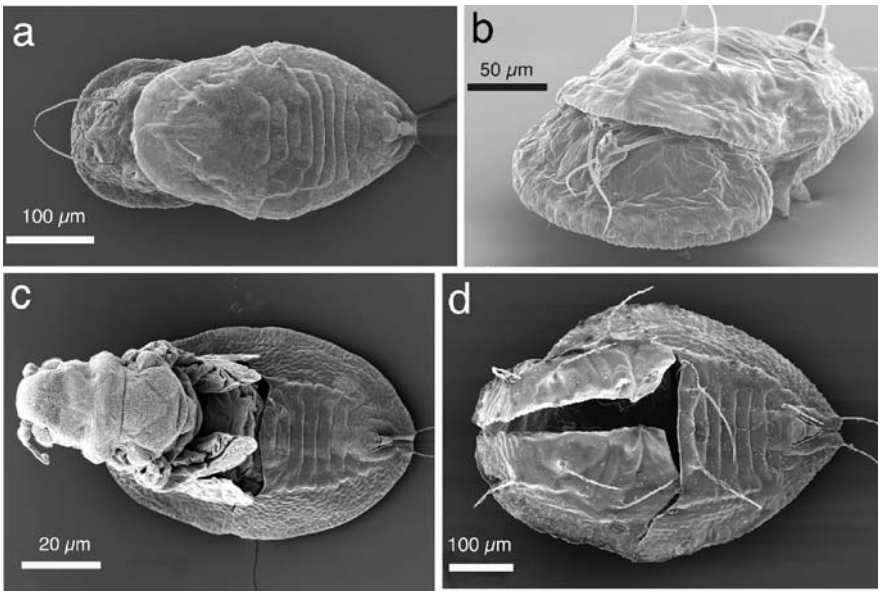



**Fig. 4.13** Molting (SEM). (**a** and **b**) Nymphs in process of a molt. (**c**) Adult emerging from pupal case. (**d**) Pupal exuvium showing T-shaped ecdysial suture

each other (Weber 1935). The four stylets extend ventrally from their bases before joining together into a stylet bundle prior to emerging from the ventral extremity of the head between the epipharynx and hypopharynx. The maxillary and mandibular stylets are approximately the same length, about 217 μm (Freeman et al. 2001). The two maxillae are interlocked by two parallel longitudinal seams that extend almost their entire length and function very much like the seams of a zip-lock plastic bag (Fig. 4.14a). These seams keep the two maxillae tightly joined together while still allowing the two maxillae to slide forward and back relative to each other during penetration of the plant tissue. The conjoined maxillae enclose the food canal and salivary canal (Fig. 4.14a). The food canal is about 3 times wider in diameter (0.657 ± 0.09 μm; mean ± SE) than the salivary canal (0.215 ± 0.03 μm) (Rosell et al. 1995), a ninefold cross sectional area difference. Both canals are separate and parallel from the base of the conjoined maxillae to about 0.5 μm from the apex where they join together into a single canal that extends to an opening at the tip (Fig. 4.14c) (Rosell et al. 1995). All food and saliva enter and leave through this apical opening.

The mandibular stylets are external to and tightly appressed against the inner maxillary stylets, which they partially enclose (Fig. 4.14a). Most of the contact between the stylet bundle and plant tissue is with the mandibular stylets; small barb-like ridges at the apex of the mandibular stylets are believed to aid the piercing process, and to anchor the stylets into the plant tissue during feeding (Fig. 4.14b) (Rosell et al. 1995; Hunter et al. 1996). The mandibular stylets contain a pair

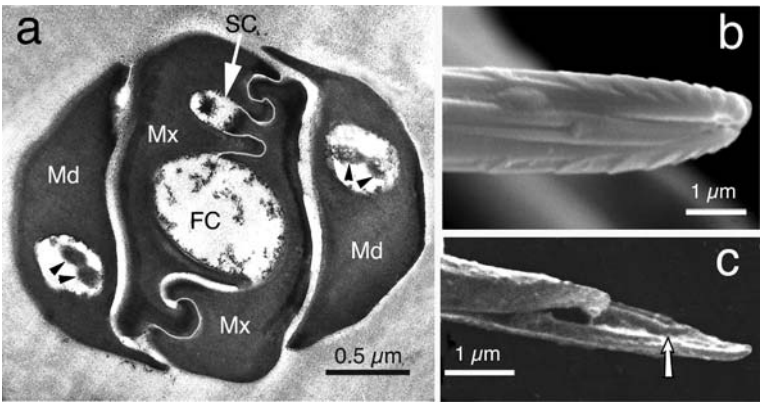



**Fig. 4.14** Stylets. (**a**) Cross section of stylet bundle; Md, mandibular stylet; Mx, maxillary stylet; FC, food canal; SC, salivary canal; *arrowheads*, mandibular dendrites (modified from Rosell et al. 1995; in the original, the mandible on the right was displaced and in this modification was put back in its correct position relative to the stylet bundle; also delineation between the two maxillary stylets is enhanced in this modification) (TEM). (**b**) Apex of stylet bundle showing serrated ridges on mandibular stylets (SEM) (**c**) apex of conjoined maxillary stylets showing where the food and salivary canals converge (*arrow*) (SEM) (from Rosell et al. 1995)

of mechanosensory dendrites (Forbes 1972; Rosell et al. 1995) (Fig. 4.14a) that probably provide the insect with information on stylet movement (Wensler 1974; Tjallingii 1978; Backus and McLean 1982). Whitefly maxillary stylets lack these internal dendrites (Forbes 1972; Rosell et al. 1995) (Fig. 4.14a). The stylets do not have chemosensory sensilla contrary to some earlier literature (e.g., Forbes 1972).

The labium is an elongate tube comprised of four linearly arranged segments (Figs. 4.7 and 4.15). A deep longitudinal furrow called the labial groove extends on the anterior side from the base of the second segment to the apex of the fourth. The margins of the furrow press tightly against each other near the outer surface so that the labial groove appears shallow in external view. However, the groove widens deeper beneath the surface and forms a longitudinal lumen that extends the full length of the groove. At rest, the stylet bundle resides in this lumen. During feeding, the stylet bundle emerges from the opening of the labial groove at the apex of the labium (Fig. 4.15a, d). The apex of the labium bears seven pairs of sensillae, four of which are mechanosensory, and three of which are either chemosensory or combined chemo- and mechanosensory (Fig. 4.15d) (Walker and Gordh 1989).

The labrum is a small appendage at the distal end of the clypeus. The stylet bundle emerges from the head behind the base of the labrum and then enters the labial groove. The labrum appears to play a role in keeping the base of the stylet bundle in the labial groove (Fig. 4.15c) (Freeman et al. 2001).

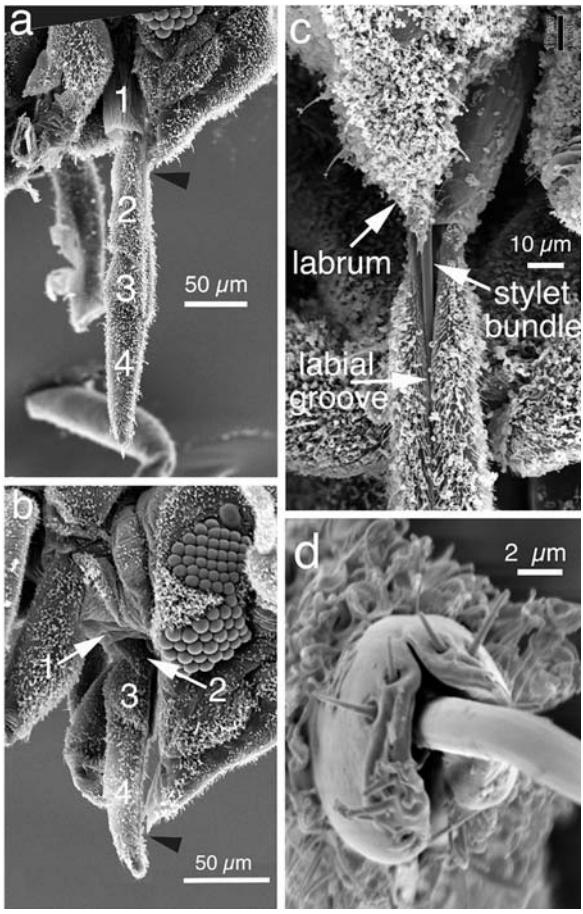



**Fig. 4.15** Adult labium (SEM). (**a**) Labium at initiation of stylet penetration; stylet bundle almost completely enclosed by extended labium. (**b**) Labium retracted into the head during deep stylet penetration (stylets broken off during specimen preparation); **a** and **b** are Figs. 4.9 and 4.10 from Freeman et al. (2001) (reprinted courtesy of the Entomological Society of America); *numbers* indicate labial segment; *black arrowheads* indicate where the stylets emerge from the head enter the labial groove. (**c**) Stylet bundle entering labial groove just below the ventral-most extremity of the labrum. (**d**) Apex of labium showing apical sensillae and the stylet bundle emerging

## Nymphs

The stylet bundle of nymphs is presumed to be essentially the same as in the adult. The major anatomical differences between adult and nymphal mouthparts occur in the labium and crumena. The labium of nymphs is two-segmented, very short, and is not nearly long enough to house the entire length of the stylet bundle (Fig. 4.5). Prior to entering the labial groove, the stylet bundle first enters the crumena, which in whitefly nymphs is a pouch-like invagination that extends internally from the base of the labium. The stylet bundle forms a loop within the crumena and exits the same opening that it entered. It then extends into the labial groove (Weber 1934; Freeman et al. 2001, Fig. 4.16). Stylet length increases with each nymphal instar (Pollard 1955) and was estimated at 114, 135, 142 and 159 μm for the four nymphal instars (Freeman et al. 2001). However, these measurements are likely underestimates as

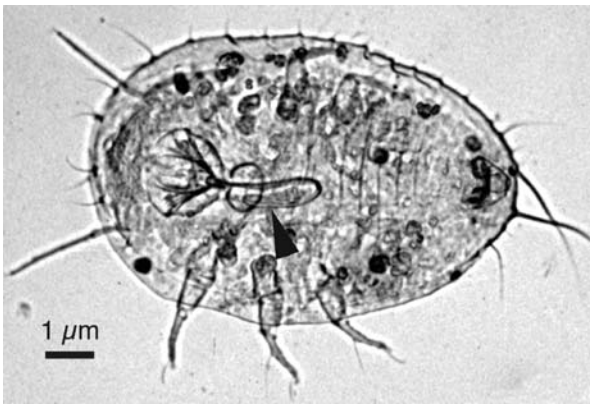



**Fig. 4.16** Cleared specimen of *Bemisia* first instar nymph showing the stylet bundle (*arrowhead*) looped into the crumena (light microscopy)

they did not account for any portion of the stylet bundle that may still have been within the crumena.

## *Mechanics of Stylet Penetration*

### Adults

Stylet penetration is initiated by extending the labium more-or-less perpendicularly to the body and pressing the apex of the labium against the plant surface. Contractions of the mandibular and maxillary protractor muscles advance the stylet bundle past the apex of the labium and into plant tissue (Fig. 4.15a, d). Contractions of these muscles advance the stylets only very short distances. Consequently, stylet penetration is an incremental process achieved by repeated cycles of contraction and relaxation of the protractor muscles (Bradley et al. 1962). To counter the mechanical resistance against penetration in the plant tissue, the head pushes downward as the protractor muscles contract. This downward push probably supplies much of the penetration force (Freeman et al. 2001). Since the stylet bases are in the head, in order for the stylets to penetrate deeper into the plant, the head pushes downward, closer to the plant surface. As it does so, the entry point of the stylets in the labial groove (at the ventral apex of the head where the labrum contacts the labial groove) slides down the labial groove toward the plant surface. Prior to plant penetration, the entry point of the stylet bundle in the labial groove is near the base of the second labial segment. At maximal length of stylet penetration, the entry point of the stylets in the labial groove moves downward almost to the apex of the fourth labial segment (Fig. 4.15b) so that virtually the entire length of the stylet bundle is inserted in the plant tissue. The length of stylet penetration in the plant at any time during stylet penetration is equal to the distance that the labrum (i.e., the entry point of

the stylet bundle in the labial groove) slides down the labial groove (Freeman et al. 2001). This can be measured by making a video recording of the labium at high magnification and measuring the distance that the labrum moves down the labium. A simple calculation is needed to compensate for the labium not being perfectly perpendicular to the optical axis of the camera (Jiang and Walker 2001).

As the head pushes the stylet bundle down the labial groove, the labium itself retracts. Labial retraction occurs primarily by the first labial segment invaginating into itself longitudinally and by the second labial segment being pulled upward into the invaginated first segment (Fig. 4.15b). The third and fourth labial segments undergo little or no change in dimensions during stylet penetration. The labium probably plays an important mechanical role by enclosing the portion of the stylet bundle between the head and the plant surface and thus preventing the stylet bundle from buckling as the protractor muscles and the downward push of the head force the stylets into the plant tissue.

The stylets can be retracted in a similar incremental process by the mandibular and maxillary retractor muscles. In addition, the stylet bundle also can be pulled out of the plant by extension of the labium. Extension of labium pushes the head away from the plant surface, pulling the stylet bundle out of the plant. At full labial extension, the stylets once again become completely enclosed in the labial groove and the entry point of the stylets in the labial groove has slid back up to the second labial segment.

Extension of the labium is caused by muscles operating on the crumena. The crumena, which in the nymphal stage is a pouch-like invagination housing the coiled stylets, transforms in the adult stage into a stiff rod-like invagination (i.e., an apodeme) that extends dorsally from the inner wall of the labium up into the prothorax (Fig. 4.17a). Retraction of the labium during stylet advancement forces the crumena upward into the prothorax, stretching the crumena protractor muscles that extend from the dorsal head of the crumena down to the base of the hypopharynx. When these muscles contract, the crumena is pulled downward causing the labium to extend (Weber 1935). Retraction of the stylets by this mechanism can be very rapid (< 1 s) and may be particularly important for quick escape when the whitefly has its stylets deep in the plant tissue.

During stylet penetration in whiteflies and aphids, the mandibular stylets lead the way ahead of the maxillary stylets; therefore, most of the penetration force is provided by the mandibular stylets (Bradley et al. 1962). Aphid maxillary stylets advance after the mandibular stylets advance; as a result, the apex of the maxillary stylets is slightly behind the apices of the mandibular stylets during stylet penetration. The same is presumably true for whiteflies (Rosell et al. 1995).

The stylet bundle of whiteflies is quite flexible and weaves its way between and around cells on its route to the phloem (Pollard 1955; Walker 1985; Walker and Perring 1994, Fig. 4.18). The steering mechanism directing stylet movement has not yet been described for whiteflies but is likely to be similar to other homopterans (Miles 1968). Mandibular stylets typically curve inward at their apex (Fig. 4.19), a feature that holds true for whiteflies (Rosell et al. 1995). If the two mandibular stylets advance simultaneously or advance alternately for very short distances, the

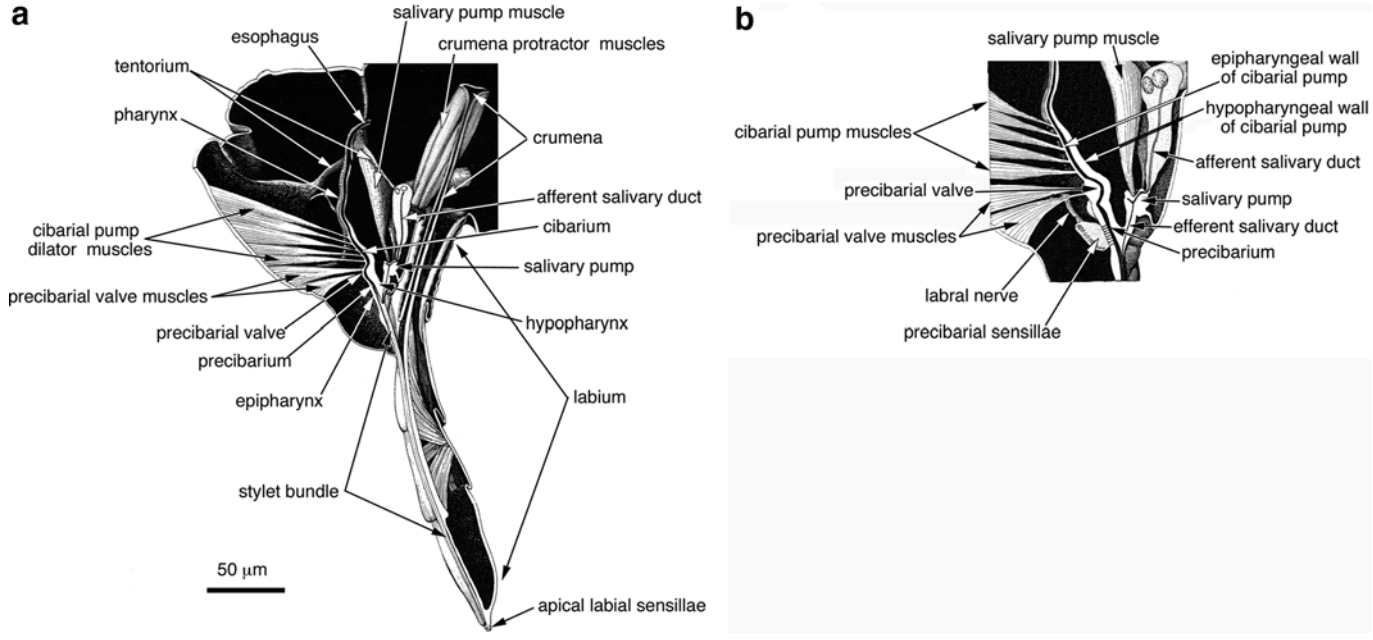



**Fig. 4.17** (**a**) Diagrammatic illustration of sagittal section through an adult whitefly head and prothorax showing the stiff, rod-like crumena, the precibarium, cibarium, precibarial valve, salivary pump, their associated musculature, and the pharynx (**b**) diagrammatic illustration of cross section through the cibarial pump (both redrawn and modified from Weber 1935; printed with permission from E. Schweizerbart'sche Verlagsbuchhandlung (http://www.schweizerbart.de))

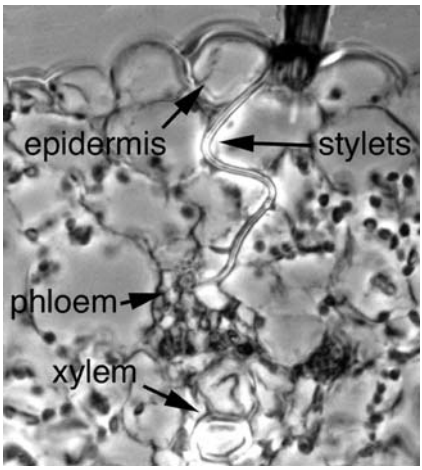



**Fig. 4.18** Light micrograph of adult *Bemisia* stylets weaving through tissue of a lima bean leaf from the leaf surface to the phloem

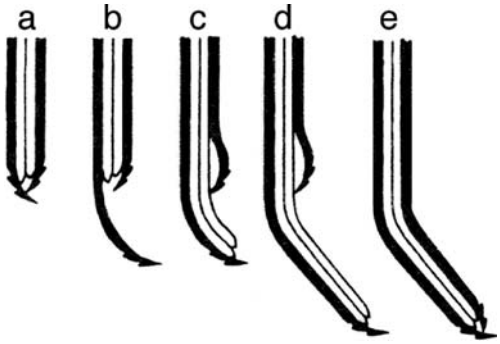



**Fig. 4.19** Diagrammatic illustration of how a piercing-sucking insect can control direction of stylet movement. (**a**) Starting position prior to turning right. (**b**) Left mandibular stylet advances and its natural curvature forces it to the right. (**c** and **d**) Maxillary stylets follow the curved path cut by the left mandibular stylet. (**e**) Right mandibular stylet advances; the tendency of the right mandibular stylet to curve left is blocked by the left mandibular stylet and maxillary stylets, so the right mandibular stylet follows the path already established by these other stylets. Modified from Miles (1968); printed with permission from Annual Reviews

tendency to curve inward by one mandible is countered by the tendency of the opposite mandible to curve inward, and the resulting movement is straight ahead. However, if one mandible advances by itself for a considerable distance, it will cut a path that curves inward, and when the opposite mandible advances, it will follow this path of least resistance (Fig. 4.19). This mechanism allows the mandibles to turn either right or left relative to their sagittal axis. By rotating the stylet bundle, thus changing the orientation of its sagittal axis, the stylets can be turned in essentially any direction. During stylet penetration, whiteflies frequently rotate their body around the penetration point on the leaf surface, which would presumably rotate the

axis of the stylets. There are also multiple protractor and retractor muscles that insert on the base of each stylet; consequently, differential contraction of these muscles on the same stylet might induce a torque that could twist the stylet's axis although this has yet to be confirmed.

### Nymphs

In nymphs, the stylet bundle forms a loop in the crumena; consequently, any force generated by contraction of the mandibular and maxillary protractor muscles would be absorbed by the loop rather than be directly translated to the apex of the stylets forcing them into the plant. Thus, nymphs most likely employ a different mechanism of stylet penetration than adults. Although we are unaware of any studies specifically on whitefly nymphs, Heriot (1934) examined stylet penetration in several scale insects that also have a very short labium and long stylets looped in a crumena. The stylet bundle of scale insects passes through two vice-like structures, one at the base and one at the apex of the short labium. The basal vise grips the stylet bundle and then protracts, forcing the stylet bundle forward. The apical vice is pressed close to the plant surface, and provides a tight fitting collar around the stylet bundle but does not fit so tight as to prevent stylet protraction. The short distance between the two vices prevents the stylet bundle from buckling when the basal vice protracts. Once the basal vice completes its protraction, it releases its grip on the stylet bundle, retracts, and once again grips the stylet bundle and protracts, repeating the process over and over. The labium of whitefly nymphs also appears to have two vice-like structures (Fig. 4.5c, d), making it likely that a similar method of stylet advancement is used.

## *Precibarium and Cibarial Pump*

The structures of the cibarial pump, precibarium, and precibarial valve have been studied primarily in adult whiteflies and this section refers to adults. These same structures in nymphs are probably more-or-less similar to adults (Weber 1934).

Proximal to their emergence from the head, the stylets diverge from their tight interlocked appression to each other and no longer enclose the food canal. At this point, the epipharynx and hypopharynx press tightly against each other, enclosing a channel whose lumen is continuous with the maxillary food canal distally, and with the pharynx basally (Fig. 4.17). The proximal part of this channel is the cibarium (referred to as the pharynx by Weber 1935) and forms the sucking pump; the distal part is the precibarium (Hunter et al. 1996), also referred to as the hypocibarium (Harris et al. 1996b), which connects the maxillary food canal to the cibarial pump.

The posterior wall of the cibarial pump is the hypopharynx and is shaped as a longitudinal trough with its concave surface facing anteriorly (Harris et al. 1996b; Hunter et al. 1996). The anterior wall of the pump is the epipharynx, which is opposite the concave surface of the hypopharyngeal wall, and abuts against it when at rest. The cibarial dilator muscles originate on the inner surface of the postclypeus,

and insert in a longitudinal apodeme on the anterior side of the epipharyngeal wall (Harris et al. 1996b; Hunter et al. 1996) (Fig. 4.17). Contraction of the dilator muscles pulls the epipharyngeal wall away from the hypopharyngeal wall, creating suction to draw food up through the maxillary food canal and precibarium. When the cibarial dilator muscles relax, the epipharyngeal wall snaps back into the hypopharyngeal trough, forcing fluid out of the pump.

A valve (termed precibarial by Hunter et al. 1996, and cibarial by Harris et al. 1996b) at the proximal end of the precibarium regulates the direction of fluid flow as the cibarial dilator muscles cycle between contraction and relaxation. The valve consists of a thickening of the epipharyngeal wall that is opposite a shallow depression in the hypopharyngeal wall (Fig. 4.17). The precibarial valve muscle originates on the inner surface of the postclypeus and inserts in the epipharyngeal wall of the valve. Similar to the cibarial dilator muscles, contraction of the precibarial valve muscle pulls the epipharyngeal wall of the precibarial valve away from the depression in the hypopharyngeal wall, opening the valve. When the muscle relaxes, the epipharyngeal wall snaps back into the depression on the hypopharyngeal wall, closing the valve. The precibarial valve muscles and cibarial dilator muscles cycle between contraction and relaxation in synchrony, so when the cibarial dilator muscles contract, creating suction, the precibarial valve muscles also contract, opening the valve and allowing food to be sucked up from the food canal into the cibarium. When the cibarial dilator muscles relax, forcing food out of the cibarium, the precibarial valve muscle also relaxes, closing the valve, so that the food cannot escape back into the food canal but instead is forced up into the pharynx.

The precibarium has 10 chemosensory sensilla in contact with its lumen, and the cibarium has eight (Hunter et al. 1996). They serve a gustatory function and are the primary taste organs of the whitefly. Because the stylets have no chemosensory sensillae, plant sap has to be imbibed at least as far up as the precibarial sensillae before any assessment of its chemistry can be made.

## *Saliva*

Salivation plays a major role in the feeding behavior of whiteflies. The salivary glands of whiteflies as well as all other Sternorrhyncha, Auchenorrhyncha, and many Heteroptera, are highly specialized and produce two distinct kinds of saliva: sheath saliva and watery saliva.

### Salivary Glands

The salivary glands of whiteflies have been examined in detail only for the adult stage (Weber 1935; Harris et al. 1996b; Ghanim et al. 2001). *Bemisia* adults have two pairs of salivary glands; the principle and accessory salivary glands. The principle glands are larger (Harris et al. 1996b) and are comprised of at least 13 cells which vary in their ultrastructure and staining, suggesting cellular specialization for production of different salivary components (Ghanim et al. 2001). The accessory glands are simpler in structure and consist of 4 cells, all similar in appearance. In

aphids, the principle glands appear to be largely responsible for producing sheath saliva, while the accessory glands appear to be largely responsible for producing watery saliva (Tjallingii 2006). The division of labor between the principle glands and accessory gland has not been examined in whiteflies. Cicero and Brown (2008) found a *Bemisia*-transmitted *Begomovirus*, *Squash leaf curl virus*, in the primary salivary gland of *Bemisia* but not in the accessory salivary gland, thus implicating the primary salivary gland in inoculation of this virus. Medina et al. (2006) found another *Bemisia*-transmitted *Begomovirus*, *Tomato yellow leaf curl Sardinia virus*, in the principle salivary glands, but they did not mention anything about the accessory glands. In contrast, aphid-transmitted luteoviruses are inoculated via the accessory salivary glands (Gildow and Rochow 1980; Shukle and Quiroz 1994; Gray and Gildow 2003).

The ducts from the principle and accessory gland on the same side fuse to form a lateral salivary duct. Further downstream, the two lateral salivary ducts fuse to form a single afferent salivary duct. This duct empties into the salivary pump, which is located posterior to the cibarial pump, and pumps saliva into the salivary canal of the maxillae (Fig. 4.17) (Harris et al. 1996b).

### Sheath Saliva

As the stylet bundle advances through the plant tissue, sheath saliva is secreted from its apex. Secretion is incremental and in synchrony with advancement of the stylet bundle. A small volume of sheath saliva is secreted and quickly gels, enclosing the apex of the stylet bundle. The stylets then advance a few microns, pushing their way through the gelled sheath saliva, and the process is repeated as the stylets advance. Each increment forms a bead of sheath saliva that adheres to the previously produced bead so that the entire length of the stylet bundle, from the leaf surface to its terminal end, is enclosed in a continuous sheath of gelled saliva with a beaded appearance (Fig. 4.20a, b). The portion of the salivary sheath on the plant surface, at the point of initial penetration, is referred to as a salivary flange (Fig. 4.20a, c). Salivary sheaths remain in the plant after the stylets are withdrawn and are easily stained, providing a convenient record of everywhere that the whitefly probed with its stylets (McLean and Kinsey 1967; Walker 1985; Backus et al. 1988; Cohen et al. 1996b, c).

Production of a salivary sheath by hemipterans has been known for a very long time (Büsgen 1891), but its function(s) remain speculative. Hypothesized functions include: (1) keeping the two mandibular stylets and conjoined maxillary stylets in a cohesive stylet bundle; (2) providing a relatively frictionless sheath to facilitate advancement and retraction of the stylet bundle through the plant tissue; (3) sealing the puncture where the tips of the stylets penetrate a phloem sieve element to prevent loss of turgor pressure in the sieve element (see “Feeding Behavior” below for importance of preventing loss of turgor pressure in sieve elements); (4) providing a relatively inert barrier between the moving stylets and the plant tissue to minimize plant wounding responses.

The chemistry of the salivary sheath of several other hemipterans has been examined including aphids which are likely to be similar to whiteflies in this regard (Miles

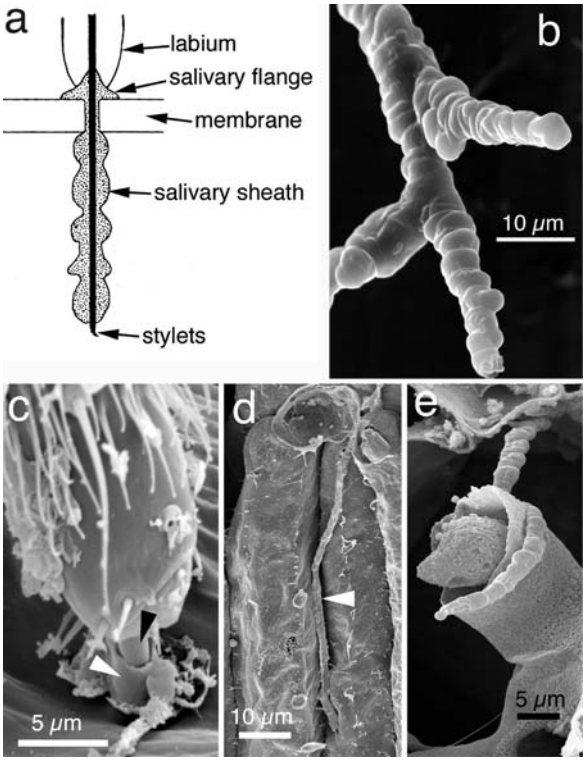



**Fig. 4.20** (**a**) Diagrammatic illustration of stylet penetration and sheath saliva into an artificial diet covered by a Parafilm® membrane (from Miles 1968; reprinted with permission from Annual Reviews) (**b**) branching salivary sheath clearly showing the beaded nature of the salivary sheath that reflects its incremental secretion (SEM) (**c**) apex of *Bemisia* labium slightly pulled back from leaf surface where the stylets are penetrating; *black arrowhead*: stylet bundle; *white arrowhead*: salivary flange (SEM) (Fig. 4.2 from Freeman et al. 2001; reprinted courtesy of the Entomological Society of America) (**d**) *Bemisia* salivary sheaths in passing between tightly packed palisade mesophyll (SEM) (**e**) *Bemisia* salivary sheaths passing through spongy mesophyll with its abundant air spaces (SEM) (**d** and **e** from Cohen et al. 1998)

1972, 1999). Sheath saliva is mainly protein and contains about 10% phospholipid and probably some conjugated carbohydrate. In addition to the physical structure formed by sheath saliva, enzymatic activity, specifically phenoloxidase and peroxidase, has also been reported in the salivary sheath of some aphid species (Miles and Peng 1989; Urbanska et al. 1998; Cherqui and Tjallingii 2000).

### Watery Saliva

Watery saliva contains salivary enzymes and metabolites and does not gel upon secretion into the plant. In aphids, watery saliva appears to be secreted intermittently during stylet penetration through intercellular space. It has been hypothesized

that watery saliva injected during stylet penetration is quickly sucked back up into the food canal, carrying soluble components from the plant up to the precibarial taste organs (see above), presumably providing the insect with chemosensory information regarding host plant suitability as well as possible cues to help locate the phloem sieve elements (Miles 1999). *Bemisia* appears to acquire chemosensory cues from intercellular space during stylet penetration (Isaacs et al. 1999), but whether they acquire this information in the same manner as hypothesized for aphids is unknown. Alternatively, it is possible that the apical labial chemoreceptors (present in whiteflies, absent in aphids) that are pressed into the salivary flange during feeding may detect internal plant chemicals that diffuse through the salivary sheath up to the salivary flange.

Both aphids and adult whiteflies inject large amounts of watery saliva into phloem sieve elements, which is how circulative plant viruses are inoculated (Prado and Tjallingii 1994; Nault 1997; Jiang et al. 2000). Ingestion of phloem sap by aphids and adult whiteflies is always preceded by injection of watery saliva into the sieve element (Tjallingii 1994, 1995, 2006; Wilkinson and Douglas 1998; Jiang et al. 1999), and once ingestion is initiated, it can be periodically interrupted by bouts of salivation into the sieve element (van Helden and Tjallingii 1993; Tjallingii 1994, 2006; Wilkinson and Douglas 1998; Sauge et al. 1998). In addition, secretion of watery saliva occurs simultaneously with ingestion in aphids (Tjallingii 2006) and probably also in whiteflies. However, saliva secreted during ingestion is believed to be caught up in the stream of sap flowing up the common canal where the food and salivary canals join near the apex of the maxillae and consequently the saliva gets ingested without having entered the sieve element (Tjallingii 2006). Potential roles for watery saliva are discussed below under "Feeding Behavior."

## Salivary Components

In the earliest study on whitefly saliva, Butler (1938) found evidence of amylase and invertase in the saliva of the cabbage whitefly, *Aleurodes brassicae* using oxidation-reduction reactions; however, such assays may produce false positives due to the general oxidative properties of hemipteran saliva (Miles 1999). More recently, Cohen and Hendrix (1994) detected α-amylase in *B. tabaci* in their assay of whole body homogenates, but provided no evidence for a salivary origin. Nonetheless, they argued that amylase is a salivary component injected into the plant while feeding. They also used the presence of amylase to hypothesize that whiteflies were facultative mesophyll feeders (a view they have since refuted – Cohen et al. 1996b, c) under the false assumption that phloem sieve elements lack starch. However, phloem sieve elements do contain starch plastids (Evert 1990; Eleftheriou 1990). Therefore, even if amylase does occur in saliva, it could still interact with sieve elements.

Cohen and Hendrix (1993) cite unpublished data indicating the presence of pectinase in whole-body homogenates of *Bemisia*. While again presence of an enzyme in whole body homogenates does not necessarily imply its presence in saliva, pectin is an insoluble polysaccharide found primarily in plants, not animals; therefore, it seems likely that if whiteflies produce a pectinase, it would have to be injected into

the plant via the saliva in order to have an effect. Furthermore, pectinases have been detected in the saliva of many, although not all, aphid species that have been examined (Adams and McAllan 1958; McAllan and Adams 1961; Campbell and Dreyer 1985; Miles 1999; Cherqui and Tjallingii 2000). Pectinases have been hypothesized to aid stylet penetration through the pectin-rich middle lamella that serves as the intercellular cement between adjacent cell walls in plant tissue (McAllan and Adams 1961; Campbell and Dreyer 1985), although this hypothesis has been challenged (Tjallingii and Hogen Esch 1993). To date, the only enzyme definitively proven to be a component of whitefly saliva is alkaline phosphatase that was detected in adult *Bemisia* principal and accessory salivary glands (especially the former) as well as in saliva secreted into an artificial medium (Funk 2001). However, its function is unknown.

Many of the additional components mentioned in a thorough review of aphid saliva (Miles 1999) may also occur in whitefly saliva, although confirmation is still lacking. Salivary interactions with the host plant play a critical role in the specialized feeding mechanism of hemipterans, and in many cases, probably determine host plant susceptibility/resistance (Miles 1999). The paucity of information on whitefly salivary components demonstrates the great need for future research in this area.

## *Feeding Behavior*

All whiteflies studied are obligate phloem-feeders (Byrne and Bellows 1991; Cohen et al. 1996b, c). *Bemisia* feeds primarily on phloem in minor veins, which they usually access from the abaxial leaf surface (Cohen et al. 1996b, c; Jiang and Walker 2007). Phloem is the plant's primary transport system for photosynthates, and phloem sap generally has high concentrations of sugars (Raven 1983; Girousse et al. 1991; van Helden and Tjallingii 1994), usually sucrose or raffinose, or sugar alcohols (Ziegler 1975). Amino acids and other organic molecules are also transported primarily in the phloem, usually in much lower concentrations than sugars (Ziegler 1975; Girousse et al. 1991; van Helden and Tjallingii 1994; Calatayud et al. 1996).

Phloem is a tissue comprised of several cell types and phloem sieve elements are the highly specialized cells through which phloem sap is transported. Whiteflies ingest sap specifically from sieve elements. Sieve elements are elongate and anastomose end-to-end with cytoplasmic continuity that allows sap to flow from one sieve element to the next. Collectively, the interconnected sieve elements comprise the sieve tubes, which are the conduits for long-distance transport of nutrients from one part of the plant to another. Consequently, whiteflies tap into a pipeline with a continuous flow of nutrients, and as the whitefly extracts the sap from a sieve element, the sap is continually replaced. As a result, whitefly larvae can complete an entire nymphal instar feeding from a single sieve element (Lei et al. 1996b).

Sieve elements have a very high turgor pressure (0.2–1 MPa – Kingsolver and Daniel 1995) due to their high sugar concentration. When the stylets pierce a sieve

element, the turgor pressure forces the sap up into the food canal, even without sucking. The precibarial valve, described previously, may play an important role in regulating the flow of sap from the sieve element to the insect. Too much flow could cause the sieve element to lose turgor pressure, triggering a sealing mechanism that would isolate the affected sieve elements and cut off its connection to the transport pipeline (Knoblauch and van Bel 1998).

Adult whiteflies also ingest sap from the plant's other transport system: xylem (Janssen et al. 1989; Lei et al. 1997, 2001; Jiang et al. 1999). Xylem sap is very dilute and usually low in organic nutrients (Raven 1983). Nonetheless, many insects that feed on phloem sap also occasionally ingest xylem sap. In aphids, this behavior is believed to occur primarily when the insects are dehydrated and has been referred to as "drinking" (Spiller et al. 1990). Adult whiteflies are not known to engage in significant ingestion of any plant cells other than phloem sieve elements and xylem tracheary elements. In the few studies on whitefly nymphs, only ingestion from phloem sap has been reported and, in contrast to adults, no ingestion of xylem sap has been detected (Lei et al. 1996a, b; Jiang and Walker 2003, 2007).

To initiate feeding, an adult whitefly first lowers its labium and usually rubs the apex of the labium over the leaf surface. It then presses the apex against the leaf surface, secretes a small amount of sheath saliva (the salivary flange), and begins to insert its stylets as described previously. Stylet penetration usually is initiated in the anticlinal grooves between adjacent epidermal cells (Freeman et al. 2001). Mechanosensory sensillae at the apex of the labium are probably involved in locating these grooves (Nault and Gyrisco 1966). The apex of the labium also bears chemosensory sensillae (Walker and Gordh 1989); however, whether or not they play a role in providing gustatory information from the leaf surface is unknown.

The stylet pathway from the leaf surface to the phloem and xylem is primarily intercellular (Capoor 1949; Pollard 1955; Walker 1985; Janssen et al. 1989; Walker and Perring 1994; Lei et al. 1996a, b; Jiang et al. 1999; Jiang and Walker 2003). The stylets are remarkably flexible and weave their way around and between cells from the plant surface to their target: a phloem sieve element (Fig. 4.18), or a xylem tracheary element, if the insect is dehydrated. Intercellular space is occupied by cell walls, by a pectin-rich middle lamella between adjacent cell walls, and by air spaces; the latter especially common in the spongy mesophyll. In aphids, there are three variations of intercellular penetration: through the middle lamella between adjacent cell walls, between adjacent parallel cellulose lamellae within cell walls, and between the cell wall and the plasmalemma (cell membrane). The latter is referred to as *intramural* penetration because it occurs on the inner side of the cell wall but external to the cell (Spiller et al. 1985; Kimmins 1986; Tjallingii and Hogen Esch 1993). Scanning electron microscopy (SEM) of fractured leaf sections indicates that in mesophyll, whitefly stylet penetration between adjacent cell walls and through intercellular air spaces is very common (Fig. 4.20d, e, Cohen et al. 1998). In tightly appressed tissues, transmission electron microscopy (TEM) is required to distinguish among the three variations of intercellular penetration. The only available TEM image of *Bemisia* stylets through tightly appressed tissue shows a stylet path apparently through the middle lamella in a cotton leaf (Fig. 4.21), but how frequent

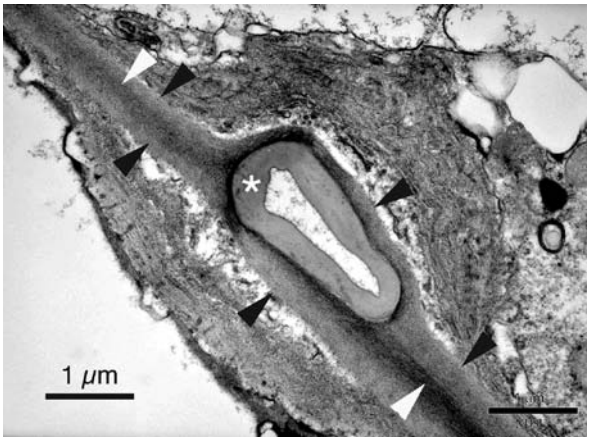



**Fig. 4.21** Transmission electron micrograph showing an adult *Bemisia* salivary sheath apparently in the middle lamella of phloem tissue in a cotton leaf, pushing the two adjacent cell walls apart; *black arrowheads*: cell walls; *white arrowheads*: middle lamella; *asterisk*: salivary sheath which is hollow because stylet bundle was pulled out prior to sectioning (TPF unpublished)

this mode of penetration is compared to other modes of intercellular penetration in tightly packed tissue remains unknown.

Although the stylet pathway to the phloem is primarily intercellular, plant cells are periodically punctured along the way. This phenomenon has been best studied in aphids, which like whiteflies, also penetrate the plant in mostly an intercellular route on their way to the phloem (Tjallingii 1985, 2006; Tjallingii and Hogen Esch 1993). Aphid stylets frequently make deviations from their intercellular route to make brief (usually <10 s) intracellular punctures with their maxillary stylet tips (Tjallingii 1985, 1995; Kimmins and Tjallingii 1985; Tjallingii and Gabrys 1999). Upon withdrawal of the stylet tips, the cell plasmalemma spontaneously seals over the puncture site and the punctured cell seems little affected (Tjallingii and Hogen Esch 1993). During these brief intracellular punctures, aphids secrete a small amount of watery saliva into the cell and then suck up a small volume of cell sap, presumably for sensory evaluation by the precibarial chemosensillae (Martín et al. 1997; Powell 2005). It is during brief intracellular punctures, especially those near the beginning of a stylet penetration, that non-persistent viruses are transmitted by aphid vectors (Lopez-Abella et al. 1988; Powell 1991; Powell et al. 1995; Perez et al. 1996; Collar et al. 1997; Martín et al. 1997).

In contrast to aphids, intracellular punctures by whiteflies are much less frequent, and generally occur only after the stylets have penetrated deep into the leaf tissue (Janssen et al. 1989; Lei et al. 1998; Jiang et al. 1999; Johnson and Walker 1999). Furthermore, salivation and ingestion behavior during intracellular punctures has not been detected in whiteflies. The difference in the frequency and behaviors occurring during these intracellular punctures may explain why the great majority of vectors of non-persistently transmitted plant viruses are aphids and not whiteflies (Nault 1997).

There are conflicting reports regarding the mode of penetration through the epidermis at the onset of stylet penetration. Scanning electron micrographs indicate that

in most cases, the stylet bundle passes directly through the epidermal cells rather than between epidermal cells (Cohen et al. 1998; Freeman et al. 2001) whereas electrical penetration graph (EPG) studies do not detect intracellular penetration through the epidermis (Janssen et al. 1989; Lei et al. 1998; Jiang et al. 1999; Johnson and Walker 1999). Several reasons might account for this discrepancy. The cell membrane (plasmalemma) does not show up in these SEM images; therefore, if the stylets penetrate through the epidermis along the inner surface of the cell wall without puncturing the plasmalemma (i.e., intramural penetration), the pathway would appear to be intracellular in SEM images. However, in at least some of the SEM images, the stylets penetrate away from the cell wall's inner surface, which would make intramural penetration unlikely in these specific cases. Alternatively, the discrepancy between the SEM and EPG studies could be because EPGs detect intracellular penetrations by recording the cell membrane electrical potential which is a property of living cells. If penetration through epidermal cells causes rapid cell death, then the membrane electrical potential would rapidly deteriorate and intracellular penetration might not be detected. This explanation implies that the interaction between stylets and epidermal cells is different from other cells which survive penetration. Although there is conflicting evidence regarding the mode of penetration through the epidermis, all studies, SEM and EPG, are in agreement that once past the epidermis, the pathway is primarily intercellular until the sieve element is penetrated.

Penetrating the leaf and locating a sieve element is a time-consuming and often unsuccessful process. After landing on a leaf, a whitefly usually makes many stylet penetrations before successfully locating a sieve element (Lei et al. 1997, 2001; Jiang et al. 1999, 2001). Salivary sheaths often have many branches indicating probes in multiple directions before the insect successfully locates a sieve element or gives up and withdraws its stylets (Walker 1985; Cohen et al. 1998; Freeman et al. 2001) (e.g., Fig. 4.20b). The average time interval from the beginning of a successful probe to the initiation of phloem phase (sieve element salivation and ingestion) varies from 16 to 42 min in various whitefly/plant species combinations (Table 4.1). Considering that many unsuccessful probes usually precede the first successful probe, it may take an hour or more after landing on a leaf before the whitefly adult begins ingesting phloem sap. Only one study reports the time required for a successful probe by a whitefly nymph (*T. vaporariorum*) to reach the phloem, and it was much longer (average of 74 min) than the adult takes on the same plant (Table 4.1, Lei et al. 1996b).

TEM studies of sieve element penetration by aphids indicate that the mandibular stylets and salivary sheath stop at the outer surface of the cell wall and only the tips of the maxillary stylets penetrate the sieve element (Evert et al. 1973; Kimmins and Tjallingii 1985; Tjallingii and Hogen Esch 1993). Although TEM studies of sieve element penetration by whiteflies are lacking, TEM data are available for whitefly penetration of a xylem cell, and similar to sieve element penetration by aphids, the mandibular stylets and salivary sheath stopped at the cell wall and only the tips of the maxillary stylets penetrated the cell (Janssen et al. 1989).

**Table 4.1** Time from the beginning of a probe required for whiteflies to attain phloem phase

| Whitefly species/strain | Plant | Time to reach phloem | |
|---|---|---|---|
| | | Phase within a probe | Reference |
| Adult whiteflies | | | |
| *Bemisia argentifolii* | Lima bean | 15.6 ± 8.3 min (mean ± SD) | Walker and Perring (1994) |
| *Bemisia tabaci* (strain B) | Tomato | 18.3 ± 12.9 min (mean ± SD) | Jiang et al. (1999) |
| *Bemisia tabaci* (strain Q) | Tomato | 23.7 ± 14.9 min (mean ± SD) | Jiang et al. (1999) |
| *Bemisia tabaci* (strain B) | Tomato (with *Mi* resistance) | 39.0 ± 25.8 min (mean ± SD) | Jiang et al. (2001) |
| *Bemisia tabaci* (strain B) | Tomato (without *Mi* resistance) | 21.2 ± 10.7 min (mean ± SD) | Jiang et al. (2001) |
| *Parabemisia myricae* | Lemon and rough lemon | 24.1 ± 17.7 min (mean ± SD) | Walker and Perring (1994) |
| *Trialeurodes vaporariorum* | Cucumber | 19.7 ± 12.9 and 24.8 ± 18.6 min (mean ± SD of two different experimental treatments) | Lei et al. (1997) |
| *Trialeurodes vaporariorum* (strain T) | Cucumber | 34.9 ± 23.3 min (mean ± SD) | Lei et al. (1998) |
| *Trialeurodes vaporariorum* (strain C) | Cucumber | 41.9 ± 28.8 min (mean ± SD) | Lei et al. (1998) |
| *Trialeurodes vaporariorum* | Cucumber | 34.5 min (mean) | Lei et al. (2001) |
| *Trialeurodes vaporariorum* (strain T) | Tomato | 23.2 ± 17.9 min (mean ± SD) | Lei et al. (1998) |
| *Trialeurodes vaporariorum* (strain C) | Tomato | 16.1 ± 13.4 min (mean ± SD) | Lei et al. (1998) |
| *Trialeurodes vaporariorum* (strain T) | Tomato (susceptible variety) | 23.2 ± 17.8 min (mean ± SD) | Lei et al. (1999) |
| *Trialeurodes vaporariorum* (strain T) | Tomato (resistant variety) | 17.8 ± 7.4 and 21.4 ± 24.0 min (mean ± SD of two different resistant cultivars) | Lei et al. (1999) |
| *Trialeurodes vaporariorum* | Tomato | 27.2 min (mean) | Lei et al. (2001) |
| *Trialeurodes vaporariorum* (strain T) | Sweet pepper | 19.3 ± 21.2 min (mean ± SD) | Lei et al. (1999) |

**Table 4.1** (continued)

| Whitefly species/strain | Plant | Time to reach phloem | |
|---|---|---|---|
| | | Phase within a probe | Reference |
| *Trialeurodes vaporariorum* | Sweet pepper | 25.8 min (mean) | Lei et al. (2001) |
| *Trialeurodes vaporariorum* | Gerbera | 25.6 min (mean) | Lei et al. (2001) |
| Whitefly nymphs *Trialeurodes vaporariorum* | Cucumber | 74.2 min (mean, n = 7) | Lei et al. (1996b) |

After penetration of a sieve element, watery saliva is secreted into the sieve element for a period of up to several minutes before the onset of ingestion (Jiang et al. 1999, 2000, 2001). Similar behavior occurs in aphids where it has been studied in more detail (Tjallingii 1994, 1995, 2006; Wilkinson and Douglas 1998). The function of this salivation has been hypothesized to "condition the sieve element" for ingestion; specifically to prevent, or reverse, the sealing response by the penetrated sieve element (Tjallingii 2006; Will et al. 2007).

Sieve elements are very sensitive to perturbations and respond to even minor injury by sealing their connections to adjacent sieve elements (Eschrich 1975; Knoblauch and van Bel 1998). Such a response would cut off the aphid or whitefly's access to the phloem sap pipeline. Plants have redundant systems to seal damaged sieve elements and thus avoid "bleeding to death" every time damage occurs. The two most common systems are callose synthesis which closes off the sieve pores that connect one sieve element to another and P-protein which undergoes an almost instantaneous change in structural state in response to damage, and the change causes it to clog the sieve pores (Eschrich 1975). Phloem-feeding insects such as whiteflies and aphids have to avoid triggering these plant responses or reverse them if they occur, and salivation into the sieve element seems the most likely way that this occurs. This hypothesis recently has gained experimental support, where the saliva of the aphid *Megoura viciae* Buckton was shown in vitro to be able to change the structural state of P-protein from a sieve-plate clogging state to a non-clogging state (Will et al. 2007). Secretion of watery saliva into the sieve element occurs not only prior to the onset of ingestion, but periodically ingestion is interrupted by bouts of salivation (Sauge et al. 1998; Tjallingii 2006). Again the function has been hypothesized to be interference with the sieve elements' sealing mechanisms. P-protein coagulation would be a problem not only if it occurred in the sieve elements, but also would be problematic for the insect if it coagulated in the stylet food canal during ingestion. Prevention of coagulation in the food canal has been hypothesized as the function of watery saliva that is secreted during ingestion and is immediately caught up in the inward flow of sap without ever entering the sieve element (Tjallingii 2006).

Salivation into sieve elements, as detected by electrical penetration graphs (EPGs), has been confirmed only for adult whiteflies (Jiang et al. 2000). Whitefly nymphs produce EPG patterns similar to those associated with phloem salivation in adults (Lei et al. 1996a, b; Jiang and Walker 2003) but definitive experiments to verify association of these nymphal EPG patterns with salivation have not yet been conducted. However, assuming the likelihood that these nymphal EPG patterns also indicate salivation, then whitefly nymphs continually cycle between ingestion and salivation in the sieve elements. Cycles lengthen in progressive instars with bouts of ingestion averaging 26, 30, and 54 min and salivation averaging 13, 17 and 25 min for instars 1–3, respectively (Jiang and Walker 2003). Nymphs of greenhouse whitefly can apparently remain feeding from the same sieve element, alternating between ingestion and presumed salivation, for an entire instar without ever withdrawing their stylets (Lei et al. 1996a, b). While adult whiteflies always precede phloem sap ingestion with salivation (Jiang et al. 1999), nymphs often begin ingestion almost as soon as the sieve element in penetrated (Lei et al. 1996a, b; Jiang and Walker 2003).

## Alimentary Canal

Phloem sap is the plant's main vehicle for transport of organic nutrients; however, the balance of those nutrients is far from optimal for animal nutrition. There usually is a great excess of sugar and water in addition to relatively low concentrations of other required nutrients such as amino acids (Raven 1983). This creates at least three major problems: (1) absorption of nutrients in low concentration is problematic; (2) the nitrogen/carbon balance is suboptimal for nutrition; and (3) the high concentration of sugar creates a strong osmotic gradient that potentially could dehydrate the insect. Consequently, a diet of phloem sap requires significant modification of the gut. In most phloem feeding insects, including whiteflies, part of the gut is modified to form a "filter chamber." The filter chamber differs among different phloem-feeders (Goodchild 1966) suggesting multiple independent origins. However, the principle of its operation is generally similar. The midgut forms a loop, and its anterior end, near the foregut/midgut junction, is in close contact with its posterior end, near the midgut/hindgut junction. The tissues in this area of contact form the filter chamber, which shunts excess water and/or sugar directly to the hindgut where it is rapidly excreted. The remaining ingested material continues passage through the midgut where it can be absorbed.

The whitefly gut is very complex anatomically and descriptions of its structure, function, and homology in the literature are not always consistent. However, there is agreement that: (1) the pharynx and esophagus form a long narrow tube connecting the cibarial pump to the filter chamber; (2) the proximal end of the midgut is dilated and then the midgut narrows into a long tube that forms a loop so that the distal end of the tube-like portion of the midgut attaches to the dilated proximal end of the midgut (the first part of the tube that extends away from the dilated proximal end of the midgut is referred to as the descending midgut/ventriculus and

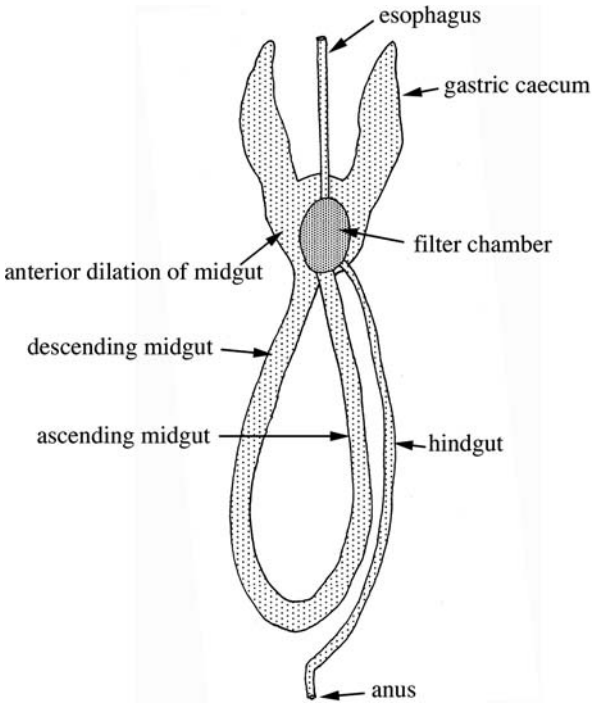



**Fig. 4.22** Overview of the gross anatomy of the whitefly alimentary canal based on consensus in the literature. The esophagus enters the filter chamber. The filter chamber adheres to the proximal end of the midgut, which is dilated at this point (Ghanim et al. 2001 refer to the dilated proximal end of the midgut as the "connecting chamber"). How the filter chamber connects to this dilation and to the esophagus are major points of contention in the literature. Distally, the midgut narrows, becoming what is referred to as the descending midgut, and extends away from the dilated proximal end of the midgut. The midgut then loops forward, becoming the ascending midgut, and its distal end enters the filter chamber. The hindgut emerges from the filter chamber and extends to the anus. The lumina of the gastric caecae and descending midgut are continuous with the dilated proximal end of the midgut; however, how the dilated proximal end of the midgut, the distal end of the ascending midgut, the basal end of the hindgut, and distal end of the esophagus are all interconnect in the filter chamber is a point of contention between the two published transmission electron microscopy studies (Cicero et al. 1995; Ghanim et al. 2001) (see text and Fig. 4.23)

the second part which loops back and attaches at its distal end to the dilated proximal end of the midgut is referred to as the ascending midgut/ventriculus); (3) the filter chamber is located at the point of contact between the dilated proximal end of the midgut and distal end of the ascending midgut; (4) the hindgut extends from the filter chamber to the anus; and, (5) the gut has two large blind-end diverticulae (Fig. 4.22). Weber (1935) considered the diverticulae to be Malpighian tubules, although he did point out that the cells of the diverticulae and midgut were identical. Like Weber, Tremblay (1959) and Harris et al. (1996a, b) used light microscopy to examine the whitefly gut and followed Weber's interpretation that the diverticulae were Malpighian tubules. However, the more detailed studies of Cicero et al. (1995)

and Ghanim et al. (2001) using electron microscopy concluded that the diverticulae are gastric caecae which anatomically are part of the midgut; thus, Weber's statement that the diverticulae cells are identical to midgut cells seems like a premonition, despite his misinterpretation of the diverticulae as Malpighian tubules. Distinction between gastric caecae and Malpighian tubules is critical to interpreting the direction of flow through the midgut since gastric caecae are located at the anterior end of the midgut, and Malpighian tubules are located at the posterior end at the midgut-hindgut junction. Thus, the designation of which part of the midgut loop is the descending midgut and ascending midgut is reversed in Harris et al. (1996a, b); correspondingly, the conventional direction of flow through the midgut (from proximal end to distal end) is the opposite of what was described.

The filter chamber itself is complex, and details of its structure and function require transmission electron microscopy (TEM). There are only two TEM studies on the whitefly filter chamber (Cicero et al. 1995; Ghanim et al. 2001). These two studies are in agreement on several aspects of the whitefly gut but also have some very significant differences.

Both studies agree that the filter chamber is convoluted and is located in the region where the distal end of the ascending midgut attaches to the dilated proximal end of the midgut. Both studies also agree that the filter chamber is a tube within the midgut lumen and that the filter chamber lumen mostly (Cicero et al. 1995) or completely (Ghanim et al. 2001) enveloped by two layers of cells (Fig. 4.23). The inner cell layer faces the lumen of the filter chamber and its apical surface facing the lumen has a brush border. The outer cell layer is comprised entirely (Cicero et al. 1995) or mostly (Ghanim et al. 2001) of modified midgut epithelium, which has a brush border on its apical surface, which faces midgut lumen in which the filter chamber is enclosed. Thus, the apical brush border surfaces of these two cell layers face opposite directions; the inner cell layer toward the filter chamber lumen and the outer cell layer toward the surrounding midgut lumen. These two cell layers interface on their basal surfaces, which are highly infolded and interdigitate with each other, thus increasing the surface area of their contact. This suggests movement of materials that do not freely pass through cell membranes (e.g., sugar) and require specialized ports in the membranes. Cicero et al. (1995) consider the inner lumen of the filter chamber to be the Malpighian lumen while Ghanim et al. (2001) consider it to be the ileum (hindgut) lumen. However, both studies show a lack of cuticular lining of this lumen, which is more consistent with a Malpighian interpretation than with an ileum interpretation. Both studies describe Malpighian cells or "Malpighian-like cells" in the filter chamber, but Cicero et al. (1995) consider almost all the cells of the inner cell layer to be Malpighian cells whereas Ghanim et al. (2001) place Malpighian-like cells interspersed among the modified midgut cells in the outer cell layer (Fig. 4.23).

Both studies indicate that the distal end of the esophagus is in close proximity to the filter chamber, but they are in significant disagreement regarding how it interfaces with the filter chamber (Fig. 4.23). Cicero et al. (1995) found a very intimate connection between the esophagus and filter chamber where the basal surface of some of the esophageal epithelial cells bordered part of the lumen of the

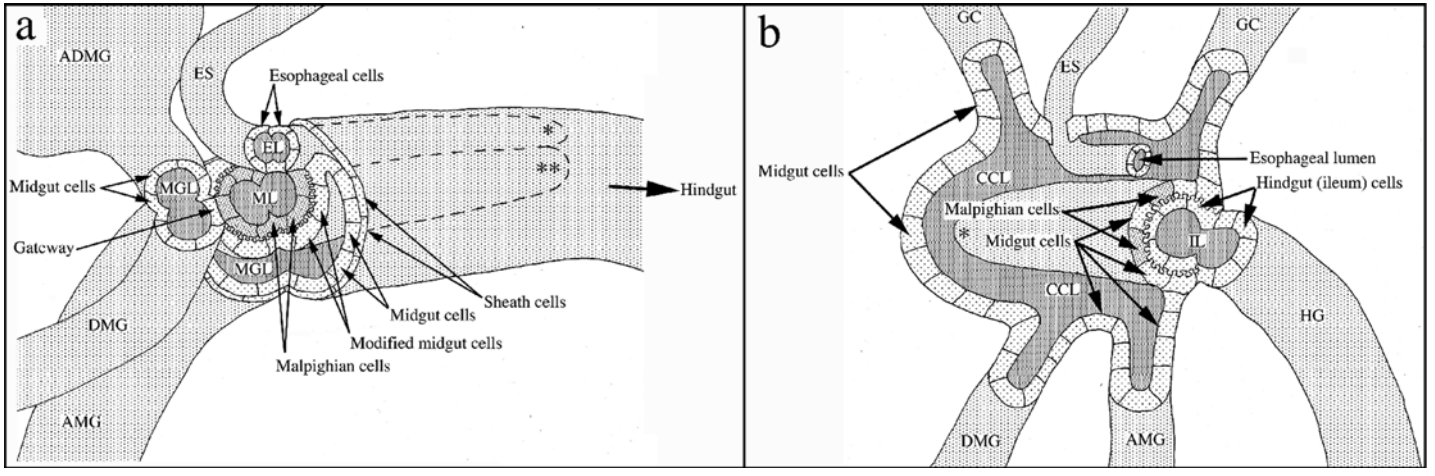



**Fig. 4.23** Models of the whitefly filter chamber (**a**) according to Cicero et al. (1995); (**b**) according to Ghanim et al. (2001). In (**a**), the distal end of the esophagus is blind-ended (*asterisk*) as well as the far *right* end of the Malpighian lumen (*double asterisk*). In addition, the far *left* end of the Malpighian lumen is also blind-ended just to the *left* of where it is cut open in the diagram (far *left* end not shown); consequently, the Malpighian lumen is completely closed at both ends. The esophageal and Malpighian lumina are in very close contact, with a single cell layer separating them at the point of contact. The gateway occurs where there is a breach in the wall of the distal end of the ascending midgut; at the gateway, the basal surfaces of epithelial cells of the proximal end of the midgut tightly adhere to the basal surfaces of some of the Malpighian cells; however, there is no contact among the Malpighian lumen and the lumina of the proximal midgut and ascending midgut at this point (for ease of illustration, the position of the gateway on the proximal midgut is drawn slightly distal to the anterior dilation of the midgut which is its actual location). The gateway is the only point of contact between the proximal end of the midgut and distal end of the ascending midgut. The lumen of the ascending midgut is continuous with the lumen of the hindgut. In (**b**), the basal end of the ileum (anterior end of hindgut) is blind-ended (*asterisk*) and embedded in the anterior dilation of the midgut (referred to as the connecting chamber lumen [CCL] by Ghanim et al). Also unlike the model of Cicero et al., in the model of Ghanim et al., the lumen of the distal end of the ascending midgut reconnects with the lumen of the anterior dilation of the midgut while the lumen of the ileum is not continuous with the lumen of the ascending midgut. Note that the lumen referred to as the Malpighian lumen (ML) by Cicero et al. (1995) is the same as and referred to as the ileum lumen (IL) by Ghanim et al. (2001); this lumen is mostly (Cicero et al. 1995) or entirely (Ghanim et al. 2001) enclosed by two cell layers that interdigitate with each other on their basal surfaces. ADMG, anterior dilation of the midgut, AMG, ascending midgut; CCL, connecting chamber lumen, DMG, descending midgut; EL, esophageal lumen; ES, esophagus; GC, gastric caecum; HG, hindgut; IL, ileum lumen; MGL, midgut lumen; ML, Malpighian lumen.

filter chamber (Fig. 4.23a). Thus, in the model of Cicero et al. (1995), the lumen of the filter chamber is mostly enclosed by a double cell layer, as described previously, but in places, esophageal epithelial cells interrupt the double cell layer and border the lumen of the filter chamber on one side and the esophageal lumen on the opposite side. These cells lack a brush border facing either lumen. Cicero et al. (1995) could not find a connection between the esophageal lumen and any of the other lumina (midgut, hindgut or Malpighian) despite specifically looking for such a connection, and concluded that the esophagus terminated in a blind end within the filter chamber (Fig. 4.23a). Thus in their model, ingested sap must cross these specialized esophageal epithelium cells from the esophageal lumen to the lumen of the filter chamber. While Ghanim et al. (2001) also describe a close contact between the distal end of the esophagus and filter chamber (Fig. 4.23b), they did not detect specialized esophageal epithelium cells bordering both the esophageal lumen and lumen of the filter chamber as reported by Cicero et al. (1995). An even more significant difference between the two studies is that Ghanim et al. (2001) indicate that the esophagus is not blind-ended, but rather it opens into the dilated proximal end of the midgut, which they refer to as the "connecting chamber" (Fig. 4.23b). They refer this as the connecting chamber because in their model, the lumina of the esophagus, basal end of the descending midgut, distal end of the ascending midgut and the gastric caecae all connect together in this chamber forming what they refer to as "the continuous lumen" of all these organs (Fig. 4.23b). In Ghanim et al.'s model, the filter chamber and the distal end of the esophagus are embedded in the connecting chamber at the point where the distal end of the ascending midgut opens into the connecting chamber. While the esophagus opens into the connecting chamber, the lumen of the filter chamber does not (Fig. 4.23b).

In Ghanim et al.'s interpretation of whitefly gut anatomy, the lumen of the filter chamber is blind-ended basally and continuous with the hindgut distally (in fact they consider the filter chamber to be the ileum region of the hindgut) (Fig. 4.23b). In summary, the path of ingested sap in the model of Ghanim et al. (2001) is as follows: Ingested sap enters the connecting chamber via the esophagus and is circulated through the continuous lumen by peristalsis and contractions of the gastric caecae. Sap flows over the filter chamber as it leaves the esophagus and as it circulates through the continuous lumen. As sap flows over the filter chamber, materials such as water and sugar are selectively transported through the double cell layer of the filter chamber and into its lumen (the ileum according to Ghanim et al.). These materials then proceed through the remainder of the hindgut to the anus for excretion.

Cicero et al.'s interpretation of whitefly gut anatomy differs in five very significant ways (Fig. 4.23): (1) As already noted, Cicero et al. believe that the esophagus terminates blind-ended; (2) The lumen of the filter chamber (Malpighian lumen according to Cicero et al.) is completely closed at both ends; (3) The midgut lumen is continuous with the hindgut lumen at the distal end of the ascending midgut; (4) Although the midgut forms a loop and its distal end attaches to its proximal end, the lumina do not reconnect at this point; (5) At this point where the distal end of the ascending midgut contacts the dilated proximal end of the midgut, there is

a breach in the wall of the ascending midgut and some of the epithelial cells of the proximal end of the midgut tightly adhere to the basal surface of some of the Malpighian cells in the filter chamber (Fig. 4.23a); however, despite this breach and the tight contact between epithelial cells of the proximal end of the midgut and the Malpighian cells that enclose the Malpighian lumen, the Malpighian lumen and midgut lumina remain isolated from each other. Cicero et al. refer to this point of contact as the "gateway" and is a point where ingested materials can be transported between the proximal end of the midgut lumen and the Malpighian lumen by passing through these two cell layers. As noted previously, the dilated proximal end of the midgut is what Ghanim et al. refer to as the connecting chamber although according to Cicero et al., this is not a "connecting chamber" because the lumina of the proximal end of the midgut and distal end of the ascending midgut do not reconnect here. Cicero et al. propose two models of how the filter chamber might function. Their first model hypothesizes that the muscular sheath which surrounds the entire gut is differentiated as sphincters at two locations; one sphincter around the ascending midgut at the point where the filter chamber is located and a second sphincter near the midgut/hindgut junction slightly distal to the filter chamber (Fig. 4.24). These two sphincters are proposed to contract alternately. When the posterior sphincter closes and the anterior sphincter opens, sap entering the filter chamber via the esophagus is prevented from going to the hindgut and is thus diverted into the midgut (Fig. 4.24b). After the midgut is filled, sphincter contraction reverses: the posterior sphincter opens and the anterior sphincter closes. Then water in the midgut moves through the gateway into the filter chamber and out the distal end of the filter chamber into the hindgut (Fig. 4.24c). In their alternative model, ingested sap enters the filter chamber via the esophagus and from there water

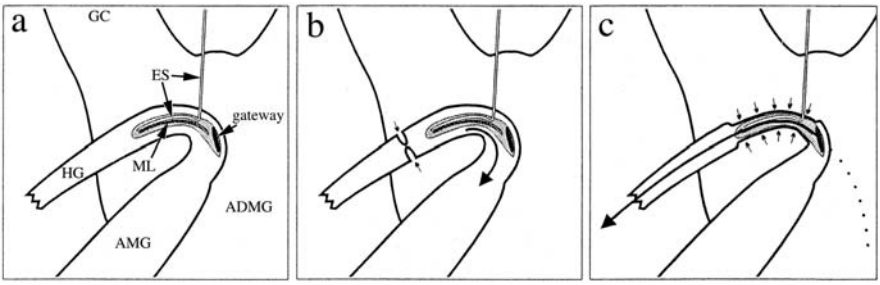



**Fig. 4.24** One model of function of the whitefly filter chamber proposed by Cicero et al. (1995). (**a**) Overview of filter chamber region with anatomical components labeled. Ingested sap enters the filter chamber (*shaded gray*) from the esophagus; direction of sap movement out of the filter chamber is controlled by two sphincters. (**b**) Sphincter (*small arrows*) closes entry to the hindgut so that ingested sap leaving the filter chamber (*large arrow*) is diverted to the midgut. (**c**) Sphincter surrounding the midgut in the region of the filter chamber closes, squeezing off the ascending midgut lumen and closing the connection between midgut and hindgut lumina; water and excess sugar (*large arrow*) from the anterior dilation of the midgut then filter through the gateway, into the Malpighian lumen, and into the hindgut for excretion. ADMG, anterior dilation of the midgut, AMG, ascending midgut; ES, esophagus; GC, gastric caecum; HG, hindgut; ML, Malpighian lumen (*solid black*). From Cicero et al. (1995)

is shunted to the hindgut in one direction and solutes in the other direction to the anterior midgut via the gateway.

In conclusion, the differences in the interpretations of the whitefly gut by Cicero et al. (1995) and Ghanim et al. (2001) are very significant, thus leaving an understanding of the structure and function of the whitefly gut an open question. Consequently, additional studies are needed before unambiguous conclusions can be made.

## Courtship and Mating

Limited research has been reported on whitefly mating behavior. However, it is clear that whiteflies participate in a series of complex and elaborate behaviors in selecting, courting, and copulating with the opposite sex of their own species or biotype. Our present knowledge comes primarily from studies on the greenhouse whitefly, *T. vaporariorum* (Las 1979; Ahman and Ekbom 1981; Li and Maschwitz 1985; Kanmiya 1996, 2006) and research conducted on *B. tabaci*, biotype A, (Li et al. 1989), biotype B (= *Bemisia argentifolii*) (Perring and Symmes 2006), a biotype native to China referred as ZHJ1 (Zang and Liu 2007) and interactions between biotypes B and ZHJ1 (Liu et al. 2007). Perring and Symmes (2006) observed that duration of antennal drumming, synchronous abdominal undulations, and body angle between males and females during copulation of biotype B were similar to biotype A and to *T. vaporariorum*. In contrast, other behaviors were unique to the various whiteflies. For example, *B. tabaci* biotype B did not exhibit body pushing behavior, *B. tabaci* biotype A used 2 wings to cover the female abdomen while the other whiteflies used 4 wings, and the antennal drumming position was unique for *T. vaporariorum*. The functionality of many of these behaviors is unknown. However, Kanmiya (2006) presented evidence of distinct courtship vibratory signals that differ among whitefly species and among *B. tabaci* biotypes. He noted that these signals were clearly linked to abdominal undulations in *T. vaporariorum* and suggested that other behaviors like antennal drumming, body pushing, and wing flicks also may be involved in auditory communication between males and females.

In this section we cover specific mating behaviors that have been identified for *B. tabaci* biotypes A, B, and ZHJ1, present comparative data on time allotted to these behaviors, discuss the mating cascade hypothesis and its relationship with mate selection, and conclude with studies on mating interactions between biotype B and non-B biotypes that favor the successful invasion of biotype B.

## Specific Mating Behaviors of Three *Bemisia tabaci* Biotypes

### Male Searching and Initial Contact Between Sexes

*Bemisia tabaci* males and females exhibit a number of precise and complex behaviors that begin with searching. Typically only males actively search for females

(Perring and Symmes 2006; Zang and Liu 2007) although Li et al. (1989) reported that a few biotype A females approached males. Zang and Liu (2007) noted that males of biotypes B and ZHJ1 actively move around the leaves until they encounter a female, designating this as "searching time." Li et al. (1989) reported a similar behavior for biotype A males with more detail. They also described males as more active than females, moving randomly over the leaf until they came within 2–3 mm of the females. The male then encircled the female before placing his fore-tarsi on the edge of her wing, or touching her with his antennae. Perring and Symmes (2006) reported that only 19% of biotype B males initiated contact with their tarsi, while 68% initiated contact with their antennae, the remaining 13% were unclear. The behavior suggests that receptors on the male antennae or fore-tarsi receive cues to initiate courtship.

### Parallel Positioning

In their study of biotype A, Li et al. 1989 reported that, following initial contact, the male positioned himself parallel to the female provided no other males interfered (Fig. 4.25a). The average time from female detection to parallel positioning was <1 min. This begins what Zang and Liu (2007) refer to as "courting time" in biotype ZHJ1. Perring and Symmes (2006) noted that once a biotype B male made initial contact with a female, 91% maintained contact with their antennae, tarsi, or both, while quickly moving into parallel orientation. On a few occasions, biotype A females refused the male by flapping her wings, pushing him away with her legs, or flying away (Li et al. 1989).

### Antennal Drumming

The next behavior described after parallel positioning is antennal drumming. Li et al. (1989) reported that both sexes raised the antennae, holding them forward and out to the side at a 45º angle to the longitudinal axis of the body. The male then drummed


**Fig. 4.25** Aspects of whitefly mating behavior. (**a**) *Bemisia tabaci* biotype B male (*upper*) and female whitefly in the early phase of parallel positioning, the first step in the courtship cascade. (**b**) Male (*lower*) and female *B. tabaci*, biotype B in copulation. (**c**) Two *B. tabaci* biotype B males courting a single female (*middle*). Notice the aggressive wing behavior of the male on the *right* against the male on the *left*

the medial section of the female flagellum with the middle segments of his own antennae. Perring and Symmes (2006) found similar behavior in biotype B, further clarifying that the antennae closest to the opposite sex were used for drumming. They also noted that males sometimes drummed the head, thorax, or nearest foreleg of the female.

### Male Abdominal Undulation

This behavior consisted of males raising and lowering the abdomen in a vertical plane, apparently contacting the leaf surface with each undulation. This behavior occurred in synchrony with the up and down movement of antennal drumming, and increased in frequency as biotype A whiteflies continued to court (Li et al. 1989). In contrast, biotype B males did not begin abdominal undulations until antennal drumming ceased (Perring and Symmes 2006). Occasionally, biotype B females also undulated their abdomens, but this was not reported for biotype A. Kanmiya (1996) reported that the male abdominal movements produced vibrations that were transmitted to the females through the leaf substrate suggesting that these acoustic signals are part of the specific mate recognition system of whiteflies.

### Body Pushing

Li et al. (1989) reported that about 15% of courting biotype A males increased the frequency of their antennal drumming, while pushing the females with the sides of their bodies in preparation for copulation. The result of this behavior was that the couple moved in a circular fashion, pivoting around the female mouthparts that were inserted in the leaf. This behavior was not observed in biotype B (Perring and Symmes 2006).

### Male Positioning

The males raise their wings and move their abdomen under the female abdomen in preparation for copulation, his wings covering hers. Li et al. (1989) reported that only the pair of wings adjacent to the biotype A female were used, but Perring and Symmes (2006) clearly indicated that biotype B males used all 4 of wings as does biotype ZHJ1, although the pair of wings furthest from the female often times did not cover her body (Zang and Liu 2007).

### Copulation

The male claspers open and the aedeagus protrudes in attempts to clasp the female terminalia (Li et al. 1989) If she accepts, the claspers grab onto the ovipositor and the terminal flap covering the female's gonopore is pulled open by the claspers. The male abdomen along with the aedeagus bend upward at a 90º angle prior to insertion into the gonopore. Zang and Liu (2007) reported that biotype ZHJ1 males vibrated their wings at a high frequency during copulation.

### Post-copulation

The female terminated copulation by prying the male free with her middle and hind legs (Li et al. 1989). They observed biotype A males resting for 1–2 min and females for 2–5 min. Perring and Symmes (2006) observed biotype B pairs remaining parallel for variable lengths of time after copulation (5–18,000 s) while feeding and self-grooming.

### Male Interference

Aggressive interruption of courting pairs by individual males has been observed "sometimes" (Li et al. 1989) for biotype A or infrequently (17 of 132 courtships) for biotype B (Perring and Symmes 2006). The intruding male most often approached the female from the opposite side of the courting male (Fig. 4.25c). Sometimes, however, the intruding male would approach from the same side as the courting male. The intruding male quickly took up a parallel position spending little time touching the courting whiteflies with his antennae. The original courting male responded by flicking his wings, covering the female with his wings and touching the intruding male with his wings (Perring and Symmes 2006). The three whiteflies remained in courtship, with periodic wing flicking by both males. In 8 out of the 17 cases, the female left the two males; in 6 cases, the intruding male replaced the original male; and in the other 3 cases, the first male succeeded in maintaining contact with the female. However, none of these interrupted courtships resulted in mating (Perring and Symmes 2006).

## *The Mating Behavior Cascade and Mate Discrimination*

Successful copulation is seldom the outcome of courtship by *B. tabaci*. Perring and Symmes (2006) reported that only 16 out of 132 (12%) biotype B pairs that initiated courtship actually copulated. Li et al. (1989) reported a success rate of just 7.7% for biotype A. These low success rates are consistent with complex mating behaviors and indicate a high degree of discrimination. Complex courtship behaviors aid in the recognition of mates belonging to the appropriate gender and species. Matthews and Matthews (1978) discussed insect courtship as a "ratchet mechanism," which progresses in a sequential fashion, each step leading to the next in a precise order to achieve successful copulation. This mechanism prevents mating if the defined sequence of courtship behaviors is not followed and thus can lead to reproductive isolation, (Butlin 1995). Of the successful biotype B matings, most (81.3%) went directly from pre-courtship, through the various stages of courtship, and into copulation, without reverting back to previous stages during the courtship sequence. For these whitefly pairs, the sending and receiving of positive mating cues occurred from the early phases of courtship through mating. For the other whitefly pairs, positive mating interactions declined as courtship progressed. Most (94%) of

the biotype B pairs that reached antennal drumming advanced to the next behavior, usually to abdominal undulation (Perring and Symmes 2006). However, only 46% moved onto male positioning of which 18% finally copulated. The final tally from abdominal undulation to copulation was 6%. Zang and Liu (2007) also found that courtships of biotype ZHJ1 frequently ended during the abdominal undulation phase. Similarly, only 23% of biotype A pairs advanced from male positioning to copulation (Li et al. 1989). The increased failure rate suggests increasing discrimination in signals between sexes at successive steps along the cascade (Perring and Symmes 2006).

Auditory signals are likely to be important for advancement through the mating cascade. Vibrational sounds produced by *T. vaporariorum* are correlated to specific male behaviors, such as antennal drumming, abdominal undulations, and wing flicking of competing males (Kanmiya 1996). He described these signals as "characteristic temporal patterns having chirps composed of a train of pulses" with unique frequency and sound amplitude characteristics. A later study showed that female *T. vaporariorum* and both sexes of *B. tabaci*, biotypes A and B also produce such signals (Kanmiya 2006). Acoustic signals are exchanged between females and males, and the rate of female response increases at the pair progresses toward copulation (Fig. 4.26).

Various studies have reported that males and females of reproductively incompatible *B. tabaci* biotypes, or even species, will invest time courting each other (Costa et al. 1993; Perring et al. 1993; Bedford et al. 1994; DeBarro and Hart 2000; Maruthi et al. 2004; Pascual 2006; Liu et al. 2007). The similarity of courtship behavior among the different *B. tabaci* biotypes likely contributes to this unproductive courting. On the other hand, behavioral differences between biotypes may be caused by critical junctions in the sending and receiving of signals between sexes of different biotypes or species. Kanmiya (2006) clearly demonstrated extreme differences in the acoustic signals produced by biotype A and biotype B (Fig. 4.26) concluding that the two are different biological species.

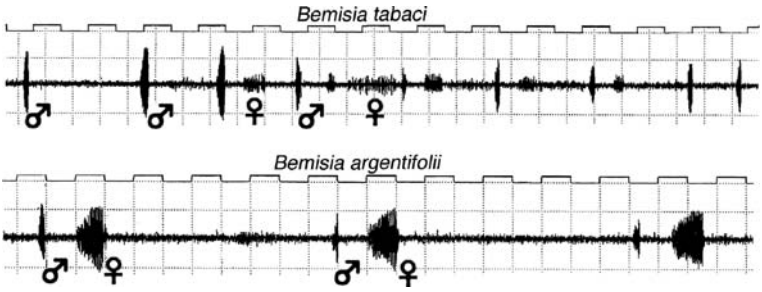



**Fig. 4.26** Oscillograms of vibratory signals produced by whiteflies during courtship by *Bemisia tabaci* and *Bemisia argentifolii* (= *B. tabaci* biotype B) Male and female responses are labeled. *Upper square* waves indicate a 1 Hz. oscillation. From Kanmiya (2006)

### *Mating Behavior and Competitive Advantage of Biotype B*

As scientists have gained a more complete understanding of whitefly mating behavior, and studied interactions between *B. tabaci* biotypes, a clearer picture has emerged of how these behaviors have contributed to the displacement of native non-B biotypes by the B biotype (see Naranjo et al. Chapter 6). Displacement of indigenous biotypes by biotype B has occurred multiple times. For example, in the early 1990s, biotype B displaced the A biotype in the southwestern United States (Liu et al. 1992; Brown et al. 1995; Perring 1996); recent surveys in the eastern United States shows the displacement of biotype A by biotype B in that region as well (McKenzie et al. 2004). Area-wide displacement of an indigenous Australian biotype (AN), and an indigenous biotype in China (ZHJ1), was clearly presented in Liu et al. (2007). In addition, Rekha et al. (2005) reported that three indigenous biotypes in India have been displaced by biotype B.

Perring (1996) noted several features of the interactions between biotypes A and B that may have contributed to displacement of biotype A in the USA. One of the most interesting observations was that biotype B males courted biotype A females longer than biotype A males courted their own females. In an environment dominated by biotype B individuals, the greater "persistence" of biotype B males would result in competition for biotype A female resources and thus, fewer successful biotype A matings. Likewise, biotype B developed higher densities than biotype Q in mixed culture (Pascual and Callejas 2004). Subsequent studies determined that biotype B males spent more time in courtship with biotype Q females than biotype Q males spent in courtship with their own females. However, biotype B males spent more time courting their own females than biotype Q males spent courting biotype B females (Pascual 2006). This situation would result in fewer biotype Q female offspring when the two biotypes were sympatric as a result of the haploid-diploid sex determination mechanism of whiteflies.

More recent controlled experiments have demonstrated interference between biotypes favoring biotype B. Zang and Liu (2007) found that reproductive success (% female offspring) of a single biotype B female increased from 0.44 to 0.74 when the number of males mating with her was increased from one to two. However, the same experiment with biotype ZHJ1 increased female progeny only slightly from 0.45 to 0.49. In mixed biotype experiments (Liu et al. 2007), adding an extra biotype ZHJ1 male to courting biotype B whiteflies had no impact on the number of B copulations or proportion of female offspring, whereas adding an extra B male to a courting ZHJ1 couple significantly decreased the number of copulation events and reduced the number of female offspring.

## Conclusions

Summarizing the work presented clearly indicates that mating interactions afford a distinct advantage for biotype B when it is introduced into new geographic areas where non-B biotypes exist. Reitz and Trumble (2002) would categorize this under

"reproductive interference" because one species competes with the other for mates. In our case, the unique biology of biotype B males appears to provide an advantage in the competition for females of other biotypes. The mating behavior signals these males receive from, and perhaps send to females are unique among biotypes that have been studied to date. It would be interesting to conduct detailed mating behavior studies and determine the outcomes of those mating interactions with representative whiteflies in the 12 major genetic groups that have recently been proposed for the *B. tabaci* species complex (Boykin et al. 2007). This work would be an important contribution to the basic biological database that will become important as whitefly researchers continue to study the relationships among distinct groups.

**Acknowledgements** We thank Dr. Joseph Cicero (School of Plant Sciences, University of Arizona), Dr. Murad Ghanim (Institute of Plant Protection, Department of Entomology, The Volcani Center, Bet Dagan, Israel), and Dr. Rosemarie Rosell (Department of Biology, University of St. Thomas, Houston, Texas) for clarification on the structure and function of the alimentary canal in their respective models. This chapter is dedicated to the memory of Dr. Jackie Blackmer, a superb scientist and whitefly researcher and more importantly, a wonderful human being. She is missed.

## Literature Cited

Adams JB, McAllan JW. 1958. Pectinase in certain insects. *Can. J. Zool.* 36:305–308.

Ahman I, Ekbom BS. 1981. Sexual behavior of the greenhouse whitefly (*Trialeurodes vaporariorum*): orientation and courtship. *Entomol. Exp. Appl.* 29:330–338.

Azab AK, Megahed MM, El-Mirsawi HD. 1969. Effect of degree of pubescence of host-plant on the number and distribution of dorsal spines in pupa of *Bemisia tabaci* (Genn.). *Bull. Soc. Entomol. Égypte* 53:353–357.

Backus EA, Hunter WB, Arne CN. 1988. Technique for staining leafhopper (Homoptera: Cicadellidae) salivary sheaths and eggs within unsectioned plant tissue. *J. Econ. Entomol.* 81:1819–1823.

Backus EA, McLean DL. 1982. The sensory systems and feeding behavior of leafhoppers. I. The aster leafhopper, *Macrosteles fascifrons* Stål (Homoptera: Cicadellidae). *J. Morphol.* 172: 361–379.

Bedford ID, Markham PG, Brown JK, Rosell RC. 1994. Geminivirus transmission and biological characterization of whitefly (*Bemisia tabaci*) biotypes from different world regions. *Ann. Appl. Biol.* 125:311–325.

Bethke JA, Paine TD, Nuessly GS. 1991. Comparative biology, morphometrics, and development of two populations of *Bemisia tabaci* (Homoptera: Aleyrodidae) on cotton and poinsettia. *Ann. Entomol. Soc. Am.* 84:407–411.

Blackmer JL, Byrne DN. 1993a. Flight behaviour of *Bemisia tabaci* in a vertical flight chamber: effect of time of day, sex, age and host quality. *Physiol. Entomol.* 18:223–232.

Blackmer JL, Byrne DN. 1993b. Environmental and physiological factors influencing phototactic flight of *Bemisia tabaci*. *Physiol. Entomol.* 18:336–342.

Boykin LM, Shatters RG, Rosell RC, McKenzie CL, Bagnall RA, DeBarro P, Frohlich DR. 2007. Global relationships of *Bemisia tabaci* (Hemiptera: Aleyrodidae) revealed using Bayesian analysis of mitochondrial COI DNA sequences. *Mol. Phylogenet. Evol.* 44: 1306–1319.

Bradley RHE, Sylvester ES, Wade CV. 1962. Note on the movements of the mandibular and maxillary stylets of the aphid, *Myzus persicae* (Sulzer). *Can. Entomol.* 94:653–654.

Brown JK, Coats SA, Bedford ID, Markham PG, Bird J, Frohlich DR. 1995. Characterization and distribution of esterase electromorphs in the whitefly, *Bemisia tabaci* (Genn.) (Homoptera, Aleyrodidae). *Biochem. Gen.* 33:205–214.

Buckner JS, Freeman TP, Ruud RL, Chu CC, Henneberry TJ. 2002. Characterization and functions of the whitefly egg pedicel. *Arch. Insect Biochem. Physiol.* 49:22–33.

Büsgen M. 1891. Der honigtau, biologsiche studien an pflanzen und pflanzenläusen. *Jena Z. Naturwiss.* 25:340–428.

Butler CG. 1938. On the ecology of *Aleurodes brassicae* Walk. (Hemiptera). *Trans. R. Entomol. Soc. London* 87:291–311.

Butlin R. 1995. Genetic variation in mating signals and responses. In *Speciation and the Recognition Concept, Theory and Application*, ed. DM Lambert, HG Spencer, pp. 327–366. Baltimore, MD: The Johns Hopkins University Press.

Byrne DN, Bellows TS. 1991. Whitefly biology. *Annu. Rev. Entomol.* 36:431–457.

Byrne DN, Cohen AC, Draeger EA. 1990. Water uptake from plant tissue by the egg pedicel of the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae). *Can. J. Zool.* 68:1193–1195.

Byrne DN, Hadley NF. 1988. Particulate surface waxes of whiteflies: morphology, composition and waxing behaviour. *Physiol. Entomol.* 13:267–276.

Calatayud PA, Nardon C, Rahbé Y. 1996. A new technique to immobilize an aphid or a mealybug on plants using a high-frequency microcautery unit. *Entomol. Exp. Appl.* 80:239–241.

Campbell BC, Dreyer DL. 1985. Host-plant resistance of sorghum: differential hydrolysis of sorghum pectic substances by polysaccharases of greenbug biotypes (*Schizaphis graminum*, Homoptera: Aphididae). *Arch. Insect Biochem. Physiol.* 2:203–215.

Capoor SP. 1949. Feeding methods of the cotton white-fly. *Curr. Sci.* 18:82–83.

Castañé C, Savé R. 1993. Leaf osmotic potential decrease: a possible cause of mortality of greenhouse whitefly eggs. *Entomol. Exp. Appl.* 69:1–4.

Cherqui A, Tjallingii WF. 2000. Salivary proteins of aphids, a pilot study on identification, separation and immunolocalisation. *J. Insect Physiol.* 46:1177–1186.

Cicero JM, Brown JK. 2008. Squash leaf curl virus localizes in primary salivary gland compartments, and at midgut and filter chamber brush border cells in viruliferous *Bemisia tabaci*. *J. Insect Sci.* 8:4. http://www.insectscience.org/8.04

Cicero JM, Hiebert E, Webb SE. 1995. The alimentary canal of *Bemisia tabaci* and *Trialeurodes abutilonea* (Homoptera, Sternorrhynchi): histology, ultrastructure and correlations to function. *Zoomorphology* 115:31–39.

Cohen AC, Chu CC, Henneberry TJ, Freeman T, Buckner J, Nelson D. 1996a. Cotton leaf surface features serve as behavioral cues to silverleaf whiteflies. *Southwest. Entomol.* 21: 377–385.

Cohen AC, Chu CC, Henneberry TJ, Freeman T, Nelson D, Buckner J, Margosan D, Vail P, Aung LH. 1998. Feeding biology of the silverleaf whitefly (Homoptera: Aleyrodidae). *Chinese J. Entomol.* 18:65–81.

Cohen A, Hendrix D. 1993. Demonstration and preliminary characterization of α-amylase in sweetpotato whiteflies, *Bemisia tabaci* (Aleyrodidae: Homoptera). In *Proceedings Beltwide Cotton Production Research Conferences*, ed. DJ Herber, DA Richter, pp. 955–958. Memphis, TN: National Cotton Council.

Cohen AC, Hendrix DL. 1994. Demonstration and preliminary characterization of α-amylase in the sweetpotato whitefly, *Bemisia tabaci* (Aleyrodidae: Homoptera). *Comp. Biochem. Physiol.* 109B:593–601.

Cohen AC, Henneberry TJ, Chu CC. 1996b. Geometric relationships between whitefly feeding behavior and vascular bundle arrangements. *Entomol. Exp. Appl.* 78:135–142.

Cohen A, Henneberry TJ, Freeman T, Margosan D, Vail P, Chu CC. 1996c. Microstructure of feeding in nymphal *Bemisia argentifolii* in cotton and cantaloupe. In *Proceedings Beltwide Cotton Production Research Conferences*, ed. P Dugger, DA Richter, pp. 740–743. Memphis, TN: National Cotton Council.

Collar JL, Avilla C, Fereres A. 1997. New correlations between aphid stylet paths and nonpersistent virus transmission. *Environ. Entomol.* 26:537–544.

Coombe PE. 1982. Visual behaviour of the greenhouse whitefly, *Trialeurodes vaporariorum*. *Physiol. Entomol.* 7:243–251.

Costa HS, Brown JK, Sivasupramaniam S, Bird J. 1993. Regional distribution, insecticide resistance, and reciprocal crosses between the 'A' and 'B' biotypes of *Bemisia tabaci*. *Insect Sci. Appl.* 14:127–138.

Costa HS, Toscano NC, Hendrix DL, Henneberry TJ. 1999. Patterns of honeydew droplet production by nymphal stages of *Bemisia argentifolii* (Homoptera: Aleyrodidae) and relative composition of honeydew sugars. *J. Entomol. Sci.* 34:305–313.

Costa HS, Toscano NC, Henneberry TJ. 1996. Mycetocyte inclusion in the oocytes of *Bemisia argentifolii* (Homoptera: Aleyrodidae). *Ann. Entomol. Soc. Am.* 89:694–699.

DeBarro PJ, Hart PJ. 2000. Mating interactions between two biotypes of the whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Australia. *Bull. Entomol. Res.* 90:103–112.

Domenichini G. 1981. Strutture di *Trialeurodes vaporariorum* (Westw.) e loro funzioni. *Mem. Soc. Ent. Ital. Genova* 60:169–176.

Eleftheriou EP. 1990. Monocotyledons. In *Sieve Elements: Comparative Structure, Induction and Development*, ed. H-D Behnke, RD Sjolund, pp. 139–159. Berlin: Springer-Verlag.

Eschrich W. 1975. Sealing systems in phloem. In *Encyclopedia of Plant Physiology, New Series Vol. 1. Transport in Plants I. Phloem Transport*, ed. MH Zimmermann, JA Milburn, pp. 39–56. Berlin: Springer-Verlag.

Evert RF. 1990. Dicotyledons. In *Sieve Elements: Comparative Structure, Induction and Development*, ed. H-D Behnke, RD Sjolund, pp. 103–137. Berlin: Springer-Verlag.

Evert RF, Eschrich W, Eichhorn SE, Limbach ST. 1973. Observations on penetration of barley leaves by the aphid *Rhopalosiphum maidis* (Fitch). *Protoplasma* 77:95–110.

Forbes AR. 1972. Innervation of the stylets of the pear psylla, *Psylla pyricola* (Homoptera: Psyllidae), and the greenhouse whitefly, *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). *J. Entomol. Soc. British Columbia* 69:27–30.

Freeman TP, Buckner JS, Nelson DR, Chu CC, Henneberry TJ. 2001. Stylet penetration by *Bemisia argentifolii* (Homoptera: Aleyrodidae) into host leaf tissue. *Ann. Entomol. Soc. Am.* 94:761–768.

Funk CJ. 2001. Alkaline phosphatase activity in whitefly salivary glands and saliva. *Arch. Insect Biochem. Physiol.* 46:165–174.

Gelman DB, Blackburn MB, Hu JS. 2005. Identification of the molting hormone of the sweet potato (*Bemisia tabaci*) and greenhouse (*Trialeurodes vaporariorum*) whitefly. *J. Insect Physiol.* 51:47–53.

Gelman DB, Blackburn MB, Hu JS, Gerling D. 2002. The nymphal-adult molt of the silverleaf whitefly (*Bemisia argentifolii*): timing, regulation, and progress. *Arch. Insect Biochem. Physiol.* 51:67–79.

Gelman DB, Gerling D. 2003. Host plant pubescence: effect on silverleaf whitefly, *Bemisia argentifolii*, fourth instar and pharate adult dimensions and ecdysteroid titer fluctuations. *J. Insect Sci.* 3:1–8.

Gerling D. 1990. *Whiteflies: Their Bionomics, Pest Status and Management*. Andover, Hants: Intercept Ltd.

Gerling D, Mayer RT. (ed.) 1996. *Bemisia 1995: Taxonomy, Biology, Damage Control and Management*. Andover, Hants: Intercept Ltd.

Ghanim M, Rosell RC, Campbell LR, Czosnek H, Brown JK, Ullman DE. 2001. Digestive, salivary, and reproductive organs of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) B Type. *J. Morphol.* 248:22–40.

Gildow FE, Rochow WF. 1980. Role of accessory salivary glands in aphid transmission of *Barley yellow dwarf virus*. *Virology* 104:97–108.

Gill RJ. 1990. The morphology of whiteflies. In *Whiteflies: Their Bionomics, Pest Status and Management*, ed. D Gerling, pp. 13–46. Andover, Hants: Intercept Ltd.

Girousse C, Bonnemain J-L, Delrot S, Bournoville R. 1991. Sugar and amino acid composition of phloem sap of *Medicago sativa*: a comparative study of two collecting methods. *Plant Physiol. Biochem.* 29:41–48.

Goodchild AJP. 1966. Evolution of the alimentary canal in the Hemiptera. *Biol. Rev.* 41:97–140.

Gray S, Gildow FE. 2003. Luteovirus-aphid interactions. *Annu. Rev. Phytopathol.* 41: 539–566.

Guershon M, Gerling D. 2001. Effect of foliar tomentosity on phenotypic plasticity in *Bemisia tabaci* (Hom., Aleyrodidae). *J. Appl. Entomol.* 125:449–453.

Harris KF, Esbroeck ZP-V, Duffus JE. 1996a. Anatomy of a virus vector. In *Bemisia 1995: Taxonomy, Biology, Damage Control and Management*, ed. D Gerling, RT Mayer, pp. 289–318. Andover, Hants: Intercept Ltd.

Harris KF, Esbroeck ZP-V, Duffus JE. 1996b. Morphology of the sweet potato whitefly, *Bemisia tabaci* (Homoptera, Aleyrodidae) relative to virus transmission. *Zoomorphology* 116:143–156.

Heriot AD. 1934. The renewal and replacement of the stylets of sucking insects during each stadium, and the method of penetration. *Can. J. Res.* 11:602–612.

Hunter WB, Hiebert E, Webb SE, Polston JE, Tsai JH. 1996. Precibarial and cibarial chemosensilla in the whitefly, *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae). *Int. J. Insect Morph. Embryol.* 25:295–304.

Isaacs R, Cahill M, Byrne DN. 1999. Host plant evaluation behaviour of *Bemisia tabaci* and its modification by external or internal uptake of imidacloprid. *Physiol. Entomol.* 24:101–108.

Janssen JAM, Tjallingii WF, van Lenteren JC. 1989. Electrical recording and ultrastructure of stylet penetration by the greenhouse whitefly. *Entomol. Exp. Appl.* 52:69–81.

Jiang YX, de Blas C, Barrios L, Fereres A. 2000. Correlation between whitefly (Homoptera: Aleyrodidae) feeding behavior and transmission of tomato yellow leaf curl virus. *Ann. Entomol. Soc. Am.* 93:573–579.

Jiang YX, Lei H, Collar JL, Martin B, Muñiz M, Fereres A. 1999. Probing and feeding behavior of two distinct biotypes of *Bemisia tabaci* (Homoptera: Aleyrodidae) on tomato plants. *J. Econ. Entomol.* 92:357–366.

Jiang YX, Nombela G, Muñiz M. 2001. Analysis by DC-EPG of the resistance to *Bemisia tabaci* on an *Mi*-tomato line. *Entomol. Exp. Appl.* 99:295–302.

Jiang YX, Walker GP. 2001. Pathway phase waveform characteristics correlated with length and rate of stylet advancement and partial stylet withdrawal in AC electrical penetration graphs of adult whiteflies. *Entomol. Exp. Appl.* 101:233–246.

Jiang YX, Walker GP. 2003. Electrical penetration graphs of the nymphal stage of *Bemisia argentifolii*. *Entomol. Exp. Appl.* 109:101–111.

Jiang YX, Walker GP. 2007. Identification of phloem sieve elements as the site of resistance to silverleaf whitefly in resistant alfalfa genotypes. *Entomol. Exp. Appl.* 125:307–320.

Johnson DD, Walker GP. 1999. Intracellular punctures by the adult whitefly *Bemisia argentifolii* on DC and AC electronic feeding monitors. *Entomol. Exp. Appl.* 92:257–270.

Kanmiya K. 1996. Discovery of male acoustic signals in the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae). *Appl. Entomol. Zool.* 31:255–262.

Kanmiya K. 2006. Mating behavior and vibratory signals in whiteflies (Hemiptera: Aleyrodidae). Mating behavior and vibratory signals in whiteflies (Hemiptera: Aleyrodidae). In *Insect Sounds and Communication: Physiology, Behaviour, Ecology and Evolution*, ed. S Drosopoulos, MF Claridge, pp. 365–376. Boca Raton, FL: CRC Press, Taylor and Francis Group.

Kimmins FM. 1986. Ultrastructure of the stylet pathway of *Brevicoryne brassicae* in host plant tissue, *Brassica oleracea*. *Entomol. Exp. Appl.* 41:283–290.

Kimmins FM, Tjallingii WF. 1985. Ultrastructure of sieve element penetration by aphid stylets during electrical recording. *Entomol. Exp. Appl.* 39:135–141.

Kingsolver JG, Daniel TL. 1995. Mechanics of food handling by fluid-feeding insects. In *Regulatory Mechanisms in Insect Feeding*, ed. RF Chapman, G deBoer, pp. 32–73. New York: Chapman and Hall.

Knoblauch M, van Bel AJE. 1998. Sieve tubes in action. *Plant Cell* 10:35–50.

Las A. 1979. Male courtship persistence in the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae). *Behavior* 72:107–125.

Lei H, Tjallingii WF, van Lenteren JC. 1996a. Recording electrical penetration graphs and honeydew excretion of the greenhouse whitefly. In *Bemisia 1995: Taxonomy, Biology, Damage Control and Management*, ed. D Gerling, RT Mayer, pp. 53–68. Andover, Hants: Intercept Ltd.

Lei H, Tjallingii WF, van Lenteren JC. 1997. Effect of tethering during EPG recorded probing by adults of the greenhouse whitefly. *J. Appl. Entomol.* 121:211–217.

Lei H, Tjallingii WF, van Lenteren JC. 1998. Probing and feeding characteristics of the greenhouse whitefly in association with host-plant acceptance and whitefly strains. *Entomol. Exp. Appl.* 88:73–80.

Lei H, Tjallingii WF, van Lenteren JC, Xu RM. 1996b. Stylet penetration by larvae of the greenhouse whitefly on cucumber. *Entomol. Exp. Appl.* 79:77–84.

Lei H, van Lenteren JC, Tjallingii WF. 1999. Analysis of resistance in tomato and sweet pepper against the greenhouse whitefly using electronically monitored and visually observed probing and feeding behaviour. *Entomol. Exp. Appl.* 92:299–309.

Lei H, van Lenteren JC, Xu RM. 2001. Effects of plant tissue factors on the acceptance of four greenhouse vegetable host plants by the greenhouse whitefly: an electrical penetration graph (EPG) study. *Eur. J. Entomol.* 98:31–36.

Li TY, Maschwitz U. 1985. Sexual behavior in the whitefly *Trialeurodes vaporariorum* Westw. *Acta. Entomol. Sinica* 28:233–236.

Li TY, Vinson SB, Gerling D. 1989. Courtship and mating behavior of *Bemisia tabaci* (Homoptera: Aleyrodidae). *Environ. Entomol.* 18:800–806.

Liu HY, Cohen S, Duffus JE. 1992. The use of isozyme patterns to distinguish sweetpotato whitefly (*Bemisia tabaci*) biotypes. *Phytoparasitica* 20:187–194.

Liu SS, DeBarro PJ, Luan JB, Zang LS, Ruan YM, Wan FH. 2007. Asymmetric mating interactions drive widespread invasion and displacement in a whitefly. *Science* 318:1769–1772.

Lopez-Abella D, Bradley RHE, Harris KF. 1988. Correlation between stylet paths made during superficial probing and the ability of aphids to transmit nonpersistent viruses. *Adv. Dis. Vector Res.* 5:251–285.

MacDowall FDH. 1972. Phototactic action spectrum for whitefly and the question of colour vision. *Can. Entomol.* 104:299–307.

Martín B, Collar JL, Tjallingii WF, Fereres A. 1997. Intracellular ingestion and salivation by aphids may cause the acquisition and inoculation of non-persistently transmitted plant viruses. *J. Gen. Virol.* 78:2701–2705.

Maruthi MN, Colvin J, Thwaites RM, Banks GK, Gibson G, Seal SE. 2004. Reproductive incompatibility and cytochrome oxidase I gene sequence variability amongst host-adapted and geographically separate *Bemisia tabaci* populations (Hemiptera: Aleyrodidae). *Syst. Entomol.* 29:560–568.

Matthews RW, Matthews JR. 1978. *Insect Behavior*. New York: John Wiley & Sons.

McAllan JW, Adams JB. 1961. The significance of pectinase in plant penetration by aphids. *Can. J. Zool.* 39:305–310.

McKenzie CL, Anderson PK, Villarreal N. 2004. An extensive survey of *Bemisia tabaci* (Homoptera: Aleyrodidae) in agricultural ecosystems in Florida. *Fla. Entomol.* 87:403–407.

McLean DL, Kinsey MG. 1967. Probing behavior of the pea aphid, *Acyrthosiphon pisum*. I. Definitive correlation of electronically recorded waveforms with aphid probing activities. *Ann. Entomol. Soc. Am.* 60:400–406.

Medina V, Pinner MS, Bedford ID, Achon MA, Gemeno C, Markham PG. 2006. Immunolocalization of *Tomato yellow leaf curl Sardinia virus* in natural host plants and its vector *Bemisia tabaci*. *J. Plant Pathol.* 88:297–306.

Mellor HE, Anderson M. 1995a. Antennal sensilla of whiteflies: *Trialeurodes vaporariorum* (Westwood), the glasshouse whitefly, *Aleyrodes proletella* (Linnaeus), the cabbage whitefly, and *Bemisia tabaci* (Gennadius), the tobacco whitefly (Homoptera: Aleyrodidae). Part 1: External morphology. *Int. J. Insect Morph. Embryol.* 24:133–143.

Mellor HE, Anderson M. 1995b. Antennal sensilla of whiteflies: *Trialeurodes vaporariorum* (Westwood), the glasshouse whitefly, and *Aleyrodes proletella* (Linnaeus), the cabbage whitefly, (Homoptera: Aleyrodidae). Part 2. Ultrastructure. *Int. J. Insect Morph. Embryol.* 24:145–160.

Mellor HE, Bellingham J, Anderson M. 1997. Spectral efficiency of the glasshouse whitefly *Trialeurodes vaporariorum* and *Encarsia formosa* its hymenopteran parasitoid. *Entomol. Exp. Appl.* 83:11–20.

Miles PW. 1968. Insect secretions in plants. *Annu. Rev. Phytopathol.* 6:137–164.

Miles PW. 1972. The saliva of Hemiptera. *Adv. Insect Physiol.* 9:183–255.

Miles PW. 1999. Aphid saliva. *Biol. Rev.* 74:41–85.

Miles PW, Peng Z. 1989. Studies on the salivary physiology of plant bugs: detoxification of phytochemicals by the salivary peroxidase of aphids. *J. Insect Physiol.* 35:865–872.

Moericke VV, Schneiders H, Vogt B. 1966. Flughemmung und fallreflexhaltung als reaktion auf gelbreiz bei *Trialeurodes vaporariorum* (Westwood). *Z. Pflanzenkr. Pflanzenschutz* 73:6–14.

Mound LA. 1962. Studies on the olfaction and colour sensitivity of *Bemisia tabaci* (Genn.) (Homoptera, Aleyrodidae). *Entomol. Exp. Appl.* 5:99–104.

Nault LR. 1997. Arthropod transmission of plant viruses: a new synthesis. *Ann. Entomol. Soc. Am.* 90:521–541.

Nault LR, Gyrisco GG. 1966. Relation of the feeding process of the pea aphid to the inoculation of pea enation mosaic virus. *Ann. Entomol. Soc. Am.* 59:1185–1197.

Neal JW, Bentz J. 1999. Evidence for the stage inducing phenotypic plasticity in pupae of the polyphagous whiteflies *Trialeurodes vaporariorum* and *Bemisia argentifolii* (Homoptera: Aleyrodidae) and the *raison d'être*. *Ann. Entomol. Soc. Am.* 92:774–787.

Pascual S. 2006. Mechanisms in competition, under laboratory conditions, between Spanish biotypes B and Q of *Bemisia tabaci* (Gennadius). *Span. J. Agric. Res.* 4:351–354.

Pascual S, Callejas C. 2004. Intra- and interspecific competition between biotypes B and Q of *Bemisia tabaci* (Hemiptera: Aleyrodidae) from Spain. *Bull. Entomol. Res.* 94:369–375.

Paulson GS, Beardsley JW. 1985. Whitefly (Hemiptera: Aleyrodidae) egg pedicel insertion into host plant stomata. *Ann. Entomol. Soc. Am.* 78:506–508.

Perez P, Tjallingii WF, Fereres A. 1996. Probing behaviour of *Myzus persicae* during transmission of potato virus Y to pepper and tobacco plants. *Z. Pflanzenkr. Pflanzenschutz* 103:246–254.

Perring TM. 1996. Biological differences of two species of *Bemisia* that contribute to adaptive advantage. In *Bemisia 1995: Taxonomy, Biology, Damage Control and Management*, ed. D Gerling, RT Mayer, pp. 3–16. Andover, Hants: Intercept Ltd.

Perring TM, Cooper AD, Rodriguez RJ, Farrar CA, Bellows TS. 1993. Identification of a whitefly species by genomic and behavioral studies. *Science* 259:74–77.

Perring TM, Symmes EJ. 2006. Courtship behavior of *Bemisia argentifolii* (Hemiptera: Aleyrodidae) and whitefly mate recognition. *J. Econ. Entomol.* 99:598–606.

Pollard DG. 1955. Feeding habits of the cotton whitefly, *Bemisia tabaci* Genn. (Homoptera: Aleyrodidae). *Ann. Appl. Biol.* 43:664–671.

Pope RD. 1983. Some aphid waxes, their form and function (Homoptera: Aphididae). *J. Nat. Hist.* 17:489–506.

Powell G. 1991. Cell membrane punctures during epidermal penetrations by aphids: consequences for the transmission of two potyviruses. *Ann. Appl. Biol.* 119:313–321.

Powell G. 2005. Intracellular salivation is the aphid activity associated with inoculation of non-persistently transmitted viruses. *J. Gen. Virol.* 86:469–472.

Powell G, Pirone T, Hardie J. 1995. Aphid stylet activities during potyvirus acquisition from plants and an in vitro system that correlate with subsequent transmission. *Eur. J. Plant Pathol.* 101:411–420.

Prado E, Tjallingii WF. 1994. Aphid activities during sieve element punctures. *Entomol. Exp. Appl.* 72:157–165.

Price JF, Taborsky D. 1992. Movement of immature *Bemisia tabaci* (Homoptera: Aleyrodidae) on poinsettia leaves. *Florida Entomol.* 75:151–153.

Raven JA. 1983. Phytophages of xylem and phloem: a comparison of animal and plant sap-feeders. *Adv. Ecol. Res.* 13:135–234.

Reitz SR, Trumble JT. 2002. Competitive displacement among insects and arachnids. *Annu. Rev. Entomol.* 47:435–465.

Rekha AR, Maruthi MN, Muniyappa V, Colvin J. 2005. Occurrence of three genotypic clusters of *Bemisia tabaci* and the rapid spread of the B biotype in south India. *Entomol. Exp. Appl.* 117:221–233.

Rosell RC, Lichty JE, Brown JK. 1995. Ultrastructure of the mouthparts of adult sweetpotato whitefly, *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae). *Int. J. Insect Morph. Embryol.* 24:297–306.

Sauge M-H, Kervella J, Rahbé Y. 1998. Probing behaviour of the green peach aphid *Myzus persicae* on resistant *Prunus* genotypes. *Entomol. Exp. Appl.* 89:223–232.

Shukle RH, Quiroz C. 1994. Use of the electronic monitor for the study of aphids as vectors of barley yellow dwarf virus. In *History, Development, and Application of AC Electronic Insect Feeding Monitors*, ed. MM Ellsbury, EA Backus, DL Ullman, pp. 106–121. Lanham, MD: Thomas Say Publications in Entomology, Entomological Society of America.

Spiller NJ, Kimmins FM, Llewellyn M. 1985. Fine structure of aphid stylet pathways and its use in host plant resistance studies. *Entomol. Exp. Appl.* 38:293–295.

Spiller NJ, Koenders L, Tjallingii WF. 1990. Xylem ingestion by aphids – a strategy for maintaining water balance. *Entomol. Exp. Appl.* 55:101–104.

Summers CG, Newton AS, Estrada D. 1996. Intraplant and interplant movement of *Bemisia argentifolii* (Homoptera: Aleyrodidae) crawlers. *Environ. Entomol.* 25:1360–1364.

Tjallingii WF. 1978. Mechanoreceptors of the aphid labium. *Entomol. Exp. Appl.* 24:731–737.

Tjallingii WF. 1985. Membrane potentials as an indication for plant cell penetration by aphid stylets. *Entomol. Exp. Appl.* 38:187–193.

Tjallingii WF. 1994. Sieve element acceptance by aphids. *Eur. J. Entomol.* 91:47–52.

Tjallingii WF. 1995. Regulation of phloem sap feeding by aphids. In *Regulatory Mechanisms in Insect Feeding*, ed. RF Chapman, G de Boer, pp. 190–209. New York: Chapman and Hall.

Tjallingii WF. 2006. Salivary secretions by aphids interacting with proteins of phloem wound responses. *J. Exp. Bot.* 57:739–745.

Tjallingii WF, Gabrys B. 1999. Anomalous stylet punctures of phloem sieve elements by aphids. *Entomol. Exp. Appl.* 91:97–103.

Tjallingii WF, Hogen Esch Th. 1993. Fine structure of aphid stylet routes in plant tissues in correlation with EPG signals. *Physiol. Entomol.* 18:317–328.

Tremblay E. 1959. Osservazioni sulla simbiosi endocellulare di alcuni Aleyrodidae (*Bemisia tabaci* Gennad., *Aleurolobus olivinus* Silv., *Trialeurodes vaporariorum* West.). *Bollet. Lab. Entomol. Agraria Filippo Silvestri Portici* 17:210–246.

Urbanska A, Tjallingii WF, Dixon AF, Leszczynski B. 1998. Phenol oxidising enzymes in the grain aphid's saliva. *Entomol. Exp. Appl.* 86:197–203.

Vaishampayan SM, Waldbauer GP, Kogan M. 1975. Visual and olfactory responses in orientation to plants by the greenhouse whitefly, *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). *Entomol. Exp. Appl.* 18:412–422.

van Helden M, Tjallingii WF. 1993. Tissue localization of lettuce resistance to the aphid *Nasonovia ribisnigri* using electrical penetration graphs. *Entomol. Exp. Appl.* 68:269–278.

van Helden M, Tjallingii WF. 1994. Phloem sap collection from lettuce (*Lactuca sativa* L.): Chemical comparison among collection methods. *J. Chem. Ecol.* 20:3191–3206.

van Lenteren JC, Noldus LPJJ. 1990. Whitefly-plant relationships: behavioural and ecological aspects. In *Whiteflies: Their Bionomics, Pest Status and Management*, ed. D Gerling, pp. 47–89. Andover, Hants: Intercept Ltd.

Walker GP. 1985. Stylet penetration by the bayberry whitefly, as affected by leaf age in lemon, *Citrus limon*. *Entomol. Exp. Appl.* 39:115–121.

Walker GP, Gordh G. 1989. The occurrence of apical labial sensilla in the Aleyrodidae and evidence for a contact chemosensory function. *Entomol. Exp. Appl.* 51:215–224.

Walker GP, Perring TM. 1994. Feeding and oviposition behavior of whiteflies (Homoptera: Aleyrodidae) interpreted from AC electronic feeding monitor waveforms. *Ann. Entomol. Soc. Am.* 87:363–374.

Weber H. 1931. Lebensweise und umweltbeziehungen von *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleurodina). *Z. Morphol. Ökol. Tierre* 23:575–753.

Weber H. 1934. Die postembryonale entwicklung der aleurodinen (Hemiptera-Homoptera). Ein beitrag zur kenntnis der metamorphosen der insekten. *Z. Morphol. Ökol. Tierre* 29:268–305.

Weber H. 1935. Der Bau der Imago der Aleurodinen. Ein Beitrag zur vergleichenden Morphologie des Insektenkörpers. *Zoologica*, Heft 89, Stuttgart. English translation (The anatomy of adult aleyrodids), ed. DL Hendrix, RJ Gill, EW Davidson. Stuttgart: E. Schweizerbart'sche Verlagsbuchhandlung (Nägele u. Obermiller). 1995.

Wensler RJD. 1974. Sensory innervation monitoring movement and position in the mandibular stylets of the aphid *Brevicoryne brassicae*. *J. Morphol.* 143:349–364.

Wilkinson TL, Douglas AE. 1998. Plant penetration by pea aphids (*Acyrthosiphon pisum*) of different plant range. *Entomol. Exp. Appl.* 87:43–50.

Will T, Tjallingii WF, Thönnessen A, van Bel AJE. 2007. Molecular sabotage of plant defense by aphid saliva. *Proc. Natl. Acad. Sci.* 104:10536–10541.

Zang LS, Liu SS. 2007. A comparative study on mating behaviour between the B biotype and non-B biotype of *Bemisia tabaci* (Hemiptera: Aleyrodidae) from Zhejiang, China. *J. Insect Behav.* 20:157–171.

Ziegler H. 1975. Nature of transported substances. In *Encyclopedia of Plant Physiology, New Series Vol. 1. Transport in Plants I. Phloem Transport*, ed. MH Zimmermann, JA Milburn, pp. 59–100. Berlin: Springer-Verlag.

# Chapter 5
# Mutualistic and Dependent Relationships with Other Organisms

**Rosemarie C. Rosell, Jacquelyn L. Blackmer, Henryk Czosnek, and Moshe Inbar**

## Introduction

Whiteflies in general – and *Bemisia tabaci* (Gennadius) in particular – are involved in complex interactions with the host plant, various microorganisms and arthropods (herbivores and natural enemies). These relationships are not only important to the ecology and evolution of *B. tabaci* but are also essential to understanding and developing innovative control strategies. Thus, we have included in this chapter a discussion of symbiotic relationships, the functional roles of microbial symbionts in whiteflies, the role of endosymbionts in begomovirus transmission, and the intra- and interspecific relationships that occur between whiteflies, other herbivores, and their host plants. Much new information has been generated since the previous *Bemisia* books (Gerling 1990; Gerling and Mayer 1996); thus, we have focused this work on new findings and the potential they hold for developing new control tactics.

## Symbiotic Relationships

Much has changed since Hooke's (1665) observations of the symbiotic organ in the human louse, and the initial findings of Blochmann (1884, 1888) and Šulc (1909) on symbiotic associations in cockroaches and cicadids, respectively. The term "symbiosis" was originally defined as the intimate "living together" of two distinct species (De Bary 1879). These interactions have since been classified as mutualistic (beneficial), parasitic (harmful) or commensal (neutral). However,

R.C. Rosell (✉)
Department of Biology, University of St. Thomas, Houston, TX 77006, USA
e-mail: rrosell@stthom.edu

Authorship of main chapter sections
Rosell and Blackmer: Symbiotic Relationships
Czosnek: Role of whitefly endosymbionts in virus transmission
Inbar: Whiteflies engaged with intra- and interspecific interactions

P.A. Stansly, S.E. Naranjo (eds.), *Bemisia*: Bionomics and Management of a Global Pest,
DOI 10.1007/978-90-481-2460-2_5, 

distinguishing types of symbioses can be difficult due to the complex and changing interactions between the symbiont and the host. Regardless of their nature, symbiotic relationships are now known to be major drivers of diversity and evolutionary novelty (Moran and Telang 1998; O'Neill et al. 1997; Hunter et al. 2003; Wernegreen et al. 2003).

Microbial symbionts have been shown to be extremely widespread in nature and particularly prevalent in arthropods. Endosymbiosis refers to the intimate cohabitation of two partners, where the one is taken up into the body of the other more highly organized partner. These associations between insect hosts and bacteria range from obligate mutualism to reproductive parasitism (Buchner 1965; Ishikawa 2003; O'Neill et al. 1997; Wernegreen et al. 2003). These partnerships may be obligate for the host, the symbiont, both or neither. In fact, it is rarely clearly defined as to how beneficial a mutualism is to both partners in the relationship. Even though the advantage to the insect host is easily discerned (usually nutritional), the benefit to the microbe is not as apparent.

Reduction or elimination of the endosymbiotic organisms from insects by antibiotic therapy, heat, or lysozyme treatments results in reduced insect growth, death, or lack of reproduction (Mittler 1971; Sinha and Peterson 1972; Costa et al. 1993b, 1997). Recent studies have shown a positive relationship between elimination of some bacteria and development and surviorship (Ruan et al. 2006). However, it has been suggested that genetic changes in insect symbiotic organisms may play a functional role in the development of insect biotypes, which may result in pesticide resistance or in the ability of an insect biotype to utilize a new host species (Nardon and Grenier 1991; Campbell 1989, 1993; Nardon and Nardon 1998). Other symbiotic relationships have been shown to be protective in that the symbionts or symbiont-associated bacteriophages produce protective toxins (Moran et al. 2005a).

## *Bacteriocyte Associated Endosymbionts*

A central tenet put forth by Buchner (1965) is that symbiosis in animals is driven by the nutritional needs of the host and is therefore common in insects that feed on restrictive diets. This hypothesis has been supported over the years; some of the most remarkable symbiotic systems are found in the Hemiptera, especially in the Auchenorrhyncha and Sternorrhyncha suborders (see review Baumann 2005). Whiteflies, members of the Sternorrhyncha that feed exclusively on phloem sap, harbor bacterial endosymbionts within specialized insect cells, which aggregate in the abdominal cavity to form a specialized organ (Costa et al. 1993b, 1995; Szklarzewicz and Moskal 2001; Zchori-Fein and Brown 2002; Thao and Baumann 2004a). The insect cells (referred to as mycetocytes in literature before 2000) are now called bacteriocytes. Similiarly, the organs, or bacteriomes, which house those bacteriocytes, were previously referred to as mycetomes.

Most of what we know comes from studies on various aphid and sharpshooter species; considerably less is known about the endosymbionts of their close relatives,

the whiteflies. Consequently, some of what is presented here relies heavily on the findings for aphids and sharpshooters, which may or may not be analogous to what is occurring in whiteflies.

## *Types of Endosymbiotic Relationships*

The bacteria found within insects are divided into two general types. Primary endosymbionts (P-endosymbionts) are defined as being present in all host species individuals, vertically transmitted and essential for the survival of the host (Koch 1967; Houk and Griffiths 1980; Baumann et al. 1995, 1997; Baumann 2005). In addition, phylogenetic trees based on P-endosymbiont and host genes are congruent in most insects; thus these relationships appear to be the result of a single ancient infection of a direct ancestor followed by co-speciation of the insect host (Baumann et al. 1993; Baumann et al. 1997; Moran and Baumann 2000; Moran et al. 2005b). Most insect P-endosymbionts have been shown to have static, reduced genomes (Moran 1996; Wernegreen et al. 2000; Moran and Mira 2001; Moran et al. 2003; van Ham et al. 2004), which has led to the hypothesis that these associations appear to be a domestication of the endosymbiont by the host and probably applies to all ancient endosymbiotic associations (Baumann 2005). It has been proposed for aphids that this genome reduction resulted from large deletions and chromosomal rearrangements, which occurred early in the establishment of the endosymbiotic relationship (Moran and Mira 2001; Silva et al. 2001).

P-endosymbionts, and a number of secondary symbionts, are housed in the bacteriocytes, clustered together to form the bacteriome (reviewed in Baumann 2005). The obligate relationship between the insect host and P-endosymbiont is so intimate that it appears that one cannot survive without the other. Survival is perpetuated by the transfer of the P-endosymbionts to subsequent generations through vertical transmission from mother to offspring (Buchner 1965; Hinde 1971, Costa et al. 1996; Wilkinson et al. 2003).

The P-endosymbionts of many insects belong to the $\gamma$ subdivision of the Proteobacteria; however, the primary endosymbionts of whiteflies evolved from a lineage distinct from other Hemiptera including those of aphids (Clark et al. 1992; Campbell 1993; Costa et al. 1993b; von Dohlen and Moran 1995; Lerat et al. 2003). The primary endosymbionts are *Buchnera aphidicola* (Munson et al. 1991) in aphids and *Candidatus* Portiera aleyrodidarum (Thao and Baumann 2004a) in whiteflies. It has been hypothesized that the pleomorphic bacteria that lack cell walls and are present in whitefly bacteriocytes are the P-endosymbionts (Thao and Baumann 2004a; Costa et al. 1993b, 1995). The P-endosymbiont phylogeny of whiteflies has been reconstructed based on either 16S rDNA or 16S–23S rDNA; results support separation of P-endosymbionts into two subfamilies as well as congruency between the whitefly host and the endosymbionts (Thao and Baumann 2004a; Rosell, Frohlich and DeBarro unpublished). However, phlyogenetic analyses of the P-endosymbionts (16S rDNA) of many closely related *B. tabaci* biotypes

(based on mtCO1 DNA) did not show a strict congruence, leading Zchori-Fein and Brown (2002) to hypothesize that different *B. tabaci* biotypes may have different complements of P-endosymbionts. More analyses are needed to clarify these points.

In most Hemiptera, these P-endosymbionts are absolutely essential to the survival of the insect; it has been shown that one of their major roles is the provisioning of essential amino acids that are unbalanced or in short supply in their phloem-sap diet (Buchner 1965; Lai et al. 1994; Liadouze et al. 1995; Sasaki and Ishikawa 1995; Moran et al. 2005b; Douglas 2006). Similarly, a related type of association has been identified in the glassy-winged sharpshooter (*Homalodisca coagulata,* now *vitripennis,* see Takiya et al. 2006). Two bacteria, *Bacteriodetes* species, *Sulcia muelleri,* and the γ-proteobacteria*, Baumannia cicadellinicola*, appear to be co-evolving with the sharpshooter. These endosymbionts occupy the same bacteriome, have the same characteristics as P-endosymbionts and, thus, are classified as *co-primary* endosymbionts. They are both required for host survival, vertically transmitted, and appear to be phylogenetically convergent with their host (Takiya et al. 2006). Using DNA sequencing, Wu et al. (2006) have shown that there is functional dependency between the two unrelated bacterial species, in that *S. muelleri* provides the essential amino acids needed by the sharpshooter, while *B. cicadellinicola* is responsible for the biosynthesis of vitamins and cofactors lacking in the host.

Insects house other morphologically diverse bacteria that do not appear to perform essential regulatory functions in insects. These are designated as secondary endosymbionts (S-endosymbionts) if located in the bacteriome, or as secondary symbionts (S-symbionts) if the location is unknown (Baumann 2005). Most phylogenetic trees show no similarity between S-endosymbiont and host genes; therefore, these prokaryotes have probably been acquired more recently and/or acquired by horizontal transmission (O'Neill et al. 1993; Fukatsu and Nikoh 2000; Fukatsu et al. 2000; Thao et al. 2003; Thao and Baumann 2004b; Baumann 2005). These associations are less fixed than those between the host and P-endosymbionts. They may act as "portals for the import of new genes that affect the host ecology" and thus have a more dynamic genome content and subject the host to greater evolutionary forces (Moran 2005; Moran and Degnan 2006).

In an examination of the ultrastructure of the bacteriocytes and bacteriomes of *B. tabaci* and *Trialeurodes vaporariorum*, Costa et al. (1993b) described two morphologically distinct types of microorganisms. The predominant type was pleomorphic (designated P, later determined to be the primary symbiont) and similar in both species of whiteflies. Coccoid microorganisms were also described (designated C, later determined to be secondary endosymbionts), which were morphologically distinct in the two whitefly species. It was later determined that all *B. tabaci* from various locations contained P-endosymbionts (Costa et al. 1995). However, the number of secondary symbionts differed with biotype (see also Chiel et al. 2007); the B-biotype from Florida, Arizona, and Hawaii, USA had one additional type of microorganism, while the A-biotype from Arizona and Mexico, and the Jatropha population from Puerto Rico, contained two additional morphological types. More recent molecular studies with 20 populations of *B. tabaci* from a variety of hosts

from around the world, representing potentially different biotypes, indicated that all populations contained P-endosymbionts, 13 contained secondary symbionts, and seven harbored *Wolbachia,* an organism that alters reproduction in its host (Zchori-Fein and Brown 2002). The primary endosymbionts of whiteflies, like aphids, are vertically transmitted through bacteriocyte inclusions into the oocyte (Costa et al. 1996); however, unlike most other organisms, intact bacteriocytes of whiteflies migrate into the ovaries (Szklarzewicz and Moskal 2001). This is an elaborate mechanism suggestive of a long and important evolutionary history.

Secondary symbionts, on the other hand, are not found in all individuals in a population, or for that matter, in all populations. These associations evolved much later, and while most are transmitted vertically from mother to offspring, others are transmitted horizontally between species (Sandstrom et al. 2001; Russell et al. 2003; Russell and Moran 2005; Gottlieb et al. 2006).

Based on phylogenetic analysis of eubacterial 16S rDNA sequences, *B. tabaci* houses several secondary symbionts (Table 5.1). Structurally, most of these species are believed to be coccoid organisms, with inner and outer cell membranes characteristic of Gram-negative bacteria (Costa et al. 1995). It is as yet unclear which of these genetic identities match the organisms observed within whitefly bacteriomes. This diverse microflora based on phylogenic sequence analysis is consistent with similar analyses from other homopterans (Moran and Telang 1998; Dobson et al. 1999; Zchori-Fein et al. 2001; Baumann 2005). This evidence suggests that there are detectable differences between endosymbiont populations in *B. tabaci* biotypes (see Section IV; Chiel et al. 2007), and warrants further study of additional populations to determine the variability among and between populations and other whitefly species.

**Table 5.1** Symbionts identified in *Bemisia tabaci*

| Type | Scientific name | Classification | References |
|---|---|---|---|
| *Primary* | *Candidatus* Portiera aleyrodidarum | γ-proteobacteria | Thao and Baumann (2004a) |
| *Secondary* | *Candidatus* Hamiltonella defensa | γ-proteobacteria | Moran et al. (2005a); Chiel et al. (2007) |
| | *Arsenophonus* | γ-proteobacteria | Thao and Baumann (2004b); Zchori-Fein et al. (2004); Chiel et al. (2007) |
| | *Candidatus* Cardinium hertigii | Bacteriodetes | Baumann et al. (2004); Zchori-Fein et al. (2004); Chiel et al. (2007) |
| | *Candidatus* Fritschea bemisiae | Chlamydiales | Zchori-Fein and Brown (2002); Thao et al. (2003); Weeks et al. (2003); Everett et al. (2005) |
| | *Rickettsia bellii*-like | α-proteobacteria | Gottlieb et al. (2006); Chiel et al. (2007) |
| | *Wolbachia* spp. | α-proteobacteria | Zchori-Fein and Brown (2002); Nirgianaki et al. (2003); Li et al. (2007); Chiel et al. (2007) |

## Endoymbionts' Role in Nutrient Provisioning

### *Provisioning in Aphids*

Experiments with artificially-reared aphids gave rise to the idea that symbionts supplied nutrients to the insect host (Campbell 1989). Aphids with intact primary endosymbionts could be reared on diets lacking the ten 'rat' essential amino acids (Dadd 1985), but aphids that had their symbionts selectively removed with antibiotics did not survive on diets lacking essential amino acids. For insects that feed on phloem, the quantity and quality of nitrogen is often deficient. Not all essential amino acids are present in the phloem, and the ratio of essential:non-essential amino acids (E:NE) is skewed in favor of the latter (1:4–1:20). The ratio of E:NE amino acids in the insect is approximately 1:1, suggesting a mechanism of homeostasis where non-essential amino acids are converted to essential amino acids (Liadouze et al. 1995; Douglas 2006). Insects lack the ability to synthesize ten of the 20 common amino acids, and if just one of these essential amino acids is lacking, the growth and fecundity of the animal will be negatively impacted and their offspring will generally be sterile (Houk and Griffiths 1980). There is now considerable evidence based on nutritional, physiological, and genomic studies that the shortfalls and imbalances in phloem amino acids are compensated for in aphids by *Buchnera* (Buchner 1965; Baumann et al. 1995; Liadouze et al. 1995; Douglas 1998, 2006; Moran and Telang 1998; Bernays and Klein 2002; Moran et al. 2003; Moran and Degnan 2006).

Additional roles for symbionts in aphids have been proposed, including the production of lipids, such as sterols (see Campbell 1989). In some coleopterans, yeast-like symbionts have taken up this role (Nasir and Noda 2003), but in homopterans, sterol-producing yeasts have only been reported in a few families (Noda and Mittler 1983; Noda and Koizumi 2003). However, the Gram negative bacteria that are found in aphids and whiteflies are not capable of synthesizing sterols (Campbell 1989), and plant sterols have been found in the honeydew of some species of aphids, making it doubtful that the known symbionts of aphids and whiteflies are involved in sterol production. Although there is much less empirical evidence, the symbionts are believed to provide other dietary components and cofactors, including pigments, antibiotics, and some or all of the B-complex vitamins (Houk and Griffiths 1980; Campbell 1989; Nakabachi and Ishikawa 1999). There are contradictory findings on these additional contributions from symbionts (Campbell and Nes 1983; Walters et al. 1994; Douglas 1998), but the increasing use of recombinant DNA techniques will hopefully resolve many of the questions that remain on the biochemical roles of insect symbionts.

### *Provisioning in Whiteflies*

Aside from the host plant, nutrient provisioning in whiteflies appears to be carried out primarily by its P-endosymbiont, *Candidatus* Portiera aleyrodidarum. However, no suitable artificial diet has been developed for rearing whiteflies through multiple

generations (Blackmer et al. 2002), so speculations on the function of symbionts have been drawn from indirect methods (e.g., use of antibiotics to reduce or eliminate symbionts). The difficulty in working with whiteflies is reflected in the paucity of literature (relative to the aphid literature) on whitefly-symbiont interactions; however, given the fact that whiteflies are mostly sessile during their immature stage, and consequently unable to relocate if the nutritional status of the plant declines, it seems likely that their symbionts will play an even more important role than those found in aphids.

Costa et al. (1993a, 1997) were the first to provide evidence that endosymbiotic bacteria in whiteflies are involved in growth and development in a manner similar to other homopterans. When the antibiotics oxytetracycline hydrochloride or rifampicin were supplied to females via their food, oviposition was reduced, growth and development of offspring was negatively impacted, bacteriome size decreased and percent emergence to the adult stage was reduced when compared to the offspring of untreated females. Further clarification of the role of the P-endosymbionts in whiteflies will require the development of an adequate artificial diet, or novel techniques for quantitatively reducing P-endosymbiont numbers.

## The Role of Secondary Symbionts in Whitefly Biology

Numerous secondary microorganisms have now been identified in aphids and whiteflies, and they appear to be more closely related to one another than are their respective P-endosymbionts (Darby et al. 2001; Gottlieb et al. 2006). The diversity of S-symbionts in whiteflies is most probably as important as aphid S-symbionts in shaping the ecology and evolution of whiteflies. While these facultative symbionts are not essential to the survival of the insect, some of them apparently participate in supplying the host with nutrients under certain conditions (Koga et al. 2003; Gottlieb et al. 2006; Douglas et al. 2006; Wilkinson et al. 2007), or otherwise assist the insect in exploiting novel host plants, thus potentially leading to new biotypes or host races (Tsuchida et al. 2004). There is some indication that these secondary symbionts may biosynthesize and supply enzymes for host-plant adaptation (Campbell and Dreyer 1985; Dreyer and Campbell 1987; Campbell 1989) and may directly or indirectly be associated with the induction of toxic disorders such as squash silverleaf (Costa et al. 1993a). Other S-symbionts are involved in heat tolerance (Montllor et al. 2002), dispersal and mating (Leonardo and Mondor 2006), virus protection and persistence (Morin et al. 2000, see below) and in resistance to parasitoid or fungal attack (Oliver et al. 2003, 2005; Ferrari et al. 2004; Scarborough et al. 2005). In some cases, negative fitness effects have been reported, which vary depending on environmental and/or host-plant species or host-plant quality (O'Neill et al. 1997; Sakurai et al. 2005; Oliver et al. 2006; Wilkinson et al. 2007).

The potential role of these S-symbionts may be easier to discern in whiteflies than is the definitive role of the P-endosymbionts because it is possible to use selective antibiotics to rid the whiteflies of their S-symbionts while leaving the primaries intact. In addition, due to their facultative nature, S-symbiont-free whitefly colonies

can be maintained. Ruan et al. (2006) used three antibiotics, tetracycline, ampicillin trihydrate, and rifampicin to selectively kill S-symbionts in two *B. tabaci* biotypes (B and non B ZHJ-1). The B-biotype was known to harbor *Hamiltonella defensa*, while the non B-biotype harbored *Arsenophonus* and *Wolbachia* (Ruan and Liu 2005). The use of tetracycline or ampicillin on B-biotype adults accelerated development and increased survival of their offspring, while treatment with rifampicin retarded development but not survival of offspring. In the non B-biotype (ZHJ-1) whitefly, treatment of adults with tetracycline or ampicillin also accelerated development but did not increase survival of offspring, while treatment with rifampicin retarded development and survival of offspring. Their findings illustrate that removal of the S-symbionts can have both beneficial and detrimental effects.

Most of the roles of these various symbionts remain to be determined. It will be extremely challenging to unravel their specific function and how interactions between and among the various symbionts are influenced by the dynamics of the host, host plant, and environmental conditions. The increased use of recombinant DNA techniques for identifying types and biochemistry of symbionts will provide much more reliable information (Baumann et al. 2004), and the recently completed whitefly genome project (Leshkowitz et al. 2006) will provide an important tool for the identification of genes involved in the life-history of *B. tabaci* and the function of their symbionts.

## Potential for Symbiont Manipulation and Pest Management

Very early on, when it was realized how dependent insects were on their symbionts, the use of symbionticides was attempted. Behrenz and Technau (1959), in an effort to control the common furniture beetle, *Anobium punctatum* Deg., infused wood with the antibacterial agent, sulfonamide, to eliminate the symbionts from the beetle larvae. The technique worked, although symbiont resistance to antibiotics (Brooks and Richards 1955a, b) made this technique untenable in the long run. Costa et al. (1997) suggested the use of transgenic plants that produce antibacterial proteins, but this will require a thorough understanding of the potential for the bacteria to develop resistance in this setting.

Alternatively, it may be possible to manipulate the host plant and indirectly influence symbiont numbers. It has been known for a long time that the lack of manganese or the addition of certain fatty acids in the diet influences the transmission of symbionts to the offspring (Brooks 1960, 1962); however, the technology for accomplishing this was unavailable until recently. Furthermore, fluctuations in nutrient levels – specifically nitrogen – are known to influence symbiont numbers. Wilkinson et al. (2007) found that aphid P-endosymbiont numbers increased with higher levels of nitrogen in the host plant. On the other hand, on low nutrient regimes, P-endosymbiont numbers decreased while the numbers of the facultative S-symbiont, *Serratia symbiotica*, increased. This S-symbiont has been shown to reduce parasitism by the aphid parasitoid, *Aphidius ervi* by 22% (Oliver et al. 2003).

Another S-symbiont, *H. defensa,* was found to reduce parasitism in aphids by 42%. As previously stated, *H. defensa* has been found in *B. tabaci*, and it is closely related to the aphid symbiont based on a 16S rRNA gene-based phylogeny (Moran et al. 2005a). Whether it also confers resistance to whitefly parasitoids is yet to be determined. However, if there is a similar relationship in whiteflies, it might be feasible to manipulate plant nitrogen levels, and simultaneously control selective S-symbionts numbers, making the whitefly more susceptible to its natural enemies.

Perhaps one of the most promising potential control strategies involves the use of facultative/parasitic symbionts, especially *Wolbachia* (Zabalou et al. 2004). These microorganisms infect a wide range of arthropods, manipulating host reproduction in a number of ways, including cytoplasmic incompatibility, parthenogenesis, male killing and feminization (Stouthamer et al. 1999; O'Neill et al. 1997). Depending on the detection mechanism employed, natural infection levels of *Wolbachia* in *B. tabaci* populations range from approximately 33% to more than 80% (Zchori-Fein and Brown 2002; Nirgianaki et al. 2003; Li et al. 2007). How and/or if *Wolbachia* manipulate reproductive isolation in *B. tabaci* has not been explored. However, Chiel et al. (2007) have shown that *Wolbachia* (and *Arsenophonus*, also associated with reproductive phenotypes) were found in most Israeli Q biotype populations, but absent in the all B biotype populations tested. Thus, these microorganisms may contribute to biotype isolation or even speciation (Chiel et al. 2007). It has been proposed that *Wolbachia* could be used as a tool to spread a particular genotype, as a vector for specific genes, as a tool to control insects similar to the sterile insect technique, and as a means to directly suppress populations (Sinkins et al. 1997; Bourtzis and O'Neill 1998; Zabalou et al. 2004). The presence of *Wolbachia* in such a high percentage of whitefly populations indicates that it would probably be a good candidate for many of these techniques.

## Role of Whitefly Endosymbionts in Virus Transmission

### *Begomovirus GroEL-Relationships*

Begomoviruses (family *Geminiviridae*) are small plant viruses infecting many important agricultural crops and ornamentals worldwide. They are characterized by a 22 × 38 nm geminate particle encapsidating circular single-stranded DNA genome molecules ~2,700–2,800 nucleotides in length (see recent review by Rojas et al. 2005). Begomoviruses are transmitted in a circulative manner by their vector, the whitefly *B. tabaci* (Rosell et al. 1999; Ghanim et al. 2001a). Virus particles ingested through the stylets enter the esophagus and filter chamber, cross the gut into the haemolymph, reach the salivary glands and are excreted with the saliva during feeding (Harris et al. 1996; Rosell et al. 1999; Ghanim et al. 2001a, b). The passage from the gut into the salivary system is particularly perilous because the haemolymph is an open system that contains numerous enzymes and cells (Crossley 1975; Brehélin 1982).

Whitefly endosymbionts can play a central role in the safe transit of plant viruses in the haemolymph. The role of chaperonins, protein complexes that aid in protein folding and are synthesized by insect endosymbiotic bacteria, was first demonstrated in aphids. Interaction between *Potato leafroll virus* (PLRV) and a *Buchnera* endosymbiotic ~63 kDa GroEL-like chaperonin from *Myzus persicae*, a member of the chaperonin-60 family (Gupta 1995), was shown to be essential for virus transmission (van der Heuvel et al. 1994). Disturbing the GroEL-PLRV interaction in vivo impaired virus survival, and thereby, virus transmission. Similarly, the survival of begomoviruses in the haemolymph of *B. tabaci* has been shown to depend on the interaction of a GroEL homologue produced by the whitefly *P. aleyrodidarum* endosymbiotic bacteria and the virions (Morin et al. 1999).

Whitefly and aphid GroEL proteins share high homology in their amino acid sequence. GroEL is present in the haemolymph of *B. tabaci* as a native 14-mer unit, each subunit having a mass of 63 kDa (Filichkin et al. 1997; Morin et al. 1999). Interaction between whitefly endosymbiotic GroEL and begomoviruses has been studied for the *Tomato yellow leaf curl virus* (TYLCV). TYLCV particles displayed affinity for the *B. tabaci* endosymbiont GroEL homologue in a virus overlay assay. Moreover, the TYLCV coat protein (CP) and *B. tabaci* GroEL interacted physically in the yeast two-hybrid system (Morin et al. 2000). Interestingly, *B. tabaci* GroEL interacted as well with the CP of the non-transmissible *Abutilon mosaic begomovirus* (AbMV), indicating that the amino acid residues at position 124, 149 and 174, which prevented AbMV from crossing into the insect haemolymph (Höhnle et al. 2001), did not prevent binding to GroEL.

The similar involvement of a GroEL in the transmission of luteoviruses by aphids and of begomoviruses by whiteflies suggests a conserved mechanism underlying circulative transmission of viruses by their insect vector. Knowing that the relative frequency of the different types of microorganism within whitefly bacteriocytes differs significantly between biotypes (Costa et al. 1995; Chiel et al. 2007), will be of great interest to determine whether the micro-fauna of whiteflies affects their competence to transmit begomoviruses (Fares et al. 2005; Pascual 2006; Jiu et al. 2006).

## *Use of* B. tabaci *Endosymbiotic GroEL in Diagnostic Tests*

The ability of endosymbiotic GroEL to bind circulative plant viruses was exploited to devise a tool for trapping and identifying plant viruses in vitro (Akad et al. 2004). Because of the scarcity of native GroEL, the gene from *B. tabaci P. aleyrodidarum* (GenBank accession number AF130421), was cloned and expressed in *E. coli* cells.

The technique was first developed for diagnosis of TYLCV. PCR tubes coated with GroEL were incubated with cleared sap of leaves from infected plants. A PCR mixture containing TYLCV-specific primers was added and TYLCV DNA

was amplified demonstrating that this virus was efficiently trapped by GroEL. The exquisite power of the GroEL-based tests was demonstrated by detecting TYLCV in a single whitefly. The same procedure has been successfully used to detect several other whitefly-transmitted geminiviruses, such as AbMV and *African cassava mosaic virus* (ACMV) (Akad et al. 2004). The spectrum of plant viruses tested was extended to circulative and non-circulative viruses, which lead Akad et al. (2004) to predict the characteristics of the viruses that have the capacity to interact with GroEL. These characteristics include a globular (or geminate) shape, a CP with a high positive charge, a high percentage of arginine, and a high isoelectric point.

## *Transgenic Plants Expressing the GroEL Confer Broad-Range Virus Resistance*

Since whitefly GroEL binds to viruses from different taxonomic families, Akad et al. (2004) exploited this phenomenon to generate transgenic tomato plants expressing the whitefly GroEL under the control of a phloem-specific promoter (Truernit and Sauer 1995). They expected that once inoculated by their vector, phloem-limited circulative viruses would be trapped in the plant phloem. As a result, proper invasion of phloem-associated cells and long distance movement could be significantly inhibited, rendering the plants resistant to the virus.

This approach was first attempted by infecting GroEL-expressing transgenic tomato plants with TYLCV (Akad et al. 2007). Homozygous plants expressing the GroEL gene showed no or mild symptoms upon whitefly-mediated TYLCV inoculation and yielded fruit, while the non-transgenic plants were symptomatic, stunted and did not yield. Using the GroEL virus trapping and detection test described above, Akad et al. (2007) could identify GroEL-TYLCV complexes in the sap of infected transgenic tomato plants.

Recently, this work was extended to generate transgenic *Nicotiana benthamiana* plants expressing the whitefly GroEL (Edelbaum and Czosnek unpublished data). It was expected that, once inoculated, viruses that are able to bind to GroEL in in vitro tests (as described above) would be trapped by GroEL expressed in transgenic *N. benthamiana*, thus rendering the plants tolerant to the virus. GroEL-expressing transgenic *N. benthamiana* plants were inoculated with two viruses binding GroEL (TYLCV and *Cucumber mosaic virus* [CMV]) and two viruses that do not bind GroEL (*Tobacco mosaic virus* [TMV] and *Grapevine virus A* [GVA]) in vitro. As predicted, the GroEL-expressing plants were tolerant to TYLCV and CMV (mild or no symptoms) but not to TMV and GVA (fully symptomatic). The amount of TYLCV and CMV (but not of GVA and TMV) in the inoculated transgenic plants was reduced by three orders of magnitude compared to inoculated non-transgenic plants. Virus-GroEL complexes were detected in TYLCV- and CMV-inoculated transgenic *N. benthamiana*, but not in plants inoculated with TMV- and GVA (data

**Table 5.2** Viruses and their properties used in GroEL-expressing transgenic *N. benthamiana* plants and summarized data from inoculated plants

| | Tobacco mosaic virus | Grapevine virus A | Cucumber mosaic virus | Tomato yellow leaf curl virus |
|---|---|---|---|---|
| *Viruses and properties* | | | | |
| Genus family | Tobamovirus Not assigned | Vitivirus *Flexiviridae* | Cucumovirus *Bromoviridae* | Begomovirus *Geminiviridae* |
| Shape | Filamentous | Filamentous | Globular | Geminate |
| Coat protein: Pi charge | 4.8 –2.0 | 8.4 1.7 | 10.3 12.2 | 10.4 22.7 |
| Symptoms on *N. benthamiana* | Death within 1 week | Stunting, mottling | Stunting, curly leaves | Stunting, leaf curling, yellowing |
| Virion binding to GroEL in vitro | No | No | Yes | Yes |
| *Virus-inoculated GroEL-expressing* N. benthamiana | | | | |
| Inoculation method | Mechanical with purified virions | Mechanical with sap of infected tobacco | Mechanical with sap of infected melon | Whitefly and agroinfection |
| Symptoms | Death within 1 week | Stunting, mottling | Mild symptoms and recovery | Symptomless |
| GroEL-virion complexes in sap | None | None | Detected | Detected |

summarized in Table 5.2). Hence, tolerance or susceptibility to viruses could be predicted following a simple in vitro GroEL-virus binding assay. Plants expressing the chaperonin were tolerant to viruses that bound GroEL in vitro.

## Intra- and Interspecific Interactions with Herbivores

### *Importance of Intra- and Interspecific Interactions in* B. tabaci *Biology*

The extreme polyphagy of *B. tabaci* creates the opportunity to form dependent interactions with numerous herbivorous arthropods on a variety of plant species and habitats. One would expect that the wide distribution of *B. tabaci* and its severe effects on the plant would yield extensive information on intra-and interspecific interactions with conspecifics and other herbivores. However, because most research on *B. tabaci* has centered primarily on pest management, information on such interactions is rather limited.

Herbivorous insects may interact directly or indirectly via the host plant (Ohgushi 2005). The interactions between (and among) whiteflies and other herbivores are normally plant-mediated due to the sessile development of immatures and their phloem-feeding behavior, which cause minimal physical damage to the plant tissue (Inbar and Gerling 2008). The importance of the plant as a mediator is enhanced by the ability of *B. tabaci* to induce fundamental changes in the infested plant including reducing photosynthesis, depleting plant reserves and inducing physiological disorders. They cause additional damage through honeydew excretion and transmission of viral diseases (Brown and Czosnek 2002; Buntin et al. 1993; Inbar and Gerling 2008; Gerling 1990). *B. tabaci* also triggers induced defense responses in the plant, some of which are highly specific (Mayer et al. 1996; van de Ven et al. 2000). Here we describe the main evidence on intra- and interspecific interactions (with herbivores) in which *B. tabaci* is involved.

## *Positive Interactions of* Bemisia *feeding*

Phloem feeders act as sinks for assimilates that may compete with natural plant sink organs (e.g. fruits). Aggregations of phloem feeders can strengthen their ability to draw assimilates and improve their nutrition by altering the level and composition of amino acids in the sap. Whiteflies may benefit from such aggregation as long as density does not lead to competition. Such positive interactions (facilitation) among *B. tabaci* were demonstrated on *Cucumis melo* (Blackmer and Byrne 1999). They showed that during the plant's vegetative growth, *B. tabaci* aggregations elevated the total concentrations of free amino acids and altered their composition. Such modifications, however, diminished once the plant shifted to the reproductive stage (Blackmer and Byrne 1999). De Barro et al. (2006) describe increased fecundity when *B. tabaci* B and AN biotypes fed on the same plants. The mechanism in this case remains unclear. Facilitation has also been reported with non-phloem feeding insects. It has been suggested that damage by *Heliothis* larvae to cotton increased vegetative growth and availability of young leaves, which are more suitable for the development of *B. tabaci* (Baumgärtner et al. 1986).

Because plant viruses may modify the suitability of plants, whiteflies may influence each other (and potentially other herbivores) via their transmission (Colvin et al. 2006; Costa et al. 1991; McKenzie et al. 2002). Virus-mediated intra-specific facilitation appears to be species-(virus and plant) specific. For example, although leaves of begomovirus-infected plants had higher amino acid levels compared with healthy controls, the levels were not strictly associated with higher *B. tabaci* performance (Costa et al. 1991). Clear positive effects of viruses on *B. tabaci* performance have been reported in other studies (Colvin et al. 2006; Mayer et al. 2002; McKenzie 2002; McKenzie et al. 2002). Both direct effects of the viruses on the whiteflies and plant-mediated effects may be involved. At least on cassava, facilitation is related to the higher free amino acid concentrations in the phloem in the virus-infected plants (Colvin et al. 2006). Jiu et al. (2007) speculates that mutualism between viruses and

the *B. tabaci* B biotype may be one of the factors that promote its host utilization and spread.

## *Competition Among Whiteflies*

We expect that competition will be important in agroecosystems, where plant quality declines especially during outbreaks of *B. tabaci*. Nevertheless, the nature of intra- and interspecific competition has been rarely evaluated. Studies that examined speciation and spread of several biotypes of *B. tabaci* revealed that intraspecific competition plays a major role in whitefly biology and evolution (Brown et al. 1995; De Barro 2005; De Barro et al. 2006; Jiu et al. 2007; Perring 1996).

*Bemisia tabaci* B biotype invaded China while suppressing the indigenous biotypes (Jiu et al. 2007). In the laboratory, the B biotype completely displaced non-B biotypes in only a few generations, depending on the plant species (Zang et al. 2006). Several mechanisms may promote the competitive superiority of the B biotype. Its overall performance on several host plants was much higher than those of the native biotypes (Zang et al. 2006). The B biotype interferes with, and may actually block courtship and mating of other biotypes (Zang and Liu 2007; see also Perring 1996; De Barro and Hart 2000). Additionally, Liu et al. (2007) showed that asymmetric mating interactions coupled with facilitation (B biotype females producing more female offspring in the presence of indigenous males) were essential in the successful invasion of B biotype whiteflies into China (see Naranjo et al. Chapter 6). Biotype-specific mutualism with plant viruses (see above) is probably also involved (Jiu et al. 2007).

*Bemisia tabaci* B and Q biotypes are common in several Mediterranean countries. Under laboratory conditions, the Q is displaced by the B biotype (Pascual and Callejas 2004) often in a single generation (Pascual 2006). Mating interference, female reproductive capacity, and better host utilization are probably involved. Under specific circumstances in the field, however, the B is displaced by the Q biotype because the latter is more resistant to certain insecticides (Horowitz et al. 2005, Pascual 2006). Interestingly, Chiel et al. (2007) recently found that these biotypes differ in the symbionts that they harbor. The secondary symbiont *Hamiltonella* was detected in (all) B biotype individuals whereas *Wolbachia* and *Arsenophonus* were detected only in Q. The role of these symbionts in biotype performance and competitive abilities is yet to be demonstrated.

The polyphagous whiteflies *B. tabaci* and *T. vaporariorum* may share the same host plant in greenhouses. However, they tend to be spatially separated by occupying different leaves (Tsueda and Tsuchida 1998). On poinsettia and green beans the two species did not coexist on the same leaf for more than two generations (ca. 2 months) (Liu et al. 1994). *B. tabaci* was more dominant on poinsettia, and *T. vaporariorum* prevailed on green bean. Although the mechanism of competition between these two genera of whiteflies was not examined, it has been suggested that species dominancy is related to reciprocal impacts on feeding and ovipositional preference and immature performance (Liu et al. 1994).

### *Competition Between Whiteflies and Other Herbivores*

*Bemisia tabaci* often share the same plants with other herbivores (e.g., Hare and Elle 2002; Inbar et al. 1999a). Unlike competition among whiteflies, interspecific competition between whiteflies and other herbivores has been largely overlooked. Circumstantial field surveys suggest that such competition might be common. For example, increased leafhopper populations on cotton were associated with lower *B. tabaci* densities (Patil 1996; see also Rao and Reddy 1994). When feeding on collard leaves previously infested with *B. tabaci*, 1st instar larvae of the cabbage looper, *Trichoplusia ni*, tended to feed on the adaxial (whitefly-free) side of the leaf. Consequently, larval developmental duration was prolonged and their relative growth rate and survival were reduced (Inbar et al. 1999b). Interestingly, late instar caterpillars consume immature whiteflies while chewing the leaf (Inbar et al. 1999b). *B. tabaci* negatively affected the preference and performance of the leafminers *Liriomyza trifolii* and *L. sativae* (Diptera: Agromyzidae) (Inbar et al. 1999b; Zhang et al. 2005). The negative effects of *B. tabaci* on the leafminers persisted even when both insects developed on different leaves of the same plant. Thus, *B. tabaci* may affect other herbivores via induced systemic resistance mechanisms in the shared host (Inbar et al. 1999a) to which they appear to be less sensitive (see also Inbar et al. 2001). On the other hand, Agrawal et al. (2000) found that induced resistance in cotton seedlings to spider mite (*Tetranychus turkestani*) feeding reduced *B. tabaci* densities. The reciprocal effects of whiteflies on mites were not tested. The interactions between mites and *B. tabaci* are even more complex because some have established phoretic associations (Fan and Petitt 1998; Soroker et al. 2003). The mites use whiteflies as a vehicle to passively disperse between plants and habitats.

## Conclusions

The symbiotic relationships of whiteflies and microbes are as diverse as those found in other Hemiptera. These ancient associations have been shown to be important in a variety of ecological and evolutionary processes in whiteflies. Thus, symbiotic relationships between whiteflies and bacterial species have taken on preeminent importance in this time of increased whitefly resistance to insecticides. New information concerning the diversity of S-symbionts, the variations in associations with different *B. tabaci* biotypes and the relationship between symbiont products, such as GroEL, and begomoviruses have shown the potential for manipulation and/or control of whiteflies using molecular mechanisms to generate transgenic plants or modified symbionts.

Intensive research on the *B. tabaci*-virus association and the spread of whitefly biotypes clearly demonstrate the important role of intraspecific interactions, which may facilitate development via improved plant suitability. Population densities and consequently economic damage as well as biotype spread may be largely dependent on such inter-biotype dynamics. Competition between biotypes may lead

to displacement and eventually may accelerate speciation in *B. tabaci*. Although intraspecific interactions are obviously intense, interspecific interactions may affect *B. tabaci* and other pests that feed on the same plant. Further research is needed to evaluate the magnitude and direction of competition. Extending our knowledge of the role of the host plant in such interactions (Inbar and Gerling 2008) will shed light on the mechanisms contributing to the ecological success of *B. tabaci*. Understanding the complex webs that link *Bemisia*, host plants, microorganisms, other herbivores and natural enemies should provide better tools for efficient and sustainable pest management strategies.

## Literature Cited

Agrawal AA, Karban R, Colfer RG. 2000. How leaf domatia and induced plant resistance affect herbivores, natural enemies and plant performance. *Oikos* 89:70–80.

Akad F, Dotan N, Czosnek H. 2004. Trapping of Tomato yellow leaf curl virus (TYLCV) and other plant viruses with a GroEL homologue from the whitefly *Bemisia tabaci*. *Arch. Virol.* 149:1481–1497.

Akad F, Eybishtz A, Edelbaum D, Gorovits R, Dar-Issa O, Iraki N, Czosnek H. 2007. Making a friend from a foe: expressing a GroEL gene from the whitefly *Bemisia tabaci* in the phloem of tomato plants confers resistance to Tomato yellow leaf curl virus. *Arch. Virol.* 152:1323–1339.

Baumann P. 2005. Biology of bacteriocyte-associated endosymbionts of plant sap-sucking insects. *Annu. Rev. Microbiol.* 59:155–189.

Baumann P, Baumann L, Lai C-Y, Rouhbakhsh D, Moran NA, Clark MA. 1995. Genetics, physiology, and evolutionary relationships of the genus *Buchnera*: intracellular symbionts of aphids. *Annu. Rev. Microbiol.* 49:55–94.

Baumann P, Moran NA, Baumann L. 1997. The evolution and genetics of aphid endosymbionts. *Bioscience* 47:12–20.

Baumann P, Munson MA, Lai C-Y, Clark MA, Baumann L, Moran NA, Campbell BC. 1993. Origin and properties of bacterial endosymbionts of aphids, whiteflies, and mealybugs. *ASM News* 59:21–24.

Baumann L, Thao ML, Funk CJ, Falk BW, Ng JCK, Baumann P. 2004. Sequence analysis of DNA fragments from the genome of the primary endosymbiont of the whitefly *Bemisia tabaci*. *Curr. Microbiol.* 48:77–81.

Baumgärtner J, Delucchi V, Von Arx R, Rubli D. 1986. Whitefly (*Bemisia tabaci* Genn., Stern.: Aleyrodidae) infestation patterns as influenced by cotton, weather and *Heliothis*: hypotheses testing by using simulation models. *Agric. Ecosyst. Environ.* 17:49–59.

Behrenz W, Technau G. 1959. Versuche zur Bekämpfung von *Anobium punctatum* mit Symbionticiden. *Z. Angew. Entomol.* 44:22–28.

Bernays EA, Klein BA. 2002. Quantifying the symbiont contribution to essential amino acids in aphids: the importance of tryptophan for *Uroleucon ambrosiae*. *Physiol. Entomol.* 27:275–284.

Blackmer JL, Byrne DN. 1999. The effect of *Bemisia tabaci* on amino acid balance in *Cucumis melo*. *Entomol. Exp. Appl.* 93:315–319.

Blackmer JL, Lee LL, Henneberry TJ. 2002. Factors affecting egg hatch, development and survival of *Bemisia argentifolii* (Homoptera: Aleyrodidae) reared on an artificial feeding system. *Environ. Entomol.* 31:306–312.

Blochmann F. 1884. Über die Metamorphose der Kerne in den Ovarialeiern und über den Beginn Der Blastodermbildung bei den Ameisen. *Verhandl. Naturhist. Med. Ver. Heidelberg [N.S.]* 3:243–247.

Blochmann F. 1888. Über das regelmässige Vorkommen von bakterienähnlichen Gebilden in den Geweben und Eiern verschiedener Insekten. *Z. Biol.* 24:1–15.

Bourtzis K, O'Neill S. 1998. *Wolbachia* infections and arthropod reproduction. *Bioscience* 48:287–293.

Brehélin M. 1982. Comparative study of structure and function of blood cells from two *Drosophila* species. *Cell Tissue Res.* 221:607–615.

Brooks MA. 1960. Some dietary factors that affect ovarial transmission of symbiotes. *Proc. Helminthol. Soc. Wash., D.C.* 27:212–220.

Brooks MA. 1962. The relationship between intracellular symbiotes and host metabolism. *Symp. Genet. Biol. Ital.* 9:456–463.

Brooks MA, Richards AG. 1955a. Intracellular symbiosis in cockroaches. I. Production of aposymbiotic cockroaches. *Biol. Bull.* 109:22–39.

Brooks MA, Richards AG. 1955b. Intracellular symbiosis in cockroaches. II. Mitotic division of mycetocytes. *Science* 122:242.

Brown JK, Czosnek H. 2002. Whitefly transmission of plant viruses. *Adv. Bot. Res.* 36:65–100.

Brown JK, Frohlich DR, Rosell RC. 1995. The sweetpotato or silverleaf whiteflies: biotypes of *Bemisia tabaci* or a species complex? *Annu. Rev. Entomol.* 40:511–534.

Buchner P. 1965. *Endosymbiosis of Animals with Plant Microorganisms*. New York: Interscience, 901 pp.

Buntin GD, Gilbertz DA, Oetting RD. 1993. Chlorophyll loss and gas exchange in tomato leaves after feeding injury by *Bemisia tabaci* (Homoptera, Aleyrodidae). *J. Econ. Entomol.* 86:517–522.

Campbell BC. 1989. On the role of microbial symbiotes in herbivorous insects. In *Insect-Plant Interactions*, Vol. I, ed. EA Bernays. Boca Raton, FL: CRC Press.

Campbell BC. 1993. Congruent evolution between whiteflies (Homoptera: Aleyrodidae) and their bacterial endosymbionts based on respective 18S and rDNAs. *Curr. Microbiol.* 26:129–132.

Campbell BC, Dreyer DL. 1985. Host-plant resistance of sorghum: differential hydrolysis of pectic substances by polysaccharases of greenbug biotypes (*Schizaphis graminum*, Homoptera: Aphididae). *Arch. Insect Biochem. Physiol.* 2:203–215.

Campbell BC, Nes WD. 1983. A reappraisal of sterol biosynthesis and metabolism in aphids. *J. Insect Physiol.* 29:149–156.

Chiel E, Gottlieb Y, Zchori-Fein E, Mozes-Daube N, Katzir N, Inbar M, Ghanim M. 2007. Biotype-dependent secondary symbiont communities in sympatric populations of *Bemisia tabaci*. *Bull. Entomol. Res.* 97:1–7.

Clark MA, Baumann L, Munson MA, Baumann P, Campbell BC, Duffus JE, Osborne LS, Moran NA. 1992. The eubacterial endosymbionts of whiteflies (Homoptera: Aleyrodoidea) constitute a lineage distinct from the endosymbionts of aphids and mealybugs. *Curr. Microbiol.* 25:119–123.

Colvin J, Omongo CA, Govindappa MR, Stevenson PC, Maruthi MN. 2006. Host-plant viral infection effects on arthropod-vector population growth, development and behaviour: management and epidemiological implications. *Adv. Virus Res.* 67:419–452.

Costa HS, Brown JK, Byrne DN. 1991. Life history traits of the whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae) on six virus-infected or healthy plant species. *Environ. Entomol.* 20:1102–1107.

Costa HS, Henneberry TJ, Toscano NC. 1997. Effects of antibacterial materials on *Bemisia argentifolii* (Homoptera: Aleyrodidae) oviposition, growth, survival, and sex ratio. *J. Econ. Entomol.* 90:333–339.

Costa HS, Toscano NC, Henneberry TJ. 1996. Mycetocyte inclusion in the oocytes of *Bemisia argentifolii* (Homoptera: Aleyrodidae). *Ann. Entomol. Soc. Am.* 89:694–699.

Costa HS, Ullman DE, Johnson MW, Tabashnik BE. 1993a. Antibiotic oxytetracycline interferes with *Bemisa tabaci* (Homoptera: Aleyrodidae) oviposition, development and ability to induce squash silverleaf. *Ann. Entomol. Soc. Am.* 86:740–748.

Costa HS, Westcot DM, Ullman DE, Johnson MW. 1993b. Ultrastructure of the endosymbionts of the whitefly, *Bemisia tabaci* and *Trialeurodes vaporariorum*. *Protoplasma* 176:106–115.

Costa HS, Westcot DM, Ullman DE, Rosell RC, Brown JK, Johnson MW. 1995. Morphological variation in *Bemisia* endosymbionts. *Protoplasma* 189:194–202.

Crossley AC. 1975. The cytophysiology of insect blood. *Adv. Ins. Physiol.* 11:117–222.

Dadd RH. 1985. Nutrition: organisms. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, Vol. 4, ed. GA Kerkut, LI Gilbert. Oxford: Pergamon Press.

Darby AC, Birkle LM, Turner SL, Douglas AE. 2001. An aphid-borne bacterium allied to the secondary symbionts of whitefly. *FEMS Microbiol. Ecol.* 36:43–50.

De Barro PJ. 2005. Genetic structure of the whitefly *Bemisia tabaci* in the Asia-Pacific region revealed using microsatellite markers. *Mol. Ecol.* 14:3695–3718.

De Barro PJ, Bourne A, Khan SC, Brancatini V. 2006. Host plant and biotype density interactions – their role in the establishment of the invasive B biotype of *Bemisia. Biol. Invasions* 8:287–294.

De Barro PJ, Hart PJ. 2000. Mating interactions between two biotypes of the whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Australia. *Bull. Entomol. Res.* 90:103–112.

DeBary A. 1879. *Die Erscheinung der Symbiose*. Strassburg: Verlag von Karl J. Trubner.

Dobson SL, Kostas B, Braig HR, Jones BF, Zhou W, Rousset F, O'Neill SL. 1999. *Wolbachia* infections are distributed throughout insect somatic and germ line tissues. *Insect Biochem. Mol. Biol.* 29:153–160.

Douglas AE. 1998. Nutritional interactions in insect-microbial symbioses: aphids and their symbiotic bacteria *Buchnera*. *Annu. Rev. Entomol.* 43:17–37.

Douglas AE. 2006. Phloem-sap feeding by animals: problems and solutions. *J. Exp. Bot.* 57:747–754.

Douglas AE, François CLMJ, Minto LB. 2006. Facultative 'secondary' bacterial symbionts and the nutrition of the pea aphid, *Acyrthosiphon pisum*. *Physiol. Entomol.* 31:262–269.

Dreyer DL, Campbell BC. 1987. Chemical basis of host-plant resistance to aphids. *Plant Cell Environ.* 10:353–361.

Everett KDE, Thao M, Horn M, Dyszynski GE, Baumann P. 2005. Novel chlamydiae in whiteflies and scale insects: endosymbionts '*Candidatus* Fritschea bemisiae' strain Falk and '*Candidatus* Fritschea eriococci' strain Elm. *Int. J. Syst. Evol. Microbiol.* 55:1581–1587.

Fan YC, Petitt FL. 1998. Dispersal of the broad mite, *Polyphagotarsonemus latus* (Acari: Tarsonemidae) on *Bemisia argentifolii* (Homoptera: Aleyrodidae). *Exp. Appl. Acarol.* 22:411–415.

Fares MA, Moya A, Barrio E. 2005. Adaptive evolution in GroEL from distantly related endosymbiotic bacteria of insects. *J. Evol. Biol.* 18:651–660.

Ferrari J, Darby AC, Daniell RJ, Godfray HCJ, Douglas AE. 2004. Linking the bacterial community of pea aphids with host-plant use and natural enemy resistance. *Ecol. Entomol.* 29:60–65.

Filichkin SA, Brumfield S, Filichkin TP, Young MJ. 1997. In vitro interactions of the aphid endosymbiont symL chaperonin with barley yellow dwarf virus. *J Virol.* 71:569–577.

Fukatsu T, Nikoh N. 2000. Endosymbiotic microbiota of the bamboo pseudococcid *Antonina crawii* (Insecta, Homoptera). *Appl. Environ. Microbiol.* 66:643–650.

Fukatsu T, Nikoh N, Kawai R, Koga R. 2000. The secondary endosymbiotic bacterium of the pea aphid *Acyrthosiphon pisum* (Insecta: Homoptera). *Appl. Environ. Microbiol.* 66:2748–2758.

Gerling D. (ed.) 1990. *Whiteflies: Their Bionomics, Pest Status and Management*. Andover, Hants: Intercept Ltd, 348pp.

Gerling D, Mayer RT. (ed.) 1996. *Bemisia 1995: Taxonomy, Biology, Damage, Control and Management*. Andover, Hants: Intercept Ltd, 684pp.

Ghanim M, Morin S, Czosnek H. 2001a. Rate of Tomato yellow leaf curl virus (TYLCV) translocation in the circulative transmission pathway of its vector, the whitefly *Bemisia tabaci*. *Phytopathology* 91:188–196.

Ghanim M, Rosell RC, Campbell LR, Czosnek H, Brown JK, Ullman DE. 2001b. Digestive, salivary and reproductive organs in *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) B type. *J. Morphol.* 248:22–40.

Gottlieb Y, Ghanim M, Chiel E, Gerling D, Portnoy V, Steinberg S, Tzuri G, Horowitz AR, Mozes-Daube N, Kontsedalov S, Gershon M, Gal S, Katzir N, Zchori-Fein E. 2006. Identification and localization of a *Rickettsia* sp. in *Bemisia tabaci* (Homoptera: Aleyrodidae). *Appl. Environ. Microbiol.* 72:3646–3652.

Gupta RS. 1995. Evolution of the chaperonin families (Hsp60, Hsp10 and Tep-1) of proteins and the origin of eukaryotic cells. *Mol. Microbiol.* 15:1–11.

Hare DJ, Elle E. 2002. Variable impact of diverse insect herbivores on dimorphic *Datura wrightii*. *Ecology* 83:2711–2720.

Harris KF, Pesic-Van Esbroeck Z, Duffus JE. 1996. Morphology of the sweet potato whitefly, *Bemisia tabaci* (Homoptera, Aleyrodidae) relative to virus transmission. *Zoomorphology* 116:143–156.

Hinde R. 1971. The control of the mycetocyte symbionts of the aphids *Brevicoryne brassicae, Myzus persicae*, and *Macrosiphum rosae*. *J. Insect Physiol.* 17:1791–1800.

Höhnle M, Höfer P, Bedford ID, Briddon RW, Markham PG, Frischmuth T. 2001. Exchange of three amino acids in the coat protein results in efficient whitefly transmission of a nontransmissible *Abutilon mosaic virus* isolate. *Virology* 290:164–171.

Hooke R. 1665. *Micrographia*. London: The Royal Society, 384pp.

Horowitz AR, Kontsedalov S, Khasdan V, Ishaaya I. 2005. Biotypes B and Q of *Bemisia tabaci* and their relevance to neonicotinoid and pyriproxyfen resistance. *Arch. Insect Biochem. Physiol.* 58:216–225.

Houk EJ, Griffiths GW. 1980. Intracellular symbiotes of the Homoptera. *Annu. Rev. Entomol.* 25:161–187.

Hunter MS, Perlman SJ, Kelly SE. 2003. A bacterial symbiont in the *Bacteroidetes* induces cytoplasmic incompatibility in the parasitoid wasp, *Encarsia pergandiella*. *Proc. R. Soc. Lond. B Biol. Sci.* 270:2185–2190.

Inbar M, Doostdar H, Leibee GL, Mayer RT. 1999a. The role of plant rapidly induced responses in asymmetric interspecific interactions among insect herbivores. *J. Chem. Ecol.* 25:1961–1979.

Inbar M, Doostdar H, Mayer RT. 1999b. The effects of sessile whitefly nymphs (Homoptera: Aleyrodidae) on leaf chewing larvae (Lepidoptera: Noctuidae). *Environ. Entomol.* 28: 353–357.

Inbar M, Doostdar H, Mayer RT. 2001. Suitability of stressed and vigorous plants to various insect herbivores. *Oikos* 94:228–235.

Inbar M, Gerling D. 2008. Plant-mediated interactions between whiteflies, herbivores and natural enemies. *Annu. Rev. Entomol.* 53:431–448.

Ishikawa H. 2003. Insect symbiosis: an introduction. In *Insect Symbiosis*, ed. TA Miller, pp. 1–21. Boca Raton, FL: CRC Press.

Jiu M, Zhou X-P, Liu S-S. 2006. Acquisition and transmission of two begomoviruses by the B and a non-B biotype of *Bemisia tabaci* from Zhejiang, China. *J. Phytopathol.* 154:587–591.

Jiu M, Zhou XP, Tong L, Xu J, Yang X, Wan F-H, Liu S-S. 2007. Vector-virus mutualism accelerates population increase of an invasive whitefly. *PLoS ONE* 2:e182. DOI: 10.1371/journal.pone.0000182

Koch A. 1967. Insects and their endosymbionts. In *Symbiosis II*, ed. SM Henry, pp. 291–296. New York: Academic Press.

Koga R, Tsuchida T, Fukatsu T. 2003. Changing partners in an obligate symbiosis: a facultative endosymbiont can compensate for loss of the essential endosymbiont *Buchnera* in an aphid. *Proc. R. Soc. Lond. B Biol. Sci.* 270:2543–2550.

Lai CH, Baumann L, Baumann P. 1994. Amplification of *trpEG:* adaptation of *Buchnera aphidicola* to an endosymbiotic association with aphids. *PNAS* 91:3819–3823.

Leonardo TE, Mondor EB. 2006. Symbiont modifies host life-history traits that affect gene flow. *Proc. R. Soc. Lond. B Biol. Sci.* 273:1079–1084.

Lerat E, Daubin V, Moran NA. 2003. From gene trees to organismal phylogeny in prokaryotes: the case of γ-proteobacteria. *PLoS Biol.* 1:e101. DOI: 10.1371/journal.pbio.0000019.

Leshkowitz D, Gazit S, Reuveni E, Ghanim M, Czosnek H, McKenzie C, Shatters RL, Jr, Brown JK. 2006. Whitefly (*Bemisia tabaci*) genome project: analysis of sequenced clones from egg, instar, and adult (viruliferous and non-viruliferous) cDNA libraries. *BMC Genomics* 7:79–94.

Li ZX, Lin HZ, Guo XP. 2007. Prevalence of *Wolbachia* infection in *Bemisia tabaci*. *Curr. Microbiol.* 54:467–471.

Liadouze I, Febvay G, Guillaud J, Bonnot G. 1995. Effect of diet on the free amino acid pools of symbiotic and aposymbiotic pea aphids, *Acyrthosiphon pisum*. *J. Insect Physiol.* 41:33–40.

Liu SS, De Barro PJ, Xu J, Luan J-B, Zang L-S, Ruan Y-M, Wan F-H. 2007. Asymmetric mating interactions drive widespread invasion and displacement in a whitefly. *Science* 318:1769–1772.

Liu TX, Oetting RD, Buntin GD. 1994. Evidence of interspecific competition between *Trialeurodes vaporariorum* (Westwood) and *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) on some greenhouse-grown plants. *J. Entomol. Sci.* 29:55–65.

Mayer RT, Inbar M, McKenzie CL, Shatters R, Borowicz V, Albrecht U, Powell CA, Doostdar, H. 2002. Multitrophic interactions of the silverleaf whitefly, host plants, competing herbivores, and phytopathogens. *Arch. Insect Biochem. Physiol.* 51:151–169.

Mayer RT, McCollum TG, McDonald RE, Polston JE, Doostdar H. 1996. *Bemisia* feeding induces pathogenesis-related proteins in tomato. In Bemisia *1995: Taxonomy, Biology, Damage, Control and Management*, ed. D Gerling, RT Mayer, pp. 179–188. Andover, Hants: Intercept Ltd.

McKenzie CL. 2002. Effect of tomato mottle virus (ToMoV) on *Bemisia tabaci* biotype B (Homoptera: Aleyrodidae) oviposition and adult survivorship on healthy tomato. *Fla. Entomol.* 85:367–368.

McKenzie CL, Shatters RG, Jr, Doostdar H, Lee SD, Inbar M, Mayer RT. 2002. Effect of Geminivirus infection and *Bemisia* infestation on accumulation of pathogenesis-related proteins in tomato. *Arch. Insect Biochem. Physiol.* 49:203–214.

Mittler TE. 1971. Some effects on the aphid *Myzus perisicae* of ingesting antibiotics incorporated into artificial diets. *J. Insect Physiol.* 17:1333.

Montllor CB, Maxmen A, Purcell AH. 2002. Facultative bacterial endosymbionts benefit pea aphids *Acyrthosiphon pisum* under heat stress. *Ecol. Entomol.* 27:189–195.

Moran NA. 1996. Accelerated evolution and Muller's ratchet in endosymbiotic bacteria. *PNAS* 93:2873–2878.

Moran NA. 2005. Symbiosis. *Curr. Biol.* 16(20):R866–R871.

Moran NA, Baumann P. 2000. Bacterial endosymbionts in animals. *Curr. Opin. Microbiol.* 3:270–275.

Moran NA, Degnan PH. 2006. Functional genomics of *Buchnera* and the ecology of aphid hosts. *Mol. Ecol.* 15:1251–1261.

Moran NA, Mira A. 2001. The process of genome shrinkage in the obligate symbiont, *Buchnera aphidicola*. *Genome Biol.* 2:20. DOI: 10.1186/gb-2001-2-12-research0054.

Moran NA, Plague GR, Sandstrom JP, Wilcox JL. 2003. A genomic perspective on nutrient provisioning by bacterial symbionts in insects. *PNAS* 100:14543–14548.

Moran NA, Russell JA, Koga R, Fukatsu T. 2005a. Evolutionary relationships of three new species of *Enterobacteriaceae* living as symbionts of aphids and other insects. *Appl. Environ. Microbiol.* 71:3302–3310.

Moran NA, Telang A. 1998. Bacteriocyte-associated symbionts of insects. *Bioscience* 48:295–304.

Moran NA, Tran P, Gerardo NM. 2005b. Symbiosis and insect diversification: an ancient symbiont of sap-feeding insects from the bacterial phylum Bacteroidetes. *Appl. Environ. Microbiol.* 71:8802–8810.

Morin S, Ghanim M, Sobol I, Czosnek H. 2000. The GroEL protein of the whitefly *Bemisia tabaci* interacts with the coat protein of transmissible and non-transmissible begomoviruses in the yeast two-hybrid system. *Virology* 276:404–416.

Morin S, Ghanim M, Zeidan M, Czosnek H, Verbeek M, van den Heuvel JFJM. 1999. A GroEL homologue from endosymbiotic bacteria of the whitefly *Bemisia tabaci* is implicated in the circulative transmission of *Tomato yellow leaf curl virus*. *Virology* 256:75–84.

Munson MA, Baumann P, Clark MA, Baumann L, Moran NA, Voegtlin DJ, Campbell BC. 1991. Evidence for the establishment of aphid-eubacterium endosymbiosis in an ancestor of four aphid families. *J. Bacteriol.* 173:6321–6324.

Nakabachi A, Ishikawa H. 1999. Provision of riboflavin to the host aphid, *Acyrthosiphon pisum*, by endosymbiotic bacteria, *Buchnera*. *J. Insect Physiol.* 45:1–6.

Nardon P, Grenier AM. 1991. Serial endosymbiosis theory and weevil evolution: the role of symbiosis. In *Symbiosis as a Source of Evolutionary Innovation*, ed. L Margulis, R Fester, pp. 153–169. Cambridge: Massachusetts Institute of Technology.

Nardon P, Nardon C. 1998. Morphology and cytology of symbiosis in insects. *Ann. Soc. Entomol. Fr. (N. S.)* 34:105–134.

Nasir H, Noda H. 2003. Yeast-like symbiotes as a sterol source in anobiid beetles (Coleoptera, Anobiidae): possible metabolic pathways from fungal sterols to 7-dehydrocholesterol. *Arch. Insect Biochem. Physiol.* 52:175–182.

Nirgianaki A, Banks GK, Frohlich DR, Veneti Z, Braig HR, Miller TA, Bedford ID, Markham PG, Savakis C, Bourtzis K. 2003. *Wolbachia* infections of the whitefly *Bemisia tabaci*. *Curr. Microbiol.* 47:93–101.

Noda H, Koizumi Y. 2003. Sterol biosynthesis by symbiontes: cytochrome P450 sterol C-22 desaturase genes from yeastlike symbionts of rice planthoppers and anobiid beetles. *Insect Biochem. Mol. Biol.* 33:649–658.

Noda H, Mittler TE. 1983. Sterols biosynthesis by symbiontes of aphids and leafhoppers. In *Metabolic Aspects of Lipid Nutrition in Insects*, ed. TE Mittler RH Dadd. Boulder CO: Westview Press.

Ohgushi T. 2005. Indirect interaction webs: herbivore-induced effects through trait change in plants. *Annu. Rev. Ecol. Syst.* 36:81–105.

Oliver KM, Moran NA, Hunter MS. 2005. Variation in resistance to parasitism in aphids is due to symbionts not host genotype. *PNAS* 102:12795–12800.

Oliver KM, Moran NA, Hunter MS. 2006. Costs and benefits of a superinfection of facultative symbionts in aphids. *Proc. R. Soc. Lond. B Biol. Sci.* 273:1273–1280.

Oliver KM, Russell JA, Moran NA, Hunter MS. 2003. Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. *PNAS* 100:1803–1807.

O'Neill SL, Gooding RH, Aksoy S. 1993. Phylolgenetically distant symbiotic microorganisms reside in *Glossina* midgut and ovary tissues. *Med. Vet. Entomol.* 7:377–383.

O'Neill SL, Hoffmann AA, Werren JH. 1997. *Influential Passengers. Inherited Microorganisms and Arthropod Reproduction*. New York: Oxford University Press, 230pp.

Pascual S. 2006. Short communication. Mechanisms in competition, under laboratory conditions, between Spanish biotypes B and Q of *Bemisia tabaci* (Gennadius). *Span. J. Agric. Res.* 4:351–354.

Pascual S, Callejas C. 2004. Intra- and interspecific competition between biotypes B and Q of *Bemisia tabaci* (Hemiptera: Aleyrodidae) from Spain. *Bull. Entomol. Res.* 94:369–375.

Patil BV. 1996. Competitive displacement of *Bemisia* with leafhoppers and aphids in a cotton ecosystem. In *Bemisia 1995: Taxonomy, Biology, Damage, Control and Management*, ed. D Gerling, RT Mayer, pp. 243–245. Andover, Hants: Intercept Ltd.

Perring TM. 1996. Biological differences of two species of *Bemisia* that contribute to adaptive advantage. In *Bemisia 1995: Taxonomy, Biology, Damage, Control and Management*, ed. D Gerling, RT Mayer, pp. 3–16. Andover, Hants: Intercept Ltd.

Rao NV, Reddy AS. 1994. Incidence of whitefly, *Bemisia tabaci* Genn., in relation to other sucking pests on cotton. *Indian J. Entomol.* 56:104–106.

Rojas MR, Hagen C, Lucas WJ, Gilbertson RL. 2005. Exploiting chinks in the plant's armor: evolution and emergence of geminiviruses. *Annu. Rev. Phytopathol.* 43:361–394.

Rosell RC, Torres-Jerez I, Brown JK. 1999. Tracing the geminivirus-whitefly transmission pathway by polymerase chain reaction in whitefly extracts, saliva, hemolymph, and honeydew. *Phytopathology* 89:239–246.

Ruan YM, Liu SS. 2005. Detection and phylogenetic analysis of prokaryotic endosymbionts in *Bemisia tabaci*. *Acta Entomol. Sin.* 48:859–865.

Ruan YM, Xu J, Liu SS. 2006. Effects of antibiotics on fitness of the B biotype and a non-B biotype of the whitefly *Bemisia tabaci*. *Entomol. Exp. Appl.* 121:159–166.

Russell JA, Moran NA. 2005. Horizontal transfer of bacterial symbionts: heritability and fitness effects in a novel aphid host. *Appl. Environ. Microbiol.* 71:7987–7994.

Russell JA, Torre LA, Sabater-Muñoz B, Moya A, Moran NA. 2003. Side-stepping secondary symbionts: widespread horizontal transfer across and beyond the Aphididoidea. *Mol. Ecol.* 12:1061–1075.

Sakurai M, Koga R, Tsuchida R, Meng X-Y, Fukatsu T. 2005. *Rickettsia* symbiont in the pea aphid *Acyrthosiphum pisum*: novel cellular tropism, effect on host fitness, and interaction with the essential symbiont *Buchnera*. *Appl. Environ. Microbiol.* 71:4069–4075.

Sandstrom JP, Russell JA, White JP, Moran NA. 2001. Independent origins and horizontal transfer of bacterial symbionts of aphids. *Mol. Ecol.* 10:217–228.

Sasaki T, Ishikawa H. 1995. Production of essential amino acids from glutamate by mycetocyte symbionts of the pea aphid, *Acyrthosiphon pisum*. *J. Insect Physiol.* 41:41–46.

Scarborough CL, Ferrari J, Godfray HCJ. 2005. Aphid protected from pathogen by endosymbiont. *Science* 310:1781.

Silva FJ, Latorre A, Moya A. 2001. Genome size reduction through multiple events of gene disintegration in *Buchnera* APS. *Trends Genet.* 17:615–618.

Sinha RC, Peterson EA. 1972. Uptake and persistence of oxytetracycline in aster plants and vector leafhoppers in relation to inhibition of clover phyllody agent. *Phytopathology* 62:377–383.

Sinkins SP, Curtis CF, O'Neill SL. 1997. The potential application of inherited symbiont systems to pest control. In *Influential Passengers. Inherited Microorganisms and Arthropod Reproduction*, ed. SL O'Neill, AA Hoffmann, JH Werren, pp. 155–175. New York: Oxford University Press.

Soroker V, Nelson DR, Bahar O, Reneh S, Yablonski S, Palevsky E. 2003. Whitefly wax as a cue for phoresy in the broad mite, *Polyphagotarsonemus latus* (Acari: Tarsonemidae). *Chemoecology* 13:163–168.

Stouthamer R, Breeuwer JAJ, Hurst GDD. 1999. *Wolbachia pipientis*: microbial manipulator of arthropod reproduction. *Annu. Rev. Microbiol.* 53:71–102.

Šulc K. 1909. O biologii kvasnic a jejich symbiose s hmysem. (Über die Biologie der Hefepilze und ihre Symbiose mit Insekten, Vortrag). *Vers. Naturw. Ges. Mahrisch-Ostrau* 5, November. (Sbornik Prirodovedecke Spolecnosti Mor. Ostrave Vol. 2, 1923).

Szklarzewicz T, Moskal A. 2001. Ultrastructure, distribution, and transmission of endosymbionts in the whitefly *Aleurochiton aceris* Modeer (Insecta, Hemiptera, Aleyrodinea). *Protoplasma* 218:45–53.

Takiya DM, McKamey SH, Cavichioli RR. 2006. Validity of *Homalodisca* and of *H. vitripennis* as the name for glassy-winged sharpshooter (Hemiptera: Cicadellidae: Cicadellinae). *Ann. Entomol. Soc. Am.* 99:648–655.

Thao ML, Baumann P. 2004a. Evolutionary relationships of primary prokaryotic endosymbionts of whiteflies and their hosts. *Appl. Environ. Microbiol.* 70:3401–3406.

Thao ML, Baumann P. 2004b. Evidence for multiple acquisition of *Arsenophonus* by whitefly species (Sternorrhyncha: Aleyrodidae). *Curr. Microbiol.* 48:140–144.

Thao ML, Baumann L, Hess JM, Falk BW, Ng JC, Gullan K, Baumann PJ. 2003. Phylogenetic evidence for two new insect-associated chlamydia of the family *Simkaniaceae*. *Curr. Microbiol.* 47:46–50.

Truernit E, Sauer N. 1995. The promoter of the *Arabidopsis thaliana SUC2* sucrose-$H^+$ symporter gene directs expression of ß-glucuronidase to the phloem: evidence for phloem loading and unloading by *SUC2*. *Planta* 196:564–570.

Tsuchida T, Koga R, Fukatsu R. 2004. Host plant specialization governed by facultative symbiont. *Science* 303:1989.

Tsueda H, Tsuchida K. 1998. Differences in spatial distribution and life history parameters of two sympatric whiteflies, the greenhouse whitefly (*Trialeurodes vaporariorum* Westwood) and the silverleaf whitefly (*Bemisia argentifolii* Bellows and Perring), under greenhouse and laboratory conditions. *Appl. Entomol. Zool.* 33:379–383.

van den Heuvel J, Verbeek M, van der Wilk F. 1994. Endosymbiotic bacteria associated with circulative transmission of potato leafroll virus by *Myzus persicae*. *J. Gen. Virol.* 75:2559–2565.

van Ham RCHJ, Moya A, Latorre A. 2004. The evolution of symbiosis in insects. In *Evolution: From Molecules to Ecosystems*, ed. A Moya, E Font, pp. 94–105. Oxford: Oxford University Press.

van de Ven WTG, LeVesque CS, Perring TM, Walling LL. 2000. Local and systemic changes in squash gene expression in response to silverleaf whitefly feeding. *Plant Cell* 12:1409–1423.

von Dohlen CD, Moran NA. 1995. Molecular phylogeny of the Homoptera: a paraphlyetic taxon. *J. Mol. Evol.* 41:211–223.

Walters FS, Mullin CA, Gildow FE. 1994. Biosynthesis of sorbic acid in aphids: an investigation into symbiont involvement and potential relationship with aphid pigments. *Arch. Insect Biochem. Physiol.* 26:49–67.

Weeks AR, Velten R, Stouthamer R. 2003. Incidence of a new sex-ratio-distorting endosymbiotic bacterium among arthropods. *Proc. R. Soc. Lond. B Biol. Sci.* 270:1857–1865.

Wernegreen JL, Degnan PH, Lazarus AB, Palacios C, Bordenstein SR. 2003. Genome evolution in an insect cell: distinct features of an ant-bacterial partnership. *Biol. Bull.* 204:221–231.

Wernegreen JJ, Ochman H, Jones IB, Moran NA. 2000. Decoupling of genome size and sequence divergence in a symbiotic bacterium. *J. Bacteriol.* 182:3867–3869.

Wilkinson TL, Koga R, Fukatsu T. 2007. Role of host nutrition in symbiont regulation: impact of dietary nitrogen on proliferation of obligate and facultative bacterial endosymbionts of the pea aphid *Acyrthosiphon pisum*. *Appl. Environ. Microbiol.* 73:1362–1366.

Wilkinson TL, Kukatsu T, Ishikawa H. 2003. Transmission of symbiotic bacteria *Buchnera* to parthenogenetic embryos in the aphid, *Acyrhosiphon pisum* (Hemiptera: Aphididae). *Arth. Struct. Dev.* 32:241–245.

Wu D, Daugherty SC, Van Aken SE, Pai GH, Watkins KL, Khouri H, Tallon LJ, Zaborsky JM, Dunbar HE, Tran PL, Moran NA, Eisen JA. 2006. Metabolic complementarity and genomics of the dual bacterial symbiosis of sharpshooters. *PLoS Biol.* 4(6):e188. DOI: 10.1371/journal.pbio.0040188

Zabalou S, Riegler M, Theodorakopoulou M, Stauffer C, Savakis C, Bourtzis K. 2004. *Wolbachia*-induced cytoplasmic incompatibility as a means for insect pest population control. *PNAS* 101:15042–15045.

Zang L, Chen WQ, Liu SS. 2006. Comparison of performance on different host plants between the B biotype and a non-B biotype of *Bemisia tabaci* from Zhejiang, China. *Entomol. Exp. Appl.* 121:221–227.

Zang L, Liu SS. 2007. A comparative study on mating behaviour between the B biotype and a non-B biotype of *Bemisia tabaci* (Hemiptera: Aleyrodidae) from Zhejiang, China. *J. Insect Behav.* 20:157–171.

Zchori-Fein EY, Brown JK. 2002. Diversity of prokaryotes associated with *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). *Ann. Entomol. Soc. Am.* 95:711–718.

Zchori-Fein EY, Gottlieb S, Kelly E, Brown JK, Wilson JM, Karr TL, Hunter MS. 2001. A newly discovered bacterium associated with parthenogenesis and a change in host selection behavior in parasitoid wasps. *PNAS* 98:12555–12560.

Zchori-Fein EY, Perlman SJ, Kelly SE, Katzir N, Hunter MS. 2004. Characterization of "Bacteriodetes" symbionts in *Encarsia* wasps (Hymenoptera: Aphelinidae): proposal of "*Candidatus* Cardinium hertigii". *Int. J. Syst. Evol. Microbiol.* 54:961–968.

Zhang LP, Zhang GY, Zhang YJ, Zhang WJ, Liu Z. 2005. Interspecific interactions between *Bemisia tabaci* (Hem., Aleyrodidae) and *Liriomyza sativae* (Dipt., Agromyzidae). *J. Appl. Entomol.* 129:443–446.

# Chapter 6
# Population Dynamics, Demography, Dispersal and Spread of *Bemisia tabaci*

**Steven E. Naranjo, Steven J. Castle, Paul J. De Barro, and Shu-Sheng Liu**

## Introduction

Understanding the population-level processes of any pest insect is central to predicting temporal and spatial changes in abundance and occurrence, as well as in developing effective pest management strategies, whether on single crops on individual farms or multiple crops within agricultural landscapes. Four components drive population dynamics in the time-space continuum: birth rates, death rates, immigration rates, and emigration rates. This deceptively simple fact becomes immensely complex, however, when one considers all the interacting biotic and abiotic factors that influence each of these key population rates. The challenge becomes even greater for a broadly polyphagous and non-diapausing pest like *Bemisia tabaci* that can thrive and reproduce on multiple host plants over the entire year in areas it inhabits (Mound and Halsey 1978; Watson et al. 1992; Naranjo et al. 2004).

A wide variety of methods can be used to study the population ecology and dynamics of an organism, many of which have been employed for the study of *B. tabaci*. These methods include observation and survey, experimental manipulation of biotic and abiotic factors, life tables, mark-recapture, simulation models, and more recently, molecular ecology methods. Several reviews have summarized past research in the area (Butler et al. 1986; Baumgärtner and Yano 1990; Byrne and Blackmer 1996; Riley et al. 1996; Drost et al. 1998; Henneberry and Castle 2001). In addition to the increasing impact of *B. tabaci* over a much greater geographic range – and greater diversity of crops – the breadth and depth of scientific literature regarding this pest has expanded, with nearly 7,000 non-abstract citations

S.E. Naranjo (✉)
USDA-ARS, Arid-Land Agricultural Research Center, 21881 North Cardon Lane, Maricopa, AZ 85138 , USA
e-mail: steve.naranjo@ars.usda.gov

Authorship of primary chapter sections
Naranjo: Sampling Populations; Demography; Dispersal, Migration and Seasonality; Population Modeling
Castle: Demography; Dispersal, Migration and Seasonality; Population Outbreaks
De Barro and Liu: Invasion and Spread

P.A. Stansly, S.E. Naranjo (eds.), *Bemisia*: Bionomics and Management of a Global Pest,
DOI 10.1007/978-90-481-2460-2_6, 

reported since the late 1800s, and roughly 310 new papers published each year since 1996 (Naranjo et al. 2007). The most recent large-scale review of the bionomics and management of this pest was based on a workshop held in 1994 (Gerling and Mayer 1996). It was followed by a more focused review of pest management issues in 2001 (Naranjo and Ellsworth 2001). Excluding articles on insecticide efficacy testing, there have been nearly 800 papers published since 1996 focusing attention on population level processes. It would be untenable to summarize all of this literature. Instead, this review will focus on advances within several broad categories of population ecology: sampling, demography, dispersal, invasion and spread, as well as population outbreak and modeling, which largely derive from these more basic fields of study. Focus will be placed primarily on advances made in the past 10–12 years, but we will also re-evaluate and re-interpret some historical research in the field with the intent of clarifying and expanding our understanding of complex factors governing the abundance and spread of *B. tabaci*.

## Sampling Populations

In order to effectively study population level processes in agroecosystems – whether in the open field or protected enclosures such as greenhouses – it is first necessary to have the proper tools and techniques for reliably assessing population density. Several reviews have summarized efforts, up to approximately 1996, on crops such as cotton, cassava, peanut, cantaloupe, tomato, and various ornamentals and vegetables (Butler et al. 1986; Ohnesorge and Rapp 1986; Parrella et al. 1989; Ekbom and Rumei 1990; Naranjo 1996). Historically, cotton has been the focus of extensive sampling research, and well-defined methods and plans have been developed for both research and pest management application (reviewed in Naranjo 1996).

Recent effort has focused on a number of other crops including tomato (Miranda et al. 1998; Arno et al. 2006; Gusmao et al. 2005, 2006), cantaloupe (Gould and Naranjo 1999; Lin et al. 2006), cucumber, watermelon and muskmelon (de Moura et al. 2003; Shen et al. 2005a), grapes (Haji et al. 2001), peppers (Fernandez et al. 2002; Lacasa-Plasencia et al. 2004), sesame (Laurentin and Pereira 2002), common bean (Pereira et al. 2004a, b), eggplant (Shen et al. 2005a) and gerbera (Yao and Zheng 2007). Cotton also continues to be a focus of sampling research (e.g., Ahmad and Aslam 2002; Atakan and Canhilal 2004; Gencsoylu 2007; Karut and Kazak 2007). As in the past, much of the research is centered on comparing and contrasting various methods and techniques for measuring relative density – e.g., on-plant counts, beat trays, sticky traps – describing the within and between plant distributions of various whitefly stages, selection of appropriate sample units and sampling techniques, and development of sampling plans, including sequential and binomial sampling plans. Researchers also continue to evaluate the use of various kinds of sticky traps and non-sticky traps (e.g. Chu et al. 1998) as a means of monitoring general population trends despite research demonstrating the poor and inconsistent predictive power of these methods for estimating densities in the crop itself (see Naranjo et al. 1995). This lack of correlation between trap catches

and field populations also has been demonstrated recently for parasitoids attacking *B. tabaci* (Hoelmer and Simmons 2008). Such trapping methods will continue to play a role in larger scale monitoring of pest incidence, but probably not in the estimation of density in either research or pest management application.

Almost without exception, the focus of sampling research with *B. tabaci* has been on developing relative sampling methods and plans. These methods continue to play an important role in pest management by providing consistent and reliable means for measuring pest density as well as in determining the need for control, and for comparative experimental studies. However, they are not adequate for detailed population studies or studies across different crops and host plants. For this application absolute – number per unit area – methods are generally required. The continued focus on relative methods is not surprising, given the large number of host plants that *B. tabaci* infests and the high population densities with which it occurs on many hosts. The techniques, and associated cost of methods, for estimating absolute density are prohibitive for many systems. Nonetheless, the increasing focus on a landscape level perspective encompassing multiple crops in a region creates an urgent need for developing absolute methods that will permit population density to be compared and analyzed within this broader spatial context. For the sessile immature stages, this will invariably involve counting insects on the whole plant, or on portions of the plant that can be reliably related to whole plant density (e.g., Ohnesorge and Rapp 1986; Abisgold and Fishpool 1990; Naranjo and Flint 1994). For mobile adults, leaf counts can be related to whole-plant counts (Naranjo and Flint 1995). Vacuum devices can also be used to remove a large fraction of the population on the whole plant if plants are small and distinct enough (Gerling and Horowitz 1984). Crops with more complex and prostrate canopy structures – such as cantaloupes – and those with complex canopy architecture – e.g., ornamental lantana – represent a greater challenge.

Some progress has been made in automating insect counting that could reduce effort, and increase accuracy, in both relative and absolute sampling. For example, research initiated in the early 1990s attempted to automate counting whitefly nymphs on leaves with digital video and image processing (Carruthers 1993). The method showed promise, but was not pursued further because of technical and funding issues. More recently, Bauch and Rath (2005) developed a prototype system to automate the collection and counting of adult whiteflies using mechanical aspirators and digital image analysis. The system may have promise for greenhouse application.

## Demography

Demography is defined as the study of birth and death rates and how they affect population age structure and rates of change in population size (Ricklefs 1979). Movement is also an important feature in determining population size, but that topic will be discussed in more detail in the next section. *B. tabaci* has six developmental stages: egg, four nymphal instars, and the adult. All are intimately connected to

the surface of their host plant and all but the adult stage and brief, early 1st instar crawler are functionally immobile (see Walker et al. Chapter 4 for more detail).

## *Life History Studies*

Numerous studies have examined multiple life history traits of *B. tabaci* on many host plants under a variety of environmental conditions in the laboratory, greenhouse and field (Table 6.1). One or more life history parameters have been measured in total on 51 different crops or other host plants. Under highly favorable conditions, generation times can be on the order of several weeks, resulting in upwards of 12 generations or more per year in tropical and subtropical areas of the world (Butler et al. 1986). In addition, adult females can produce hundreds of eggs within their 2–3 weeks lifespan (Drost et al. 1998; Henneberry and Castle 2001). In general, fecundity has been vastly underestimated in existing laboratory studies due to various methodological factors. Fecundity of well over 500 progeny per female has been demonstrated for *B. tabaci* feeding on cantaloupe, a favored host plant (Castle unpublished data). Overall, high fecundity and rapid development underlie the capacity of *B. tabaci* to build huge population densities over short periods of time; it also accounts, in part, for the insect's notorious pest status (see Population Outbreaks below).

## *Sex Ratio*

Sex ratio variation in *B. tabaci* populations has been observed in different studies spanning many decades, yet little is understood about the governing mechanisms or effects on population growth. Arrhenotokous sex determination often yields biased sex ratios favoring one or the other sex, depending on a range of conditions (Krainacker and Carey 1990; Wrensch 1993). Too few field studies of *B. tabaci* sex ratios have been conducted to determine a general pattern. In Israel, Horowitz and Gerling (1992) found that female:male ratios of 3:1 early season (May-June) shifted to parity by mid September, and then late season became male-biased. A similar pattern was reported for *B. tabaci* on tobacco in India (Pruthi and Samuel 1942), other reports have indicated male-biased ratios (Trehan 1944; Khalifa and El Khidir 1964).

Skewed sex ratios arise through various mechanisms affecting either the relative birth or death rates of the sexes. For example, early biological studies of *B. tabaci* indicated that life spans are longer for females (Avidov 1956; Khalifa and El Khidir 1964; Azab et al. 1971). The sex ratio of emerging adults is therefore of potentially greater interest in terms of revealing variation among populations. Differences in developmental success between the sexes are insufficient to skew sex ratios because the quality of resources available to developing cohorts of nymphs should be similar. As with other polyphagous pests, such as spider mites (Wrensch and Young 1983), it is unlikely that differential mortality – at either the beginning or end of the life

**Table 6.1** Summary of life history studies conducted on *Bemisia tabaci*

| Host species | Common name | Study type[a] | Temperature profile[b] | Development[c] | Survival | Reproduction[d] | Sex ratio | Citation[e] |
|---|---|---|---|---|---|---|---|---|
| **Cultivated crops** | | | | | | | | |
| *Beta vulgaris* | Sugarbeet | GH | S | E, N | | X | | 6 |
| *Brassica alboglabra* | Chinese kale | L | | E, N | E, N, A | X | X | 12 |
| *Brassica oleracea* | Cabbage | L, GH | S | E, N | E, N, A | X | | 6, 53, 56 |
| *Brassica oleracea* | Broccoli | L, GH | S | E, N | E, N, A | X | | 15, 55 |
| *Cajanus cajan* | Pigeonpea | L | S | | N | X | | 37 |
| *Capsicum annuum* | Pepper | L | S, M | E, N | E, N, A | X | | 15, 26, 33, 35, 53 |
| *Citrullus lanatus* | Watermelon | L | S, M | E, N | N | | | 4, 15 |
| *Cucumis melo* | Cantaloupe | L, GH | S, M | E, N | E, N, A | X | | 4, 13, 15, 55 |
| *Cucumis sativus* | Cucumber | L, GH, F | S, M | E, N | E, N, A | X | X | 4, 15, 23, 26, 38, 39, 40, 43, 48, 49, 53 |
| *Cucurbita maxima* | Pumpkin | L | S | E, N | N, A | X | | 13, 15 |
| *Cucurbita pepo, C.* spp. | Squash | L, GH, F | S, M | E, N | E, N, A | X | X | 4, 6, 13, 23, 43, 48, 55, 56 |
| *Daucus carota* | Carrot | L | S | E, N | | | | 15 |
| *Dolichos lablab* | Hyacinth bean | L, F | S | E, N | A | X | | 18, 27 |
| *Glycine max* | Soybean | L | S | E, N | E, N, A | X | | 1, 11, 36 |
| *Gossypium hirsutum, G. barbadense* | Cotton | L, GH, F | S, M | E, N | E, N, A | X | X | 1, 5, 8, 9, 12, 13, 15, 22, 23, 25, 27, 39, 40, 42, 43, 44, 45, 46, 50, 51, 55, 56, 57 |
| *Ipomoea batatas* | Sweet potato | L | S, M | E, N | E, N, A | X | X | 3, 12, 15, 17, 49 |
| *Lactuca sativa* | Lettuce | L, GH | S | E, N | N, A | X | | 9, 13, 14, 15 |
| *Solanum lycopersicum* | Tomato | L, GH, F | S, M | E, N | E, N, A | X | X | 7, 12, 13, 15, 16, 23, 24, 26, 46, 47, 48, 49, 53, 54 |
| *Manihot esculenta* | Cassava | L | S | E, N | A | X | | 10 |
| *Medicago sativa* | Alfalfa | L, GH | S | E, N | E, N | X | | 15, 55 |
| NA | NA | L | M | E, N | E, N, A | X | | 29 |
| *Nicotiana tabacum* | Tobacco | L, GH | S, M | E, N | E, N, A | X | X | 12, 19, 47, 53, 56 |
| *Parthenium argentatum* | Guayule | L | S | E, N | | | | 15 |

**Table 6.1** (continued)

| Host species | Common name | Study type[a] | Temperature profile[b] | Development[c] | Survival | Reproduction[d] | Sex ratio | Citation[e] |
|---|---|---|---|---|---|---|---|---|
| *Phaseolus angularis* | Adzuki bean | L | S | E, N | A | X | | 11 |
| *Phaseolus vulgaris* | Common Bean | L, GH | S | E, N | E, N, A | X | X | 10, 15, 46, 48, 49, 53, 56 |
| *Solanum melongena* | Eggplant | L, GH | S, M | E, N | E, N, A | X | X | 2, 15, 20, 26, 41, 43, 47, 48, 49, 52, 53 |
| *Vigna mungo* | Black gram | L | S | | N | X | | 37 |
| *Vigna radiata* | Mung bean | L | S | E, N | N, A | X | | 1, 11, 37 |
| *Vigna sinensis* | Cowpea | L | S | E, N | E, N, A | X | | 36 |
| *Vigna unguiculata* | Blackeyed pea | L | | | N | X | | 37 |
| **Wild/ornamental hosts** | | | | | | | | |
| *Amaranthus retroflexus* | Redroot amaranth | F | S | E, N | E, N, A | X | | 21 |
| *Brassica kaber* | Wild mustard | L, GH | S | E, N | N, A | X | | 34 |
| *Capsella bursa-pastoris* | Shepherd's purse | L, GH | S | E, N | N, A | X | | 34 |
| *Chromolaena odorata* | Jack in the bush | F | S | E, N | E, N, A | X | | 21 |
| *Codiaeum variegatum* | Garden croton | L | S | E, N | E, N, A | X | | 30, 31 |
| *Convolvulus arvensis* | Field bindweed | L | S | E, N | | | | 14 |
| *Cyamopsis tetragonoloba* | Guar | L | S | E, N | | | | 15 |
| *Datura stramonium* | Jimsonweed | L | M | E, N | | | | 16 |
| *Desmodium tortuosum* | Florida beggarweed | F | S | E, N | E, N, A | X | | 21 |
| *Euphorbia characias* | Albanian spurge | L | M | E, N | | | | 16 |
| *Euphorbia pulcherrima* | Poinsettia | L | S, M | E, N | E, N, A | X | X | 5, 10, 16, 19, 28, 30, 46 |
| *Hibiscus mutabilis* | Dixie rosemallow | L | S | E, N | E, N, A | X | | 30 |
| *Hibiscus rosa-sinensis* | Hibiscus | L | S | E, N | E,N, A | X | X | 30, 32, 46 |
| *Jatropha gossypifolia* | Bellyache bush | L | S | E, N | A | X | | 10 |

**Table 6.1** (continued)

| Host species | Common name | Study type[a] | Temperature profile[b] | Development[c] | Survival | Reproduction[d] | Sex ratio | Citation[e] |
|---|---|---|---|---|---|---|---|---|
| *Lactuca serriola* | Prickly lettuce | L, GH | S | E, N | N, A | X | | 34 |
| *Lantana camara* | Lantana | L, GH | M | E, N | A | X | X | 47 |
| *Linum usitatissimum* | Common flax | L | S | E, N | | | | 15 |
| *Malva parviflora* | Cheeseweed mallow | L, GH | S | E, N | N, A | X | | 34 |
| *Malvastrum coromandelianum* | Threelobe false mallow | F | S | E, N | E, N, A | X | | 21 |
| *Sonchus asper* | Spiny sow thistle | L | S | E, N | | | | 14 |

[a] L, laboratory; GH, greenhouse; F, field or semi-field.
[b] Experimental conditions under which the study was conducted. S denotes either a single controlled temperature or an uncontrolled variable temperature such as in a greenhouse or field setting; M denotes controlled multiple temperatures potentially useful in developing rate models.
[c] E, egg stage; N, nymphal stages; A, adult stage for development and survival.
[d] Reproductive traits include oviposition rate, total fecundity, and age at first reproduction.
[e] Numbers listed here are associated with numbers following a given citation in the Literature Cited section.

cycle – can alone account for the degree of female-bias often observed in sex ratios of *B. tabaci*. There is also the possibility that inseminated female whiteflies control fertilization of eggs much as it is thought to occur in arrhenotokous Hymenoptera (King 1993; Nadel and Luck 1985). However, Li et al. (1989) showed that both sexes mate multiple times; Liu et al. (2007) also found that multiple matings were required to sustain female progeny production in both biotype B and the indigenous Chinese ZHJ1. This represents a departure from parasitic Hymenoptera and suggests that differences between *B. tabaci* and the better-studied Hymenoptera need to be explored.

## *Life Tables*

Several recent laboratory studies have estimated such basic demographic parameters as generation time, net reproductive rate, and intrinsic rates of increase (e.g., Tsai and Wang 1996, Chen et al. 2003a; Xu et al. 2003; Samih 2005; Kakimoto et al. 2007). All point to the known potential for extremely rapid population growth. However, the general usefulness of these measures in understanding and predicting field population dynamics is limited because they measure reproduction, development, and survival under ideal circumstances. Although it is possible to accurately measure birth processes, such as development and reproduction, under controlled conditions in a laboratory, greenhouse, or even a semi-field setting, it is impossible to meaningfully measure death processes in a similar manner due to the large number of both biological and ecological factors affecting survival. Consequently, the only way to accurately measure survival is through life tables or other manipulative approaches in the insect's actual habitat. Relatively few such studies have been attempted.

Life tables typically emphasize the characterization and measurement of mortality components affecting populations (Carey 1993). Although multiple and overlapping generations of *B. tabaci* pose significant problems, the sessile nature of immature stages provides an avenue for developing robust, cohort-specific life tables (Naranjo and Ellsworth 2005). To date, field-based life tables have been developed for *B. tabaci* on cotton (Horowitz et al. 1984, Naranjo and Ellsworth 2005; Naranjo et al. 2004; Naranjo, Ellsworth and Cañas unpublished data; Karut and Naranjo 2009), cassava (Asiimwe et al. 2007), soybean (de Albergaria et al. 2003), cucumber (Shen et al. 2005b), cantaloupe, broccoli, alfalfa, ornamental lantana, and various annual weeds (Naranjo et al. 2004; Naranjo, Ellsworth and Cañas unpublished data). Hoddle and van Driesche (1996) developed life tables for *B. tabaci* on poinsettias, but these were done in experimental greenhouses to assess the impact of augmentative biological control with parasitoids. Two general approaches were used in these studies, both of which were time consuming. One approach established artificial cohorts on plants, started with adults in leaf bags. This method was followed by repeated samplings to assess mortality over time and use of mathematical techniques to estimate stage survivorship (e.g. Horowitz et al. 1984). A second approach was based on natural cohorts

in field populations, also followed by repeated observations to determine death rate. However, this method also allowed the cause of death for each life stage to be determined, thus providing quantification of mortality factors as well (e.g. Naranjo and Ellsworth 2005).

Most life table studies document high levels of preimaginal mortality in *B. tabaci* populations on a number of host plants (Fig. 6.1). Mean estimates have exceeded 93% with the exception of soybean in Brazil, spring cantaloupes in Arizona, USA, and cucumber in China. However, generational mortality closer to 99% would probably be necessary to control or regulate populations in most systems, given the insect's reproductive capacity (Naranjo and Ellsworth 2005). Field-based life table studies to estimate adult mortality or fecundity in the field are lacking for all crops.

Multiple mortality factors affecting immature stages of *B. tabaci* have been identified. They include such natural enemies as predators, parasitoids and fungi, inviability of eggs, dislodgement from the plant surface due to weather events and predators, and physiological and other unknown agents (Fig. 6.1a). Collectively, natural enemies inflict high levels of mortality on *B. tabaci* on all host plants that have been examined with the exception of winter-planted broccoli in Arizona, USA where desiccation takes the greatest toll (Naranjo et al. 2004). Predation has been found to be a key factor in Arizona cotton, cantaloupes, annual weeds, alfalfa, and ornamental lantana (Naranjo and Ellsworth 2005; Naranjo, Ellsworth and Cañas unpublished data). However, parasitism by aphelinids was shown to be the key factor

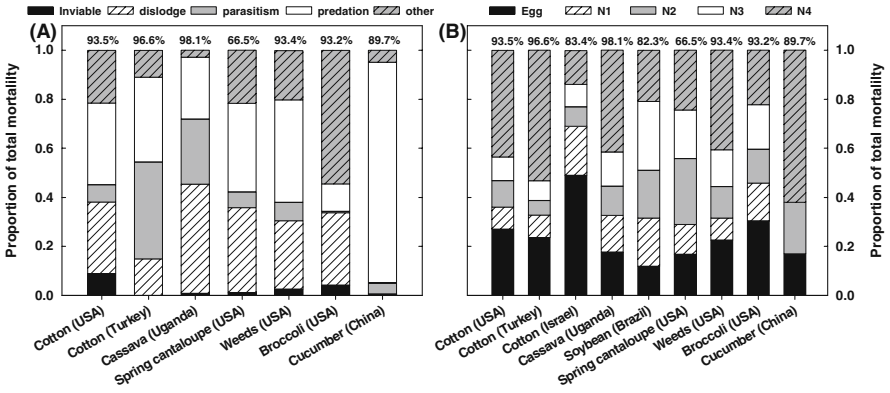



**Fig. 6.1** (**a**) Summary of partial, field-based life table studies of immature (eggs and nymphs). *B. tabaci* on various host plants. Results are based on mean contributions of various mortality agents over multiple generations. Numbers above the bars denote mean generational mortality. Studies on cotton in Turkey did not report egg inviability, and this mortality is partially reflected in "other". Studies on cucumber in China did not report dislodgement, but it is assumed that estimates of predation include dislodgement. (**b**) Summary of partial, field-based life table studies of immature (eggs and nymphs) *B. tabaci* on various host plants. Results are based on mean mortalities within each developmental stage over multiple generations. Numbers above the bars denote mean generational mortality. Studies on cucumber in China partitioned mortality by eggs, small nymphs (N1 + N2), and large nymphs (N3 + N4). See text for study citations

in Turkish cotton (Karut and Naranjo 2009) and cassava in Uganda (Asiimwe et al. 2007). Dislodgement from the plant surface can also provide significant mortality. Naranjo and Ellsworth (2005) determined that high winds and blowing dust were a major cause of death for most life stages, while rainfall contributed to the dislodgement of eggs and young nymphs. Chewing predators, such as beetles, caused most dislodgement of larger nymphs. Anecdotal observations of the negative impact of weather on *B. tabaci* population dynamics have been noted by early workers (e.g. Khalifa and El Khidir 1964; Avidov and Harpaz 1969).

In general, mortality rates are highest during the fourth nymphal stadium followed by the egg stage (Fig. 6.1b). These are the longest stages of the immature life cycle, so it is not surprising that they sustain the highest death rates. However, mortality appeared to be more evenly distributed among the five immature life stages on soybean in Brazil and spring cantaloupes, and winter broccoli in the USA (Fig. 6.1b).

While life tables provide critical information on the causes and rates of mortality affecting populations, additional data, such as immigration and emigration rates, are needed to fully understand the factors regulating population growth. Density-dependence of mortality factors is another important facet but is extremely difficult to assess, in part because of the spatial scale over which it might occur. For this reason, life table studies of *B. tabaci* have not attempted to asses density-dependence except for Naranjo and Ellsworth (2005) who failed to demonstrate it at the plant level for any of the mortality factors they examined in their life tables.

Failure to identify density-dependence does not preclude its existence (Bellows et al. 1992), nor would density dependence necessarily regulate a population below economic threshold. Still, we know that certain factors can suppress populations for extended periods while not necessarily regulating them within defined boundaries. For example, several studies have documented resurgence of *B. tabaci* populations following the use of broad-spectrum insecticides (e.g. Abdelrahman and Munir 1989; Devine et al. 1998) suggesting that natural enemies are capable of suppressing *B. tabaci* populations under some circumstances. The role of density-dependent action by natural enemies in these outcomes is unknown. In general, historical evidence suggests a central role of insecticide overuse in the promotion of *B. tabaci* as a pest (Eveleens 1983), but a re-appraisal of the data suggests other governing factors (Castle 1999, also see Population Outbreaks below). A significant role of natural enemies – primarily predators – is also suggested by suppression of *B. tabaci* over long periods following the use of selective insecticides to quell population growth initiated by immigration into early season Arizona cotton (Ellsworth and Martinez-Carrillo 2001; Naranjo 2001; Naranjo and Ellsworth 2009). Although density-dependent action of mortality agents cannot be ruled out in these instances, patterns of population change in *B. tabaci* suggest a strong influence of seasonal host related factors (e.g., crop senescence) in controlling growth and movement of populations in both individual fields and within the broader landscape (see discussion of seasonality below).

## Dispersal, Migration and Seasonality

*B. tabaci* is a multivoltine insect that has no diapause or quiescent stage. As a result, populations are sustained through the continual exploitation of multiple host resources, both wild and cultivated, over the annual cycle. Dispersal is therefore an integral component of the ecology of *B. tabaci* that enables host finding and colonization in a constantly shifting environment. Dispersal is also a critical mechanism enabling *B. tabaci* to vector plant viruses, distribute insecticide resistance genes, and escape natural enemies.

### *Measuring Movement and Flight Behavior*

The ability of *B. tabaci* to disperse has been noted from the time it was first recognized as a pest. Early literature is replete with observational and anecdotal evidence of the displacement and dispersion of populations from one host plant to another and the capture of adults on traps removed from crop areas (e.g., Husain and Trehan 1933; Khalifa and El Khidir 1964; Costa 1975; Naresh and Nene 1980; Gerling and Horowitz 1984; Stansly 1996). However, more carefully controlled experimental studies have occurred only in the past several decades. Investigations of dispersal by *B. tabaci* have been conducted in laboratory and field settings in order to address specific questions about flight duration (Blackmer and Byrne 1993a; Blackmer et al. 1995a), distances travelled (Byrne et al. 1996; Isaacs and Byrne 1998), exogenous cues (Blackmer and Byrne 1993b; Isaacs and Byrne 1998), and endogenous cues (Blackmer et al. 1995a, b) that govern flight – or its cessation – as well as individual variation in flight behavior (Blackmer and Byrne 1993a; Blackmer et al. 1995a). Insight gained from decades of aphid flight and migration studies have shown various strategies of dispersal in terms of migratory vs. foraging flight (Kennedy 1985). This prompted similar interests in *B. tabaci* with studies on wing morphology suggesting the presence of multiple flight morphs (Byrne and Houck 1990). A follow-up study failed to confirm significant differences in wing morphology, but did identify differences in flight behavior with respect to duration of flight in different proportions of the population (Blackmer et al. 1995a). Further investigation using a vertical flight chamber demonstrated individual differences in response times to vegetative cues presented at intervals within the flight chamber. Whiteflies responding within 5 min (76%) were considered trivial flyers, those flying $\geq$15 min (6%) were considered migratory, and the remaining 16% were grouped intermediate (Blackmer and Byrne 1995a). The inclusion of an intermediate group fits well in the context of the assertion by Dingle (1996) that flight distance from a source is more appropriately considered as a continuum of responses rather than simply binary *sensu* Kennedy (1985).

Mark and recapture studies have demonstrated a strong directional component to dispersal by *B. tabaci*. Whiteflies marked the evening before with DayGlo® dust were consistently trapped the following day as far as 2.7 km from the source field

in Yuma, AZ, USA (Byrne et al. 1996). Whether individuals trapped at this distance represented foraging or migratory flyers was uncertain, but the regularity in which at least some individuals attained the 2.7 km distance prompted the suggestion that migrating whiteflies were capable of exceeding this distance (Isaacs and Byrne 1998). The conclusion was supported by Cohen et al. (1988) who found that whiteflies marked on the weed *Cynanchum acutum* were subsequently recaptured 6 days later on traps near tomato fields some 7 km away. In another field study, a series of traps was positioned at four heights from ground level up to 7.2 m at six equidistant locations out to 100 m from the source field. A gradual decline in numbers of whiteflies caught with increasing distance out to 100 m was observed for both males and females. Less than 5% of marked whiteflies were caught at the 7.2 m height, indicating steep ascending flights by a small number of individuals. A significant relationship between egg load and trap height but not trap distance was observed, indicating a modest trade-off between flight dispersal and oogenesis as suggested by Blackmer et al. (1995a) and substantiated by Isaacs and Byrne (1998).

It has been suggested that short-range flights are of greater importance to weak flyers, such as aphids and whiteflies, than longer distance migratory flights (Loxdale et al. 1993). Although *B. tabaci* may be a weak flyer, it is nonetheless highly adaptable at moving considerable distances within its environment. Large swarms of flying *B. tabaci* in Brazil were noted in small towns several kilometers from soybean fields where they originated, prompting Costa (1975) to suggest that *B. tabaci* moves farther than is generally recognized. During the early to mid 1990s, it was not unusual to see huge numbers of adults within the Phoenix, Arizona metropolitan area, during the later summer and early fall, many kilometers removed from potential cotton source fields (SEN, SJC per. obs.). In regions – such as the irrigated desert valleys of the southwestern United States where high populations of *B. tabaci* have developed – the authors observed large numbers of whiteflies moving at heights of 1–3 m above the ground. Dispersal flights are most conspicuous during the first few hours of daylight on summer days before temperatures reach prohibitive levels.

Movement across the landscape to new reproductive hosts is dependent on staying aloft long enough to be carried by prevailing breezes; it is not uncommon to observe whiteflies in deep grass, or on other non-reproductive hosts during the intense heat of mid-day and late afternoon, before embarking on subsequent short-range flights in search of suitable hosts. Other dispersers have been observed in deep crevices in the ground of recently irrigated fallow fields – gaining shelter from sun and heat – but without any food source. A small-bodied insect like *B. tabaci* is unlikely to have sufficient body fat or water reserves to survive in the desert heat without deriving water and nutrition from its temporary host. Soluble carbohydrates in phloem are metabolized into sorbitol at rapid rates providing protection during high temperature exposures (Wolfe et al. 1998; Salvucci 2000). In addition to this physiological mechanism, the behavioral adaptability of dispersing *B. tabaci* to find shelter and derive sustenance allows incremental movements to be made by extending the range that can be covered – foraging flight – and increasing the probability of host finding. Experiments to measure survival rates during dispersal have not

been attempted because of the difficulty of simulating natural conditions, leaving unknown how long an adult *B. tabaci* can survive using temporary hosts, or how many short-range flights it can make before finding a permanent host or expiring. These observations point to the fact that the dispersal process is complicated by many biological and ecological facets, increasing the challenge of understanding and predicting movement by this insect even further.

Although several models have been developed to study spatial dynamics (see below), and predict the redistribution of *B. tabaci* in the landscape based on simple rules or diffusion processes (Wilhoit et al. 1994; Brewster et al. 1997), no studies have directly measured rate of emigration and immigration. As a consequence, our full understanding of *B. tabaci* population dynamics is limited to anecdotal observations, indirect experiments, and inferences drawn from untested models. Von Arx et al. (1983a) were the first to show the quantitative importance of movement to the population dynamics of *B. tabaci*. Their simulation model for the Sudanesse cotton system was based on temperature-dependent insect dynamics and a physiologically-based plant model. Immigration and emigration were fitted by simulation experiments to adjust for the result that within-field birth and death rates alone would have projected population densities several orders of magnitude greater than field populations by season's end (p. 357, Fig. 11). A similar approach was used by Naranjo and Ellsworth (2005) when they input their field estimates of generational death rates in a simulation model based on temperature-dependent birth rates (DeGrandi-Hoffman and Naranjo 1996) to project the quantitative contribution of immigration near the beginning of the season and emigration near season's end in Arizona cotton (Fig. 6.2). Although neither study provides mechanistically-based estimates of immigration and emigration rates, both demonstrate the critical importance of these population processes and highlight the need for more detailed research specifically addressing the measurement of these key rates and their relationship to both biotic and abiotic factors.

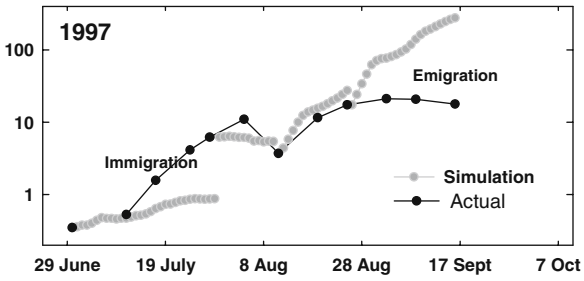



**Fig. 6.2** Comparison of simulated and actual population densities for *B. tabaci* on cotton in central Arizona, USA. Simulations are based on a temperature-dependent, stage-structured model that was initiated with actual insect densities (eggs, nymphs, and adults) and observed rates of immature mortality at the establishment of each of four cohorts. These discrepancies during early and late season reflect the important contribution of immigration and emigration. Modified from Naranjo and Ellsworth (2005) with permission from Blackwell Publishing

## *Seasonality and Metapopulations*

The complex processes outlined above – plus other facets of the insect's biology – underlie the seasonal host usage pattern and regional metapopulation dynamics of *B. tabaci* within the landscape (Fig. 6.3). Details vary from region to region depending on host plant composition and descriptions are limited to relatively few systems in the world and derived from surveys of crop and uncultivated hosts. Watson et al. (1992) described a typical seasonal pattern for the indigenous Biotype A of *B. tabaci* based on survey data from the Yuma Valley of Arizona in the late 1980s. Whitefly populations were at their lowest level in late winter, subsisting primarily on a variety of weeds. They moved onto cantaloupe and then cotton in spring and continued building to high levels in cotton during the summer and early fall. Early winter found them in vegetables like broccoli, cauliflower, and lettuce, after which they were left with only weeds in which to begin the cycle anew. These survey studies also pointed to the importance of distance between potential hosts, as well as the role of wind, in shaping seasonal patterns of occurrence. For example, Watson et al. (1992) showed a negative relationship between densities of *B. tabaci* on lettuce in the late fall with increasing distance from cotton source crops. They also showed the definitive role of wind with down-wind sink crops being disproportionately infested.

Similar patterns were described for *B. tabaci* infesting cotton in central Arizona (Butler and Henneberry 1984), the Central Valley of California (Godfrey et al. 1995), Israel (Gerling 1984), southern Florida (Stansly 1996) and southern India (Murugan and Uthamasamy 2001) even though the particular spring, summer, fall, and winter host plants can vary. Perennial hosts such as alfalfa and landscape ornamentals also play a role in several of these systems, even though their contribution is less well understood (Yee et al. 1997; Naranjo et al. 2004). There is a clear connection between quickly growing populations of *B. tabaci* on spring cantaloupe and the subsequent invasion of cotton in central Arizona (Kelly-Johnson et al. 1995; Naranjo and Ellsworth 2005); this pattern is exacerbated by the low level of natural mortality of *B. tabaci* on cantaloupe during this portion of the season (Naranjo et al. 2004,

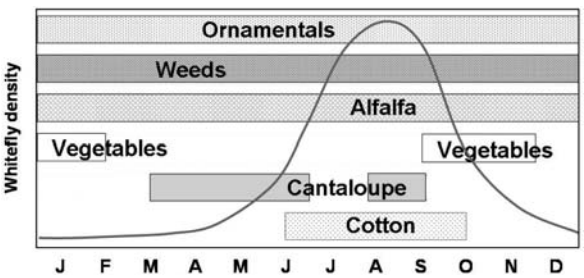



**Fig. 6.3** Example of the seasonal cycle and metapopulation dynamics of *B. tabaci* in an Arizona, USA cropping landscape. The *line graph* denotes the relative population size of the insect which is very low during winter months and reaches a peak during the summer on crops such as cotton

see Fig. 6.1). A similar pattern has been suggested by Legaspi et al. (1997) for cotton grown in the Lower Rio Grande Valley of Texas. Özgür et al. (1989) describes a slightly different seasonal pattern for *B. tabaci* infesting cotton in the Çukurova plain of Turkey. Here they found that the wild perennial shrub *Cistus* spp., which grows in the foothills adjacent to the plains, hosts populations of *B. tabaci* during the winter which then acts as a direct source for cotton and other vegetable crops in the plains. Their patterns show that spring vegetables are not a necessary bridge to cotton as they appear to be in other regions. There is a negative relationship between distance to *Cistus* spp. stands and *B. tabaci* infestations in spring crops in the plains, but not between spring vegetables and cotton. A variation on the theme was seen in south Florida, where declining whitefly populations are hosted by weeds during the wet summer fallow period (Stansly 1996). Populations then build up during fall in fruiting vegetables – especially tomato – although declining temperatures slow growth rates. The jump to overlapping spring vegetables initiates rapidly growing populations which, when good post harvest sanitation is practiced, have only the surrounding weeds for hosts after termination of the crop. Overall, these seasonal cycles are critical to the continued persistence of *B. tabaci* in agricultural landscapes and hold many clues for effective population management.

Much of metapopulation theory and application has focused on understanding the dynamics of relatively rare species that occupy fragmented habitats (e.g., Marquet 2002; Hanski 2004). However, some attention has been directed to control of mobile pests, and therefore may have relevance to *B. tabaci*. Levins (1969) used simple models to demonstrate that a control action applied synchronously to sub populations would lead to the greatest reduction in metapopulation size, mainly as a result of eliminating refuges that permit persistence of local populations. Such a phenomenon may explain the success of a whitefly management program introduced for Arizona cotton in 1996 (Ellsworth and Martinez-Carrillo 2001; Naranjo and Ellsworth 2009) based on sampling, economic thresholds, effective insecticides, and grower education. The program resulted in the relatively synchronous suppression of pest populations in individual fields over a wide area and an overall lowering of *B. tabaci* metapopulation density. A similar result was seen in south Florida, merely but encouraging growers to remove crop residues after harvest in late spring, thereby denying whitefly populations favorable hosts during summer (Stansly and Schuster 1990; Stansly 1996).

More complex metapopulation models have also included the role of natural enemies. For instance, Ives and Settle (1997) showed that natural enemies and rates of movement, by both the pest and natural enemies, can influence the degree to which synchronous or asynchronous control might produce the lowest metapopulation densities. Although we are beginning to understand the role and contribution of natural enemies to *B. tabaci* dynamics (Naranjo and Ellsworth 2005; also see Arnó et al. Chapter 15) our knowledge of natural enemy movement is even more rudimentary than that of the pest itself. Overall, expanded research at the metapopulation scale would be greatly beneficial to the development of more robust, sustainable, and predictable management systems for *B. tabaci*.

## Invasion and Spread

*B. tabaci* has a well-deserved reputation for being invasive, mostly because biotype B has spread – over the past 20 or so years – from its origins in the Middle East–Asia Minor region to at least 50 countries in Africa, the Americas, Asia, Australia and Europe via the trade in ornamentals (Cheek and Macdonald 1994; Dalton 2006). The Q biotype has also begun to invade from its origin in countries bordering the Mediterranean Basin to at least 10 countries in Africa, the Americas, Asia and Europe (Genbank, Zhang et al. 2005; Dalton 2006; Ian Scott personal communication; De Barro P unpublished data) again via the trade in ornamental species. The invasive ability and damage potential of the B biotype has earned it a place as one of the world's top 100 insidious species (http://www.issg.org) of global agriculture. The focus here will be on the underlying processes of invasion and establishment (Table 6.2).

In most instances, the history of invasion by *B. tabaci* has been poorly documented. Unfortunately, this is a typical characteristic of biological invasions (Reitz 2007). In the USA, for example, the displacement of the indigenous A biotype by B biotype was essentially over by the time it was detected (Hamon and Salguero 1987;

**Table 6.2** Summary and example citations of mechanisms identified as contributing to invasion

| Factors influencing competitive displacement | Genetic groups | References |
|---|---|---|
| Differential female fecundity | Mediterranean/Asia Minor/Africa (B) vs Mediterranean (Q) | Pascual and Callejas (2004) |
| Differential insecticide resistance | Mediterranean/Asia Minor/Africa (B) vs Mediterranean (Q) | Horowitz et al. (2003, 2005); Pascual and Callejas (2004); Pascual (2006) |
| Differential resource utilization | Mediterranean/Asia Minor/Africa (B) vs Mediterranean (Q), Mediterranean/Asia Minor/Africa (B) vs Australia (AN) | Pascual and Callejas (2004); De Barro et al. (2006) |
| Courting and mating interference | Mediterranean/Asia Minor/Africa (B) vs Mediterranean (Q), Mediterranean/Asia Minor/Africa (B) vs New World (A), Mediterranean/Asia Minor/Africa (B) vs Australian (AN), Mediterranean/Asia Minor/Africa (B) vs Asia II (ZHJ1) | Pascual and Callejas (2004); Perring et al. (1994); De Barro and Hart (2000); Liu et al. (2007) |
| Increased frequency of copulation | Mediterranean/Asia Minor/Africa (B) vs Asia II (ZHJ1), Mediterranean/Asia Minor/Africa (B) vs Australia (AN) | Liu et al. (2007) |
| Indirect effects of begomovirus infection in the host plant | Mediterranean/Asia Minor/Africa (B) vs Asia II (ZHJ1) | Jiu et al. (2007) |

Perring 1996). Establishment and displacement by B biotype was better monitored in the later invasions of Australia and China (Liu et al. 2007), presumably because to be forewarned is to be forearmed, in this case with the tools and knowledge to support a monitoring program.

B biotype was first detected in Australia in 1994 (Gunning et al. 1995). Quarantine trace-back and records of insecticide control failures suggested that entry occurred no earlier than mid-1993 and that spread was again facilitated by the ornamental trade industry (De Barro 1995). Analysis of field collections suggested that displacement of the indigenous population by B biotype was complete in 3–4 years in northern Queensland and 4–5 years in southern Queensland and New South Wales (Fig. 6.4). Climex modeling (Sutherst et al. 1999; Sutherst and De Barro unpublished data) and observation indicated displacement was complete in 19–45 generations (Fig. 6.4). However, in Katherine (Northern Territory), B biotype cycles between occasional establishment during the dry winter months and local extinction during the wet summer. Biotype B also failed to establish in roadside vegetation (primarily *Euphorbia cyathophora*) around Bargara, a coastal town near Bundaberg, Queensland. Mowing patterns and differential utilization of young and old foliage between the native species and invasive biotypes were cited as possible contributing factors (De Barro PJ and Liu SS unpublished data). In Zhejiang,

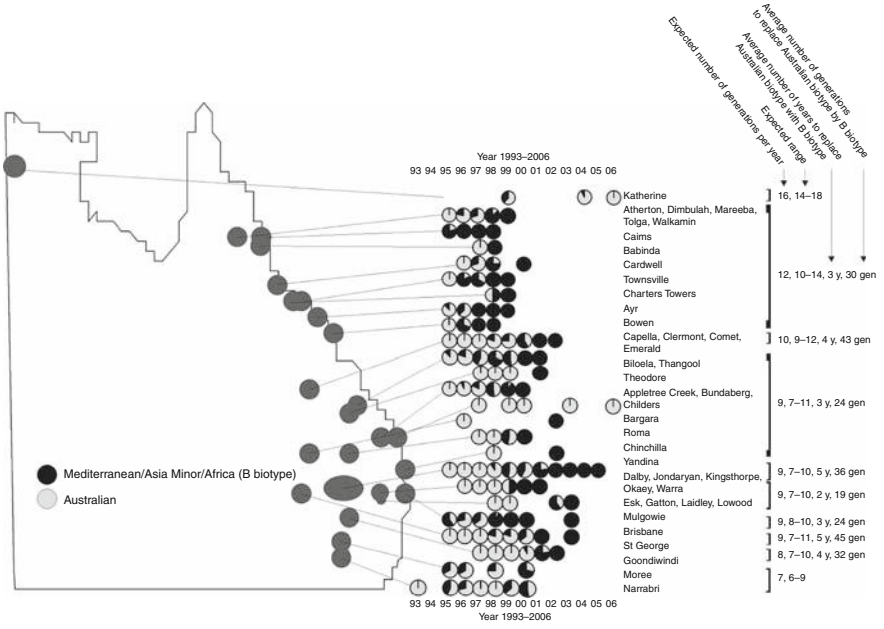



**Fig. 6.4** Map of north eastern Australia detailing the process of displacement of the indigenous Australian *B. tabaci* by the invasive B biotype and the number of generations and length of time between incursion and full displacement. Map based on Liu et al. (2007)

China, the earliest invasions by biotype B occurred in locations with the most frequent transport of ornamentals. Displacement of the indigenous ZHJ1 biotype in these areas was complete by 2005.

## *The Invasion Process*

The allopatric phylogeographical structure of *B. tabaci* (Boykin et al. 2007) raises two questions in regard to invasion capacity: (1) how has this pattern persisted for most groups, and (2) why have the sibling biotypes B and Q shown such a remarkable capacity to spread? Although geographic barriers may explain some allopatry, the global distribution of *B. tabaci* types does not generally correspond to obvious climatic regions. It would therefore appear that abiotic factors are unlikely to explain the observed patterns. Furthermore, the apparent failure of many genetic groups to invade and establish in neighboring regions suggests that the capacity for *B. tabaci* to invade is uncommon. The failure of biotype B to establish permanently in southern Spain (Simon et al. 1999) may indicate the difficulty of invading a neighboring territory because the populations have evolved the necessary characters to compete in response to interaction along the border (Kniskern and Rausher 2001). In contrast, species transplanted into a new geographic space may exhibit novel traits that confer a selective advantage against the indigenous population (Cox 2004). For this reason, invasive organisms often thrive at the expense of indigenous, closely related organisms occupying the same niche (Odum 1971). Therefore, detailed assessments of behavioral mechanisms may be necessary to gain insight into the causes of animal invasions (Holway and Suarez 1999).

## *Mating Interactions*

Li et al. (1989) showed for the first time that single males were capable of interrupting courtship. Perring et al. (1994) and Perring and Symmes (2006) later reported that biotype B males actively interfered with the courting and mating of biotype A females. This behavior is often found in the incompletely isolated mate recognition system of two closely related species (Butlin 1995). Lack of discrimination during courting and mating is one factor contributing to competitive displacement through reproductive interference (Reitz and Trumble 2002).

While mating interference is a normal mate competition mechanism within *B. tabaci,* it appears that there is considerable variability in the intensity of the interactions when compared across different genetic groups. This variability sets up the potential for asymmetry in genetic group interactions. Asymmetrical mating interference has been identified as yet another mechanism for competitive displacement (Reitz and Trumble 2002). A number of studies have also observed mating interactions of this nature (Perring et al. 1994; Mabbett 2004; Pascual 2006; Perring and Symmes 2006; Ruan et al. 2007; Zang and Liu 2007; also see Walker et al. Chapter 4).

In a comprehensive study that combined long-term field monitoring of the invasion process, population experiments under caged conditions, and detailed behavioral observations in both China and Australia, Liu et al. (2007) showed that rapid invasion and displacement by biotype B were associated with significant changes in the sex ratio of both the indigenous and alien populations. Populations of biotype B, on cotton alone in Zhejiang, had female ratios of 60–70% compared with 50–60% for the indigenous ZHJ1. During displacement, however, female ratios in biotype B increased to 70–85% while those in ZHJ1 declined to 35–45%. Similar patterns were observed between biotype B and the Australian AN biotype. Follow-up cage studies showed that significant changes in sex ratios leading to rapid displacement of indigenous biotypes occurred when mixed cohorts of ≈10% of the B biotype and 90% indigenous biotype were reared continuously for multiple generations on equally suitable host plants.

It was observed through video recording (Ruan et al. 2007; Liu et al. 2007) that individuals of biotype B and an indigenous biotype placed on the same leaf exhibited intensive mating interactions which lead to very different consequences for each (Fig. 6.5). A biotype B pair increased frequency of courtships and copulations when second male of either B or an indigenous biotype was added. However, the indigenous male interfered little with the B couple, whereas a supplementary B biotype male interrupted 16–28% of the courtships leading to a significant reduction of copulation events, even though the indigenous couple did increase their activity somewhat. These changes in copulation events resulted in corresponding changes in the sex ratio of the progeny while the numbers of progeny produced remained unchanged. Critical to this interaction was the fact that biotype B responded independently of whether males were all biotype B, or a mix of biotype B and indigenous individuals. Therefore, both indigenous males and invader males helped to promote copulation among the invaders and consequently increased the invaders' competitive capacity. In contrast, indigenous females did not respond to increased numbers of adult males and copulation was more frequently interrupted by biotype B males.

Skewed sex ratios resulting from asymmetric mating interactions have an obvious impact on the numerical changes of the invader and indigenous populations. Given this mechanism, even a small number of biotype B in a new location may succeed by rapidly producing female progeny and interfering with the mating of indigenous individuals, leading to rapid population growth of the invader population while driving the indigenous population to local extinction.

## *Host Plant Effects*

Differential female fecundity is another mechanism contributing to competitive displacement; in addition, it is linked to differential resource acquisition allowing one species to have higher growth rates and greater survivorship (Reitz and Trumble 2002). Host plant range, and high variability in reproductive potential as a function of host plant (see Table 6.1) play a role in invasion and displacement. The capability of the B biotype to utilize a much wider range of crops and ornamental hosts than

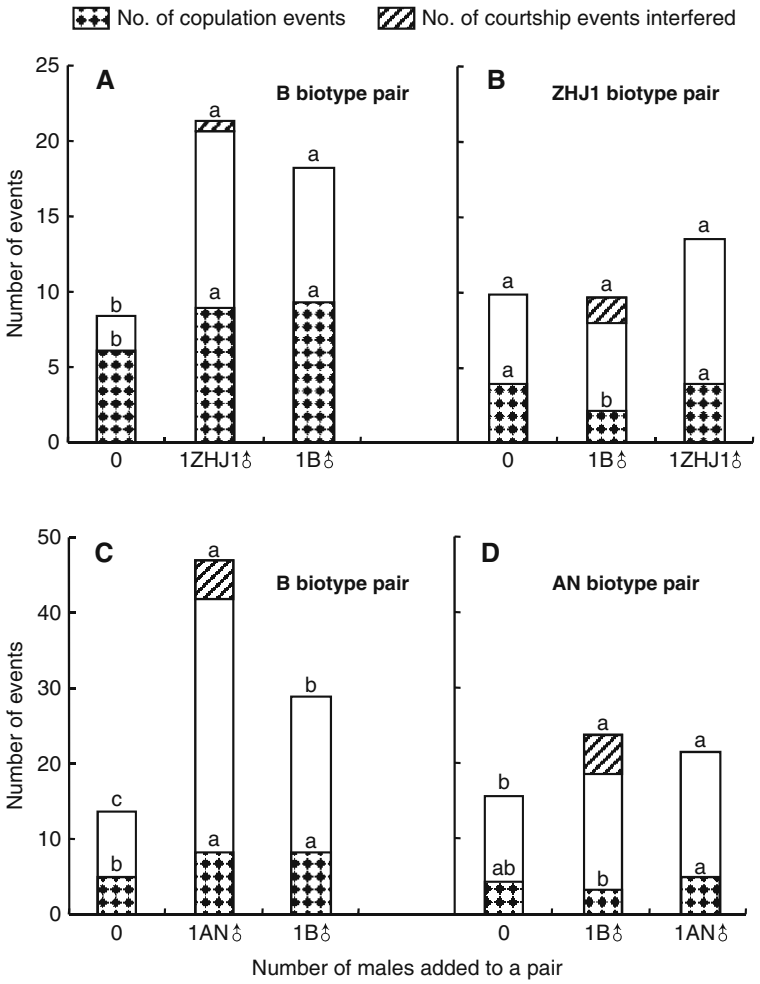



**Fig. 6.5** Behavioral elements that caused changes in events of copulation in the B biotype (**a**, **c**) or the ZHJ1 biotype (**b**) or AN biotype (**d**) when a pair of ♀×♂ of a given biotype was supplemented with one ♀ of the B or the ZHJ1/AN biotype. The individual entire columns present the mean total number of courtship events per B male (**a**, **c**) or per ZHJ1/AN male (**b**, **d**) with the female in each of the treatments. *Different letters* above the *bars* indicate significant difference. Figures based on data from Liu et al. (2007); $n$=14–30 per treatment

the indigenous biotype A of North America contributed to the rapid invasion that took place there from the mid-1980s onward. The novel infestation on poinsettias and other ornamental crops in Florida by *B. tabaci* was soon followed by squash silverleaf symptoms and other plant growth disorders diagnostic of the B biotype (Hamon and Salguero 1987; Yokomi et al. 1990). Within 5 years, novel infestations on broccoli, cauliflower, cantaloupe, alfalfa and even citrus to a limited extent, were

noted in the Imperial Valley of California (Perring et al. 1991). Complete displacement of the indigenous A biotype by the B biotype occurred in this region within a short time period. In China also, biotype B showed a much greater capacity to use host plants in 5 different families, compared to the indigenous ZHJ1 that could complete development on cotton and squash, but not on tobacco, cabbage, or kidney bean (Zang et al. 2005a).

Host plant can also contribute to displacement rate. For example, Zang et al. (2005b) found that displacement took six generations on cotton but only two on squash starting with equal numbers of biotype B and the indigenous biotype ZHJ1. Similarly, De Barro et al. (2006) showed that the minimum number of invaders relative to indigenous individuals required for establishment was lower with cotton – a host more suited to the invader, than with *E. cyathophora* – a host well suited to both biotypes B and indigenous AN biotypes. There are many examples of biotype B tending to outperform its competitors on the same host plant (e.g., Bethke et al. 1991; Costa and Brown 1991; Bedford et al. 1994; Nombela et al. 2001; Pascual and Callejas 2004; Zang et al. 2006). These examples demonstrate that differential host acceptability can influence the capacity for the invader to either interfere or not within a landscape consisting of patches of suitable and unsuitable hosts. Thus, the mosaic of host patches may favor the invader or enable it to escape interference, permitting more ready establishment.

## *Insecticide Resistance*

The role of insecticides in influencing the invasion and persistence of different genetic groups of *B. tabaci* has been considered largely in regard to the interaction between the biotypes B and Q, and to a lesser extent, biotypes B and A. Costa et al. (1993) and Coats et al. (1994) both suggested that the capacity of biotype B to invade the USA was linked to its higher level of insecticide resistance relative to the indigenous genetic group. Horowitz et al. (2003, 2005) found that higher levels of resistance to pyriproxyfen and neonicotinoids in biotype Q were associated with the greater abundance of biotype Q relative to B in Israel. Similarly, the apparent disappearance of invading biotype B in southern Spain in favor of the indigenous biotype Q (Guirao et al. 1997; Cervera et al. 2000) has been linked to resistance of the latter to pyriproxyfen and neonicotinoids (Nauen et al. 2002; Rauch and Nauen 2003; Pascual 2006). However, whether insecticides actually influence invasion on a regional scale is difficult to determine. Our understanding of the genetic structure of *B. tabaci,* and biotypes B and Q specifically, began only after the widespread use of pyriproxyfen and neonicotinoids in the region surrounding the Mediterranean Basin, so it is not known what the regional genetic structures were prior to the use of these pesticides. The considerable genetic differences between biotypes B and Q likely evolved well before agriculture, so insecticide resistance cannot have driven these evolutionary relationships, although it may contribute to local dominance of one over the other.

### *Whitefly–Begomovirus Interactions*

An additional factor that can aid invasion and displacement is the acceleration of population increase by the invader through mutualism with begomoviruses (Costa et al. 1991; McKenzie 2002; Zhang et al. 2000; Jiu et al. 2007). Costa et al. (1991) observed that the B biotype showed greater egg-to-adult survival on pumpkin infected with the watermelon curly mottle strain of *Squash leaf curl virus* compared with uninfected plants. Zhang et al. (2000) used modeling techniques to investigate increased vector fecundity, as well as vector density for *B. tabaci* feeding on cassava infected with *Cassava mosaic virus*, speculating that the interaction between virus and vector accelerated the spread of the vector and therefore the virus. Similarly, the B biotype laid more eggs on tomato plants infected with *Tomato mottle virus* than they did on uninfected plants (McKenzie 2002). Jiu et al. (2007) showed that invasive B biotype increased its fecundity and longevity 12- and 6-fold, respectively, when feeding on plants infected with *Tobacco curly shoot virus*, and 18- and 7-fold, respectively, when feeding on *Tomato yellow leaf curl China virus*-infected plants compared with healthy plants. In contrast, Rubinstein and Czosnek (1997) found that *B. tabaci* viruliferous with *Tomato yellow leaf curl virus* had reduced adult longevity and fecundity, suggesting that the whitefly-virus interaction may not always benefit the insect. Still further, biotype ZHJ1 performed similarly on both healthy and virus-infected plants. Both viruses have become widespread in south China following the invasion and establishment of the B biotype.

### *Future Invasion Threats*

The mechanisms identified by Liu et al. (2007) provide a construct within which to consider the process of invasions that lead to competitive displacement, and additionally a framework for speculating on future potential invasion within the *B. tabaci* complex. The accumulated evidence suggests that biotypes B and Q possess similar capacities to invade. One can pose the question, given their close genetic origins, as to whether this capacity to invade is shared by other close relatives, i.e., the Indian Ocean and Sub-Saharan Africa silverleafing genetic groups (Boykin et al. 2007). If so, then consideration should be given to the access these two genetic groups may have via the ornamental nursery plant invasion pathway, a route that has proven an effective means of invasion and spread.

## Population Outbreaks

The dispersion of *B. tabaci* around the globe offers the opportunity to examine patterns of infestation in regions that vary in management practices, climates, and cropping systems. While many regions in the world have been impacted by severe infestations of *B. tabaci* in the past few decades, they represent only the latest in a series of worldwide outbreaks dating back to the 1920s. While locations where

epidemic populations occur usually command attention, it should not be overlooked that relatively benign, endemic populations are more common. Even in regions experiencing chronic outbreaks – such as the Imperial Valley of California – there are times that populations remain below levels requiring control despite the availability of suitable host crops. This suggests complexity in population processes beyond simple mismanagement issues. Overall, observations – historical and modern, epidemic and endemic – need to be examined collectively to better comprehend the principal factors determining the size and notoriety of *B. tabaci* populations.

The term "outbreak" is often used loosely to describe pest populations causing economic damage (e.g. Avidov 1956) that can be related to specific economic thresholds, which can vary according to crop, yield, and market standards. It is more valid, however, to think of a population outbreak as a biologically based phenomenon where population growth is excessive, i.e., beyond expectation, exponential in pattern, occuring over a broad, metapopulation scale, and is prolonged over multiple generations. The assessment of whitefly infestation intensity is further complicated by the lack of absolute sampling methods based on numbers per unit of area (see Sampling above). As a result, there are no objective standards for proclaiming "outbreak populations." For example, the 1991 outbreak that occurred in the Imperial Valley of California was not documented by published data on whitefly densities, but instead was based on estimates of the economic impact (Gonzalez et al. 1992; Perring et al. 1993), media reports, and a few famous photographs of whitefly clouds over fields (Fig. 6.6).

**Fig. 6.6** "Clouds" of *B. tabaci* adults over a fallow field in the Imperial Valley of California, USA during the massive outbreak in 1991. Photo by Jim Hurt

## *Historical Outbreaks*

Following accounts of *B. tabaci* outbreaks in the Sudan Gezira region in the late 1970s (Eveleens 1983; Dittrich et al. 1985), there has been a tendency to view *B. tabaci* as pesticide induced (Byrne et al. 1990; Byrne and Devonshire 1993;

Johnson et al. 1982). However, several examples suggest that it was capable of primary pest status without invoking the pesticide disruption hypothesis.

### India – 1920s

Abundant literature exists on problems associated with cotton cultivation in the Punjab region of India now part of Pakistan during the 1920s and 1930s. Cotton crop failures occurred in 1919 and 1926 with partial failures taking place in 1921, 1927, and 1928 (Roberts 1930; Husain and Trehan 1933). Various explanations were advanced, but whitefly – *B. gossypiperda* Misra and Lamba (later synonomized to *B. tabaci*, Russell 1957) – was the probable cause based on densities in excess of 50 eggs and/or nymphs per $cm^2$ of leaf (Husain and Trehan 1933), also described as completely covering the leaf surface (Misra and Lamba 1929). It was suggested that "sustained drastic measures will have to be adopted to prevent the enormous loss annually caused by the White-fly to cotton" (Misra and Lamba 1929).

### Israel – 1930s and 1940s

Huge populations of *B. tabaci* seen in regions that are now Israel during the 1930s and 1940s before the use of synthetic organic insecticides appear to be legitimately designated as outbreaks (Avidov 1956). Serious damage was first recorded in 1931 in the eastern Esdraelon Plain to eggplants, tomatoes, cabbage and cauliflower with additional widespread damage of vegetable crops in the coastal plain and the interior valleys and upper Galilee in 1935; cucumber and melon plants were particularly susceptible to attack. Silverleaf disorder was first reported from Israel (Be'eri and Kapuler 1963) although it was not attributed to *B. tabaci*. The breadth of crops attacked in Israel in the 1930s – and higher infestations in the warmer parts of the region – had all the earmarks of a biotype B attack that the rest of the world would experience beginning in the mid-1980s in North America. The most recent molecular phylogenies of *B. tabaci* collections from around the world indicate a North African/Middle East origin for biotype B (De Barro 2005; Boykin et al. 2007).

### Brazil – 1970s

The rapid increase in soybean acreage that took place in the northern areas of Paraná state, and neighboring parts of Sao Paulo state in Brazil, was considered the main factor leading to abnormal population densities of *B. tabaci* (Costa 1975). In 1973 and 1974 especially, "extremely high populations of *B. tabaci* were recorded among cultivated plants and weeds." Much of the breeding occurred on successive plantings of soybeans with the later plantings becoming the most severely infested. Costa (1975) remarked that leaves of late planted soybeans were completely covered by immature stages. Large scale dispersal of adults into adjacent towns was another hallmark of the outbreak (see Dispersal, Migration and Seasonality above). Although Costa (1975) described experimental work with insecticides against *B. tabaci*, no indication was given that insecticides were being used to treat the soybeans or other infested crops during this period.

## *Factors Contributing to Outbreaks*

More recent outbreaks of *B. tabaci* – in Sudan during the late 1970s (Eveleens 1983 Dittrich et al. 1985), the Imperial Valley of California in 1981 (Johnson et al. 1982), and again in 1991 (Perring et al. 1991), as well as south Texas in 1991 (Norman et al. 1995) – occurred under heavy insecticide pressure. Beyond the obvious failures of management, what fundamental factors drive populations of *B. tabaci* to outbreak levels in some agro-ecosytems and not others? Based on both historical and modern outbreaks, the following four factors appear to be crucial.

### Climate

There are few other agricultural pests that are as ubiquitous as *B. tabaci*. Its ability to tolerate high temperatures (Wolfe et al. 1998; Salvucci 2000) gives *B. tabaci* a large geographical range. There is also substantial evidence that *B. tabaci* populations are negatively impacted by rainfall (e.g., Husain and Trehan 1940; Sundaramurthy 1992; Naranjo and Ellsworth 2005). This knowledge has served as the basis for simulating rainfall with overhead sprinklers to significantly reduce populations in crops (Castle et al. 1996). Some of the most explosive populations have been observed in irrigated desert environments where consistently high temperatures shorten generation times, and rainfall is infrequent. Mild winters, warm spring and fall seasons, and hot summers that permit a diverse assortment of crops to be grown year round all also contribute to success of *B. tabaci* in regions like the Imperial Valley of California, Israel and Sudan Gezira.

Differences in climate between growing regions can profoundly impact populations of *B. tabaci*, even if other attributes of the environment are similar. For example, two different agricultural areas in California – the Imperial Valley and the San Joaquin Valley – are both intensively farmed regions with urban interfaces that provide diverse crops and ornamental plants capable of hosting *B. tabaci* year round. However, the pattern of destruction by *B. tabaci* populations observed in the Imperial Valley – particularly during the 1990s – has never occurred in the San Joaquin Valley. Higher temperatures in the Imperial Valley, enabling earlier and faster development of *B. tabaci* populations, is the most obvious factor. Annual heat unit accumulation in the warmer southern end of the San Joaquin Valley is still only about 2/3 that of the Imperial Valley. More importantly, early season heat unit accumulation is double in the Imperial Valley, allowing *B. tabaci* populations to grow faster on earlier planted crops such as cantaloupe and cotton as well as on earlier revived hosts like alfalfa, ornamentals, and weeds.

### Agriculture

*B. tabaci* displays many attributes of an r-selected species (Price 1997) adapted to exploit ephemeral resources. While probably occurring at generally low density in natural environments, the development of agriculture has provided new opportunities for rapid population growth as host plants became more abundant,

predictable in occurrence, and higher in quality due to the widespread use of irrigation, synthetic fertilizers and genetic improvement. Close to ideal conditions occur in places like the Imperial Valley where suitable crops grown in a seasonal succession provide continuous food resources for multiple whitefly generations (see Dispersal, Migration and Seasonality above), exacerbating the potential for population outbreak.

These considerations point to the necessity of a key change in agricultural production practices. For example, for many decades cotton was the principal cash crop during the mid-twentieth century in the Sudan Gezira, a period otherwise free of destructive outbreaks. Following independence in 1956, a program to diversify crops to better meet the needs of the nation was instituted (Simpson and Simpson 1991). Shortly afterwards, the Managil Extension of the Blue Nile was developed, greatly increasing the land area under irrigation. Cotton acreage more than doubled over the next two decades, large areas of groundnuts were planted, and other minor crops including cowpea, cucurbits, and chickpeas were grown, all of which served as suitable hosts for building *B. tabaci* populations moving into cotton (Griffiths 1984). A similar intensification of agriculture favoring *B. tabaci* populations occurred in Brazil resulting in the outbreaks of the early 1970s (Costa 1975). Although other crops and weeds were present in the outbreak region, the more critical factor was the successive plantings of soybeans that increased growth over a longer period.

The ultimate in temporal overlap of *B. tabaci* host crops may be in the irrigated desert valleys of the southwestern USA. With their hot and arid climate, there is little to interrupt *B. tabaci* populations between March and November of each year following brief and mild winters. The importance of cropping systems is highlighted by the comparison between the Imperial Valley and Coachella Valley, separated by only 120 km. While climates are similar, whiteflies attain outbreak densities only in the Imperial Valley. The Coachella Valley features three main perennial crops – table grapes, citrus and date palms, in addition to cotton. Cucurbit and vegetable are secondary crops grown on limited areas with no March through November constancy that would allow progressive development of *B. tabaci* populations.

## Biotic Potential

The intrinsic biology of *B. tabaci* further enables outbreak potential. *B. tabaci* is highly polyphagous, with the full extent of its host range possibly still undocumented. Although cotton figures prominently in the record of historical outbreaks, more suitable hosts are known, especially for biotype B. For example, *B. tabaci* reached densities >10-fold greater in cantaloupe compared to adjacent cotton (Castle 2006). In a comparison of five vegetable hosts, fecundity was highest on eggplant and lowest on cucumber (Tsai and Wang 1996). However, part of the reported variability of fecundity (Table 6.1) may be artifacts of differing methodologies as well as host plant suitability.

The biotype issue also plays a central role in the invasion of a new area (see above) as well as in its outbreak potential. Overall, the extraordinary biotic potential of biotype B expressed through enhanced polyphagy (Perring et al. 1991, De Barro et al. 2006), more aggressive mating interactions (Liu et al. 2007), sex ratio variation

favoring female progeny (Liu et al. 2007), and higher fecundity (Bethke et al. 1991) sets biotype B apart from all other *B. tabaci* biotypes, contributing to its potential for both invasion and population outbreak.

## Management

Interpretation of the failure of pest management in the Sudan Gezira in the late 1970s altered between the direct impact of insecticides on *B. tabaci* populations – i.e., resistance development (Dittrich et al. 1985) – and the indirect impact of disrupting natural enemy populations (Eveleens 1983). Resistance in a target population is readily measured with reference to unexposed laboratory populations. The challenge is to connect this information to loss of field control (Sawicki 1987). Careful study is also needed to connect the decline in natural enemy abundance to pest population resurgence. Overall, considerable effort is required to evaluate how control of *B. tabaci* populations is attained or lost. For example, life tables conducted in Arizona cotton fields attempted to partition mortality due to insecticides and other agents in assessing the contribution of each mortality factor to pest control (Naranjo 2001; Naranjo and Ellsworth 2009).

Part of the difficulty in assigning causes to population outbreak is that buildup of *B. tabaci* populations can go on for years with numerous factors contributing to the gradual increase. The transition to outbreak status can be rather arbitrary, and can occur even if there are no substantive changes in management practices from previous non-outbreak years. In contrast, evidence for the impact of altered management practices on outbreak status can be more apparent because of timely decreases in population densities following initiation of a novel control practice. The emergency registration of the insecticides buprofezin and pyriproxyfen for use in Arizona in 1996 – following the outbreak conditions of 1995 – resulted in a dramatic drop in population densities despite an early season buildup prior to the first applications that indicated another outbreak year. This trend continued for the next

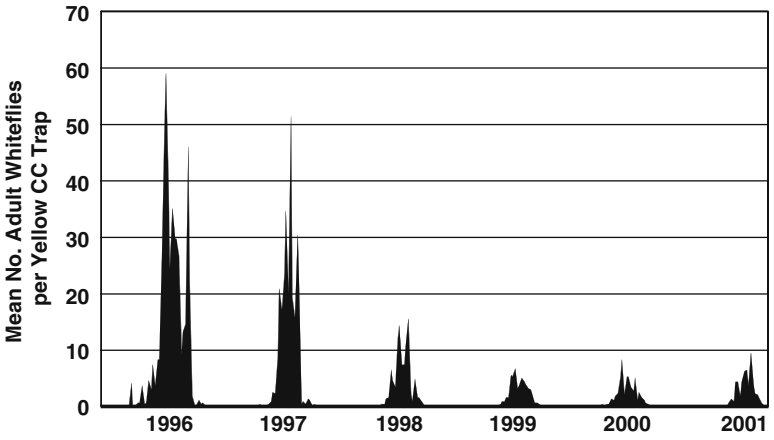



**Fig. 6.7** Decline in mean number of *B. tabaci* adults caught in a network of 26 CC traps positioned around the Imperial Valley, CA between April and November

several years and is evidenced by the decreasing number of insecticide applications for whiteflies (Ellsworth and Martinez-Carrillo 2001) between 1995 (6.6) and 1999 (0.4). Similarly, the commercialization and registration of the neonicotinoid insecticide imidacloprid for use on cucurbit and vegetable crops in the Imperial Valley of California made a substantial impact on *B. tabaci* densities in its first full year of use. Population densities of *B. tabaci* continued to drop over the next several years (Fig. 6.7) as measured by a whitefly monitoring program from 1996 to 2002 (Chu et al. 2007). The long persistence of soil-systemic applications of imidacloprid helped to suppress early buildups of *B. tabaci* in spring cucurbits that, along with other management adjustments, helped to bring the perpetual outbreak of the early 1990s under control.

## Population Models

A variety of models have been developed for *B. tabaci* to help understand the role of complex processes like dispersal and metapopulation dynamics. Some are relatively simple and developed for the purpose of predicting and forecasting (Zalom et al. 1985; Muniz 2000a; Yadav and Singh 2006). Others are aimed at guiding development of particular control tactics (van Giessen et al. 1995; Shen and Ren 2003). Still others developed for similar purposes, have also served as a means of structuring and synthesizing current biological/ecological knowledge which can in turn be used to generate hypotheses and direct future research (von Arx et al. 1983a, Wilhoit et al. 1994; DeGrandi-Hoffman and Naranjo 1996; Mills and Gutierrez 1996; Brewster et al. 1997; Crowder et al. 2006; Richards et al. 2006). The first site-specific simulation model for *B. tabaci* (Von Arx et al. 1983a) remains one of the best examples of completeness and detail, coupling temperature-dependent developmental, reproductive and survivorship models with dispersal rates calibrated from the field and a physiologically-based cotton plant model. The simulation model of Degrandi-Hoffman and Naranjo (1996) used similar temperature-dependent models to describe *B. tabaci* dynamics, but also provided the user with a general platform to model dynamics in different crops based on insect biological parameters, and allowing natural enemy dynamics (parasitoids and predators) and their effects to be included. Such site-specific models have been useful for exploring and evaluating factors potentially impacting pest population dynamics in single fields (Baumgärtner et al. 1986; Degrandi-Hoffman et al. 1997; Naranjo and Ellsworth 2005).

Mills and Gutierrez (1996) focused specifically on biological control by aphelinid parasitoids, and explored the ramifications of using heteronomous hyperparasitoids like *Encarsia* spp. on overall pest population suppression. Crowder et al. (2006) were interested in measuring the biological and ecological factors contributing to resistance of pyriproxyfen, a commonly used insect growth regulator for whitefly control. Several models have also been developed to study virus-vector-crop interactions and epidemiology, but these will not be detailed here.

Coincident with the growing interest in metapopulation dynamics and pest management at the landscape level, several spatial-temporal models have been developed (Wilhoit et al. 1994, Brewster et al. 1997). Both these models use a mapping method that assigns cells on a spatial grid to a crop or host type meant to mimic actual landscapes. Population growth within each cell is governed by a simple logistic growth model with variable carrying capacities. Intercrop movement is modeled by allowing portions of the population to move to other grid cells. In the Wilhoit et al. (1994) model, adults move only once the crop is harvested, while Brewster et al. (1997) used a variable random diffusion process driven by crop age. The Brewster model also included a simple Nicholson-Baily submodel for simulating the effect of parasitoids on *B. tabaci* dynamics.

Despite their underlying simplicity, both of these spatial models can arrive at rather complex dynamics (Wilhoit et al. 1994; Allen et al. 1996; Brewster et al. 1997). They can potentially be used to generate and test hypotheses based on real-world scenarios of crop placement, timing and management, insecticide management and biological control strategies, as well as other factors to reduce *B. tabaci* metapopulations. Attacking pests like *B. tabaci* at the landscape level represents the next but unrealized frontier of IPM articulated by Kogan (1998) over a decade ago. *B. tabaci* researchers should continue to develop empirical data and refine models to exploit and push this frontier forward.

## Conclusions

The population dynamics of *B. tabaci* is governed entirely by four rate processes – birth, death, immigration, and emigration. Each, in turn, is governed by a wide array of interacting biotic and abiotic factors that ultimately determine changing occurrence and abundance over time and space. Considerable progress has been made in understanding the population biology and ecology of *B. tabaci* in the past 10–12 years, concurrent with its expanding geographic range and impact on world agriculture. Sampling techniques and methods have been developed for several new affected crops, and additional work has refined and extended our knowledge of sampling on other crops. To date, life history studies measuring development, survival, and reproduction have been completed on 51 crop, and non-cultivated host plants, under controlled conditions. In contrast, field-based life tables, which have primarily focused on immature death rates, have been completed on only six crops, one ornamental host, and several annual weeds in only a few agroecosystems in the world. Compared to other aspects of its biology, relatively little is known about dispersal of *B. tabaci.* Some important insights were provided from studies through the mid-1990s, but little progress has been made in furthering our knowledge over the past decade. Our understanding of the processes of immigration and emigration remain largely observational and anecdotal despite their critical importance to pest management, and local and metapopulation dynamics. *B. tabaci*, particularly the B biotype, has excelled at invasion and worldwide spread. As a consequence, we are beginning to understand the multiple factors that contribute to the incursion

of *B. tabaci* into new regions and habitats, such as mating interactions, host plant related effects, insecticide resistance, and virus/vector/plant interactions. Similarly, we now understand that population outbreaks are facilitated and enabled by a variety of interacting forces including climate, agricultural intensification and change, biotic potential, and pest management practices. Finally, several useful site-specific and metapopulation models have been developed, although refinement and use of these models has slowed over the past decade with the most recent modeling activity focused on virus dynamics and epidemiology.

The following are avenues of method research and development that should be pursued in order to advance our understanding of population level processes in *B. tabaci*.

(1) Methods for the absolute estimation of *B. tabaci* population density should be developed and refined for multiple crops and hosts. Such methods are needed to support detailed population studies such as life tables that include adult survival and reproduction. Absolute methods would also be useful to metapopulation level studies that measure, compare, and predict the dynamics of populations within the landscape.
(2) Additional field-based life table studies are needed in a broader array of crops and other host plants. These studies should focus on immature development and survival as well as adult reproduction. Such data would allow the development of more accurate models which in turn would allow better estimates of endogenous population growth. Life tables would also be useful in contrasting, understanding, and predicting the interaction of multiple pest management tactics such as insecticides, biological control, and variety selection.
(3) Improved methods for the study of movement need to be developed. This would include more refined methods for mark-recapture studies leading to better estimates of movement rates both within and among crops in the landscape. Also, the use of models developed in #2 could be employed to better estimate rates of immigration and emigration. Landscape level analyses similar to those applied to other pests (e.g. Carriere et al. 2006) might provide important insights into source-sink relationships for metapopulations of *B. tabaci* and its natural enemies.
(4) The forecasting of population outbreaks – and more importantly, a clearer understanding of the confluence of factors leading to such outbreaks – would be useful from both a pest management perspective, and as a means of gaining better insight into population regulation.
(5) Our understanding of the invasion process should be refined and expanded to permit better prediction of future invasion and displacement events. More comparative studies on the biological characteristics – and interactions between invasive and indigenous genetic groups – may provide better understanding of the behavioral and ecological mechanisms underlying invasion and displacement. Predicting the spread of established invasions would depend on knowledge gained about movement and population growth (see Liebhold and Tobin 2008).

These broad areas of inquiry emphasize only a few of the many facets upon which a more complete and necessary understanding of *B. tabaci* population dynamics can be built. Coupled with knowledge of the insect's biology, its complex interactions with other organisms, and the myriad of control tactics being developed and used to suppress populations, this should enable more robust, environmentally sound and sustainable management strategies aimed at mitigating *B. tabaci's* impact on world agriculture.

## Literature Cited

Abdel-Baky NF, El-Naga AMA, El-Nagar ME, Heikal GAM. 2004. Population density and host preference of the silverleaf whitefly, *Bemisia argentifolii* Perring & Bellows, among three important summer crops. *Egypt. J. Biol. Pest Control* 14:251–258. (#1)

Abdelrahman AA, Munir B. 1989. Sudanese experience in integrated pest management of cotton. *Insect Sci. Appl.* 10:787–794.

Abisgold JD, Fishpool LDC. 1990. A method for estimating population sizes of whitefly nymphs (*Bemisia tabaci* Genn.) on cassava. *Trop. Pest Manage.* 36:287–292.

Ahmad M, Aslam M. 2002. Evaluation of different pest scouting methods for monitoring whitefly population in cotton fields at Multan (Pakistan). *Arab J. Plant Prot.* 20:52–54.

Allen JC, Brewster CC, Paris JF, Riley DG, Summers CG. 1996. Spatiotemporal modeling of whitefly dynamics in a regional cropping system using satellite data. In Bemisia *1995: Taxonomy, Biology, Damage, Control and Management*, ed. D Gerling, RT Mayer, pp. 111–124. Andover, Hants: Intercept Ltd.

Arno J, Albajes R, Gabarra R. 2006. Within-plant distribution and sampling of single and mixed infestations of *Bemisia tabaci* and *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae) in winter tomato crops. *J. Econ. Entomol.* 99:331–340.

Asiimwe P, Ecaat JS, Otim M, Gerling D, Kyamanywa S, Legg JP. 2007. Life-table analysis of mortality factors affecting populations of *Bemisia tabaci* on cassava in Uganda. *Entomol. Exp. Appl.* 122:37–44.

Atakan E, Canhilal R. 2004. Evaluation of yellow sticky traps at various heights for monitoring cotton insect pests. *J. Agric. Urban Entomol.* 21:15–24.

Avidov Z. 1956. Bionomics of the tobacco whitefly (*Bemisia tabaci* Genn.) in Israel. *Khavim (English edition)* 7:25–41. (#2)

Avidov Z, Harpaz I. 1969. *Family Aleyrodidae. Plant Pests of Israel*. Jerusalem: University Press, pp. 76–84.

Azab AK, Megahed MM, El-Mirsawi HD. 1971. On the biology of *Bemisia tabaci* (Genn.) (Hemiptera-Homoptera: Aleyrodidae). *Bull. Entomol. Soc. Egypt* 55:305–315. (#3)

Bauch C, Rath T. 2005. Prototype of a vision based system for measurements of white fly infestation. *Acta Hortic.* 2:773–779.

Baumgärtner J, Delucchi V, Von Arx R, Rubli D. 1986. Whitefly (*Bemisia tabaci* Genn., Stern.: Aleyrodidae) infestation patterns as influenced by cotton, weather and *Heliothis*: hypotheses testing by using simulation models. *Agric. Ecosyst. Environ.* 17:49–59.

Baumgärtner J, Yano E. 1990. Whitefly population dynamics and modelling. In *Whiteflies: Their Bionomics, Pest Status and Management*, ed. D Gerling, pp. 123–146. Andover, Hants: Intercept Ltd.

Bayhan E, Ulusoy MR, Brown JK. 2006. Effects of different cucurbit species and temperature on selected life history traits of the 'B' biotype of *Bemisia tabaci*. *Phytoparasitica* 34:235–242. (#4)

Bedford ID, Briddon RW, Brown JK, Rosell RC, Markham PG. 1994. Geminivirus transmission and biological characterisation of *Bemisia tabaci* (Gennadius) biotypes from different geographic regions. *Ann. Appl. Biol.* 125:311–325.

Be'eri Y, Kapuler B. 1963. Summer squash. In *Growing of Vegetables, Part B*, ed. YM Margalit, pp. 235–240. Tel Aviv (in Hebrew): Sifriyyat Hassadeh Publications.

Bellows TS, Jr, Van Driesche RG, Elkinton JS. 1992. Life-table construction and analysis in the evaluation of natural enemies. *Annu. Rev. Entomol.* 37:587–614.

Bethke JA, Paine TD, Nuessly GS. 1991. Comparative biology, morphometrics, and development of two populations of *Bemisia tabaci* (Homoptera: Aleyrodidae) on cotton and poinsettia. *Ann. Entomol. Soc. Am.* 84:407–411. (#5)

Blackmer JL, Byrne DN. 1993a. Flight behaviour of *Bemisia tabaci* in a vertical flight chamber: effect of time of day, sex, age and host quality. *Physiol. Entomol.* 18:223–232.

Blackmer JL, Byrne DN. 1993b. Environmental and physiological factors influencing phototatic flight of *Bemisia tabaci*. *Physiol. Entomol.* 18:336–342.

Blackmer JL, Byrne DN, Tu Z. 1995a. Behavioral, morphological, physiological traits associated with migratory *Bemisia tabaci* (Homoptera: Aleyrodidae). *J. Insect Behav.* 8:251–267.

Blackmer JL, Lindley VA, Byrne DN. 1995b. Histological examination of flight muscle development and breakdown in *Bemisia tabaci* (Homoptera: Aleyrodidae): relationship to age and flight behavior. *J. Morphol.* 226:213–221.

Blua MJ, Yoshida HA, Toscano NC. 1995. Oviposition preference of two *Bemisia* species (Homoptera: Aleyrodidae). *Environ. Entomol.* 24:88–93. (#6)

Bonato O, Lurette A, Vidal C, Fargues J. 2007. Modelling temperature-dependent bionomics of *Bemisia tabaci* (Q-biotype). *Physiol. Entomol.* 32:50–55. (#7)

Boykin LM, Shatters RG, Jr, Rosell RC, McKenzie CL, Bagnall RA, DeBarro P, Frohlich DR. 2007. Global relationships of *Bemisia tabaci* (Hemiptera: Aleyrodidae) revealed using Bayesian analysis of mitochondrial COI DNA sequences. *Mol. Phylogenet. Evol.* 44:1306–1319.

Brewster CC, Allen JC, Schuster DJ, Stansly PA. 1997. Simulating the dynamics of *Bemisia argentifolii* (Homoptera: Aleyrodidae) in an organic cropping system with a spatiotemporal model. *Environ. Entomol.* 26:603–616.

Butler GD, Jr, Henneberry TJ. 1984. *Bemisia tabaci* as a cotton pest in the desert cotton-growing areas of the southwestern United States. In *Proceedings Beltwide Cotton Production Research Conferences*, ed. JM Brown, pp. 195–197. Memphis, TN: National Cotton Council.

Butler GD, Jr, Henneberry TJ, Clayton TE. 1983. *Bemisia tabaci* (Homoptera: Aleyrodidae): development, oviposition, and longevity in relation to temperature. *Ann. Entomol. Soc. Am.* 76:310–313. (#8)

Butler GD, Jr, Henneberry TJ, Hutchison WD. 1986. Biology, sampling and population dynamics of *Bemisia tabaci*. In *Agricultural Zoology Reviews*, ed. GE Russell, pp. 167–195. Andover, Hants: Intercept Ltd.

Butlin R. 1995. Genetic variation in mating signals and responses. In *Speciation and the Recognition Concept, Theory and Application*, ed. DM Lambert, HG Spencer, pp. 327–366. Baltimore, MD: The Johns Hopkins University Press.

Byrne DN, Bellows TS, Parrella MP. 1990. Whiteflies in agricultural systems. In *Whiteflies: Their Bionomics, Pest Status and Management*, ed. D Gerling, pp. 227–261. Andover, Hants: Intercept Ltd.

Byrne DN, Blackmer JL. 1996. Examination of short-range migration by *Bemisia*. In Bemisia *1995: Taxonomy, Biology, Damage, Control and Management*, ed. D Gerling, RT Mayer, pp. 17–28. Andover, Hants: Intercept Ltd.

Byrne FJ, Devonshire AL. 1993. Insensitive acetylcholinesterase and esterase polymorphism in susceptible and resistant populations of the tobacco whitefly, *Bemisia tabaci*. *Pestic. Biochem. Physiol.* 45:34–42.

Byrne DN, Draeger EA. 1989. Effect of plant maturity on oviposition and nymphal mortality of *Bemisia tabaci* (Homoptera: Aleyrodidae). *Environ. Entomol.* 18:429–432. (#9)

Byrne DN, Houck MA. 1990. Morphometric identification of wing polymorphism in *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae). *Ann. Entomol. Soc. Am.* 83:487–493.

Byrne DN, Rathman RJ, Orum TV, Palumbo JC. 1996. Localized migration and dispersal by the sweet potato whitefly, *Bemisia tabaci*. *Oecologia* 105:320–328.

Carabali A, Bellotti AC, Montoya Lerma J, Cuellar ME. 2005. Adaptation of *Bemisia tabaci* biotype B (Gennadius) to cassava, *Manihot esculenta* (Crantz). *Crop Prot.* 24:643–649. (#10)

Carey JR. 1993. *Applied Demography for Biologist: with Special Emphasis on Insects*. Oxford, NY: Oxford University Press.

Carriere Y, Ellsworth PC, Dutilleul P, EllersKirk C, Barkley V, Antilla L. 2006. A GIS-based approach for areawide pest management: the scales of *Lygus hesperus* movements to cotton from alfalfa, weeds, and cotton. *Entomol. Exp. Appl.* 118:203–210.

Carruthers RI. 1993. [sampling]. In *Sweetpotato Whitefly: 1993 Supplement to the 5-Year National Research and Action Plan*, U.S. Dept. Agric., Agric. Res. Serv. No. 112. p. 11.

Castle SJ. 1999. Agricultural intensification and pest outbreaks: a reappraisal of events in the Sudan Gezira. *Ann. Entomol. Soc. Am.* 92:840–852.

Castle SJ. 2006. Concentration and management of *Bemisia tabaci* in cantaloupe as a trap crop for cotton. *Crop Prot.* 25:574–584.

Castle SJ, Henneberry TJ, Toscano NC. 1996. Suppression of *Bemisia tabaci* (Homoptera: Aleyrodidae) infestations in cantaloupe and cotton with sprinkler irrigation. *Crop Prot.* 15:657–663.

Cervera MT, Cabezas JA, Simon B, Martinez-Zapater JM, Beitia F, Cenis JL. 2000. Genetic relationships among biotypes of *Bemisia tabaci* (Hemiptera: Aleyrodidae) based on AFLP analysis. *Bull. Entomol. Res.* 90:391–396.

Cheek S, MacDonald O. 1994. Statutory controls to prevent the establishment of *Bemisia tabaci* in the United Kingdom. *Pestic. Sci.* 42:135–137.

Chen YM, Chen CN, Chen WS. 2003a. Population parameters of the silverleaf whitefly (*Bemisia argentifolii* Bellows & Perring) on four legume cultivars. *Formosan Entomol.* 23:249–261. (#11)

Chen Q, Liu YS, Zhu GR, Xiao LF. 2003b. Development, survivorship, reproduction and population increase of B-biotype *Bemisia tabaci* (Homoptera: Aleyrodidae) on five host plants. *J. Shandong Agric. Univ.* 34:479–481. (#12)

Chu CC, Barnes E, Natwick ET, Chen TY, Ritter D, Henneberry TJ. 2007. Trap catches of the sweetpotato whitefly (Homoptera: Aleyrodidae) in the Imperial Valley, California, from 1996 to 2002. *Insect Sci.* 14:165–170.

Chu CC, Henneberry TJ, Natwick ET. 1998. *Bemisia argentifolii* adults caught in CC whitefly traps at different trap heights and trap catch relationships to leaf-turn counts on cotton. *Southwest. Entomol.* 23:259–268.

Coats SA, Brown JK, Hendrix DL. 1994. Biochemical characterization of biotype-specific esterases in the whitefly *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae). *Insect Biochem. Mol. Biol.* 24:723–728.

Cohen S, Kern J, Harpaz I, Ben-Joseph R. 1988. Epidemiological studies of the tomato yellow leaf curl virus (TYLCV) in the Jordan Valley, Israel. *Phytoparasitica* 16:259–270.

Costa AS. 1975. Increase in the populational density of *Bemisia tabaci*, a threat of widespread virus infection of legume crops in Brazil. In *Tropical Diseases of Legumes*, ed. J Bird, K Maramorosch, pp. 27–29. New York: Academic Press.

Costa HS, Brown JK. 1991. Variation in biological characteristics and in esterase patterns among populations of *Bemisia tabaci* Genn. and the association of one population with silverleaf symptom development. *Entomol. Exp. Appl.* 61:211–219.

Costa HS, Brown JK, Byrne DN. 1991. Life history traits of the whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae) on six virus-infected or healthy plant species. *Environ. Entomol.* 20:1102–1107. (#13)

Costa HS, Brown JK, Sivasupramaniam S, Bird J. 1993. Regional distribution, insecticide resistance, and reciprocal crosses between the 'A' and 'B' biotypes of *Bemisia tabaci*. *Insect Sci. Appl.* 14:255–266.

Coudriet DL, Meyerdirk DE, Prabhaker N, Kishaba AN. 1986. Bionomics of sweetpotato whitefly (Homoptera: Aleyrodidae) on weed hosts in the Imperial Valley, California. *Environ. Entomol.* 15:1179–1183. (#14)

Coudriet DL, Prabhaker N, Kishaba AN, Meyerdirk DE. 1985. Variation in developmental rate on different hosts and overwintering of the sweetpotato whitefly, *Bemisia tabaci* (Homoptera: Alleyrodidae). *Environ. Entomol.* 14:516–519. (#15)

Cox GW. 2004. *Alien Species and Evolution: The Evolutionary Ecology of Exotic Plants, Animals, Microbes, and Interacting Native Species*. Washington, DC: Island Press.

Crowder DW, Carriere Y, Tabashnik BE, Ellsworth PC, Dennehy TJ. 2006. Modeling evolution of resistance to pyriproxyfen by the sweetpotato whitefly (Homoptera: Aleyrodidae). *J. Econ. Entomol.* 99:1396–1406.

Dalton R. 2006. Whitefly infestations – The Christmas invasion. *Nature* 443:898–900.

de Albergaria NMMS, Cividanes FJ, Doria HOS. 2003. Tabela de vida ecologica de *Bemisia tabaci* (Genn.) biotipo B (Hemiptera: Aleyrodidae). *Neotrop. Entomol.* 32:559–563.

De Barro PJ. 1995. *Bemisia tabaci* biotype B: a review of its biology, distribution and control. CSIRO Div. Entomol. Tech. Paper No. 33, 58pp.

De Barro PJ. 2005. Genetic structure of the whitefly *Bemisia tabaci* in the Asia-Pacific region revealed using microsatellite markers. *Mol. Ecol.* 14:3695–3718.

De Barro PJ, Bourne A, Khan SA, Brancatini VAL. 2006. Host plant and biotype density interactions – their role in the establishment of the invasive B biotype of *Bemisia tabaci*. *Biol. Invasions* 8:287–294.

De Barro PJ, Hart PJ. 2000. Mating interactions between two biotypes of the whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Australia. *Bull. Entomol. Res.* 90:103–112.

de Moura MF, Picanco MC, da Silva EM, Guedes RNC, Pereira JL. 2003. Sampling plan for B-biotype of *Bemisia tabaci* in cucumber crop. *Pesq. Agro. Brasileira* 38:1357–1363.

DeGrandi-Hoffman G, Naranjo SE. 1996. Biocontrol-whitefly: a whitefly, parasite, and predator population dynamics model. In *Silverleaf Whitefly (Formerly Sweetpotato Whitefly, Strain B): 1996 Supplement to the 5-Year National Research and Action Plan*, U.S. Dept. Agric., Agric. Res. Serv. 1996–01. p. 3.

DeGrandi-Hoffman G, Naranjo SE, Gould J. 1997. Insights on *Bemisia* population dynamics from simulations with the BIOCONTROL-WHITEFLY model. In *Silverleaf Whitefly, 1997 Supplement to the 5-Year National Research and Action Plan: Progress Review, Technology Transfer, and New Research and Action Plan (1997–2001)*, U.S. Dept. Agric., Agric. Res. Serv. 1997–02. p. 65.

Demichelis S, Arno C, Bosco D, Marian D, Caciagli P. 2005. Characterization of biotype T of *Bemisia tabaci* associated with *Euphorbia characias* in Sicily. *Phytoparasitica* 33:196–208. (#16)

Devine GJ, Ishaaya I, Horowitz AR, Denholm I. 1998. Effects of piperonyl butoxide on *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae): mortality, development, parasitism and predation in Israeli cotton fields. *Crop Prot.* 17:717–726.

Dingle H. 1996. *Migration. The Biology of Life on the Move*. Oxford: Oxford University Press.

Dittrich V, Hassan SO, Ernst GH. 1985. Sudanese cotton and the whitefly: a case study of the emergence of a new primary pest. *Crop Prot.* 4:161–176.

Drost YC, van Lenteren JC, van Roermund HJW. 1998. Life-history parameters of different biotypes of *Bemisia tabaci* (Hemiptera, Aleyrodidae) in relation to temperature and host plant: a selective review. *Bull. Entomol. Res.* 88:219–229.

Ekbom BS, Rumei X. 1990. Sampling and spatial patterns of whiteflies. In *Whiteflies: Their Bionomics, Pest Status and Management*, ed. D Gerling, pp. 107–121. Andover, Hants: Intercept Ltd.

El-Helaly MS, El-Shazli AY, El-Gayar FH. 1971. Biological studies on *Bemisia tabaci* Genn. (Homopt.: Aleyrodidae) in Egypt. *Z. Angew. Entomol.* 69:48–55. (#17)

El-Khidir E. 1965. Bionomics of cotton whitefly, (*Bemisia tabaci* Genn.) in the Sudan and the effects of irrigation on population density of whiteflies. *Sudan Agric. J.* 1:8–22. (#18)

Ellsworth PC, Martinez-Carrillo JL. 2001. IPM for *Bemisia tabaci*: a case study from North America. In Special Issue: Challenges and Opportunities for Pest Management of *Bemisia tabaci* in the New Century, ed. SE Naranjo, PC Ellsworth. *Crop Prot.* 20:853–869.

Enkegaard A. 1993. The poinsettia strain of the cotton whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae), biological and demographic parameters on poinsettia (*Euphorbia pulcherrima*) in relation to temperature. *Bull. Entomol. Res.* 83:535–546. (#19)

Eveleens KG. 1983. Cotton-insect control in the Sudan Gezira: analysis of a crisis. *Crop Prot.* 2:273–287.

Fekrat L, Bor PS. 2005. A study of biology of cotton whitefly *Bemisia tabaci* (Gennadius) on three cultivars of eggplant in laboratory conditions. *Iranian J. Agric. Sci.* 36:137–143. (#20)

Fernandez P, Miguel M, Guirao P, Zamora M, Alcazar A, Oncina M, Lacasa A. 2002. Sampling systems development for *Bemisia tabaci* in greenhouses of ecological pepper production of southeastern Spain. In *Ecological Agriculture and Animal Husbandry in a Framework of Diversification and Joint Development. Gijon* [*Spain*]*, 16th to 21st of September, 2002. Proceedings of 5th Congress of Ecological Agriculture Spanish Society – 1st Iberoamerican Congress on Agroecology*, ed. E Dapena De La Fuente, JL Porcuna-Coto, pp. 897–905. Villaviciosa, Spain: Servicio Regional De Investigacion Y Desarrollo Agroalimentario.

Gachoka KK, Obeng-Ofori D, Danquah EY. 2005. Host suitability of two Ghanaian biotypes of *Bemisia tabaci* (Homoptera: Aleyrodidae) on five common tropical weeds. *Int. J. Trop. Insect Sci.* 25:236–244. (#21)

Gameel OI. 1978. The cotton whitefly *Bemisia tabaci* (Genn.) in the Sudan Gezira. In *3rd Seminar on Strategy for Cotton Pest Control in the Sudan*, Basle, Switzerland, May 8–10, 1978. pp. 111–131. (#22)

Gencsoylu I. 2007. Evaluation of yellow sticky traps on populations of some cotton pests. *Am. Eurasian J. Agric. Environ. Sci.* 2:62–67.

Gergis MF. 1994. Population growth and life table analysis in relation to temperature and host plants. In *Proceedings Beltwide Cotton Conferences*, ed. DJ Herber, DA Richter, pp. 1229–1230. Memphis, TN: National Cotton Council. (#23)

Gerling D. 1984. The overwintering mode of *Bemisia tabaci* and its parasitoids in Israel. *Phytoparasitica* 12:109–118.

Gerling D, Horowitz AR. 1984. Yellow traps for evaluating the population levels and dispersal patterns of *Bemisia tabaci* (Homoptera: Aleyrodidae). *Ann. Entomol. Soc. Am.* 77:753–759.

Gerling D, Mayer RT (eds.). 1996. *Bemisia 1995: Taxonomy, Biology, Damage, Control and Management*. Andover, Hants: Intercept Ltd.

Godfrey LD, Goodell PB, Summers CG. 1995. Silverleaf whitefly population dynamics and management in the San Joaquin Valley. In *Proceedings Beltwide Cotton Conferences*, ed. DA Richter, J Armour, pp. 95–97. Memphis, TN: National Cotton Council.

Gonzalez RA, Goldman GE, Natwick ET, Rosenberg HR, Grieshop JI, Sutter SR, Funakoshi T, Davila-Garcia S. 1992. Whitefly invasion in Imperial Valley costs growers, workers millions in losses. *Calif. Agric.* 46:7–8.

Gould, JR, Naranjo, SE. 1999. Distribution and sampling of *Bemisia argentifolii* (Homoptera: Aleyrodidae) and *Eretmocerus eremicus* (Hymenoptera: Aphelinidae) on cantaloupe vines. *J. Econ. Entomol.* 92:402–408.

Griffiths WT. 1984. A review of the development of cotton pest problems in the Sudan Gezira. M.Sc. Thesis, University of London.

Guirao P, Belitia F, Cenis JL. 1997. Biotype determination of Spanish populations of *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Bull. Entomol. Res.* 87(6):587–593.

Gunning RV, Byrne FJ, Conde BD, Connelly MI, Hergstrom K, Devonshire AL. 1995. First report of B-biotype *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) in Australia. *J. Aust. Entomol. Soc.* 34:116.

Gusmao MR, Picanco MC, Guedes RNC, Galvan TL, Pereira EJG. 2006. Economic injury level and sequential sampling plan for *Bemisia tabaci* in outdoor tomato. *J. Appl. Entomol.* 130:160–166.

Gusmao MR, Picanco MC, Zanuncio JC, Silva DJH, Barrigossi JAF. 2005. Standardized sampling plan for *Bemisia tabaci* (Homoptera: Aleyrodidae) in outdoor tomatoes. *Sci. Hortic.* 103:403–412.

Haji FNP, Moreira AN, Bleicher E, Cesar R, Ferreira F, de Alencar JA, Barbosa FR. 2001. Monitoring and determination of threshold level of whitefly *Bemisia argentifolii* on grape crops. *Cir. Tecnica Embrapa Semi Arido* 67:1–8.

Hamon A, Salguero V. 1987. *Bemisia tabaci*, sweetpotato whitefly, in Florida (Homoptera: Aleyrodidae: Aleyrodinae). Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville. Entomology Circular No. 292.

Hanski I. 2004. Metapopulation theory, its use and misuse. *Basic Appl. Ecol.* 5:225–229.

Hendi A, Abdel-Fattah MI, El-Sayed A. 1987. Biological study on the white-fly, *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae). *Bull. Entomol. Soc. Egypt* 65:101–108. (#24)

Henneberry TJ, Castle SJ. 2001. *Bemisia*: pest status, economics, biology and population dynamics. In *Virus-Insect-Plant Interactions*, ed. KF Harris, OP Smith, JE Duffus, pp. 247–278. San Diego, CA: Academic Press.

Hoddle MS, van Driesche R. 1996. Evaluation of *Encarsia formosa* (Hymenoptera: Aphelinidae) to control *Bemisia argentifolii* (Homoptera: Aleyrodidae) on poinsettia (*Euphorbia pulcherrima*): a lifetable analysis. *Fla. Entomol.* 79:1–12.

Hoelmer KA, Simmons AM. 2008. Yellow sticky trap catches of parasitoids of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in vegetable crops and their relationship to in-field populations. *Environ. Entomol.* 37:391–399.

Holway DA, Suarez AV. 1999. Animal behavior: an essential component of invasion biology. *Trends Ecol. Evol.* 14:328–330.

Horowitz AR, Gerling D. 1992. Seasonal variation of sex ratio in *Bemisia tabaci* on cotton in Israel. *Environ. Entomol.* 21:556–559.

Horowitz AR, Gorman K, Ross G, Denholm I. 2003. Inheritance of pyriproxyfen resistance in the whitefly, *Bemisia tabaci* (Q biotype). *Arch. Insect Biochem. Physiol.* 54:177–186.

Horowitz AR, Kontsedalov S, Khasdan V, Ishaaya I. 2005. Biotypes B and Q of *Bemisia tabaci* and their relevance to neonicotinoid and pyriproxyfen resistance. *Arch. Insect Biochem. Physiol.* 58:216–225.

Horowitz AR, Podoler H, Gerling D. 1984. Life table analysis of the tobacco whitefly *Bemisia tabaci* (Gennadius) in cotton fields in Israel. *Acta Oecolog.* 5:221–233.

Husain MA, Trehan KK. 1933. Observations on the life-history, bionomics and control of the white-fly of cotton (*Bemisia gossypiperda* M. & L.). *Indian J. Agric. Sci.* 3:701–753. (#25)

Husain MA, Trehan KN. 1940. Final report on the scheme of investigation on the white-fly of cotton in the Punjab. *Indian J. Agric. Sci.* 10:101–109.

Isaacs R, Byrne DN. 1998. Aerial distribution, flight behaviour and eggload: their inter-relationship during dispersal by the sweetpotato whitefly. *J. Anim. Ecol.* 67:741–750.

Ives AR, Settle WH. 1997. Metapopulation dynamics and pest control in agricultural systems. *Am. Nat.* 149:220–246.

Jiu M, Zhou XP, Tong L, Xu J, Yang X, Wan FH, Liu SS. 2007. Vector-virus mutualism accelerates population increase of an invasive whitefly. *PLoS ONE* 2:e182. DOI: 10.1371/journal.pone.0000182.

Johnson MW, Toscano NC, Reynolds HT, Sylvester ES, Kido KR, Natwick ET. 1982. Whiteflies cause problems for southern California growers. *Calif. Agric.* 9–10:24–26.

Kakimoto K, Inoue H, Yamaguchi T, Ueda S, Honda K, Yano E. 2007. Host plant effect on development and reproduction of *Bemisia argentifolii* Bellows & Perring (B-tabaci [Gennadius] B-biotype) (Homoptera: Aleyrodidae). *Appl. Entomol. Zool.* 42:63–70. (#26)

Karut K, Kazak C. 2007. Monitoring adult *Eretmocerus mundus, Encarsia lutea* and *Bemisia tabaci* with yellow sticky traps in cotton *Gossypium hirsutum*. *J. Appl. Entomol.* 131:553–558.

Karut K, Naranjo SE. 2009. Mortality factors affecting *Bemisia tabaci* populations on cotton the Çukurova plain, Turkey. *J. Appl. Entomol.* 133:367–374.

Kelly-Johnson S, Sivasupramaniam S, Watson TF. 1995. Seasonal population dynamics of sweetpotato whitefly in Maricopa County, Arizona. In *Proceedings Beltwide Cotton Conferences*, ed. DA Richter, J Armour, pp. 979–984. Memphis, TN: National Cotton Council.

Kennedy JS. 1985. Migration, behavioral and ecological. In *Contributions in Marine Science*, Suppl. 27, ed. MA Rankin, pp. 5–27. Austin, TX: Austin Marine Science Institute, University of Texas.

Khalifa A, El Khidir E. 1964. Biological study on *Trialeurodes lubia* and *Bemisia tabaci* (Aleyrodidae). *Bull. Entomol. Soc. Egypt* 48:115–129. (#27)

King BH. 1993. Sex ratio manipulation by parasitoid wasps. In *Evolution and Diversity of Sex Ratio in Insects and Mites*, ed. DL Wrensch, M Ebbert, pp. 418–441. New York: Chapman and Hall.

Kniskern J, Rausher MD. 2001. Two modes of host-enemy coevolution. *Popul. Ecol.* 43:3–14.

Kogan M. 1998. Integrated pest management: historical perspectives and contemporary developments. *Annu. Rev. Entomol.* 43:243–270.

Krainacker DA, Carey JR. 1990. Male demographic constraints to extreme sex ratio in the twospotted spider mite. *Oecologia* 82:417–423.

Lacasa-Plasencia A, Miguel-Ferreras M, Guirao-Moya P, Fernandez-Molina P. 2004. [Spatial distribution and sampling of *Bemisia tabaci* on pepper] Distribucion espacial y muestreo de *Bemisia tabaci* en pimiento. In *15. Symposium Internacional sobre Evaluacion del Riesgo de Plagas y Enfermedades. El Muestreo como Herramienta Esencial en la Proteccion Integrada*, Valencia (Espana), December 14–15, 2004.

Laurentin H, Pereira C. 2002. Distribution pattern and sampling of preimaginal whitefly *Bemisia tabaci* (Gennadius) Homoptera: Aleyrodidae in sesame (*Sesamum indicu*m L.). *Bioagro* 14:145–152.

Legaspi BC, Jr, Legaspi JC, Carruthers RI, Goolsby J, Hadman J, Jones W, Murden D, Wendel L. 1997. Areawide population dynamics of silverleaf whitefly (Homoptera: Aleyrodidae) and its parasitoids in the lower Rio Grande Valley of Texas. *J. Entomol. Sci.* 32:445–459.

Levins R. 1969. Some demographic and genetic consequences of environmental heterogeneity for biological control. *Bull. Entomol. Soc. Am.* 15:237–240.

Li T, Vinson SB, Gerling D. 1989. Courtship and mating behavior of *Bemisia tabaci* (Homoptera: Aleyrodidae). *Environ. Entomol.* 18:800–806.

Liebhold AM, Tobin PC. 2008. Population ecology of insect invasions and their management. *Annu. Rev. Entomol.* 53:387–408.

Lin L, Ren SX. 2005. Development and reproduction of 'B' biotype *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) on four ornamentals. *Insect Sci.* 12:137–142. (#30)

Lin L, Ren SX. 2005. Development and reproduction of B biotype *Bemisia tabaci* on variegated leaf crotones. *J. South China Agric.* 26:39–42. (#31)

Lin FC, Su TH, Wang CL. 1997. Effect of temperature on the development and reproduction of silverleaf whitefly (*Bemisia argentifolii* Bellows and Perring) and its population fluctuation on poinsettia. *Chinese J. Entomol.* 17:66–79. (#28)

Lin FC, Wang CL, Chen CN. 2006. Spatial pattern and optimal sample size estimation of adult *Bemisia argentifolii* (Hemiptera: Aleyrodidae) on cantaloupe in a nethouse. *Formosan Entomol.* 26:343–356.

Lin KJ, Wu KM, Wei HY, Guo YY. 2004. Effects of temperature and humidity on the development, survival and reproduction of B biotype of *Bemisia tabaci* (Homoptera: Aleyrodidae) from Beijing. *Acta Phyto. Sin.* 31:166–172. (#29)

Liu SS, DeBarro PJ, Xu J, Luan JB, Zang LS, Ruan YM, Wan FH. 2007. Asymmetric mating interactions drive widespread invasion and displacement in a whitefly. *Science* 318:1769–1772.

Liu TX, Stansly PA. 1998. Life history of *Bemisia argentifolii* (Homoptera: Aleyrodidae) on *Hibiscus rosa-sinensis* (Malvaceae). *Fla. Entomol.* 3:437–445. (#32)

Loxdale HD, Hardie J, Halbert S, Foottit R, Kidd NAC, Carter CI. 1993. The relative importance of short- and long-range movement of flying aphids. *Biol. Rev.* 68:291–311.

Mabbett T. 2004. Mating interactions of *Bemisia tabaci* biotypes in Cyprus. *Resistant Pest Manage. Newslett.* 13:3–4.

Marquet PA. 2002. Metapopulations. In *Encyclopedia of Global Environmental Change*, ed. HA Mooney, JG Canadell, pp. 411–420. Chichester: John Wiley & Sons.

McKenzie CL. 2002. Effect of tomato mottle virus (ToMoV) on *Bemisia tabaci* biotype B (Homoptera: Aleyrodidae) oviposition and adult survivorship on healthy tomato. *Fla. Entomol.* 85:367–368.

Mills NJ, Gutierrez AP. 1996. Prospective modeling in biological control: an analysis of the dynamics of heteronomous hyperparasitism in a cotton-whitefly-parasitoid system. *J. Appl. Ecol.* 33:1379–1394.

Miranda MMM, Picanco M, Leite GLD, Zanuncio JC, De Clercq P. 1998. Sampling and non-action levels for predators and parasitoids of virus vectors and leaf miners of tomato in Brazil. *Med. Fac. Landbouww. Univ. Gent* 63:519–526.

Misra CS, Lamba KS. 1929. The cotton white-fly (*Bemisia gossypiperda*, n. sp.). *Bull. Agric. Res. Inst., Pusa* 196:1–7.

Mound LA, Halsey SH. 1978. *Whitefly of the World. A Systematic Catalogue of the Aleyrodidae (Homoptera) with Host Plant and Natural Enemy Data*. London/Chichester: British Museum (Natural History)/John Wiley & Sons.

Muniz M. 2000a. Development of the B-biotype of *Bemisia tabaci* (Gennadius, 1889) (Homoptera: Aleyrodidae) on three varieties of pepper at constant temperatures. *Bol. Sanid. Veg., Plagas* 26:605–617. (#33)

Muniz M. 2000b. Host suitability of two biotypes of *Bemisia tabaci* on some common weeds. *Entomol. Exp. Appl.* 95:63–70. (#34)

Muniz M, Nombela G. 2001. Differential variation in development of the B- and Q-biotypes of *Bemisia tabaci* (Homoptera: Aleyrodidae) on sweet pepper at constant temperatures. *Environ. Entomol.* 30:720–727. (#35)

Murugan M, Uthamasamy S. 2001. Dispersal behaviour of cotton whitefly *Bemisia tabaci* (Genn.) under cotton based gardenland agroecosystem of Coimbatore, Tamil Nadu. *Madras Agric. J.* 88:1–6.

Musa PD, Ren SX. 2005. Development and reproduction of *Bemisia tabaci* (Homoptera: Aleyrodidae) on three bean species. *Insect Sci.* 12:25–30. (#36)

Nadel H, Luck RF. 1985. Span of female emergence and male sperm depletion in the female-biased, quasi-gregarious parasitoid, *Pachycrepoideus vindemiae* (Hymenoptera: Pteromalidae). *Ann. Entomol. Soc. Am.* 78:410–414.

Naranjo SE. 1996. Sampling *Bemisia* for research and pest management applications. In Bemisia *1995: Taxonomy, Biology, Damage, Control and Management*, ed. D Gerling, RT Mayer, pp. 209–224. Andover, Hants: Intercept Ltd.

Naranjo SE. 2001. Conservation and evaluation of natural enemies in IPM systems for *Bemisia tabaci*. In Special Issue: Challenges and Opportunities for Pest Management of *Bemisia tabaci* in the New Century, ed. SE Naranjo, PC Ellsworth. *Crop Prot.* 20:835–852.

Naranjo SE, Butler GD, Jr, Henneberry TJ. 2007. Bibliography *of Bemisia tabaci* and *Bemisia argentifolii*. URL: http://www.ars.usda.gov/Services/docs.htm?docid=10916

Naranjo SE, Cañas LA, Ellsworth PC. 2004. Mortalidad de *Bemisia tabaci* un sistema de cultivos múltiples [Mortality of *Bemisia tabaci* in a multiple crop system]. *Hortic. Int.* 43(February):14–21.

Naranjo SE, Ellsworth PC. 2001. Challenges and Opportunities for Pest Management of *Bemisia tabaci* in the New Century. *Crop Prot.* 20(Special Issue):707–872.

Naranjo SE, Ellsworth PC. 2005. Mortality dynamics and population regulation in *Bemisia tabaci*. *Entomol. Exp. Appl.* 116:93–108.

Naranjo SE, Ellsworth PC. 2009. 50 Years of the integrated control concept: moving the model and implementation forward in Arizona. *Pest Manage. Sci.* 65:1267–1286.

Naranjo SE, Flint HM. 1994. Spatial distribution of preimaginal *Bemisia tabaci* (Homoptera: Aleyrodidae) in cotton and development of fixed-precision sequential sampling plans. *Environ. Entomol.* 23:254–266.

Naranjo SE, Flint HM. 1995. Spatial distribution of adult *Bemisia tabaci* (Homoptera: Aleyrodidae) in cotton and development and validation of fixed-precision sampling plans for estimating population density. *Environ. Entomol.* 24:261–270.

Naranjo SE, Flint HM, Henneberry TJ. 1995. Comparative analysis of selected sampling methods for adult *Bemisia tabaci* (Homoptera: Aleyrodidae) in cotton. *J. Econ. Entomol.* 88:1666–1678.

Naresh JS, Nene YL. 1980. Host range, host preference for oviposition and development and the dispersal of *Bemisia tabaci* Gennadius, a vector of several plant viruses. *Indian J. Agric. Sci.* 50:620–623. (#37)

Nauen R, Stumpf N, Elbert A. 2002. Toxicological and mechanistic studies on neonicotinoid cross resistance in Q-type *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Pest Manage. Sci.* 58:868–875.

Nombela G, Beitia F, Muniz M. 2001. A differential interaction study of *Bemisia tabaci* Q-biotype on commercial tomato varieties with or without the Mi resistance gene, and comparative host responses with the B-biotype. *Entomol. Exp. Appl.* 98:339–344.

Norman JW, Jr, Riley DG, Stansly PA, Ellsworth PC, Toscano NC. 1995. Management of silverleaf whitefly: a comprehensive manual on the biology, economic impact and control tactics. U.S. Dept. Agric./Coop. State Res. Ext. Education Serv., Grant Publ. 93-EPIX-1-0102, 13pp.

Odum HT. 1971. *Environment Power and Society*. New York: John Wiley & Sons.

Ohnesorge B, Rapp G. 1986. Monitoring *Bemisia tabaci:* a review. *Agric. Ecosyst. Environ.* 17:21–27.

Ozgür AF, Sekeroglu E, Ohnesorge B, Göçmen H. 1989. Studies on the population dynamics of *Bemisia tabaci* Genn. (Homopt., Aleyrodidae) in Cukurova, Turkey. *J. Appl. Entomol.* 107:217–227.

Pai KF, Shih CIT. 2003. Population parameters of *Bemisia argentifolii* Bellows & Perring (Homoptera: Aleyrodidae) on cucumber. *Plant Prot. Bull. (Taipei)* 45:91–100. (#38)

Parrella MP, Jones VP, Malais MS, Heinz KM. 1989. Advances in sampling in ornamentals. *Fla. Entomol.* 72:394–403.

Pascual S. 2006. Mechanisms in competition, under laboratory conditions, between Spanish biotypes B and Q of *Bemisia tabaci* (Gennadius). *Span. J. Agric. Res.* 4:351–354.

Pascual S, Callejas C. 2004. Intra- and interspecific competition between biotypes B and Q of *Bemisia tabaci* (Hemiptera: Aleyrodidae) from Spain. *Bull. Entomol. Res.* 94:369–375.

Pereira MFA, Boica AL, Jr, Barbosa JC. 2004a. Amostragem sequencial (presenca-ausencia) para Bemisia tabaci (Genn.) biotipo B (Hemiptera: Aleyrodidae) em feijoeiro (Phaseolus vulgaris L.) [Sequential sampling (Presence-absence) for *Bemisia tabaci* (Genn.) biotype B (Hemiptera: Aleyrodidae) in the common bean (*Phaseolus vulgaris* L.)]. *Neotrop. Entomol.* 33: 499–504.

Pereira MFA, Boica AL, Jr, Barbosa JC. 2004b. Distribuicao espacial de *Bemisia tabaci* (Genn.) biotipo B (Hemiptera Aleyrodidae) em feijoeiro (*Phaseolus vulgaris* L.) [Spatial distribution of *Bemisia tabaci* (Genn.) biotype B (Hemiptera: Aleyrodidae) on bean crop (*Phaseolus vulgaris* L.)]. *Neotrop. Entomol.* 33:493–498.

Perring TM. 1996. Biological differences of two species of *Bemisia* that contribute to adaptive advantage. In *Bemisia 1995: Taxonomy, Biology, Damage, Control and Management*, ed. D Gerling, RT Mayer, pp. 3–16. Andover, Hants: Intercept Ltd.

Perring TM, Cooper A, Kazmer DJ, Shields C, Shields J. 1991. New strain of sweetpotato whitefly invades California vegetables. *Calif. Agric.* 45:10–12.

Perring TM, Cooper AD, Rodriguez RJ, Farrar CA, Bellows TS. 1993. Identification of a whitefly species by genomic and behavioral studies. *Science* 259:74–77.

Perring TM, Farrar CA, Cooper AD. 1994. Mating behavior and competitive displacement in whiteflies. In *Silverleaf Whitefly (Formerly Sweetpotato Whitefly, Strain B): 1994 Supplement to the 5-Year National Research and Action Plan*, U.S. Dept. Agric., Agric. Res. Serv. No. 125. p. 25.

Perring TM, Symmes EJ. 2006. Courtship behavior of *Bemisia argentifolii* (Hemiptera: Aleyrodidae) and whitefly mate recognition. *Ann. Entomol. Soc. Am.* 99:598–606.

Powell DA, Bellows TS, Jr. 1992. Adult longevity, fertility and population growth rates for *Bemisia tabaci* (Genn.) (Hom, Aleyrodidae) on two host plant species. *J. Appl. Entomol.* 113:68–78. (#39)

Powell DA, Bellows TS, Jr. 1992. Preimaginal development and survival of *Bemisia tabaci* on cotton and cucumber. *Environ. Entomol.* 21:359–363. (#40)

Price, PW. 1997. *Insect Ecology*, 3rd Edition. New York: John Wiley & Sons.

Pruthi HS, Samuel CK. 1942. Entomological investigations on the leaf-curl disease of tobacco in northern India. V. Biology and population of the white-fly vector [*Bemisia tabaci* (Gen.)] in relation to the incidence of the diseases. *Indian J. Agric. Sci.* 12:35–57.

Qiu BL, Ren SX, Mandour NS, Lin L. 2003. Effect of temperature on the development and reproduction of *Bemisia tabaci* B biotype (Homoptera: Aleyrodidae). *Entomol. Sin.* 10:43–49. (#41)

Rao NV, Reddy AS. 1989. Seasonal influence on developmental duration of whitefly *Bemisia tabaci* in upland cotton *Gossypium hirsutum*. *Indian J. Agric. Sci.* 59:383–385. (#42)

Rauch N, Nauen R. 2003. Identification of biochemical markers linked to neonicotinoid cross resistance in *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Arch. Insect Biochem. Physiol.* 54:165–176.

Reitz SR. 2007. Ecology: invasion of the whiteflies. *Science* 318:1733–1734.

Reitz SR, Trumble JT. 2002. Competitive displacement among insects and Arachnids. *Annu. Rev. Entomol.* 47:435–465.

Richards TJ, Eaves J, Fournier V, Naranjo SE, Chu CC, Henneberry TJ. 2006. Managing economic risk caused by insects: bug options. *Agric. Finance Rev.* 66:27–45.

Ricklefs RE. 1979. *Ecology*, 2nd Edition. New York: Chiron Press.

Riley D, Nava-Camberos U, Allen J. 1996. Population dynamics of *Bemisia* in agricultural systems. In *Bemisia 1995: Taxonomy, Biology, Damage, Control and Management*, ed. D Gerling, RT Mayer, pp. 93–109. Andover, Hants: Intercept Ltd.

Roberts W. 1930. Note on the American cotton crop failure in the Punjab. *Agric. J. India* 24: 77–81.

Ruan YM, Luan JB, Zang LS, Liu SS. 2007. Observing and recording copulation events of whiteflies on plants using a video camera. *Entomol. Exp. Appl.* 124:229–233.

Rubinstein G, Czosnek H. 1997. Long-term association of tomato yellow leaf curl virus with its whitefly vector *Bemisia tabaci*: effect on the insect transmission capacity, longevity and fecundity. *J. Gen. Virol.* 78:2683–2689.

Russell LM. 1957. Synonyms of *Bemisia tabaci* (Gennadius) (Homoptera, Aleyrodidae). *Bull. Brooklyn Entomol. Soc.* 52:122–123.

Salvucci ME. 2000. Sorbitol accumulation in whiteflies: evidence for a role in protecting proteins during heat stress. *J. Therm. Biol.* 25:353–361.

Samih MA. 2005. Comparative study on biological parameters of *Bemisia tabaci* (Genn.) collected on four host plants from Varamin-Iran. *Comm. Agric. Appl. Biol. Sci.* 70:663–670. (#43)

Samih MA, Kamali K, Talebi AA, Javaran MJ. 2003a. Comparative study on the life table parameters of local populations of cotton whitefly *Bemisia tabaci* in Iran. *J. Entomol. Soc. Iran* 23:1–19. (#44)

Samih MA, Kamali K, Talebi AA, Moharramipour S, Fathipour Y. 2003b. Comparative study on the age specific reproduction parameters of 10 regional populations of *Bemisia tabaci* in Iran. *Agric. Sci. (Tabriz)* 13:51–61. (#45)

Sanchez A, Geraud-Pouey F, Esparza D. 1997. Bionomics of the tobacco whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae) and potential for population increase on five host plant species. *Rev. Facultad Agron., Univ. Zulia (Venezuela)* 14:193–206. (#46)

Sawicki RM. 1987. Definition, detection and documentation of insecticide resistance. In *Combating Resistance to Xenobiotics*, ed. GM Ford, DW Holloman, BPS Khambay, RM Sawicki, pp. 105–117. Chichester: Ellis Horwood.

Sharaf NS, Al-Musa AM, Batta Y. 1985. Effect of different host plants on population development of the sweet potato whitefly (*Bemisia tabaci* Genn., Homoptera: Aleyrodidae). *Dirasat* 12:89–100. (#48)

Sharaf N, Batta Y. 1985. Effect of some factors on the relationship between the whitefly *Bemisia tabaci* Genn. (Homopt., Aleyrodidae) and the parasitoid *Eretmocerus mundus* Mercet (Hymenopt., Aphelinidae). *Z. Angew. Entomol.* 99:267–276. (#47)

Shen BB, Ren SX, Musa PD, Zhou JH. 2005a. The spatial distribution pattern of *Bemisia tabaci* adults. *Chinese Bull. Entomol.* 42:544–546.

Shen B, Chen C, Ren S. 2005b. Life table of natural *Bemisia tabaci* population on cucumber plants in different seasons. *Yingyong Shengtai Xuebao* 16:2473–2475.

Shen BB, Ren SX. 2003. Simulation of the control effects of different factors on *Bemisia tabaci* population. *Acta Agric. Univ. Jiangxiensis* 25:890–895.

Simon B, Moriones E, Soria C, Beitia F, Bosco D, Cenis JL. 1999. Variación genética de poblaciones de *Bemisia tabaci* Gennadius en la cuenca del Mediterráneo occidental [Genetic variation in populations of *Bemisia tabaci* Gennadius in the western Mediterranean basin]. In *Resúmenes del Congreso Nacional de Entomología Aplicada, VII Jornadas Científicas de la Sociedad Española de Entomología Aplicada*, Aguadulce, Spain, November 8–12, 1999, p. 20. Junta de Andalucía, Espana: Consejería de Agricultura y Pesca.

Simpson IG, Simpson MC. 1991. Systems of agricultural production in central Sudan and Khartoum Province. In *The Agriculture of the Sudan*, ed. GM Graig, pp. 252–279. Oxford: Oxford University Press.

Stansly PA. 1996. Seasonal abundance of silverleaf whitefly in southwest Florida vegetable fields. *Proc. Fla. State Hortic. Soc.* 108:234–242.

Stansly PA, Schuster DA. 1990. Update on sweetpotato whitefly. In *Proceedings Florida Tomato Institute, Vegetable Crops Special Series SS-VEC-001, IFAS*, University of Florida, Gainesville, ed. WM Stall, pp. 41–59.

Sundaramurthy VT. 1992. Upsurgence of whitefly *Bemisia tabaci* Gen. in the cotton ecosystem in India. *Outlook Agric.* 21:109–115.

Sutherst RW, Maywald GF, Yonow T, Stevens PM. 1999. *CLIMEX Predicting the Effects of Climate on Plants and Animals: CD-ROM and User Guide*. Collingwood: CSIRO Publishing.

Trehan KN. 1944. Further notes on the bionomics of *Bemisia gossypiperda* M. & L., the white-fly of cotton in the Punjab. *Indian J. Agric. Sci.* 14:53–63.

Tsai JH, Wang KH. 1996. Development and reproduction of *Bemisia argentifolii* (Homoptera: Aleyrodidae) on five host plants. *Environ. Entomol.* 25:810–816. (#49)

van Giessen WA, Mollema C, Elsey KD. 1995. Design and use of a simulation model to evaluate germplasm for antibiotic resistance to the greenhouse whitefly (*Trialeurodes vaporariorum*) and the sweetpotato whitefly (*Bemisia tabaci*). *Entomol. Exp. Appl.* 76:271–286.

Von Arx R, Baumgärtner J, Delucchi V. 1983a. A model to stimulate the population dynamics of *Bemisia tabaci* (Genn.) (Stern. Aleyrodidae) on cotton in the Sudan Gezira. *Z. Angew. Entomol.* 96:341–363.

von Arx RV, Baumgärtner J, Delucchi V. 1983b. Developmental biology of *Bemisia tabaci* (Genn.) (Sternorrhyncha, Aleyrodidae) on cotton at constant temperatures. *Bull. Swiss Entomol. Soc.* 56:389–399. (#50)

Wagner TL. 1995. Temperature-dependent development, mortality, and adult size of sweetpotato whitefly biotype B on cotton. *Environ. Entomol.* 24:1179–1188. (#51)

Wang KH, Tsai JH. 1996. Temperature effect on development and reproduction of silverleaf whitefly (Homoptera: Aleyrodidae). *Ann. Entomol. Soc. Am.* 89:375–384. (#52)

Watson TF, Silvertooth JC, Tellez A, Lastra L. 1992. Seasonal dynamics of sweetpotato whitefly in Arizona. *Southwest. Entomol.* 17:149–167.

Wilhoit L, Schoenig S, Supkoff D, Johnson BA. 1994. A regional simulation model for silverleaf whitefly in the Imperial Valley. In *PM 94-01, California*. Pest Management Analysis and Planning Program, 109pp.

Wolfe GR, Hendrix DL, Salvucci ME. 1998. A thermoprotective role for sorbitol in the silverleaf whitefly, *Bemisia argentifolii*. *J. Insect Physiol.* 44:597–603.

Wrensch DL. 1993. Evolutionary flexibility through haploid males or how chance favors the prepared genome. In *Evolution and Diversity of Sex Ratio in Insects and Mites*, ed. DL Wrensch, M Ebbert, pp. 118–149. New York: Chapman and Hall.

Wrensch DL, Young SSY. 1983. Relationship between primary and tertiary sex ratio in the two-spotted spider mite (Acarina: Tetranychidae). *Ann. Entomol. Soc. Am.* 76:786–789.

Xu WH, Zhu GR, Zhang YJ, Wu QJ, Xu BY, Li GL. 2003. An analysis of the life table parameters of *Bemisia tabaci* feeding on seven species of host plants. *Entomol. Knowledge* 40:453–455. (#53)

Yadav DK, Singh SK. 2006. Forecast model of major insect pests of mung bean. *Ann. Plant Prot. Sci.* 14:323–328.

Yang TC, Chi H. 2006. Life tables and development of *Bemisia argentifolii* (Homoptera: Aleyrodidae) at different temperatures. *J. Econ. Entomol.* 99:691–698. (#54)

Yao ST, Zheng YL. 2007. A study on the spatial distribution and sampling of *Bemisia tabaci* (Gennadius) adults in African daisy, *Gerbera jamesonii* Bolus, fields. *Acta Agric. Zhejiangensis* 19:233–236.

Yee WL, Toscano NC. 1996. Ovipositional preference and development of *Bemisia argentifolii* (Homoptera: Aleyrodidae) in relation to alfalfa. *J. Econ. Entomol.* 89:870–876. (#55)

Yee WL, Toscano NC, Palumbo JC, Blua MJ, Yoshida HA. 1997. Seasonal population trends of *Bemisia argentifolii* (Homoptera: Aleyrodidae) on alfalfa in southern California and Arizona. *Environ. Entomol.* 26:241–249.

Yokomi RK, Hoelmer KA, Osborne LS. 1990. relationship between the sweetpotato whitefly and the squash silverleaf disorder. *Phytopathology* 80:895–900.

Zalom FG, Natwick ET, Toscano NC. 1985. Temperature regulation of *Bemisia tabaci* (Homoptera: Aleyrodidae) populations in Imperial Valley cotton. *J. Econ. Entomol.* 78:61–64.

Zang LS, Chen WQ, Liu SS. 2006. Comparison of performance on different host plants between the B biotype and a non-B biotype of *Bemisia tabaci* from Zhejiang, China. *Entomol. Exp. Appl.* 121:221–227. (#56)

Zang LS, Liu SS. 2007. A comparative study on mating behaviour between the B biotype and a non-B biotype of *Bemisia tabaci* (Hemiptera: Aleyrodidae) from Zhejiang, China. *J. Insect Behav.* 20:157–171.

Zang LS, Liu SS, Liu YQ, Chen WQ. 2005a. A comparative study on the morphological and biological characteristics of the B biotype and a non-B biotype (China-ZHJ-1) of *Bemisia tabaci* (Homoptera: Aleyrodidae) from Zhejiang, China. *Acta Entomol. Sin.* 48:742–748. (#57)

Zang LS, Liu SS, Liu YQ, Ruan YM, Wan FH. 2005b. Competition between the B biotype and a non-B biotype of the whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae) in Zhejiang, China. *Shengwu Duoyangxing* 13:181–187.

Zhang XS, Holt J, Colvin J. 2000. A general model of plant-virus disease infection incorporating vector aggregation. *Plant Pathol.* 49:435–444.

Zhang LP, Zhang YJ, Zhang WJ, Wu QJ, Xu BY, Chu D. 2005. Analysis of genetic diversity among different geographical populations and determination of biotypes of *Bemisia tabaci* in China. *J. Appl. Entomol.* 129:121–128.

# Section III
# Biology and Epidemiology of *Bemisia*-Vectored Viruses

**Moshe Lapidot and Jane E. Polston**

## Introduction

The whitefly, *Bemisia tabaci* (Gennadius), is one of the most devastating pests in modern agriculture today. The whitefly feeds on plants by inserting the stylets into the leaf and withdrawing sap from the phloem. Feeding by whiteflies causes extensive direct damage to crops through excessive sap removal, excretion of honeydew that promotes growth of sooty mold fungi, and by inducing systemic disorders such as leaf silvering in squash and irregular ripening in tomato. However, the most devastating damage induced by whiteflies is due to virus transmission. During feeding whiteflies acquire plant viruses that are in the phloem. After moving to and feeding on another plant, the whiteflies may transmit the acquired viruses. Despite the large number of whitefly species, only three, *B. tabaci, Trialeurodes vaporariorum* and *T. abutiloneus* are known as vectors of plant viruses. *B. tabaci* is the most important of these three and has been demonstrated to vector over 150 different viruses in the tropics and subtropics, most of which belong to the *Begomovirus* genus.

## Viruses Transmitted by *Bemisia* spp

The most important *Bemisia*-transmitted viruses are from one genus in each of three families of viruses: *Geminiviridae*, genus *Begomovirus*; *Closeroviridae*, genus *Crinivirus*; and *Potyviridae*, genus *Ipomovirus*.

### *Begomoviruses*

Viruses in the genus *Begomovirus* (family *Geminiviridae*) are characterized by an approximately 20 × 28 nm twinned isometric particle containing a circular single-stranded DNA (ssDNA molecule). Most of the begomoviruses, such as *Tomato golden mosaic virus* (TGMV), are bipartite, that is have their genome split between

---

Moshe Lapidot and Jane E. Polston (Section Editors)

two different genomic molecules known as DNA-A and DNA-B, each approximately 2,600 nucleotides (nt) in size. Others, such as *Tomato yellow leaf curl virus* (TYLCV) are monopartite, having a single DNA genomic component of approximately 2,800 nt. More than 110 species of begomoviruses have been recognized by the International Committee on Taxonomy of Viruses (ICTV), making this the largest genus of whitefly-transmitted viruses.

Begomoviruses are transmitted by whiteflies in a persistent, circulative manner. That is, once the vector feeds on an infected host plant and acquires the virus, transmission can then occur following a latent period of a few hours, and may continue for the life span of the vector. However, it has been shown for a number of begomoviruses that transmission efficiency declines with time. Also, female whiteflies are more efficient than the males in transmitting begomoviruses. For example, transmission efficiency of TYLCV by female whiteflies was six fold higher than that of males (Cohen and Nitzany 1966).

When the different forms of the whitefly were tested for acquisition of begomoviruses, it was found that the TYLCV is acquired by immature stages of the whitefly following feeding on an infected plant, and that 28% of those emerging adults were able to transmit the virus (Cohen and Nitzany 1966; Cohen and Lapidot 2007). This led to another issue – is TYLCV transmitted transovarially to the whitefly progeny? This controversial issue is discussed in detail by Accotto and Sardo (Chapter 12).

During the last two decades there has been a worldwide spread of the B biotype of *B. tabaci*, accompanied by the emergence of whitefly-transmitted geminiviruses. Due to this spread of begomoviruses, we now have the complete genome sequence of a few hundred begomoviruses available. A number of begomoviruses have been the subject of extensive study due to their severe economic impact. These include a number of cassava infecting begomoviruses that have caused severe pandemics of cassava mosaic virus disease (CMD). The spread and devastating effect CMD has had in the African continent is described by Legg (Chapter 7). Another severe begomoviral disease is tomato yellow leaf curl disease (TYLCD), which is a major constraint for open field tomato production worldwide. TYLCD is caused by a number of begomovirus species and strains, and its epidemiology is described in this section by Moriones and Navas-Castillo (Chapter 8). In Chapter 9, Morales details the overall epidemiology of begomoviruses in Latin America.

## *Criniviruses*

Viruses in the genus *Crinivirus* (family *Closteroviridae*) are characterized by a flexuous rod particle averaging of 650 - 900 nm in length containing two RNA molecules of approximately 7,000–9,000 nucleotides (nt) each, termed RNA 1 and RNA 2. The 11 accepted species of criniviruses are transmitted by *Bemisia* in a semi-persistent manner. Once the virus is acquired, there is no latent period and the insect can transmit immediately following acquisition. However, the retention

time – the time from feeding that the insect is still viruliferous – is shorter than persistent viruses, and usually declines after a few days.

Another interesting characteristic of criniviruses is their symptom appearance – unlike begomoviruses, crinivirus disease symptoms are slow to appear and are evident on older leaves. For instance, following inoculation of melon plants by *Cucurbit yellow stunting disorder virus* (CYSDV), it may take over a month before the appearance of disease symptoms. Moreover, while viral induced symptoms are pronounced on the older leaves, very young leaves at the plant apex appear symptomless despite viral presence. *Crinivirus-Bemisia* interactions are discussed in detail by Wintermantel (Chapter 10).

### *Ipomoviruses*

Viruses in the genus *Ipomovirus* (family *Potyviridae*) are characterized by a rod-shaped filamentous particle, about 700 nm in length, containing a single positive sense RNA genome about 10,000 nt in length. Ipomoviruses are transmitted by *Bemisia* in a non-persistent manner, and the viral retention time is only a few hours. At the moment there are only three members of the genus Ipomovirus accepted by the ICTV (Fauquet et al. 2005). Recently a new ipomovirus, *Squash vine yellowing virus* (SqVYV), was found to be the cause of watermelon vine decline in Florida, and it is reviewed in this section by Adkins et al. (Chapter 11).

### *Other Viruses*

Less well studied are the six whitefly-transmitted viruses in the genus *Carlavirus* (*Flexiviridae*), and two viruses in the *Comoviridae* and *Luteoviridae* reported to be transmitted by *B. tabaci*. There are other less substantiated reports of viruses in other genera transmitted by *B. tabaci*, so it is likely that the diversity of whitefly-transmitted viruses will increase in the next few years.

## Whitefly Diversity and Virus Transmission

*B. tabaci* is a widely distributed and genetically variable insect vector, and a number of biological and molecular parameters have been recognized among its populations in different locations (Bedford et al. 1994; Brown et al. 1995; Boykin et al. 2007). These different populations, referred to as species, biotypes, and most recently as clades, are more than an academic curiosity. The movement of a new *B. tabaci* biotype into an area has often resulted in the appearance of new virus-host relationships, increased incidence of virus infected plants, and subsequent negative impacts on crop yields (Polston and Anderson 1997; Moffat 1999; Mansoor et al. 2003). The three mostly studied biotypes in regard to viral transmission are biotypes A (or sweetpotato), B (also known as the silverleaf whitefly) and Q.

Differences in transmission of begomoviruses were first recognized among different populations of whiteflies in Puerto Rico in the 1950s (Bird 1957). However, in-depth studies have shown that the ability to acquire and transmit a begomovirus per se does not appear to vary greatly among clades. A study of 18 different whitefly populations representing as many as 7 different clades and 15 begomoviruses collected from both Old and New World sites was conducted to determine if biotypes differed in their ability to transmit begomoviruses (Bedford et al. 1994). This study concluded that virus distribution within the host plant and the interaction of the whitefly with the plant host were the most important factors influencing the ability of a whitefly to transmit a begomovirus. However, efficiency of transmission to and from the same host has been shown to vary among the different biotypes of whiteflies (McGrath and Harrison 1995; Sanchez-Campos et al. 1999; Jiang et al. 2004).

The situation with whiteflies and criniviruses is not as clear as that of whiteflies and begomoviruses. Some studies have documented differences among whitefly clades in the transmission of criniviruses and others have shown no differences. For example, one study did not find any differences between biotype B and Q in the transmission efficiency of CYSDV to cucumber (Berdiales et al. 1999). Differences were measured in the efficiency of transmission of *Lettuce infectious yellows virus* (LIYV) by biotypes A and B (Cohen et al. 1992) and these results were supported by the disappearance of LIYV in the Imperial Valley of California when the A biotype was displaced by the B biotype (Duffus 1995).

## Conclusions

Whiteflies, and the plant viruses they transmit, have developed a relationship over millennia which effectively support the dependence of plant viruses on the presence of plant hosts. This relationship very efficiently exploits plant hosts produced in monocultures (modern agriculture) and presents a great challenge to entomologists, plant virologists and horticulturists who are trying to minimize the impact of these viruses on crop yields. The following chapters present summaries of selected examples of the current understanding of the relationship of whiteflies and the plants viruses they transmit, and how that relationship impacts agriculture.

## Literature Cited

Bedford ID, Markham PG, Brown JK, Rosell RC. 1994. Geminivirus transmission and biological characterization of whitefly *Bemisia tabaci* biotypes from different geographic regions. *Ann. Appl. Biol.* 125:311–325.

Berdiales B, Bernal JJ, Sáez E, Woudt B, Beitia F, Rodríguez-Cerezo E. 1999. Occurrence of cucurbit yellow stunting disorder virus (CYSDV) and beet pseudo-yellows virus in cucurbit crops in Spain and transmission of CYSDV by two biotypes of *Bemisia tabaci*. *Eur. J. Plant Pathol.* 105:211–215.

Bird J. 1957. A whitefly-transmitted mosaic of *Jatropha gossypifolia*. *Agric. Exp. Sta. Univ. Puerto Rico* 22:1–35.

Boykin LM, Shatters RG, Jr, Rosell RC, McKenzie CL, Bagnall RA, De Barro P, Frohlich DR. 2007. Global relationships of *Bemisia tabaci* (Hemiptera: Aleyrodidae) revealed using Bayesian analysis of mitochondrial COI DNA sequences. *Mol. Phylogenet. Evol.* 44:1306–1319.

Brown JK, Bird J, Frohlich DR, Rosell RC, Bedford ID, Markham PG. 1995. The relevance of variability within the *Bemisia tabaci* species complex to epidemics caused by subgroup III geminiviruses. In *Bemisia 1995*: *Taxonomy, Biology, Damage Control and Management*, ed. D Gerling, RT Mayer, pp. 77–89. Andover, Hants, UK: Intercept Ltd.

Cohen S, Duffus JE, Liu HY. 1992. A new *Bemisia tabaci* Biotype in the southwestern United States and its role in silverleaf of squash and transmission of lettuce infectious yellow virus. *Phytopathology* 82:86–90.

Cohen S, Nitzany FE. 1966. Transmission and host range of the tomato yellow leaf curl virus. *Phytopathology* 56:1127–1131.

Cohen S, Lapidot M. 2007. Appearance and expansion of TYLCV: a historical point of view. In *Tomato Yellow Leaf Curl Virus Disease*, ed. H Czosnek, pp. 3–12. The Netherlands: Springer.

Duffus JE. 1995. Whitefly transmitted yellowing viruses of the Cucurbitaceae. In *Cucurbitaceae '94*, ed. GE Lester, JR Dunlap, pp. 12–16. Westlaco, TX: Gateway Printing.

Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA. 2005. *Virus Taxonomy*: *Eighth Report of the International Committee on Taxonomy of Viruses*. London: Elsevier Academic Press.

Jiang YX, De Blas C, Bedford ID, Nombela G, Muniz M. 2004. Effect of *Bemisia tabaci* biotype in the transmission of *Tomato yellow leaf curl Sardinia virus* (TYLCSV-ES) between tomato and common weeds. *Span. J. Agric. Res.* 2:115–119.

McGrath PF, Harrison BD. 1995. Transmission of tomato leaf curl geminiviruses by *Bemisia tabaci*: effects of virus isolate and vector biotype. *Ann. Appl. Biol.* 126:307–316.

Moffat AS. 1999. Geminiviruses emerge as serious crop threat. *Science* 286:1835.

Mansoor S, Briddon RW, Zafar Y, Stanley J. 2003. Geminivirus disease complexes: an emerging threat. *Trends Plant Sci.* 8:128–134.

Polston JE, Anderson PK. 1997. The emergence of whitefly-transmitted geminiviruses in tomato in the Western Hemisphere. *Plant Dis.* 81:1358–1369.

Sanchez-Campos S, Navas-Castillo J, Camero R, Soria C, Diaz JA, Moriones E. 1999. Displacement of tomato yellow leaf curl virus (TYLCV)-Sr by TYLCV-Is in tomato epidemics in Spain. *Phytopathology* 89:1038–1043.

# Chapter 7
# Epidemiology of a Whitefly-Transmitted Cassava Mosaic Geminivirus Pandemic in Africa

**James P. Legg**

## Introduction

Cassava (*Manihot esculenta* Crantz) is a semi-perennial root crop that is widely grown throughout the tropics and is used either as a staple food crop, or for the production of starch and its derivatives. Sub-Saharan Africa produces more than half of the world's cassava crop. In fact, the total fresh production of more than 100 million tons (FAO 2009) of cassava is greater than that of any other crop. Cassava plants are characteristically robust and are able to provide acceptable yields even under unfavorable rainfall and soil fertility conditions. The most economically important constraints to production are pests and diseases, of which cassava mosaic virus disease (CMD) is the most damaging. Depending on the susceptibility of the cassava genotype, the disease has variable effects on the cassava plants it attacks, including virulence of the infecting virus strain(s) and local environmental conditions. In all cases, the leaves of affected plants show yellow to pale green chlorotic mosaic symptoms. The overall growth and tuberous root formation of the plant are also impaired (Storey 1936).

CMD has been known in Africa for more than a century (Warburg 1894), but it has received only sporadic research attention, in large part due to the perceived low status of the crop. Beginning in the early 1990s, this situation changed dramatically, however, as a result of two contrasting forces. The first was growing recognition that cassava has great potential for enhanced utilization and consequent commercialization; the second was the emergence of a new unusually severe form of CMD. The impact of the CMD pandemic on the growing aspirations of cassava producers gave rise to a dramatic increase in cassava research that has been sustained to the present day. Otim-Nape et al. (1996) described the early stages of the

J.P. Legg (✉)
International Institute of Tropical Agriculture (IITA), PO Box 34441, Dar es Salaam, Tanzania
e-mail: j.legg@cgair.org

P.A. Stansly, S.E. Naranjo (eds.), *Bemisia*: Bionomics and Management of a Global Pest,
DOI 10.1007/978-90-481-2460-2_7, 

severe CMD phenomenon from Uganda. The pandemic has subsequently affected at least nine countries, including: Uganda, Kenya, Tanzania, Sudan, Democratic Republic of Congo (DRC), Rwanda, Republic of Congo (ROC), Burundi and Gabon. There is extensive literature examining the various aspects of this crop virus pandemic, although it is somewhat skewed towards the virological aspects. By contrast, there is relatively little published information on the role of the *Bemisia tabaci* (Gennadius) whitefly vector, an issue that will need to be addressed in future research programs. Research findings on severe CMD in East and Central Africa have been examined in a number of review articles. Results focusing most directly on the CMD pandemic include papers by: Otim-Nape et al. (1997, 2000), Legg (1999), Legg and Fauquet (2004), Thresh and Cooter (2005) and Legg et al. (2006). Some of the most recently published information will be explored, by addressing a series of key questions on different aspects of the epidemiology of the CMD pandemic.

## An Overview of Cassava Mosaic Geminiviruses, Whiteflies and the Epidemiology of the CMD Pandemic

### *Cassava Mosaic Geminivirus Biology*

Cassava mosaic disease is caused by an array of viruses in the genus, *Begomovirus*, family *Geminiviridae*, commonly referred to as the cassava mosaic geminiviruses (CMGs). CMGs are bipartite, single-stranded, closed circular DNA viruses in which the DNA-A and DNA-B molecules are each slightly less than 2,800 nucleotides long (Harrison et al. 1977, Hong et al. 1993). The two DNA molecules are encapsidated within a geminate protein "coat" that has dimensions of approximately 35 by 22 nm. DNA-A has two virion-sense and four complementary-sense open reading frames (ORFs). Their primary function is to produce the protein coat, mediate virus replication inside the nucleus of the host cell, and modulate interactions with host genes involved with post-transcriptional gene silencing (PTGS) (Hanley-Bowdoin et al. 1999; Vanitharani et al. 2004). The single virion-sense and complementary-sense genes on DNA-B control nuclear transport and both intra-cellular and long distance movement (Pascal et al. 1994; Hanley-Bowdoin et al. 1999). CMGs recorded from sub-Saharan Africa include: *African cassava mosaic virus* (ACMV) (Bock and Woods 1983), *East African cassava mosaic virus* (EACMV) (Bock and Woods 1983), *South African cassava mosaic virus* (SACMV) (Berrie et al. 2001), *East African cassava mosaic Malawi virus* (EACMMV) (Zhou et al. 1998), *East African cassava mosaic Cameroon virus* (EACMCV) (Fondong et al. 2000), *East African cassava mosaic Zanzibar virus* (EACMZV) (Maruthi et al. 2004) and *East African cassava mosaic Kenya virus* (EACMKV) (Bull et al. 2006). The number of CMGs species recorded is greatest in the countries of East Africa (Fig. 7.1); as a result, this sub-region has been proposed as the center of African CMG diversity (Ndunguru et al. 2005). The two non-African CMG species, occurring in South Asia are

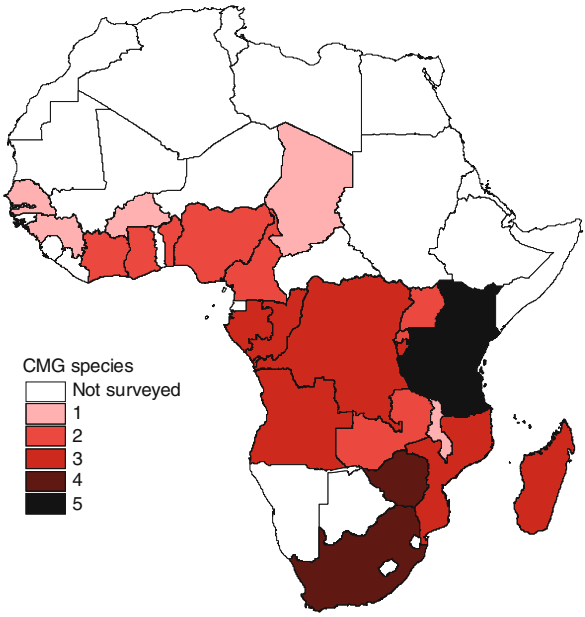



**Fig. 7.1** Numbers of CMG species reported from African countries, 2008

*Indian cassava mosaic virus* (ICMV) (Hong et al. 1993) and *Sri Lankan cassava mosaic virus* (SLCMV) (Saunders et al. 2002; Stanley 2002).

## *CMG Transmission*

CMGs can be propagated either through the use of cuttings from an infected mother plant – cutting-borne infection – or through the feeding activity of the whitefly, *B. tabaci* – whitefly-borne infection – (Storey and Nichols 1938). The balance between these two types of infection is a key characteristic of the epidemiology of these viruses. Under most circumstances, field-recorded incidences of cutting-borne infection are significantly greater than those of whitefly-borne infection (Legg and Thresh 2000) resulting in a stable overall incidence of disease. At the spreading 'front' of the severe CMD pandemic, however, whitefly-borne infection is greater than cutting-borne infection, leading to significant increases in disease incidence from one season to the next (Gibson et al. 1996; Legg and Ogwal 1998). Although it was recognized from the early years of CMD research that *Bemisia* whiteflies were the likely vector of the viruses causing CMD (Kufferath and Ghesquière 1932; Storey and Nichols 1938), it was not until the latter part of the twentieth century that the characteristics of transmission were experimentally described (Dubern 1979, 1994). *B. tabaci* was shown to be a persistent vector, retaining the capability to transmit for at least 9 days after first acquisition, although the minimum inoculation access period was as little as 10 min (Dubern 1994).

## *Vector Host Interactions*

*Bemisia tabaci* is one of the most economically important of all insect species, both because of its capability to transmit one of the largest groups of plant viruses – the begomoviruses – and because of its environmental adaptability (Byrne and Bellows 1991). The evolutionary divergence enabling this insect to exploit such diverse environments has resulted in levels of genetic and concomitant biological variation leading to the recognition of the set of populations as a species complex (Brown et al. 1995). Most populations, of which the so-called B biotype is the best documented, are polyphagous, primarily colonizing herbaceous annuals and crop plants of this type (Byrne et al. 1990). *B. tabaci* populations occurring on cassava are distinct in this respect, since cassava is a woody semi-perennial. It also appears that cassava *B. tabaci* in Africa is more or less restricted to this plant and its very close relatives (Burban et al. 1992; Legg 1996). Cassava has its origins in the New World, and was only introduced to Africa in the sixteenth century. It is assumed that high levels of cyanogenic glucosides present in the leaves have necessitated specific adaptations in colonizing *B. tabaci* populations. It is also notable that *B. tabaci* has yet to effectively colonize cassava in the Americas, and that *B. tabaci*-transmitted CMGs are absent in Latin America as well as the other major cassava-growing regions in South-East Asia. Even in Africa, where *B. tabaci* colonizes cassava in all areas where it is grown, populations are usually moderate to low, being measured in hundreds per plant rather than thousands or more. This fact, coupled with the modest transmission efficiency of CMGs – typically less than 2% (Fargette et al. 1990) – has meant that for much of the history of CMGs in Africa, incidences of CMD have been moderate to low and relatively stable. The important exceptions to this condition include the "first-encounter" epidemics of the 1920s–1930s, localised epidemics in the 1970s–1980s in Cape Verde and parts of Nigeria (Calvert and Thresh 2002), and – most significantly in recent years – the CMD pandemic in East and Central Africa (Otim-Nape et al. 1997; Legg 1999).

## *The CMD Pandemic*

Unusually severe CMD, which was first noted in north-central parts of Uganda in the late 1980s (Otim-Nape et al. 1994), spread rapidly throughout Uganda during the 1990s. Characteristics of the epidemic included: unusually severe CMD symptoms, elevated *B. tabaci* populations, and rapid disease spread giving rise to high levels of current-season whitefly-borne infection in previously lightly-affected crops. The consequences for cassava producers were yield losses typically greater than 50% (Byabakama et al. 1999; Owor et al. 2004b). The loss of "viable" stems to use for the following crop led to the widespread abandonment of cassava. These losses further resulted in widespread food shortages and localized famine resulting in fatalities (Thresh et al. 1994). Early monitoring surveys revealed the rate of spread of the epidemic front through central and southern Uganda to be 20–30 km per year (Legg and Ogwal 1998). Through the second half of the decade new reports of spread were

received from Kenya (1995), Sudan (1997), Tanzania (1998), and the DRC (1999) (Legg 1999).

The CMD pandemic – as it became known – continued to spread further to the east, south, and west. By 2005, it had affected nine countries of East and Central Africa, covering an area of more than 2.6 million sq. km. and causing losses of more than 13 million tons (Legg et al. 2006). During the early period of pandemic spread through Uganda and neighboring countries, there were significant efforts to research the basic epidemiological characteristics of the disease. As the magnitude of the social impact of the problem escalated, this emphasis changed and resources were directed almost entirely towards the development and deployment of control measures. These trends have influenced research progress in the understanding of this dynamic disease although several key research questions still remain unanswered. In the following section, some of these research themes will be examined. Important new insights will be described, but attention will also be drawn to those areas that remain poorly understood. It is hoped that by highlighting specific areas where current knowledge is weak, this review can contribute to the development of a new and expanded research agenda that will be commensurate with the ever-growing magnitude of the problem.

## The CMD Pandemic: New Insights and Research Gaps

### *What Caused the Changes in the Impact of CMD-causing Viruses on Cassava Plants?*

#### A Recombinant Virus Is Associated with the Pandemic

The two most important features of the CMD pandemic have been unusually severe symptomology and high populations of the *B. tabaci* vector (Gibson et al. 1996). Molecular evidence was presented showing that the predominant virus in epidemic-affected areas was a recombinant variant subsequently named *East African cassava mosaic virus*-Uganda (EACMV-UG) (Zhou et al. 1997). This was the first significant demonstration of recombination in the *Geminiviridae*. The recombinant was shown to have a large part of the ACMV AV1 ORF – or coat protein – inserted into DNA-A that was otherwise typical of EACMV. The origin of this recombination event remains a matter of conjecture, since parent EACMV has yet to be found in Uganda where EACMV-UG was first reported. Another possible origin for the recombined hybrid could be northeastern DRC, where both ACMV and EACMV-UG are known to occur, and which is situated at the center of the pandemic-affected zone (Legg et al. 2006). The absence of any research on CMG diversity in this region, however, currently precludes any firm conclusion from being drawn. A contrasting view is that EACMV-UG occurs widely both within and outside the pandemic-affected zone, as evidenced by reported occurrences in southern Africa (Berry and Rey 2001), but has only been triggered to rapid epidemic-like spread in East and Central Africa because of other, as yet unknown biotic or abiotic environmental factors.

This is an issue of great relevance to the epidemiological future of the CMGs in sub-Saharan Africa and warrants further study.

### Virus–Virus Synergism Enhances Symptom Severity

Single EACMV-UG infections were shown to elicit more severe CMD symptoms in cassava than ACMV. More importantly, however, dual infections with both viruses led to a synergistic interaction where the concentrations of both component viruses were significantly increased (Harrison et al. 1997; Pita et al. 2001). Dual infection and the resulting synergism have subsequently been shown to occur in other countries where the pandemic has spread, including Rwanda (Legg et al. 2001), DRC (Neuenschwander et al. 2002), Burundi (Bigirimana et al. 2004) and Kenya (Were et al. 2004). Synergistic interaction between ACMV and EACMVs – defined here to include EACMV, EACMCV, EACMKV, EACMMV, EACMZV and SACMV – is not unique to the pandemic-affected part of Africa, however. It has also been reported in Cameroon (Fondong et al. 2000) and Nigeria (Ogbe et al. 2003, Ariyo et al. 2005). Mixed infections comprising ACMV, and one or more EACMVs, have also been reported from southern African countries (Berry and Rey 2001). Importantly, these other instances of synergistic mixed infections are not associated with the rapid rates of CMD spread characteristic of the pandemic-affected zone. The reason for this remains unclear.

### Molecular Complementation Facilitates Synergism

Molecular investigations of the synergistic response elicited in mixed infections suggest that this is a consequence of the complementary suppression of post-transcriptional gene silencing by viruses of each of the two main virus groups (ACMV and EACMVs). Expression of ACMV AC4 was shown to enhance the replication of EACMCV, while AC2 expression in EACMCV had a similar synergizing effect for ACMV (Vanitharani et al. 2004). Mixed infections of EACMVs have been infrequently reported, although there is field evidence to suggest that mild strains of EACMV-UG provide a cross-protective effect inhibiting subsequent super-infection by severe EACMV-UG strains (Owor et al. 2004a). These observations have important practical implications for the future pattern of development of the pandemic, since they suggest that severe impacts may not result in regions or countries in which ACMV is absent. This includes coastal areas of Kenya, Tanzania, and northern Mozambique as well as Malawi (Legg and Fauquet 2004). Conversely, it also suggests that all other cassava-growing regions of sub-Saharan Africa not currently affected by the pandemic remain threatened by it.

### Virus-Host Dynamics Change Following the Passage of the Pandemic 'Front'

Rapid spread of CMGs occurs at the pandemic front, as initially healthy plants become dually infected by ACMV and EACMV-UG, and as elevated *B. tabaci* populations promote spread between both plants and fields. In the second season, however, the dynamics begin to change because:

(i) Most plants of the new crop are already infected through the planted cuttings taken from the previous crop.
(ii) Growers do not select the most severely diseased plants as sources of cuttings for the new crop.
(iii) Some growers decide not to plant a new cassava crop, in most cases opting to plant an alternative like the sweet potato.

These dynamics have a series of important outcomes for the CMG pathosystem. The combined effect of planting virus-infected parent material, with the exclusion of the most severely diseased stems, is a reduction in the population of the viruses present in the most severely diseased plants (dual ACMV+EACMV-UG infections). Over time, this results in the competitive exclusion of ACMV (Legg et al. 2006) as well as the gradual predominance of mild strains of EACMV-UG (Owor et al. 2004a) (Figs. 7.2 and 7.3). Reduced cultivation of cassava results in reduced *B. tabaci* populations (Legg and Ogwal 1998), which further reduces virus spread. The net result of these changes is an overall amelioration in the CMD situation, although incidence levels remain significantly greater than those of the pre-pandemic condition, and are only reduced in areas where CMD control measures are implemented (Legg et al. 2006).

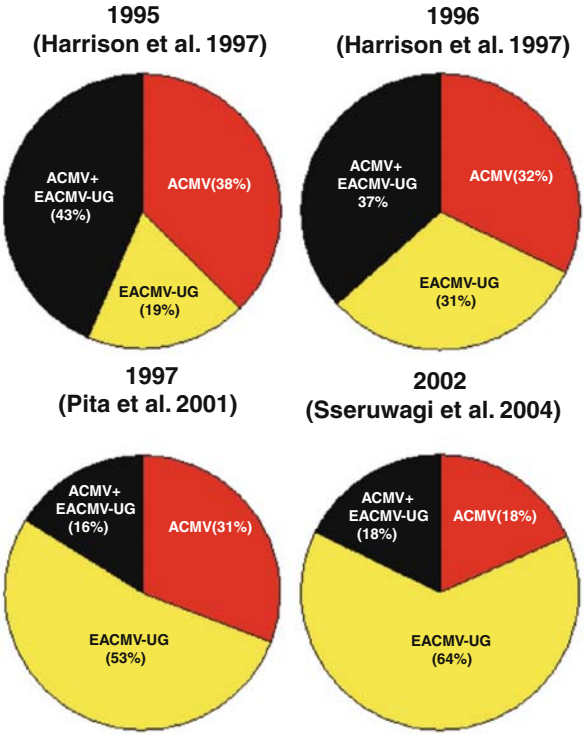



**Fig. 7.2** Changes in the incidence of CMG infections in cassava, Uganda, 1995–2002

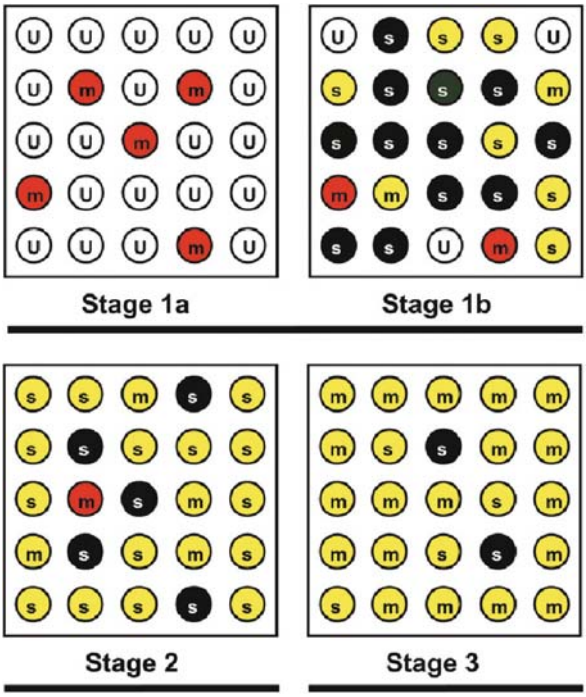



**Fig. 7.3** Diagrammatic representation of changes in virus occurrence in cassava plants over time in pandemic-affected areas of East and Central Africa. *Circles* represent individual cassava plants. *Colors* indicate virus infection: *white* – uninfected; *yellow* – EACMV-UG; *red* – ACMV; *black* – ACMV+EACMV-UG mixed infection. "u" uninfected; "m" mild symptoms; "s" severe symptoms. Stage 1a and 1b represent the sprouting and full maturity growth stages respectively of a single crop cycle. This corresponds to the first season's impact of the CMD pandemic. Stages 2 and 3 represent subsequent crop cycles, each being 1–3 years after the previous stage

## *Why Are* B. tabaci *Whiteflies Super-Abundant in the Pandemic-Affected Zone?*

### *B. tabaci* Has Adapted Rapidly to Cassava

While it is assumed that *B. tabaci* is an Old World insect, and may have its origins in Africa (Campbell et al. 1996, see also Brown, Chapter 2), cassava has a relatively recent history in Africa. Although the crop was introduced to the continent in the sixteenth century by Portuguese seafarers (Jennings 1970), it was not until the late nineteenth and early twentieth centuries that the crop became more widely cultivated. The first record of CMD in Africa from this period (Warburg 1894) demonstrates that *B. tabaci* succeeded in colonizing the crop fairly soon after its introduction. Significantly, *B. tabaci* is one of only a small number of insects that have been able to effectively colonize cassava in Africa. These findings are in contrast to the situation in Latin America where the crop supports a diverse arthropod fauna (Bellotti and Schoonhoven 1978). In spite of *B. tabaci*'s success in the initial colonization of cassava in Africa, there were no reports of levels of abundance that could cause actual physical damage to the crop, as there were with other whitefly species colonizing cassava in Latin America (Bellotti and Arias 2001). Studies in both West Africa (Burban et al. 1992), and East Africa (Legg et al. 1994), did

demonstrate an incipient specialization within cassava-colonizing *B. tabaci* populations, however; they also appear to have been the prelude for the dramatic increase in abundances reported from cassava in pandemic-affected parts of East Africa in the 1990s (Otim-Nape et al. 1996, Legg et al. 2006). Super-abundant *B. tabaci* populations, commonly numbering more than 100 adults per top five leaves, have been widely reported in recent years from pandemic-affected countries as causing physical damage to cassava (Legg et al. 2006). Features of this damage include blotchy chlorosis in the young newly-emerged leaves that support the greatest numbers of adult *B. tabaci*, as well as the growth of sooty mold and concomitant reduction in leaf size in lower leaves where populations of late instar nymphs are greatest.

## Both Genetic and Environmental Factors Have Been Proposed as Causes of Super-Abundance

Increases in *B. tabaci* populations have more or less coincided with the rapid spread of severe CMD in areas of East and Central Africa. Assuming there is no change in transmission efficiency, greater vector abundance will increase the spread of the virus. The converse situation, in which raised vector populations result from higher incidences of virus-infected cassava plants, has also been proposed as a cause for super-abundance (Colvin et al. 2006). This theory posits that virus-infected cassava plants are better hosts for cassava *B. tabaci* than uninfected plants, and that this synergistic interaction boosts vector populations. The positive feedback resulting from this type of interaction would drive up levels of both virus infection and vector abundance until resulting reductions in the level of cassava cultivation would eventually hinder vector colonization of new fields thus limiting further vector population increase. Confined cage experiments – using a single cassava variety from Uganda – suggest that CMD-infected plants can provide a better substrate for *B. tabaci* population development (Colvin et al. 2006), although there are currently no field data to support this theory. Furthermore, an examination of data collected through numerous extensive surveys across sub-Saharan Africa – and summarized in Sseruwagi et al. (2004) – showed that while mean whitefly abundance is significantly correlated with current-season whitefly-borne infection, it was not correlated with cutting-borne infection, which is equivalent to the CMD inoculum present in fields at sprouting. Moreover, for the 97 regions considered in 17 countries, only 18% of regions with high whitefly abundances – defined as having a mean adult abundance per top five leaves of greater than 5 – had high levels of cutting infection (>40%), in spite of the fact that approximately half of all regions had high levels of cutting infection. A further inconsistency in the CMD-whitefly synergism hypothesis is the fact that some of the highest abundances of *B. tabaci* have been recorded from CMD-free virus resistant varieties (Omongo 2003).

Changes in the genetic make-up of *B. tabaci* populations associated with the CMD pandemic have also been suggested as a possible cause for super-abundance. The success of the B biotype provides an obvious model (Perring et al. 1991, Brown et al. 1995). Sequences cloned from a portion of the cytochrome oxidase 1

mitochondrial DNA gene (mtCO1) of *B. tabaci* populations collected from locations behind, at, and ahead of the CMD pandemic front in Uganda pointed to the association of a distinct genotype cluster with the pandemic (Legg et al. 2002). Subsequent sampling, however, showed the situation to be more complex than was initially thought when the putative pandemic-associated genotype cluster was apparently displaced by the "local" cluster (Sseruwagi et al. 2005a). Analysis was further complicated by the fact that all populations are assumed to interbreed (Maruthi et al. 2001). Consequently, a sustained association over time between the mtCO1 marker, and genetic factors associated with super-abundance, would not be expected. Therefore, in order for the theory of a genetic cause for super-abundance to be proven, bioassays will be required to confirm or refute the suggestion that some populations are fitter than others. Molecular markers will need to be developed that are tightly linked to genes associated with this enhanced fitness, and these genes will need to be identified. In view of the complexity of the molecular elements of these tasks, genomics approaches will be of value. Data generated from the *B. tabaci* genome project (Leshkowitz et al. 2006) will clearly be of particular value here.

## *What Are the Factors Behind the Rapid Local Spread of the Pandemic?*

### Two Key Changes in the CMD Pathosystem Have Driven the Pandemic

There are a large number of factors that influence local spread of CMGs in East and Central African agricultural environments. The most important of these are briefly described here:

(i) *Virus concentration in the leaves*. This concentration is determined by the virulence of the virus species/strain, interactions between virus species/strains, and the relative sensitivity of the cassava host to infection.
(ii) *Virus movement within the plant*. Some virus strains/species are incompletely systemic within some cassava varieties. This leads to reversion or the sprouting of CMD-free plants from cuttings obtained from diseased parent plants where the virus infection was incompletely systemic.
(iii) *Planting material selection*. The selection by growers of disease-free stems for establishing a new crop. The ease with which selection can be achieved depends on a number of factors, such as the percentage of disease-free plants that are present in the parent crop, the visibility of symptoms in harvest age crops, and the knowledge of the grower about the value of selection and their motivation to implement it.
(iv) *Varietal response*. Varietal response is the relative susceptibility or resistance of the variety to infection.
(v) *Crop disposition.* Crop disposition is the spatial and temporal relationship between the grower's field and neighboring cassava crops. This is significant

because the proximity of neighboring cassava fields and staggering of planting dates both favor new crop colonization by whiteflies emigrating from mature crops.

(vi) *Vector abundance*. More whiteflies result in a greater number of new CMD infections although the relationship is not linear, most notably at high incidence levels where multiple infections become more frequent (Gregory 1948).

(vii) *Vector transmission efficiency*. Whitefly populations that have a greater efficiency of acquiring, retaining, and inoculating CMGs will promote more rapid CMG spread. Similarly, virus strains/species that have a better co-adaptation with co-occurring vector populations will be more efficiently transmitted.

(viii) *Vector irritability*. Vectors that alight on a single plant, and continue to feed and mate there, will reduce local spread while others that continually move from one plant to the next will enhance it.

(ix) *Vector flight*. Whiteflies that are able to fly longer distances, and locate new cassava crops more effectively, will enhance local CMG spread.

(x) *Vector host range*. The ability of cassava whiteflies to effectively colonize other host plants can enhance the potential for using non-cassava "stopovers" when searching for new cassava crops. However, it can also serve to reduce the concentration on cassava and "dilute down" cassava whitefly populations.

In most cases, experimental data are not available to show whether or not changes have occurred in each of these factors immediately prior to, during, or after the impact of the CMD pandemic. However, a number of them – particularly those associated with the crop, varieties, and grower practice – clearly have not changed over the period of the pandemic's initial arrival and spread (Otim-Nape et al. 1997). Growers have not changed varieties over the period of passage of the pandemic front, nor have any changes in phytosanitation practices precipitated pandemic-associated changes in the pathosystem. The two factors that very clearly have changed are virus concentration – resulting from the spread of EACMV-UG and its synergistic interaction with ACMV – and the abundance of the whitefly vector. The combination of increased virus titres with super-abundant *B. tabaci* populations inevitably results in sharply increased local virus spread. This pair of changes is such that there would be no additional requirement for more efficient *B. tabaci* transmission. This lack of difference in transmission efficiencies has been confirmed, however, through comparisons of the relative vectoring efficiencies of *B. tabaci* from India and Africa (Maruthi et al. 2002). Results from this study showed that Indian cassava *B. tabaci* were more efficient at transmitting Indian CMGs than CMGs from Africa, and that African cassava *B. tabaci* were more efficient at transmitting African CMGs than those from India. No such differences were apparent, however, when comparing the transmission efficiencies of *B. tabaci* from different parts of Africa transmitting different African CMG species.

### Evidence for the Origins of Changes in Both Virus and Vector Remains Inconclusive

It seems unlikely, however, for a change in virus and a change in vector to have occurred independently at more or less the same time and location. A change in one of the two leading to a change in the other seems more plausible. Research opinion is currently divided on whether or not *B. tabaci* super-abundance has resulted from changes in virus infection (Colvin et al. 2006; Legg et al. 2006) and further study is warranted. It also seems unlikely that a change in the vector would in itself give rise to the virus changes that have been associated with the pandemic, although greater vector abundance might increase the probability of recombination events as a consequence of more frequent mixed virus infection. Another possibility could be the simultaneous "invasive" introduction of both new virus and new vector. The closest affiliates of the "invader" genotype cluster of cassava *B. tabaci* reported from pandemic-affected parts of Uganda (Legg et al. 2002) were from Cameroon in West-Central Africa. Although EACMV-UG has yet to be reported either from Cameroon itself – or any country further west – it is present in both western and eastern parts of DRC, ROC, and eastern Gabon (Neuenschwander et al. 2002; Legg et al. 2004). Given the many uncertainties, it is unlikely that conclusions will be drawn on the origins of changed virus and vector populations until there is a much more thorough understanding of the identities and distributions of CMG strains/species and cassava *B. tabaci* genotypes throughout the cassava-growing regions of sub-Saharan Africa.

## *What Are the Factors Behind the Rapid Regional Spread of the Pandemic?*

### The Flight Capabilities of *B. tabaci* Permit Mid-range Migration

Few published studies exist on *B. tabaci* flight, a fact that is somewhat surprising given the critical importance of the insect's biology to its role as a pan-tropical pest and virus vector in diverse agricultural systems. Most of what is known has been derived either from studies conducted in open desert environments (Byrne et al. 1995; Byrne and Blackmer 1996), or under controlled conditions using flight chambers (Blackmer and Byrne 1993). This Arizona-based group demonstrated variability in flight response between individuals within *B. tabaci* populations, with most being trivial flyers. A small percentage – typically less than 5% – was of the migratory form. In the Arizona desert environment, *B. tabaci* individuals were consistently captured in traps at the maximum distance from the source – 2.7 km – suggesting that they were capable of longer flights. Similar experiments run in desert environments in Israel – where *B. tabaci* adults were marked with fluorescent dye – gave some recaptures at up to 7 km from the point of release (Cohen et al. 1988). No studies of *B. tabaci* migration have been carried out in any part of Africa. From the early stages of the CMD epidemic in Uganda, however, it was noted that the

front of severe disease was spreading at speeds of at least 20–30 km per year (Otim-Nape et al. 1996, Legg and Ogwal 1998). Since new infections occurring at the front arose from current-season whitefly-borne infection, it became evident that the novel pandemic-associated virus EACMV-UG was being carried by migrating populations of *B. tabaci*. Although some local data are available on short distance movements of *B. tabaci* within and around cassava fields (Fishpool et al. 1995), no information is available describing movements from one cassava field to another. Based on the data on *B. tabaci*'s flight capabilities elsewhere, however, and recognizing that the generation time for *B. tabaci* on cassava in Uganda ranges from 27 to 39 days (Legg 1995), it seems entirely plausible that *B. tabaci* populations could move 20–30 km over the course of a year. A review of serial datasets, collected from countries bordering Lake Victoria (Uganda, Kenya and Tanzania), allows the construction of a longer-term model for pandemic spread and assumed concomitant *B. tabaci* movement from 1995 to 2007. These data, averaged over the entire period, estimate the rate of pandemic spread resulting from mid-range *B. tabaci* migration as 38 km per year for movement down the western shore of Lake Victoria, and 24 km per year for the eastern shore (Fig. 7.4). This is the only current example in Africa of such region-level movements, in spite of the fact that there are other areas where synergistic mixed CMG infections occur.

It may be that the unusual movements are simply more apparent because of the super-abundance of the whitefly populations. An alternative possibility – that still needs to be tested, however – is that the pandemic-associated *B. tabaci* populations have a larger proportion of migratory morphs than other cassava-colonizing populations outside of the pandemic-affected zone, and that they are therefore pre-disposed to mid-range migration and CMG spread.

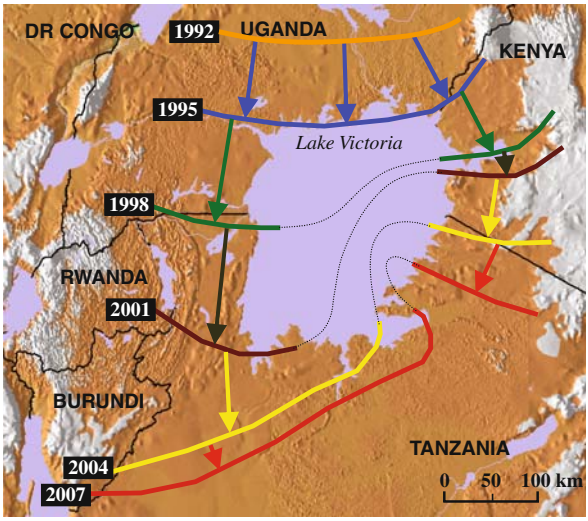



**Fig. 7.4** Diagrammatic representation of the progression of the CMD pandemic "front" in the Great Lakes region of East and Central Africa from 1995 to 2007

### Patterns of Whitefly Migration and Resulting Regional Pandemic Spread Are Influenced by Environmental Factors

Serial datasets – obtained from CMD monitoring in the Great Lakes region of East and Central Africa – point to key factors encouraging or restricting pandemic spread from 1995 to 2007 (Fig. 7.4). The map illustrating the pattern of spread clearly shows differences in the rates of spread between regions. Assuming that the biological elements of the system are more or less consistent throughout – *i.e.,* same virus, same vector in the pandemic-affected area, and same level of susceptibility in local cassava cultivars – it should be possible to categorize affected areas in terms of a number of environmental factors. For example:

- Vegetation density or relative thickness of both natural and crop vegetation in the zone; general levels include high, moderate, and low.
- Cassava cultivation intensity associated with the number, size and density of cassava plantings in the zone; levels are high, moderate and low.
- Relief or the degree of hilliness of the zone; levels are high, moderate and low.
- Impeding water body or whether there is a large lake, or part of a lake, dividing northern and southern parts of the zone; levels, in this case, are presence or absence.

A quick assessment of the levels of each of these factors and their relationship with pandemic spread distances achieved in different zones, shows that neither vegetation density nor relief appears to be related to the rate of pandemic spread (Table 7.1). By contrast, rate of spread seems to be associated with the relative

**Table 7.1** Characteristics of zones along the eastern and western shores of Lake Victoria through which the CMD pandemic spread between 1995 and 2007

| Location/time period | Vegetation density | Relief (hilliness) | Intensity of cassava cultivation | Impeding water body | Distance of pandemic spread in 3 years |
|---|---|---|---|---|---|
| Western Lakeshore | | | | | |
| 1995–1998 | High | Moderate | Moderate | No | 140 |
| 1998–2001 | Moderate | High | Moderate | No | 160 |
| 2001–2004 | Moderate | High | Moderate | No | 120 |
| 2004–2007 | Moderate | Moderate | Low | No | 35 |
| Average | | | | | 38 km/year |
| Eastern Lakeshore | | | | | |
| 1995–1998 | Moderate | Moderate | Moderate | No | 95 |
| 1998–2001 | Low | Low | Low | Yes* | 30 |
| 2001–2004 | Moderate | Moderate | Moderate | No | 90 |
| 2004–2007 | Low | Moderate | High | No | 75 |
| Average | | | | | 24 km/year |

*The Winam Gulf of Lake Victoria.

cultivation intensity of cassava. It is also notable that the zone through which spread was slowest was a zone in which a large inlet of Lake Victoria (the Winam Gulf) must have impeded north to south movement of whitefly populations, since the Gulf is 7 km wide at the narrowest point. All of the zones through which the pandemic has spread around Lake Victoria have similar altitudes (1,150–1,400 m); it is not possible, therefore, to use this scenario to assess the effect of altitude on patterns of spread. However, evidence from neighboring Rwanda and Burundi in which large cassava-growing parts of both countries lie between 1,400 and 1,800 m suggest that high altitude – and associated lower temperatures – does not impede the spread of the pandemic, as all cassava-growing parts of both countries were affected by the pandemic in a relatively short period from 2001 to 2006 (Bigirimana et al. 2004, Sseruwagi et al. 2005b, Legg et al. 2006). Above ca. 1,800 m growth conditions become unfavorable for cassava and the crop is only rarely encountered above this altitude. Significantly, the large area in central Kenya that lies above this altitudinal limit (Fig. 7.4) has provided an effective barrier to the eastward spread of the pandemic in Kenya.

### The CMD Pandemic Is not Spread by Growers Carrying Infected Cassava Cuttings from Pandemic Affected to Unaffected Regions

CMGs' are most often propagated by growers using infected cuttings to establish a new crop. It has been suggested that the pandemic's spread is caused by growers carrying diseased cuttings from pandemic-affected to unaffected regions. This is difficult to either prove or disprove, although under the subsistence conditions that characterize most cassava cultivation in East and Central Africa, cuttings are almost always taken from a grower's own field, or that of a nearby relative or neighbor. Patterns of grower-to-grower diffusion of CMD-resistant varieties in western Kenya do give some indication, however, of the general scale of the movement of cuttings carried by humans. Early pandemic control measures were first implemented in Uganda (Thresh et al. 1994). Resistant varieties were identified and disseminated widely throughout the country, but adoption was particularly widespread in the eastern region bordering Kenya. As the pandemic began to impact western Kenya in the late 1990s, many Kenyan growers along the Uganda border were able to access CMD-resistant germplasm, a process that was aided by the fact that the large "Teso" ethnic group straddles the border in this area. Official CMD-resistant multiplication and dissemination programs were initiated simultaneously in western Kenya, where planting materials were distributed more widely. Data obtained from a 2002 monitoring survey clearly demonstrated the restricted spread of the CMD-resistant variety (NASE 3 or Migyera) introduced by growers from Uganda (Fig. 7.5). By 2002 – 6 years after the initial spread of the pandemic into western Kenya from Uganda – grower-to-grower dissemination of NASE 3 had led to widespread cultivation of the variety in an area less than 35 km from the Ugandan border (Fig. 7.5). Over the same period, however, EACMV-UG and the associated severe CMD-pandemic had spread more than 200 km to the east and south. Furthermore, the pandemic front was only a short distance from the border with Tanzania. These data provide an indirect example of the lack of association between

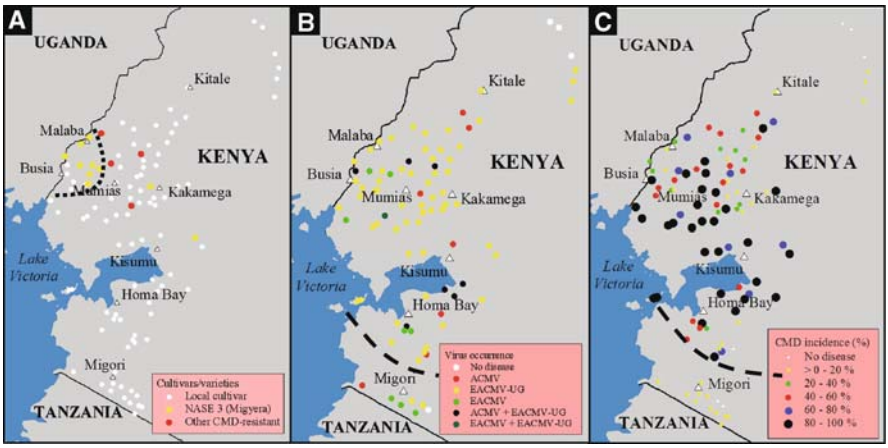



**Fig. 7.5** Distribution of local and introduced CMD-resistant varieties, CMG occurrence and CMD incidence in western Kenya, 2002. The *dotted line* in **A** represents the extent of grower to grower movement of variety NASE3 from Uganda. The *dashed lines* in **B** and **C** represent the extent of spread – south-westwards from the origin in Uganda – of the severe CMD pandemic

germplasm movements and pandemic spread. The data are further supported by the absence of a localized EACMV-UG associated epidemic outside of the contiguous range of the pandemic zone which would be anticipated if individual displacements of EACMV-UG infected cassava cuttings were able to prime epidemic development.

## *Can the Spread of the CMD Pandemic Be Halted?*

### Host Plant Resistance Provides Effective CMD Control

It was recognized from the earliest days of research into CMD that wild relatives of cassava might offer potential sources of resistance to the virus, or viruses, causing the disease (Jennings 1957). One such relative, Ceara rubber (*Manihot glaziovii* Muell.-Arg.), was successfully crossed with cultivated cassava; the progeny was triple back-crossed to produce cassava varieties combining the root quality of cultivated cassava with the CMD resistance of *M. glaziovii* (Jennings 1957; Jennings 1994). Resistance breeding efforts were subsequently continued in Nigeria, mainly at the International Institute of Tropical Agriculture (IITA) (Hahn et al. 1980). The CMD-resistant material developed there – from the late 1960s onwards – has been sent to most cassava-producing countries in sub-Saharan Africa (Manyong et al. 2000). Resistant germplasm developed in the years leading up to the early 1990s primarily made use of the *M. glaziovii* derived resistance, which was considered to be multigenic. More recently, however, use has been made of alternative sources of resistance derived from West African landraces (Dixon et al. 2003; Lokko et al. 2005). One of the sources from which molecular markers have been developed is

*CMD2* (Akano et al. 2002). Unlike the inter-specific cross derived sources, *CMD2* appears to be a single dominant gene. Future strategies will involve the exploration of both gene-pyramiding as well as the use of transgene approaches (Dixon et al. 2003; Okogbenin et al. 2007; Zhang et al. 2005).

Resistant varieties have been widely deployed in CMD management programs throughout sub-Saharan Africa (Manyong et al. 2000). Although no variety has proven to be immune under field conditions, resistance has been robust and durable for the more than 40 years that has been used against the full range of CMGs occurring in different parts of Africa (Byabakama et al. 1997; Egesi et al. 2007).

### Pre-emptive Deployment of Resistant Varieties Has Been Used to Enhance the Response Times of CMD Mitigation Programmes

CMD-resistant varieties have less CMG infection and therefore provide significantly greater yields than local CMD-susceptible landraces under the conditions of rapid spread of severe CMD that are characteristic of the pandemic (Byabakama et al. 1999). However, in areas of East and Central Africa outside the pandemic-affected zone, the impact of CMD is much less. There is consequently less incentive for growers to change to growing introduced resistant varieties. Nevertheless, concerted efforts have been made in both East and West Africa to deploy resistant germplasm in currently unaffected areas as a pre-emptive measure (Legg et al. 1999; Ogbe et al. 2006). In May 1999, 10,000 tissue culture plants of CMD-resistant varieties were transported from IITA in Nigeria to Mwanza, Tanzania – a location that was ahead of the advancing pandemic front (Legg et al. 1999). This material was hardened off and multiplied, and subsequently formed the basis for major multiplication and dissemination efforts in all parts of the Lake Zone of Tanzania. Notably, the growers' uptake of one of the principal varieties introduced in this way – TMS 4(2)1425 – was significantly less in unaffected areas than it was in pandemic-affected zones. A United States $16.5 million pre-emptive mitigation program in Nigeria was initiated in 2002 in the southern and south-eastern states of the country, at a time when the closest pandemic-affected location in north-central Republic of Congo (Ntawuruhunga et al. 2007), was still more than 950 km distant from the most south-eastern tip of Nigeria. In order to overcome possible hurdles to adoption, the multiplication and dissemination program was combined with an extensive network of on-farm varietal evaluation experiments and opportunities for post-harvest utilization and commercialization of cassava products were promoted. In Tanzania, pre-pandemic deployment of resistant material has helped to reduce the impact of the pandemic primarily through speeding up the response time. In Nigeria, the pre-emptive CMD mitigation work continued into 2009, although there was still no confirmed report of the occurrence of EACMV-UG in its eastern neighbor, Cameroon. It may therefore take several years before it is possible to judge the impact of the Nigerian program.

### Biological and Socio-political Factors Preclude the Halting of the Pandemic

Any spreading virus disease can in theory be halted by preventing movement of the vector, removing sources of inoculum – either by removing all diseased plants

and/or replacing them with immune varieties – or by removing both the crop as well as the non-crop weed host plants completely. The geographical scale which would be required is dependent upon the flight capability of the vector, the relative scale and importance of other modes of virus propagation, the intensity of cultivation of the crop, distribution of alternative virus hosts, and the historical status of epidemic development – *i.e.,* the degree to which it has already advanced prior to initiating efforts to halt further spread. Eradication has been effectively used in the control of other viruses, such as the mealybug-transmitted *Cacao swollen shoot virus* (CSSV) (Dzahini-Obiatey et al. 2006; Thresh and Owusu 1986), and the aphid-transmitted *Plum pox virus* (PPV) (Ramel et al. 2006). In both of these cases, however, spread occurs less rapidly than has been reported for the CMD pandemic. Mealybugs that transmit CSSV semi-persistently have only limited mobility, whilst aphids transmit PPV in a non-persistent manner. By contrast, *B. tabaci* is highly mobile and transmits CMGs persistently. The mobility and transmission characteristics of *B. tabaci* are such that removal of virus-infected plants would have to be practiced over large areas through mandatory roguing schemes, or cassava would have to be completely removed (together with alternative CMG hosts) from farming systems using crop-free zones/periods. Eradication programs of this type were used during the 1930s–1940s in order to control the first recorded epidemic of CMD in colonial Uganda (Jameson 1964). These programs, however, were confined to the northeastern part of the country. Furthermore, colonial authorities applied severe penalties to growers who refused to co-operate. The current pandemic affects more than 2,600,000 square kilometres of nine countries in East and Central Africa (Legg et al. 2006), and is therefore on a vastly different scale compared to the early Ugandan epidemic. Additionally, current social norms will not permit the implementation of strict laws mandating the removal of any cassava plants, healthy or diseased. Even if it were possible to implement eradication programs, logistics would be prohibitively difficult for numerous reasons. Most target regions are affected by limited transport infrastructure and inadequate resourcing and capacity of local growers, research institutions, extension services and plant quarantine authorities as well as absence of supportive private sector partners and unpredictable civil security. It is also significant that seven of the nine pandemic-affected countries have had significant periods of civil unrest in pandemic-affected areas. As a consequence it is reasonable to conclude that it is currently not possible to halt the spread of the pandemic using host plant removal, at least within the cassava-growing areas of the continental mainland of Africa. This does not mean, however, that all countries and regions as yet unaffected by the pandemic will be equally vulnerable to its future impact. Historically, previously affected countries have shown that impacts are slower and less acute in regions where cassava is not a major crop. For this reason, the effects in the maize-dominated agricultural environments of southern Africa – south of Zambia – are likely to be minimal. The absence of ACMV from coastal East Africa – running from south-east Kenya through Tanzania to northern Mozambique and Malawi – may also ensure that these areas will not experience the severe disease caused by ACMV+EACMV-UG synergism. Conversely, there does seem to be a significant likelihood that the pandemic will spread westwards, through Cameroon, then

into Nigeria and the rest of the southern coastal cassava belt of West Africa. Since this sub-region produces more than half of Africa's cassava crop, this remains a major concern, in spite of the pre-emptive mitigation measures already initiated in south-eastern Nigeria.

### The Pandemic Can Be Confined to, and Managed in Africa

Although it may not be possible to halt the spread of the CMD pandemic in Africa, effective management systems are available. With effective implementation, these systems will also help reduce the risk of African CMGs spreading to the other continents – Asia and South America – where cassava is an important crop. The deployment of host plant resistance to the CMGs has resulted in substantial reductions in CMD incidence and severity, with concomitant increases in production (Otim-Nape et al. 2000, Sserubombwe et al. 2001; Legg et al. 2006). In Uganda, the first country to be affected, total fresh weight production of cassava is now greater than pre-pandemic levels (FAO 2009), and more than half of all growers are now planting CMD-resistant varieties. Major mitigation initiatives are currently underway in other affected countries which should result in similar successes. An important example is the December 2007 approval of a $US22,000,000 grant from the Bill and Melinda Gates Foundation (United States) for cassava disease mitigation in six countries in East and Central Africa. Clearly, similar levels of effort will continue to be required for the foreseeable future.

Increasing air travel – particularly for passengers originating from African countries – will represent an important risk for the spread of CMGs to Latin America and Asia in future years. While such an eventuality may seem inevitable, the likelihood of spread occurring – following a chance introduction in either Asia or Latin America – is decreased by the fact that *B. tabaci* in South America does not currently colonize cassava (Carabali et al. 2005). Furthermore, *B. tabaci* populations in South Asia are inefficient transmitters of African CMGs (Maruthi et al. 2002). Nevertheless, it will be important for plant quarantine institutions in the major cassava-producing countries of these two continents to be fully aware of the threat posed, and to be vigilant in monitoring the health status of cassava crops in their respective countries in order to ensure that any inadvertent introductions are eradicated as quickly as possible. This will be more readily achieved in Latin America – where there are currently no geminiviruses affecting cassava – than in South Asia (India and Sri Lanka) – where both ICMV and SlCMV occur widely.

## Conclusions

The pandemic of severe cassava mosaic disease has been arguably the most important crop disease event to affect Africa over the latter part of the twentieth and early years of the twenty-first centuries. This event has had a severe impact on the largely agricultural economies of affected countries resulting in widespread food insecurity

for already vulnerable populations. Significantly, however, the phenomenon has catalyzed a resurgence of research interest in cassava, a crop that for much of its history in Africa has been given scant attention by the agricultural development and commercial farming communities. CMD management programs are currently receiving increasing support and providing effective mitigation in pandemic-affected areas, although the recovery period can range from 7 to 10 years. However, the nature of the components of the CMD pathosystem is such that there is little likelihood that the pandemic can be halted within Africa at any stage in the future. The high mobility of *B. tabaci*, its "transformation" into a super-abundant pest in the pandemic zone, the vast area over which the pandemic now extends, and the logistical difficulties in implementing control programs all serve to prevent the halting of further spread. Important lessons can nevertheless still be learned about how to improve existing control work, in addition to how to prevent the emergence of similar problems in the future. This review has highlighted a number of important areas where additional research is required. It is hoped that they will provide a basis for the development of plans for future research into the cassava mosaic geminiviruses and their *B. tabaci* whitefly vector in Africa.

## *Questions to Direct Future Research Efforts*

1. What makes CMGs and whiteflies behave differently in pandemic-affected areas than elsewhere?
2. Why does synergism between different CMG species not always result in epidemics?
3. What is the origin of EACMV-UG? Did the ACMV-EACMV recombination occur in Uganda, or is EACMV-UG an invasive introduction?
4. What is the nature and distribution of CMGs and *B. tabaci* genotypes in different cassava-growing regions of Africa? Much is now known, but there are still many gaps in our understanding.
5. Are there biological differences between distinct genotype clusters of *B. tabaci*?
6. If biological differences are present between these genotype clusters, which genes are associated with these differences?
7. Are genetic factors or synergism with CMD-diseased host plants the primary factor driving super-abundance in cassava *B. tabaci* populations?
8. What are the factors that encourage *B. tabaci* emigration from a crop? Although changes in food quality have been shown to be one such factor, does overcrowding in super-abundant cassava *B. tabaci* populations lead to increases in the proportion of migratory morphs?
9. Are whiteflies in pandemic-affected zones different from others in their migratory capabilities?
10. Can improved understanding of the factors driving the CMD pandemic aid the development of control approaches that will complement existing programs based on the deployment of host plant virus resistance?

**Acknowledgments** Thanks are expressed to colleagues from national root crops research programs in Burundi (ISABU), Democratic Republic of Congo (INERA), Kenya (KARI), Rwanda (ISAR), Tanzania (TRTCP) and Uganda (NARO) who have played a key role in much of the research discussed in this paper. Particular thanks are expressed to Mr. Hannington Obiero who led the Kenya Agricultural Research Institute (KARI) team in western Kenya that conducted the survey in 2002, some of the results from which are presented in Fig. 7.5. The technical support of IITA colleagues, Mr. Geoffrey Okao-Okuja and Mr. Robert Obonyo, is also recognized.

## Literature Cited

Akano AO, Dixon AGO, Mba C, Barrera E, Fregene M. 2002. Genetic mapping of a dominant gene conferring resistance to cassava mosaic disease. *Theor. Appl. Genet.* 105:521–535.

Ariyo OA, Koerbler M, Dixon AGO, Atiri GI, Winter S. 2005. Molecular variability and distribution of cassava mosaic begomoviruses in Nigeria. *J. Phytopathol.* 153:226–231.

Bellotti AC, Arias B. 2001. Host plant resistance to whiteflies with emphasis on cassava as a case study. *Crop Prot.* 20:813–823.

Bellotti A, Schoonhoven AV. 1978. Mite and insect pests of cassava. *Annu. Rev. Entomol.* 23:39–67.

Berrie LC, Rybicki EP, Rey MEC. 2001. Complete nucleotide sequence and host range of South African cassava mosaic virus: further evidence for recombination amongst geminiviruses. *J. Gen. Virol.* 82:53–58.

Berry S, Rey MEC. 2001. Molecular evidence for diverse populations of cassava-infecting begomoviruses in southern Africa. *Arch. Virol.* 146:1795–1802.

Bigirimana S, Barumbanze P, Obonyo R, Legg JP. 2004. First evidence for the spread of *East African cassava mosaic virus* – Uganda (EACMV-UG) and the pandemic of severe cassava mosaic disease to Burundi. *Plant Pathol.* 53(2):231.

Blackmer JL, Byrne DN. 1993. Flight behaviour of *Bemisia tabaci* in a vertical flight chamber: effect of time of day, sex, age and host quality. *Physiol. Entomol.* 18:223–232.

Bock KR, Woods RD. 1983. The etiology of African cassava mosaic disease. *Plant Dis.* 67:994–995.

Brown JK, Frohlich DR, Rosell RC. 1995. The sweetpotato/silverleaf whiteflies: biotypes of *Bemisia tabaci* (Genn.), or a species complex? *Annu. Rev. Entomol.* 40:511–534.

Bull SE, Briddon RW, Sserubombwe WS, Ngugi K, Markham PG, Stanley J. 2006. Genetic diversity and phylogeography of cassava mosaic viruses in Kenya. *J. Gen. Virol.* 87:3053–3065.

Burban C, Fishpool LDC, Fauquet C, Fargette D, Thouvenel J-C. 1992. Host-associated biotypes within West African populations of the whitefly *Bemisia tabaci* (Genn.) (Hom., Aleyrodidae). *J. Appl. Entomol.* 113:416–423.

Byabakama BA, Adipala E, Ogenga-Latigo MW, Otim-Nape GW. 1999. The effect of amount and disposition of inoculum on cassava mosaic virus disease development and tuberous root yield of cassava. *Afr. J. Plant Prot.* 5:21–29.

Byabakama BA, Adipala E, Ogenga-Latigo MW, Tusiime G, Otim-Nape GW. 1997. The resistance of improved cassava varieties to cassava mosaic disease in Uganda. *Afr. J. Plant Prot.* 7:45–57.

Byrne DN, Bellows TS, Jr. 1991. Whitefly biology. *Annu. Rev. Entomol.* 36:431–458.

Byrne DN, Bellows TS, Jr, Parrella MP. 1990. Whiteflies in agricultural systems. In *Whiteflies: Their Bionomics, Pest Status and Management*, ed. D Gerling, pp. 227–261. Andover, Hants: Intercept Ltd.

Byrne DN, Blackmer JL. 1996. Examination of short-range migration by *Bemisia*. In *Bemisia 1995: Taxonomy, Biology, Damage, Control and Management*, ed. D Gerling, RT Mayer, pp. 17–28. Andover, Hants: Intercept Ltd.

Byrne DN, Blackmer JL, Rathman RJ. 1995. Migration and dispersal by the sweetpotato whitefly. *Phytoparasitica* 22:314.

Calvert LA, Thresh JM. 2002. The viruses and virus diseases of cassava. In *Cassava: Biology, Production and Utilization*, ed. AC Bellotti, RJ Hillocks, JM Thresh, pp. 237–260. Wallingford, Oxon: CABI.

Campbell BC, Steffen-Campbell JD, Gill R. 1996. Origin and radiation of whiteflies: an initial molecular phylogenetic assessment. In *Bemisia 1995: Taxonomy, Biology, Damage, Control and Management*, ed. D Gerling, RT Mayer, pp. 29–51. Andover, Hants: Intercept Ltd.

Carabali A, Bellotti AC, Montoya-Lerma J, Cuellar ME. 2005. Adaptation of *Bemisia tabaci* biotype B (Gennadius) to cassava, *Manihot esculenta* (Crantz). *Crop Prot.* 24:643–649.

Cohen S, Kern J, Harpaz I, Ben-Joseph R. 1988. Epidemiological studies of the tomato yellow leaf curl virus, in the Jordan Valley, Israel. *Phytoparasitica* 16:259–270.

Colvin J, Omongo CA, Govindappa MR, Stevenson PC, Maruthi MN, Gibson G, Seal SE, Muniyappa V. 2006. Host-plant viral infection effects on arthropod-vector population growth, development and behaviour: management and epidemiological implications. *Adv. Virus Res.* 67:419–452.

Dixon AGO, Bandyopadhyay R, Coyne D, Ferguson M, Ferris RSB, Hanna R, Hughes J, Ingelbrecht I, Legg J, Mahungu N, Manyong V, Mowbray D, Neuenschwander P, Whyte J, Hartmann P, Ortiz R. 2003. Cassava: from a poor farmer's crop to a pacesetter of African rural development. *Chron. Hort.* 43(4):8–14.

Dubern J. 1979. Quelques propriétés de la mosaïque Africaine du manioc. I: La transmission. *Phytopathol. Zeit.* 96:25–39.

Dubern J. 1994. Transmission of African cassava mosaic geminivirus by the whitefly (*Bemisia tabaci*). *Trop. Sci.* 34:82–91.

Dzahini-Obiatey H, Akumfi Ameyaw G, Ollennu LA. 2006. Control of cocoa swollen shoot disease by eradicating infected trees in Ghana: a survey of treated and replanted areas. *Crop Prot.* 25:647–652.

Egesi CN, Ogbe FO, Akoroda M, Ilona P, Dixon A. 2007. Resistance profile of improved cassava germplasm to cassava mosaic disease in Nigeria. *Euphytica* 155:215–224.

Fargette D, Fauquet C, Grenier E, Thresh JM. 1990. The spread of African cassava mosaic virus into and within cassava fields. *J. Phytopathol.* 130:289–302.

Fishpool LDC, Fauquet C, Thouvenel J-C, Burban C, Colvin J. 1995. The phenology of *Bemisia tabaci* populations (Homoptera: Aleyrodidae) on cassava in southern Côte d'Ivoire. *Bull. Ent. Res.* 85:197–207.

Fondong V, Pita JS, Rey MEC, de Kochko A, Beachy RN, Fauquet CM. 2000. Evidence of synergism between African cassava mosaic virus and the new double recombinant geminivirus infecting cassava in Cameroon. *J. Gen. Virol.* 81:287–297.

Food and Agriculture Organization of the United Nations (FAO). 2009. Cassava production data 2007. (http://www.fao.org).

Gibson RW, Legg JP, Otim-Nape GW. 1996. Unusually severe symptoms are a characteristic of the current epidemic of mosaic virus disease of cassava in Uganda. *Ann. Appl. Biol.* 128:479–490.

Gregory PH. 1948. The multiple infection transformation. *Ann. Appl. Biol.* 35:412–417.

Hahn SK, Terry ER, Leuschner K. 1980. Breeding cassava for resistance to cassava mosaic disease. *Euphytica* 29:673–683.

Hanley-Bowdoin L, Settlage SB, Orozco BM, Nagar S, Robertson D. 1999. Geminiviruses: models for plant DNA replication, transcription and cell cycle regulation. *Crit. Rev. Plant Sci.* 18:71–106.

Harrison BD, Barker H, Bock KR, Guthrie EJ, Meredith G, Atkinson M. 1977. Plant viruses with circular single-stranded DNA. *Nature* 266:54–55.

Harrison BD, Zhou X, Otim-Nape GW, Liu Y, Robinson DJ. 1997. Role of a novel type of double infection in the geminivirus-induced epidemic of severe cassava mosaic in Uganda. *Ann. Appl. Biol.* 131:437–448.

Hong YG, Robinson DJ, Harrison BD. 1993. Nucleotide sequence evidence for the occurrence of three distinct whitefly-transmitted geminiviruses in cassava. *J. Gen. Virol.* 74:2437–2443.

Jameson JD. 1964. Cassava mosaic disease in Uganda. *East Afr. Agric. J.* 29:208–213.
Jennings D. 1957. Further studies in breeding cassava for virus resistance. *East Afr. Agric. J.* 22:213–219.
Jennings DL. 1970. Cassava in Africa. *Field Crop Abstr.* 23:271–278.
Jennings DL. 1994. Breeding for resistance to African cassava mosaic virus in East Africa. *Trop. Sci.* 34:110–122.
Kufferath H, Ghesquière J. 1932. La mosaïque du manioc. *Compte-Rendu de la Société de Biologie Belge* 109:1146–1148.
Legg JP. 1995. The ecology of *Bemisia tabaci* (Gennadius) (Homoptera), vector of African cassava mosaic geminivirus in Uganda. Doctoral Thesis, University of Reading, UK. 183pp.
Legg JP. 1996. Host-associated strains within Ugandan populations of the whitefly *Bemisia tabaci* (Genn.),(Hom., Aleyrodidae). *J. Appl. Entomol.* 120:523–527.
Legg JP. 1999. Emergence, spread and strategies for controlling the pandemic of cassava mosaic virus disease in east and central Africa. *Crop Prot.* 18(10):627–637.
Legg JP, Fauquet CM. 2004. Cassava mosaic geminiviruses in Africa. *Plant Mol. Biol.* 56(4):585–599.
Legg JP, French R, Rogan D, Okao-Okuja G, Brown JK. 2002. A distinct, invasive *Bemisia tabaci* (Gennadius) (Hemiptera: Sternorrhyncha: Aleyrodidae) genotype cluster is associated with the epidemic of severe cassava mosaic virus disease in Uganda. *Mol. Ecol.* 11(7):1219–1229.
Legg JP, Gibson RW, Otim-Nape GW. 1994. Genetic polymorphism amongst Ugandan populations of *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae), vector of African cassava mosaic geminivirus. *Trop. Sci.* 34:73–81.
Legg JP, Kapinga R, Teri J, Whyte JBA. 1999. The pandemic of cassava mosaic virus disease in East Africa: control strategies and regional partnerships. *Roots* 6(1):10–19.
Legg JP, Ndjelassili F, Okao-Okuja G. 2004. First report of cassava mosaic disease and cassava mosaic geminiviruses in Gabon. *Plant Pathol.* 53(2):232.
Legg JP, Ogwal S. 1998. Changes in the incidence of African cassava mosaic geminivirus and the abundance of its whitefly vector along south-north transects in Uganda. *J. Appl. Entomol.* 122:169–178.
Legg JP, Okao-Okuja G, Mayala R, Muhinyuza J-B. 2001. Spread into Rwanda of the severe cassava mosaic virus disease pandemic and associated Uganda variant of *East African cassava mosaic virus* (EACMV-Ug). *Plant Pathol.* 50:796.
Legg JP, Owor B, Sseruwagi P, Ndunguru J. 2006. Cassava mosaic virus disease in East and Central Africa: epidemiology and management of a regional pandemic. *Adv. Virus Res.* 67:355–418.
Legg JP, Thresh JM. 2000. Cassava mosaic virus disease in East Africa: a dynamic disease in a changing environment. *Virus Res.* 71:135–149.
Leshkowitz D, Gazit S, Reuveni E, Ghanim M, Czosnek H, McKenzie C, Shatters RL, Jr, Brown JK. 2006. Whitefly (*Bemisia tabaci*) genome project: analysis of sequenced clones from egg, instar, and adult (viruliferous and non-viruliferous) cDNA libraries. *BMC Genomics* 7:79.
Lokko Y, Danquah EY, Offei SK, Dixon AGO, Gedil MA. 2005. Molecular markers associated with a new source of resistance to the cassava mosaic disease. *Afr. J. Biotech.* 4:873–881.
Manyong VM, Dixon AGO, Makinde KO, Bokanga M, Whyte J. 2000. *Impact of IITA-Improved Germplasm on Cassava Production in Sub-Saharan Africa*. Ibadan, Nigeria: IITA.
Maruthi MN, Colvin J, Seal S. 2001. Mating compatibility, life-history traits, and RAPD-PCR variation in *Bemisia tabaci* associated with the cassava mosaic disease pandemic in East Africa. *Entomol. Exp. Appl.* 99:13–23.
Maruthi MN, Colvin J, Seal S, Gibson G, Cooper J. 2002. Co-adaptation between cassava mosaic geminiviruses and their local vector populations. *Virus Res.* 86:71–85.
Maruthi MN, Seal S, Colvin J, Briddon RW, Bull SE. 2004. *East African cassava mosaic Zanzibar virus* – a recombinant begomovirus species with a mild phenotype. *Arch. Virol.* 149:2365–2377.

Ndunguru J, Legg JP, Aveling TAS, Thompson G, Fauquet CM. 2005. Molecular biodiversity of cassava begomoviruses in Tanzania: evolution of cassava geminiviruses in Africa and evidence for East Africa being a center of diversity of cassava geminiviruses. *Virol. J.* 2:21.

Neuenschwander P, Hughes Jd'A, Ogbe F, Ngatse JM, Legg JP. 2002. The occurrence of the Uganda Variant of East African Cassava Mosaic Virus (EACMV-Ug) in western Democratic Republic of Congo and the Congo Republic defines the westernmost extent of the CMD pandemic in East/Central Africa. *Plant Pathol.* 51(3):384.

Ntawuruhunga P, Okao-Okuja G, Bembe A, Obambi M, Mvila AJC, Legg JP. 2007. Incidence, severity and viruses associated with cassava mosaic disease in the Republic of Congo. *Afr. Crop Sci. J.* 15(1):1–9.

Ogbe FO, Dixon AGO, Hughes Jd'A, Alabi OJ, Okechukwu R. 2006. Status of cassava begomoviruses and their new natural hosts in Nigeria. *Plant Dis.* 90:548–553.

Ogbe FO, Thottappilly G, Dixon AGO, Mignouna HD. 2003. Variants of East African cassava mosaic virus and its distribution in double infections with African cassava mosaic virus in Nigeria. *Plant Dis.* 87:229–232.

Okogbenin E, Porto MCM, Egesi C, Mba C, Espinosa E, Santos LG, Ospina C, Marín J, Barrera E, Gutiérrez J, Ekanayake I, Iglesias C, Fregene MA. 2007. Marker-assisted introgression of resistance to cassava mosaic disease into Latin American germplasm for the genetic improvement of cassava in Africa. *Crop Sci.* 47:1895–1904.

Omongo CA. 2003. Cassava whitefly, *Bemisia tabaci*, behaviour and ecology in relation to the spread of the cassava mosaic pandemic in Uganda. PhD Thesis. University of Greenwich, UK.

Otim-Nape GW, Bua A, Baguma Y. 1994. Accelerating the transfer of improved crop production technologies: controlling African cassava mosaic virus disease in Uganda. *Afr. Crop Sci. J.* 2:479–495.

Otim-Nape GW, Bua A, Thresh JM, Baguma Y, Ogwal S, Semakula GN, Acola G, Byabakama B, Martin A. 1997. *Cassava Mosaic Virus Disease in Uganda: The Current Pandemic and Approaches to Control*. Chatham: Natural Resources Institute.

Otim-Nape GW, Bua A, Thresh JM, Baguma Y, Ogwal S, Ssemakula GN, Acola G, Byabakama B, Colvin J, Cooter RJ, Martin A. 2000. *The Current Pandemic of Cassava Mosaic Virus Disease in East Africa and its Control*. Chatham: Natural Resources Institute.

Otim-Nape GW, Thresh JM, Fargette D. 1996. *Bemisia tabaci* and cassava mosaic virus disease in Africa. In *Bemisia 1995: Taxonomy, Biology, Damage, Control and Management*, ed. D Gerling, RT Mayer, pp. 319–350. Andover, Hants: Intercept Ltd.

Owor B, Legg JP, Obonyo R, Okao-Okuja G, Kyamanywa S, Ogenga-Latigo MW. 2004a. Field studies of cross protection with cassava in Uganda. *J. Phytopathol.* 152:243–249.

Owor B, Legg JP, Okao-Okuja G, Obonyo R, Ogenga-Latigo MW. 2004b. The effect of cassava mosaic geminiviruses on symptom severity, growth and root yield of a cassava mosaic virus disease-susceptible cultivar in Uganda. *Ann. Appl. Biol.* 145(3):331–337.

Pascal E, Sanderfoot AA, Ward BM, Medville R, Turgeon R, Lazarowitz SG. 1994. The geminivirus BR1 movement protein binds single-stranded DNA and localizes to the cell nucleus. *Plant Cell* 6:995–1006.

Perring TM, Cooper A, Kazmer DJ, Shields C, Shields J. 1991. New strain of sweetpotato whitefly invades California vegetables. *Calif. Agric.* 45:10–12.

Pita JS, Fondong VN, Sangare A, Otim-Nape GW, Ogwal S, Fauquet CM. 2001. Recombination, pseudorecombination and synergism of geminiviruses are determinant keys to the epidemic of severe cassava mosaic disease in Uganda. *J. Gen. Virol.* 82:655–665.

Ramel ME, Gugerli P, Bünter M. 2006. Plum pox virus (PPV) in Switzerland. *EPPO Bull.* 36(2):215–216.

Saunders K, Nazeera S, Mali VR, Malathi VG, Briddon R, Markham PG, Stanley J. 2002. Characterisation of *Sri Lankan cassava mosaic virus* and *Indian cassava mosaic virus*: evidence for acquisition of a DNA B component by a monopartite begomovirus. *Virology* 293:63–74.

Sserubombwe WS, Thresh JM, Otim-Nape GW, Osiru DSO. 2001. Progress of cassava mosaic virus disease and whitefly vector populations in single and mixed stands of four cassava varieties grown under epidemic conditions in Uganda. *Ann. Appl. Biol.* 138:161–170.

Sseruwagi P, Legg JP, Maruthi MN, Colvin J, Rey MEC, Brown JK. 2005a. Genetic diversity of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) populations and presence of the B biotype and a non-B biotype that can induce silverleaf symptoms in squash, in Uganda. *Ann. Appl. Biol.* 147:253–265.

Sseruwagi P, Okao-Okuja G, Kalyebi A, Muyango S, Aggarwal V, Legg JP. 2005b. Cassava mosaic geminiviruses associated with cassava mosaic disease in Rwanda. *Int. J. Pest Manage.* 51:17–23.

Sseruwagi P, Sserubombwe WS, Legg JP, Ndunguru J, Thresh JM. 2004. Methods of surveying the incidence and severity of cassava mosaic disease and whitefly vector populations on cassava in Africa: a review. *Virus Res.* 100:129–142.

Stanley J. 2002. Characterisation of *Sri Lankan cassava mosaic virus* and *Indian cassava mosaic virus*: evidence for acquisition of a DNA B component by a monopartite begomovirus. *Virology* 293:63–74.

Storey HH. 1936. Virus diseases of East African plants. VI-A progress report on studies of the disease of cassava. *East Afr. Agric. J.* 2:34–39.

Storey HH, Nichols RFW. 1938. Studies on the mosaic of cassava. *Ann. Appl. Biol.* 25:790–806.

Thresh JM, Cooter RJ. 2005. Strategies for controlling cassava mosaic virus disease in Africa. *Plant Pathol.* 54:587–614.

Thresh JM, Otim-Nape GW, Jennings DL. 1994. Exploiting resistance to African cassava mosaic virus. *Asp. Appl. Biol.* 39:51–60.

Thresh JM, Owusu GK. 1986. The control of cocoa swollen shoot disease in Ghana: an evaluation of eradication procedures. *Crop Prot.* 5:41–52.

Vanitharani R, Chellapan P, Pita JS, Fauquet CM. 2004. Differential roles of AC2 and AC4 of cassava geminiviruses in mediating synergism and suppression of posttranscriptional gene silencing. *J Virol.* 78(17):9487–9498.

Warburg O. 1894. Die kulturpflanzen usambaras. *Mitt. Deut. Schutz.* 7:131.

Were HK, Winter S, Maiss E. 2004. Occurrence and distribution of cassava begomoviruses in Kenya. *Ann. Appl. Biol.* 145:175–184.

Zhang P, Vanderschuren H, Fütterer J, Gruissem W. 2005. Resistance to cassava mosaic disease in transgenic cassava expressing antisense RNAs targeting virus replication genes. *Plant Biotech. J.* 3:385–398.

Zhou X, Liu Y, Calvert L, Munoz C, Otim-Nape GW, Robinson DJ, Harrison BD. 1997. Evidence that DNA-A of a geminivirus associated with severe cassava mosaic disease in Uganda has arisen by interspecific recombination. *J. Gen. Virol.* 78:2101–2111.

Zhou X, Robinson DJ, Harrison BD. 1998. Types of variation in DNA-A among isolates of East African cassava mosaic virus from Kenya, Malawi, Tanzania. *J. Gen. Virol.* 79:2835–2840.

# Chapter 8
# Tomato Yellow Leaf Curl Disease Epidemics

**Enrique Moriones and Jesús Navas-Castillo**

## Introduction

The emergence of begomoviruses (genus *Begomovirus*, family *Geminiviridae*) in recent decades appears to be closely related to the increased prevalence of *Bemisia tabaci* (Jones 2003; Mansoor et al. 2003; Varma and Malathi 2003), and is associated with changes in crop cultivation, increased movement of people and plants, and changes in cropping practices such as the intensive use of insecticides and/or overlapping of susceptible host species in crop rotations (Morales and Jones 2004).

Tomato yellow leaf curl disease (TYLCD) was first reported in Israel in 1931 (Cohen and Antignus 1994). Since then, epidemics have emerged and devastated tomato crops in numerous countries in Africa, the Americas, the Caribbean, Europe, the Middle East, and Southeastern Asia (Moriones and Navas-Castillo 2000; Picó et al. 1996; Varma and Malathi 2003). Accounts of the presence of TYLCD-related viruses associated with tomato epidemics continue to occur. For example, recent confirmation of the presence of TYLCD-associated viruses has been reported from the Pacific Coast of Mexico (Brown and Idris 2006), Reunion Island (Delatte et al. 2005), North Africa (Anfoka et al. 2005; Gorsane et al. 2004; Tahiri et al. 2006), and Venezuela (Zambrano et al. 2007). Yield losses of up to 100% occur frequently in affected crops. Symptoms observed in infected tomato plants vary widely depending on the time of disease onset, environmental conditions, and tomato cultivar. In addition to stunting and flower abortion, a prominent upward curling of leaflet margins is observed, along with a size reduction of leaflets and yellowing of young leaves (Fig. 8.1A, B). Reduction in size can also occur in fruits of affected plants without obvious symptoms, leading to significant reductions in yield. Plants infected at early growth stages will be severely stunted, abort blooms and not bear fruit (Fig. 8.1C). However, when infections occur at later growth stages (Fig. 8.1D), fruits present before disease arrival ripen almost normally. In addition to the direct losses caused

E. Moriones (✉)
Plant Virus Laboratory, Estación Experimental "La Mayora", Consejo Superior de Investigaciones Científicas, 29750 Algarroba-Costa, Málaga, Spain
e-mail: moriones@eelm.csic.es

P.A. Stansly, S.E. Naranjo (eds.), *Bemisia*: Bionomics and Management of a Global Pest,
DOI 10.1007/978-90-481-2460-2_8, 

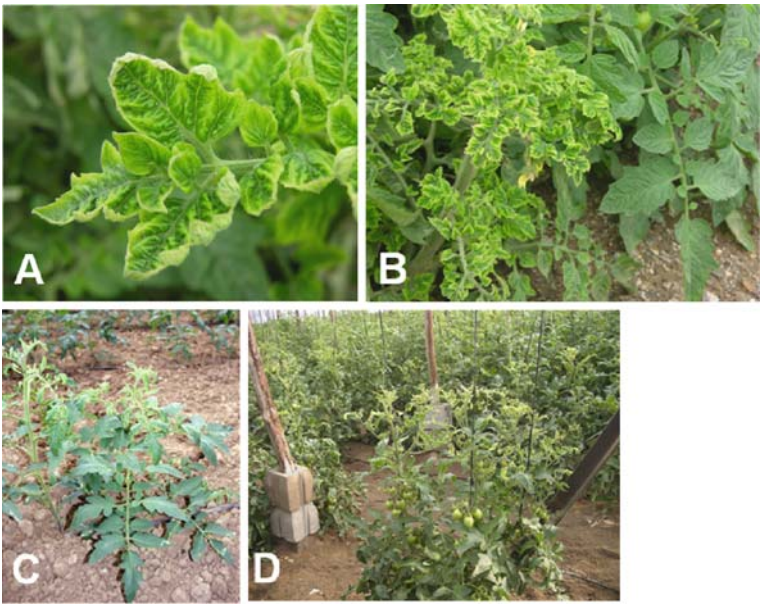



**Fig. 8.1** Symptoms of tomato yellow leaf curl disease (TYLCD). (**A**) detail of yellowing and upward curling of leaflet margins observed in leaves of a TYLCD-affected tomato plant. (**B**) stunting, yellowing and leaf curling of a TYLCD-affected plant compared to a healthy tomato plant in the background. (**C**) plants affected by TYLCD-infection during early growth stages resulting in complete yield loss, (**D**) tomato plants infected at late growth stages showing production of normal tomato fruits in the lower part of the plant

by infections, indirect losses can also result from the limitation of tomato production in areas and periods when moderate to low disease pressure occurs.

Almost 40 years of intense research on TYLCD epidemics have pursued solutions to the dramatic damage caused by this disease. This chapter provides an overview of the knowledge acquired on TYLCD epidemics that can help to design effective control strategies.

## Genetic Diversity of TYLCD-Associated Viruses

Begomoviruses are single-stranded DNA plant viruses with small twin (geminate) virions consisting of two incomplete icosahedra transmitted by the whitefly, *Bemisia tabaci* Gen. (Stanley et al. 2005). Most begomoviruses have bipartite genomes consisting of two circular DNA components of about 2,800 nucleotides (DNA A and DNA B). However, TYLCD-associated viruses usually are monopartite, having a single genomic component that resembles DNA A. The genome of monopartite begomoviruses encodes the replication-associated protein (Rep), the coat protein

(CP), proteins C4 and V2 associated with pathogenicity and virus-host interactions, as well as the replication enhancer protein (REn) and the transcription activator protein (TrAP) that participate in replication and gene expression. Open reading frames (ORFs) are organized bi-directionally and are separated by an intergenic region (IR) that contains key elements for initiating replication and transcription of the viral genome (reviewed by Gutiérrez 1999; Hanley-Bowdoin et al. 2000).

TYLCD is caused by a complex of phylogenetically related begomovirus species that produce similar symptoms when infecting tomato plants. To date, eleven different virus species have been officially recognized as being associated with TYLCD, among them *Tomato yellow leaf curl virus*, TYLCV (Fauquet et al. 2008). Almost seventy complete nucleotide sequences are available from isolates belonging to TYLCD-associated virus species (Table 8.1). With the exception of the bipartite *Tomato yellow leaf curl Indonesia virus, Tomato yellow leaf curl Thailand virus* and *Tomato yellow leaf curl Kanchanaburi virus*, all TYLCD-associated viruses have a monopartite genome. Because the symptoms caused in tomato by the different TYLCD-associated virus species are essentially the same, the nucleotide sequence identity of the complete genome was adopted as the main species demarcation criteria in this group. Thus, the *Geminiviridae* Study Group of the International Committee on Taxonomy of Viruses proposed an 89% identity threshold between complete DNA-A component nucleotide sequences to establish a new species (Fauquet et al. 2008; Stanley et al. 2005).

## Virus-Vector Interactions in the TYLCD Complex

TYLCD-associated viruses are transmitted by *B. tabaci* in a persistent manner. After entering the food canal, viruses cross the midgut barrier and are then transmitted by circulating through the insect hemocoel into the salivary glands (reviewed by Brown and Czosnek 2002; Czosnek et al. 2002). Optimal transmission is accomplished after 16–24 h of acquisition from source plants, and 20–24 h of inoculation on target plants. Males are significantly less efficient than females as transmitters, and nymphs are as efficient as adults in acquiring the virus (Caciagli et al. 1995; Cohen and Nitzany 1966). A decrease in vector capability has been observed with aging of *B. tabaci* (Rubinstein and Czosnek 1997). Transmission differences may exist among TYLCD-associated viruses, as described between TYLCV and *Tomato yellow leaf curl Sardinia virus* (TYLCSV) (Sánchez-Campos et al. 1999), and among other TYLCD-associated viruses (McGrath and Harrison 1995). Also, efficiency of transmission can vary depending on vector biotype (e.g. Sánchez-Campos et al. 1999). Recent studies indicate that a GroEL homologue produced by *B. tabaci* endosymbiotic bacteria is essential for virus transmission, interacting with particles of TYLCV in the insect hemolymph and ensuring the safe circulation of the virus (Morin et al. 1999; see Rosell et al. Chapter 5). Another important aspect for TYLCV epidemics is the suggestion that the virus could be maintained between epidemics in *B. tabaci* populations through copulation and/or transovarial

**Table 8.1** Begomovirus species associated with the tomato yellow leaf curl disease. Virus isolates for which the complete DNA A sequence is available are listed. Species names are in italic script and isolate names with strain and isolate descriptors are in roman script. Sequence accession numbers, and assigned abbreviations are also listed (Source: *Geminiviridae* Study Group, International Committee on Taxonomy of Viruses)

| *Virus species*/isolates | DNA A | DNA B | Acronym |
|---|---|---|---|
| *Tomato yellow leaf curl Axarquia virus* | | | |
| Tomato yellow leaf curl Axarquia virus – [Spain:Algarrobo:2000] | AY227892 | | TYLCAxV-[ES:Alg:00] |
| *Tomato yellow leaf curl China virus* | | | |
| Tomato yellow leaf curl China virus – Bean [China:Yunnan:Bean:2004] | DQ256460 | | TYLCCNV-Bea[CN:Yn:Bea:04] |
| Tomato yellow leaf curl China virus – Baoshan1 [China:Yunnan 10:Tobacco:2000] | AJ319675 | | TYLCCNV-Bao1[CN:Yn10:Tob:00] |
| Tomato yellow leaf curl China virus – Baoshan2 [China:Yunnan 11:Tobacco:2000] | AJ319676 | | TYLCCNV-Bao2[CN:Yn11:Tob:00] |
| Tomato yellow leaf curl China virus – Chuxiong [China:Yunnan 25:Tomato:2000] | AJ457985 | | TYLCCNV-Chu[CN:Yn25:Tom:00] |
| Tomato yellow leaf curl China virus – Chuxiong [China:Yunnan 295:Tobacco:2005] | AM260703 | | TYLCCNV-Chu[CN: Yn295:Tob:05] |
| Tomato yellow leaf curl China virus – Dali [China:Yunnan 8:Tobacco:1999] | AJ319677 | | TYLCCNV-Dal[CN:Yn8:Tob:99] |
| Tomato yellow leaf curl China virus – Dali [China:Yunnan 43:Tobacco:2001] | AJ781302 | | TYLCCNV-Dal[CN:Yn43:Tob:01] |
| Tomato yellow leaf curl China virus – Dali [China:Yunnan 5:Tobacco:1999] | AJ319674 | | TYLCCNV-Dal[CN:Yn5:Tob:99] |
| Tomato yellow leaf curl China virus – Datura [China:Yunnan 72:Datura:2005] | EF011559 | | TYLCCNV-Dat[CN:Yn72:05] |
| Tomato yellow leaf curl China virus – Honghe [China:Guangxi 102:2004] | AM050555 | | TYLCCNV-Hon[CN:Gx102:04] |
| Tomato yellow leaf curl China virus – Honghe [China:Guangxi] | AF311734 | | TYLCCNV-Hon[CN:Gx] |
| Tomato yellow leaf curl China virus – Honghe [China:Yunnan 231:Tobacco:2005] | AM260701 | | TYLCCNV-Hon[CN:Yn231:Tob:05] |
| Tomato yellow leaf curl China virus – Honghe [China:Yunnan 244:Tobacco:2005] | AM260702 | | TYLCCNV-Hon[CN:Yn244:Tob:05] |
| Tomato yellow leaf curl China virus – Honghe [China:Yunnan 322:Solanum:2005] | AM181683 | | TYLCCNV-Hon[CN:Yn322:Sol:05] |
| Tomato yellow leaf curl China virus – Honghe [China:Yunnan 36:Tobacco:2001] | AJ420316 | | TYLCCNV-Hon[CN:Yn36:Tob:01] |
| Tomato yellow leaf curl China virus – Honghe [China:Yunnan 38:Tobacco:2001] | AJ420317 | | TYLCCNV-Hon[CN:Yn38:Tob:01] |
| Tomato yellow leaf curl China virus – Honghe [China:Yunnan 64:Siegesbeckia:2001] | AJ457823 | | TYLCCNV-Hon[CN:Yn64:Sie:01] |
| *Tomato yellow leaf curl Guangdong virus* | | | |
| Tomato yellow leaf curl Guangdong virus – [China:Guangzhou 3:2003] | AY602166 | | TYLCGuV-[CN:Gz3:03] |
| *Tomato yellow leaf curl Indonesia virus* | | | |
| Tomato yellow leaf curl Indonesia virus – [Indonesia:Lembang:2005] | AF189018 | AF511528 | TYLCKaV-[ID:Lem:05] |
| *Tomato yellow leaf curl Kanchanaburi virus* | | | |
| Tomato yellow leaf curl Kanchanaburi virus – [Thailand:Kanchanaburi 1:2001] | AF511529 | AF511528 | TYLCKaV-[TH:Kan1:01] |

**Table 8.1** (continued)

| *Virus species*/isolates | DNA A | DNA B | Acronym |
|---|---|---|---|
| Tomato yellow leaf curl Kanchanaburi virus – [Thailand:Kanchanaburi 2:Eggplant:2001] | AF511530 | AF511527 | TYLCKaV-[TH:Kan2:Egg:01] |
| Tomato yellow leaf curl Kanchanaburi virus – [Vietnam:Binhduong:Eggplant:2005] | DQ641702 | | TYLCKaV-[VN:Bin:Egg:05] |
| Tomato yellow leaf curl Kanchanaburi virus – [Vietnam:2005] | DQ169054 | DQ169055 | TYLCKaV-[VN:05] |
| *Tomato yellow leaf curl Malaga virus* | | | |
| Tomato yellow leaf curl Malaga virus – [Spain:421:1999] | AF271234 | | TYLCMalV-[ES:421:99] |
| *Tomato yellow leaf curl Mali virus* | | | |
| Tomato yellow leaf curl Mali virus – Ethiopia [Ethiopia:Melkassa:2005] | DQ358913 | | TYLCMLV-ET[ET:Mel:05] |
| Tomato yellow leaf curl Mali virus – Mali [Mali:2003] | AY502934 | | TYLCMLV-ML[ML:03] |
| *Tomato yellow leaf curl Sardinia virus* | | | |
| Tomato yellow leaf curl Sardinia virus – Sardinia [Italy:Sardinia:1988] | X61153 | | TYLCSV-Sar[IT:Sar:88] |
| Tomato yellow leaf curl Sardinia virus – Sicily [Italy:Sicily] | Z28390 | | TYLCSV-Sic[IT:Sic] |
| Tomato yellow leaf curl Sardinia virus – Sicily [Israel:Rehovot:2005] | DQ845787 | | TYLCSV-Sic[IL:Reh:05] |
| Tomato yellow leaf curl Sardinia virus – Sicily [Tunisia:Bkalta 3:2002] | AY736854 | | TYLCSV-Sic[TN:Bka3:02] |
| Tomato yellow leaf curl Sardinia virus – Spain [Morocco:Agadir:2002] | AY702650 | | TYLCSV-ES[MA:Aga:02] |
| Tomato yellow leaf curl Sardinia virus – Spain [Spain:Almeria 2:1992] | L27708 | | TYLCSV-ES[ES:Alm2:92] |
| Tomato yellow leaf curl Sardinia virus – Spain [Spain:Canary] | AJ519675 | | TYLCSV-ES[ES:Can] |
| Tomato yellow leaf curl Sardinia virus – Spain [Spain:Murcia 1:1992] | Z25751 | | TYLCSV-ES[ES:Mur1:92] |
| *Tomato yellow leaf curl Thailand virus* | | | |
| Tomato yellow leaf curl Thailand virus – A [Thailand:1] | X63015 | X63016 | TYLCTHV-A[TH:1] |
| Tomato yellow leaf curl Thailand virus – A [Thailand:2] | AF141922 | AF141897 | TYLCTHV-A[TH:2] |
| Tomato yellow leaf curl Thailand virus – B [China:Yunnan 72:2002] | AJ495812 | | TYLCTHV-B[CN:Yn72:02] |
| Tomato yellow leaf curl Thailand virus – B [Myanmar:Yangon:1999] | AF206674 | | TYLCTHV-B[MM:Yan:99] |
| Tomato yellow leaf curl Thailand virus – B [Thailand:Chiang Mai] | AY514630 | AY514633 | TYLCTHV-B[TH:ChMai] |
| Tomato yellow leaf curl Thailand virus – B [Thailand:Nong Khai] | AY514631 | AY514634 | TYLCTHV-B[TH:NoK] |
| Tomato yellow leaf curl Thailand virus – C [Thailand:Sakon Nakhon] | AY514632 | AY514635 | TYLCTHV-C[TH:SaNa] |
| *Tomato yellow leaf curl Vietnam virus* | | | |
| Tomato yellow leaf curl Vietnam virus – [Vietnam:Hanoi:2005] | DQ641697 | | TYLCVNV-[VN:Han:05] |
| *Tomato yellow leaf curl virus* | | | |
| Tomato yellow leaf curl virus – Israel [China:Shangai 2:2005] | AM282874 | | TYLCV-IL[CN:SH2:05] |

**Table 8.1** (continued)

| *Virus species*/isolates | DNA A | DNA B | Acronym |
|---|---|---|---|
| Tomato yellow leaf curl virus – Israel [Cuba] | AJ223505 | | TYLCV-IL[CU] |
| Tomato yellow leaf curl virus – Israel [Dominican Republic] | AF024715 | | TYLCV-IL[DO] |
| Tomato yellow leaf curl virus – Israel [Egypt:Ismaelia] | AY594174 | | TYLCV-IL[EG:Ism] |
| Tomato yellow leaf curl virus – Israel [Egypt:Nobaria:1991] | EF107520 | | TYLCV-IL[EG:Nob:91] |
| Tomato yellow leaf curl virus – Israel [Israel:Rehovot:1986] | X15656 | | TYLCV-IL[IL:Reo:86] |
| Tomato yellow leaf curl virus – Israel [Italy:Sicily:2004] | DQ144621 | | TYLCV-IL[IT:Sic:04] |
| Tomato yellow leaf curl virus – Israel [Japan:Haruno:2005] | AB192966 | | TYLCV-IL[JR:Han:05] |
| Tomato yellow leaf curl virus – Israel [Japan:Misumi:Stellaria] | AB116631 | | TYLCV-IL[JR:Mis:Ste] |
| Tomato yellow leaf curl virus – Israel [Japan:Miyazaki] | AB116629 | | TYLCV-IL[JR:Miy] |
| Tomato yellow leaf curl virus – Israel [Japan:Omura:Eustoma] | AB116630 | | TYLCV-IL[JR:Omu:Eus] |
| Tomato yellow leaf curl virus – Israel [Japan:Omura:Ng] | AB110217 | | TYLCV-IL[JR:Omu:Ng] |
| Tomato yellow leaf curl virus – Israel [Japan:Tosa:2005] | AB192965 | | TYLCV-IL[JR:Tos:05] |
| Tomato yellow leaf curl virus – Israel [Jordan:Tomato:2005] | EF054893 | | TYLCV-IL[JO:Tom:05] |
| Tomato yellow leaf curl virus – Israel [Lebanon:Tomato:2005] | EF051116 | | TYLCV-IL[LB:Tom:05] |
| Tomato yellow leaf curl virus – Israel [Mexico:Culiacan:2005] | DQ631892 | | TYLCV-IL[MX:Cul:05] |
| Tomato yellow leaf curl virus – Israel [Morocco:Berkane:2005] | EF060196 | | TYLCV-IL[MO:Ber:05] |
| Tomato yellow leaf curl virus – Israel [Puerto Rico:2001] | AY134494 | | TYLCV-IL[PR:01] |
| Tomato yellow leaf curl virus – Israel [Spain:Almeria:Pepper:1999] | AJ489258 | | TYLCV-IL[ES:Alm:Pep:99] |
| Tomato yellow leaf curl virus – Israel [Tunisia:2005] | EF101929 | | TYLCV-IL[TN:05] |
| Tomato yellow leaf curl virus – Israel [Turkey:Mersin:2005] | AJ812277 | | TYLCV-IL[TR:Mer:05] |
| Tomato yellow leaf curl virus – Israel [United States of America:Florida:1997] | AY530931 | | TYLCV-IL[USA:Flo] |
| Tomato yellow leaf curl virus – Gezira [Sudan:1996] | AY044138 | | TYLCV-Gez[SD:96] |
| Tomato yellow leaf curl virus – Iran [Iran:Iranshahr:1998] | AJ132711 | | TYLCV-IR[IR:Ira:98] |
| Tomato yellow leaf curl virus – Oman [Oman:Al-Batinah:2005] | DQ644565 | | TYLCV-OM[OM:Alb:05] |
| Tomato yellow leaf curl virus – Mild [Israel:1993] | X76319 | | TYLCV-Mld[IL:93] |
| Tomato yellow leaf curl virus – Mild [Japan:Aichi] | AB014347 | | TYLCV-Mld[JR:Aic] |
| Tomato yellow leaf curl virus – Mild [Japan:Aichi2:2003] | DD033365 | | TYLCV-Mld[JR:Aic2:03] |
| Tomato yellow leaf curl virus – Mild [Japan:Atumi] | AB116633 | | TYLCV-Mld[JR:Atu] |
| Tomato yellow leaf curl virus – Mild [Japan:Daito] | AB116635 | | TYLCV-Mld[JR:Dai] |

**Table 8.1** (continued)

| *Virus species*/isolates | DNA A | DNA B | Acronym |
|---|---|---|---|
| Tomato yellow leaf curl virus – Mild [Japan:Kisozaki] | AB116634 | | TYLCV-Mld[JR:Kis] |
| Tomato yellow leaf curl virus – Mild [Japan:Osuka] | AB116636 | | TYLCV-Mld[JR:Osu] |
| Tomato yellow leaf curl virus – Mild [Japan:Shimizu] | AB110218 | | TYLCV-Mld[JR:Shi] |
| Tomato yellow leaf curl virus – Mild [Japan:Shizuoka] | AB014346 | | TYLCV-Mld[JR:Shz] |
| Tomato yellow leaf curl virus – Mild [Japan:Yaizu] | AB116632 | | TYLCV-Mld[JR:Yai] |
| Tomato yellow leaf curl virus – Mild [Jordan:Cucumber:2005] | EF158044 | | TYLCV-Mld[JO:Cuc:05] |
| Tomato yellow leaf curl virus – Mild [Jordan:Homra:2003] | AY594175 | | TYLCV-Mld[JO:Hom:03] |
| Tomato yellow leaf curl virus – Mild [Jordan:Tomato:2005] | EF054894 | | TYLCV-Mld[JO:Tom:05] |
| Tomato yellow leaf curl virus – Mild [Lebanon:LBA44:05] | EF185318 | | TYLCV-Mld[ILB;LBA44:05] |
| Tomato yellow leaf curl virus – Mild [Portugal:2:1995] | AF105975 | | TYLCV-Mld[PT:2:95] |
| Tomato yellow leaf curl virus – Mild [Reunion:2002] | AJ865337 | | TYLCV-Mld[RE:02] |
| Tomato yellow leaf curl virus – Mild [Spain:72:1997] | AF071228 | | TYLCV-Mld[ES:72:97] |
| Tomato yellow leaf curl virus – Mild [Spain:Almeria:1999] | AJ519441 | | TYLCV-Mld[ES:Alm:99] |

transmission (Ghanim and Czonsnek 2000; Ghanim et al. 1998), although contradictory information exists regarding infectivity of whitefly progeny after transovarial transmission (Bosco et al. 2004; Goldman and Czosnek 2002; also see Accotto and Sardo Chapter 12). Therefore, further research is needed to clarify the possible consequences of this latter phenomenon on TYLCD epidemics.

Virus-vector interactions can dramatically influence epidemics. Thus, for example, it has been demonstrated that the existence of mutualistic interactions between *B. tabaci* and *Tomato yellow leaf curl China virus* (TYLCCNV) can potentially determine the success of an invasive *B. tabaci* genotype in the whitefly population (Jiu et al. 2007). When the performance of the invasive B and the indigenous ZHJ1 whitefly biotypes on healthy and TYLCCNV-infected plants were compared, the invasive B biotype significantly increased its fecundity and longevity on infected plants, in contrast to the indigenous ZHJ1 which performed similarly on healthy and virus-infected plants. This indirect mutualism between the B biotype whitefly and TYLCCN via the host plants, and the apparent lack of such mutualism for the indigenous whitefly, might contribute to the ability of the B whitefly biotype to displace indigenous whiteflies (see Naranjo et al. Chapter 6). The consequences of these interactions for the population dynamics of both types of organisms could be crucial for the evolution of virus epidemics. Forthcoming studies with other virus-whitefly combinations in other geographical areas will help to understand aspects of the transmission process in order to better define target steps for disease management.

## Cultivated Hosts and Wild Reservoirs of TYLCD-Associated Viruses

Although the viruses associated with TYLCD affect primarily tomato, natural infections have been reported in other cultivated hosts such as common bean (Gorsane et al. 2004; Martínez-Zubiaur et al. 2002; Navas-Castillo et al. 1999), pepper (Gorsane et al. 2004; Quiñones et al. 2002; Polston et al. 2006, Reina et al. 1999), tobacco (Font et al. 2005), squash (Martínez-Zubiaur et al. 2004), *Physalis ixocarpa* (Gámez-Jiménez et al. 2009), and lisianthus (*Eustoma grandiflorum*, Cohen et al. 1995). Natural infections in wild hosts have been reported in members of the Solanaceae (*Solanum nigrum*, *S. luteum*, *Datura stramonium*, *Physalis* sp.) and other plant families such as Asclepidaceae (*Cynanchum acutum*), Caparidaceae (*Cleome viscosa*), Convolvulaceae (*Convolvulus* sp.), Compositae (*Conyza sumatrensis, Dittrichia viscosa*), Cuscutaceae (*Cuscuta* sp.), Chenopodiaceae (*Chenopodium murale*), Euphorbiaceae (*Mercurialis ambigua, Croton lobatus*, *Euphorbia* sp.), Malvaceae (*Malva parviflora, Malva* sp.), and Poligonaceae (*Polygonum* sp.) (Cohen and Antignus 1994; Davino et al. 1994; Bedford et al. 1998; Sánchez-Campos et al. 1999, 2000; Jordá et al. 2001; Salati et al. 2002).

In fresh-market bean (*Phaseolus vulgaris*), TYLCV and *Tomato yellow leaf curl Málaga virus* (TYLCMalV) cause bean leaf crumple disease (BLCD), a serious

disease first described in greenhouses of southern Spain in 1997 (García-Andrés et al. 2007a; Navas-Castillo et al. 1999). Symptoms such as thickening, epinasty, crumpling, and an upward curling of leaves and reduction of foliar area have been observed associated with this disease. Also, abnormal shoot proliferation and internode and leaf size reduction appear in young shoots of affected plants resulting in a bushy appearance. Plants infected in early growth stages show dramatic stunting and abortion of new inflorescences, and production is entirely lost. Pods already set exhibit deformations that make them unmarketable.

In pepper (*Capsicum annuum*), no correlation was observed between TYLCV detection in naturally or experimentally infected plants and symptom expression (Morilla et al. 2005; Polston et al. 2006). TYLCV is able to infect a number of genotypes from several other *Capsicum* species, although none showed symptoms after experimental infection (Polston et al. 2006). Tobacco plants found naturally infected by TYLCV in Spain were also asymptomatic (Font et al. 2005).

*Solanum nigrum* is an important reservoir of TYLCD-associated viruses in southern Spain. The "IL" and "Mld" strains of TYLCV, the "ES" strain of TYLCSV, and the recombinant species TYLCMalV and *Tomato yellow leaf curl Axarquía virus* (TYLCAxV), have all been reported infecting natural populations of *S. nigrum* in that region. Symptoms of plant stunting and upward leaf curling, yellowing, and size reduction were observed in this host (García-Andrés et al. 2006). In the Domican Republic, a wide field survey found symptomless infections of a number of species belonging to different plant families (Salati et al. 2002). In Israel, *C. acutum* was identified as a potent source of inoculum for the primary spread of TYLCV to tomato crops in the Jordan valley (Cohen et al. 1988).

## Recombination as a Key Force Driving Evolution of TYLCD-Associated Virus Populations

In the absence of mixed infections involving different begomoviruses, genetic stability has been observed in TYLCSV populations, with a progressive increase in genetic diversity over time (Sánchez-Campos et al. 1999), as has also been observed for TYLCV (Dellate et al. 2007). However, it should be highlighted that begomoviruses are prone to exchange genetic material through recombination when co-infections occur in the same cell of a host plant. Thus, genetic exchange can combine sequences from different origins providing a broader basis for viral adaptation and emergence associated with changes in virulence and host ranges. A key role of recombination in the evolution of begomoviruses has been suggested (Rybicki 1994; Padidam et al. 1999). The importance of recombination on the genetic diversification of the TYLCD-associated viruses has become evident as an increasing number of virus sequences are available (Fauquet et al. 2005). In addition to rolling circle replication (Saunders et al. 1991), homologous recombination dependent replication occurs in begomoviruses, thus recovering damaged and incomplete DNA for productive infection (Preiss and Jeske 2003). This type of replication process can then

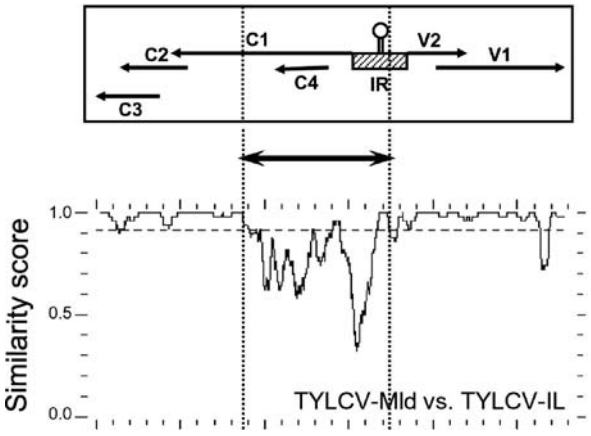



**Fig. 8.2** PLOTSIMILARITY (Devereux et al. 1984) diagram (scanning window = 50) comparing the nucleotide sequences of *Tomato yellow leaf curl virus* (TYLCV) isolates of the "Mld" (isolate ES:72:97) and "IL" (isolate IL:Reo:86) strains. Separation between regions for which differential distribution of nucleotide identity is observed is indicated by *vertical dotted lines*. Dissimilar region is indicated with a *double arrow line*. Positions of the open reading frames and the intergenic region (IR) are indicated at the *top* of the figure. *Horizontal broken lines* correspond to the mean nucleotide identitity between the sequences compared. GenBank accession number of sequences used for comparison are AF071228 (TYLCV-Mld[ES:72:97], and X15656 (TYLCV-IL[IL:Reo:86])

facilitate the occurrence of recombination between begomoviruses during mixed infections. The ability of a virus to generate new genetic variants through recombination can be relevant to understand its adaptive capacity and emergence in nature (Rybicki and Pietersen 1999; Varma and Malathi 2003).

The earliest evidence for naturally-occurring recombinants in the TYLCD-associated virus complex resulted from comparison of the genomes of the "Mld" and "IL" strains of TYLCV (Table 8.1). A clear indication of the existence of a genomic region that differed significantly between these two viral variants was obtained by comparison of their nucleotide sequences (Fig. 8.2). This genomic region was suggested to have been acquired by TYLCV-IL through genetic exchange with a tomato leaf curl-like begomovirus from Southeastern Asia (Navas-Castillo et al. 2000). Thus, the genomes of TYLCV-IL and TYLCV-Mld reflected a modular composition, with genome fragments of diverse phylogenetic origin put together after recombination events.

A similar situation was found for TYLCSV, another species of the TYLCD-associated complex. The TYLCSV clade comprises at least three different strains named "Sardinia" (Sar), "Sicily" (Sic), and "Spain" (ES) (Table 8.1). The genomes of strains "ES" and "Sic" are closely related. However, comparison of the genomes of "Sar" and "Sic" isolates from Italy indicated the presence of a region that showed significant differences between them (Fig. 8.3A). When the nucleotide sequences in this region were phylogenetically analyzed, TYLCSV-Sar was placed within

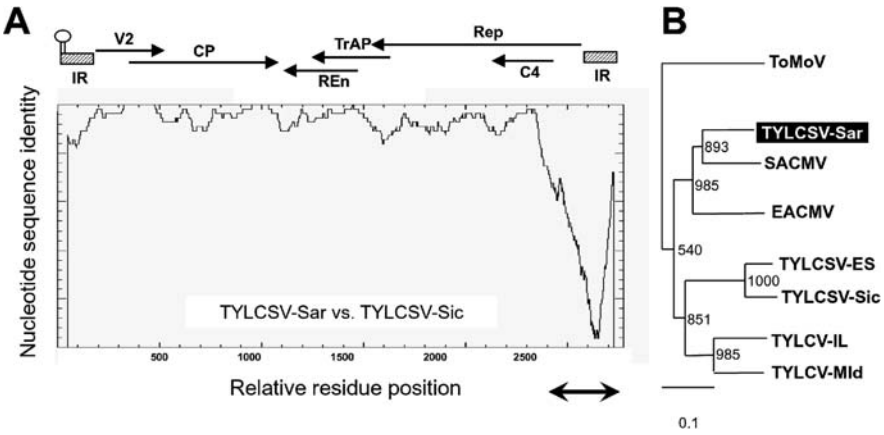



**Fig. 8.3** (**A**) PLOTSIMILARITY (Devereux et al. 1984) diagram comparing the nucleotide sequences of isolates of the "Sar" and "Sic" strains of *Tomato yellow leaf curl Sardinia virus* (TYLCSV). The region for which different nucleotide sequences are detected is indicated with a *double arrow line*. Positions of the open reading frames and the intergenic region (IR) are indicated at the *top* of the figure. (**B**) Phylogenetic relationships for viruses in the TYLCD-associated virus complex and the DNA-A component of representative isolates of viruses infecting cassava from Africa. Relationships were inferred by using the neighbor-joining method from the sequences of the TYLCSV-Sar vs. TYLCSV-Sic differential region indicated in **A**. Support for nodes in a bootstrap analysis with 1,000 replications is shown for values over 500. Horizontal branch lengths are drawn to scale with the bar indicating 0.1 nucleotide replacements per site. Abbreviations and GenBank accession numbers of genome sequences compared are as follow: SACMV, *South African cassava mosaic virus*, AF155806; EACMV, *East African cassava mosaic virus*, Z83257; TYLCSV-ES, *Tomato yellow leaf curl Sardinia virus*-Spain, Z25751; TYLCSV-Sar, *Tomato yellow leaf curl Sardinia virus*-Sardinia, X15655; TYLCSV-Sic, *Tomato yellow leaf curl Sardinia virus*-Sicily, Z28390; TYLCV-IL, *Tomato yellow leaf curl virus*-Israel, X15656; TYLCV-Mld, *Tomato yellow leaf curl virus*-Mild, X76319. An isolate of *Tomato mottle virus* (ToMoV, L14460) was used as the outgroup

the clade that included cassava-infecting begomoviruses from Africa. The closest related sequence was that of *South African cassava mosaic virus* (SACMV) (Fig. 8.3B). Therefore, these results indicated that the "Sar" strain of TYLCSV might have emerged from a genetic exchange between ancestors of TYLCSV and a cassava begomovirus that probably orginating in Africa.

The above examples of putative recombinations involving begomoviruses of the TYLCD virus complex currently present in the Mediterranean Basin and begomoviruses from Asia and Africa suggest that TYLCD-associated viruses could have traveled across the Old World making successive genomic exchanges with viruses with which they shared hosts. Direct evidence that continuous evolution of TYLCD-associated viruses through recombination is taking place in Mediterranean populations has been obtained from field studies performed during several epidemics. Complex TYLCD-associated virus populations are present in this region, involving several species and strains of these species. These complex populations are present in either cultivated (Anfoka et al. 2005; García-Andrés et al. 2007a;

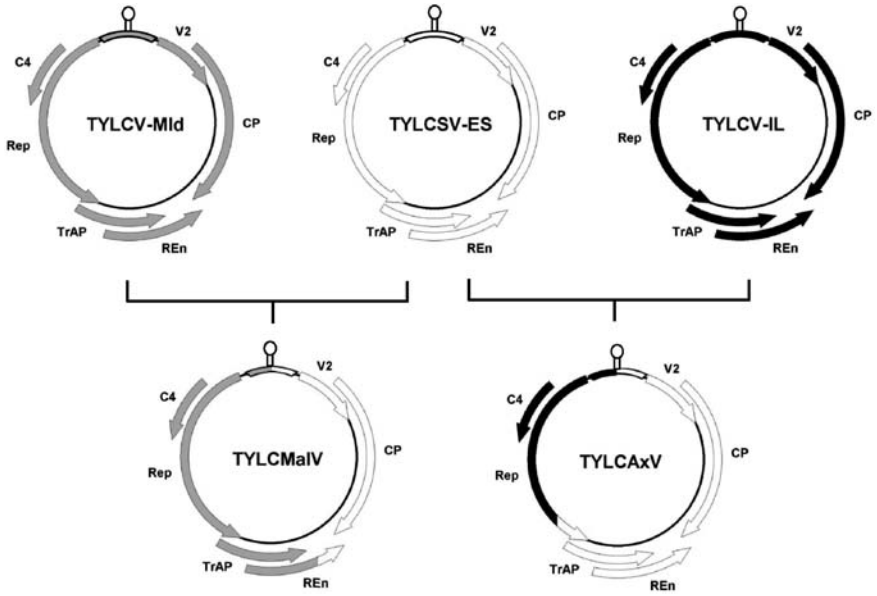



**Fig. 8.4** Schematic representation of the genomes of isolates of *Tomato yellow leaf curl Sardinia virus*-ES (TYLCSV-ES), *Tomato yellow leaf curl virus*-Mld (TYLCV-Mld) and *Tomato yellow leaf curl virus*-IL (TYLCV-IL) from Spain and the recombinant viruses *Tomato yellow leaf curl Málaga virus* (TYLCMalV) and *Tomato yellow leaf curl Axarquía virus* (TYLCAxV) derived from them by genetic exchange of the genome fragments indicated. GenBank accession number for sequences of isolate used for comparison are Z25751 (TYLCSV-ES[ES:Mur1:92]), AF071228 (TYLCV-Mld[ES:72:97]), AJ489258 (TYLCV-IL[ES:Alm:Pep:99]), AF271234 (TYLCMalV-[ES:421:99]), and AY227892 (TYLCAxV-[ES:Alg:00])

Gorsane et al. 2005; Tahiri et al. 2006) or wild species (García-Andrés et al. 2006), providing opportunities for occurrence of mixed infections. As a result, recombinant viruses with novel biological properties have emerged that suggest a step forward in the ecological adaptation to a newly invaded area. This is the case of TYLCMalV and TYLCAxV found in Spain (García-Andrés et al. 2006; Monci et al. 2002) (Fig. 8.4), The spread of these recent emerged recombinant viruses in the TYLCD population has in some cases displaced previously existing genotypes (García-Andrés et al. 2007a). These data demonstrate the extent to which genetic exchange is contributing to the diversification and adaptation of TYLCD-associated virus populations and the key role of recombination in their evolution.

As indicated above, sequence analyses of field isolates revealed evidence indicating the presence of recombinant viruses in TYLCD-associated virus populations. However, it was not clear whether recombination represents a frequent phenomenon during mixed infections of a single host plant. Mixed infections of model systems like TYLCSV-ES and TYLCV-Mld, were investigated by García-Andrés et al. (2007b). Results indicated that, after a long period of co-infection, recombinant genotypes constituted a significant proportion of the viral population (Fig. 8.5A). A wide range of recombinant viruses was recovered from mixed infected plants

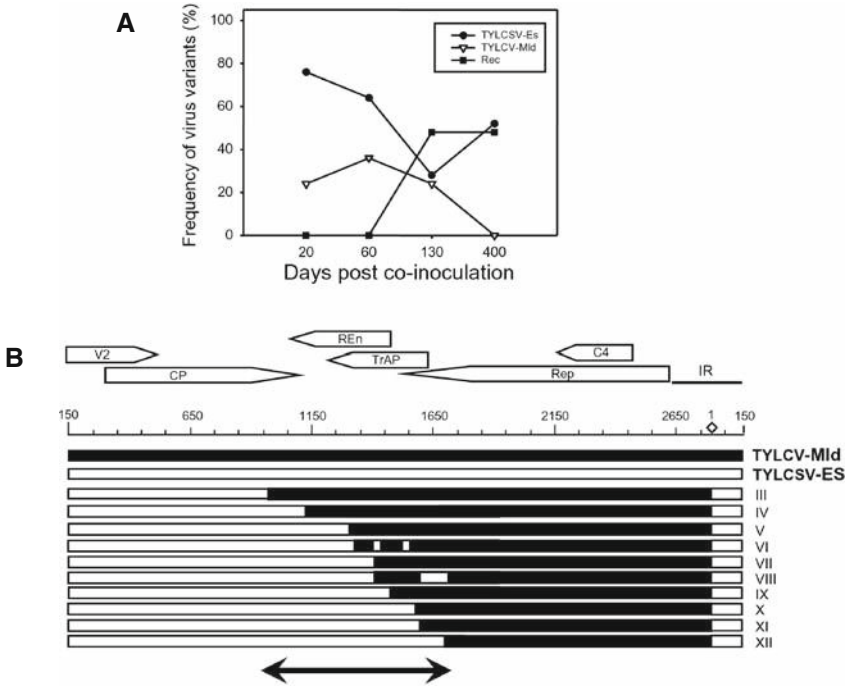



**Fig. 8.5** Frequency and type of recombinant genomes detected after co-inoculation of the isolate ES:Mur1:92 of *Tomato yellow leaf curl Sardinia virus* (TYLCSV-ES) and the isolate ES:72:97 of *Tomato yellow leaf curl virus* (TYLCV-Mld) in tomato cv. 'Moneymaker' plants. Studies were based on restriction fragment length polymorphism analysis on clones containing full-length genome fragments amplified from the virus population present in total nucleic acids extracted from the mixed-infected plants at several times post co-inoculation. (**A**) the frequency of recombinant (Rec) variants in a single tomato plant at several times post-inoculation is shown compared to parental TYLCSV-ES and TYLCV-Mld viruses. (**B**) the different types of recombinant viruses detected (III to XII) in co-infected tomatoes is schematically represented; the double arrow line in the bottom of the figure indicates the hot spot region of recombination found outside the IR

(Fig. 8.5B) that reinforced the significance of recombination as a source of genetic diversity. Not all regions of the genome seemed to contribute equally to genetic exchange. In addition to the intergenic region, a known hot spot for recombination found in all recombinants, a second crossover region could be delimited (Fig. 8.5B). Both sequence homology and secondary structures in crossover sites seemed to be involved in recombination. These features could favor discontinuous DNA replication via replication complex switching between homologous regions of DNA templates (García-Andrés et al. 2007b).

Knowledge of the genetic structure of natural virus populations is essential to design more effective and durable control strategies. For example, great differences can exist between virus genotypes in their ability to overcome host plant resistance. Thus, in a study to determine the resistance of twelve tomato advanced breeding lines derived from *S. chilense* to TYLCD-associated viruses, it was concluded that six lines exhibited resistance only to TYLCSV but not to TYLCV (de Castro

et al. 2005). In this context, emergence of recombinant genotypes can be crucial for the durability of the resistance trait. TYLCD epidemics have occurred in the western Mediterranean Basin, including Italy and Spain, since the late 1980s. A complex virus population was found, composed of three distinct species and several strains (the "Sar", "Sic" and "ES" strains of TYLCSV, the "IL" and "Mld" strains of TYLCV, and TYLCMalV), compatible with multiple introductions and spread of virus types in the region (García-Andrés et al. 2007a). In Spain, initial colonization with isolates of the "ES" strain of TYLCSV during the early 1990s resulted in a relatively stable population (Sánchez-Campos et al. 2002). However, subsequent introductions of isolates of the "IL" and "Mld" strains of TYLCV (Morilla et al. 2003; Navas-Castillo et al. 1999) resulted in a rapid displacement of TYLCSV in the population (Sánchez-Campos et al. 1999). Also, novel recombinant genotypes, like TYLCMalV and TYLCAxV emerged from genetic exchanges between isolates of the "Mld" or "IL" strains of TYLCV and isolates of the "ES" strain of TYLCSV, respectively. These recombinants exhibited biological properties that suggested they were more ecologically adapted than either parental virus (García-Andrés 2007b; Monci et al. 2002). Isolates of the "Sar" and "Sic" strains of TYLCSV were present in Sicily and Sardinia since 1989 (Crespi et al. 1995; Kheyr-Pour et al. 1991). Introduction of isolates of the "IL" strain of TYLCV was reported in 2002 in Sicily, resulting in a progressive displacement of previously existing TYLCD-associated viruses (Davino et al. 2006) and emergence of recombinant viruses (Davino et al. 2009). Information is available about the genetic structure of TYLCD-associated virus populations in other geographical regions which suggests complex populations in which recombination processes might be involved (Delatte et al. 2005; Duffy and Holmes 2007; Tahiri et al. 2006; Ueda et al. 2004; Zambrano et al. 2007).

## Diagnosis of TYLCD-Associated Viruses

Knowledge about the identity of viruses involved in epidemics is essential for implementation of correct control strategies. TYLCD-associated viruses produce distinct symptoms that can, however, be confused with these or other begomoviruses, Therefore, diagnosis cannot rely on symptomatology. Although polyclonal and monoclonal antibodies have been developed for TYLCV-associated viruses detection, cross-reactions occur among a wide range of begomoviruses due to shared motifs in the coat protein (Harrison et al. 2002). Thus, molecular diagnostic techniques have been developed for specific or generic detection of viruses involved in TYLCD infections (reviewed by Accotto and Noris 2007; Moriones and García-Andrés 2008), discussed below.

### *Polymerase Chain-Reaction*

The polymerase chain-reaction (PCR) is an extremely sensitive technique for rapid and accurate detection and subsequent identification of viruses. Begomoviruses replicate via double-stranded DNA intermediates that can serve as templates for

amplification and are therefore well suited to PCR detection and identification. Moreover, simple PCR methods have been developed that avoid the purification of nucleic acids, allowing analysis of a large number of samples in a short time (Atzmon et al. 1998; Navas-Castillo et al. 1998). Although PCR is still laborious and too expensive for large-scale identification analysis, it offers a rapid means of detection, and is a helpful and widely used technique for identification of viruses involved in TYLCD.

Primers for specific detection of TYLCD-associated viruses are designed based primarily on the IR sequences or on the sequences in the 5′-proximal end of the Rep ORF, which are the most variable regions between viral species and strains. For example, Monci et al. (2002) designed primers specific to TYLCSV or to TYLCV. Using a combination of these primers, specific PCR amplification could be obtained for these virus species or recombinants between them, such as TYLCMalV.

Generic PCR detection of TYLCD-associated viruses could also be developed based on primers designed for regions conserved among their genomes. For example, a PCR procedure based on a single primer pair designed from sequences conserved in Rep ORF has been developed for universal detection of TYLCSV and TYLCV isolates (García-Andrés et al. 2007a). The product of the amplification could then be used for determining the DNA sequence of the virus, allowing phylogenetic positioning in the genus.

## *RFLP Analysis of PCR-Amplified Products*

Methodologies have been developed for identification and differentiation of related viruses based on the combination of PCR and restriction fragment length polymorphism (RFLP) analysis. RFLP is an easy method for characterizing PCR-amplified fragments, and is useful for detecting new uncharacterized begomoviruses, or to study genetic diversity in natural populations. For TYLCD-associated viruses, a PCR-RFLP diagnosis system was developed that allows typing of isolates present in the Mediterranean Basin (Accotto et al. 2000). Samples singly infected with TYLCSV or TYLCV, or samples mixed infected with both viruses, could easily be distinguished in this way. Moreover, appearance of unexpected novel digestion patterns could be indicative of the presence of new strains or species. PCR-RFLP offers a good alternative for preliminary analysis, but further studies including other parts of the genome are needed for a definitive identification of the virus present in a given sample (e.g. Font et al. 2007).

## *Nucleic Acid Hybridization*

Nucleic acid hybridization is becoming a routine methodology for virus diagnosis. Methods involving tissue squash blot – that avoid purification of nucleic acids and allow rapid analysis of large number of samples – are frequently used. Radioactively labeled DNA probes have been used (Navot et al. 1989), although non-radioactive

labeling allows for a more user-friendly diagnostic test (Crespi et al. 1991; Noris et al. 1994). Stem or leaf-petiole transversal section squashes, or dot blots with DNA extracts, are used for detection on positively-charged nylon membranes following standard hybridization methodologies. Genomic regions that are highly variable between TYLCD-associated viruses, like the IR and the 5′ proximal end of the Rep ORF, are useful in producing DNA probes that are highly species- or strain-specific. For example, probes specific to either TYLCV or TYLCSV (Navas-Castillo et al. 1999) can be used for a preliminary classification of the viruses found in infected samples. Based on hybridization analysis, specific detection of closely related isolates or strains that differ in some portion of their genomes can be done by preparing probes specific to that portion. Thus, it is possible to distinguish infections caused by isolates of the "IL" strain of TYLCV (Navot et al. 1991) from infections caused by isolates of the "Mld" strain of TYLCV (Antignus and Cohen 1994) with this approach (Navas-Castillo et al. 2000). Mixing probes specific to several viruses will result in a more generic diagnostic system if specific detection is not the objective and wide range detection of TYLCD-associated viruses is preferred. The production and combination of full-length genome probes also gives satisfactory wide range detection capabilities for TYLCV and TYLCSV infections (e.g. Accotto et al. 2000).

### *Rolling Circle Amplification (RCA) sing Φ29 RNA Polymerase*

A procedure has been developed (Inoue-Nagata et al. 2004) to improve the diagnosis of begomoviruses based on the use of the DNA polymerase of the *Bacillus subtilis* bacteriophage Φ29. This enzyme possesses both polymerase and strand displacement-activity, allowing circular DNA to be replicated to a nearly unlimited extent by using a rolling circle amplification (RCA) mechanism (Esteban et al. 1993). Actively replicating begomoviruses are ideal substrates for this enzyme because they produce various circular DNA intermediates during the complementary strand synthesis of their circular single-stranded DNA via rolling circle replication and recombination-dependent replication.

It has been suggested that this procedure would widely improve diagnosis, and therefore help to implement quarantine measures in under-equipped laboratories from developing countries (Haible et al. 2006). RCA amplification has also been successfully used for amplification of the full genome of TYLCD-associated viruses, including recombinants that have arisen after co-inoculation of TYLCV and TYLCSV in tomato plants (García-Andrés et al. 2007b).

## Management of TYLCD Epidemics

TYLCD-associated viruses are emerging worldwide, causing severe damage to tomatoes and other important crops. Spread of TYLCV to the New World, probably following the introduction of infected tomato seedlings, resulted in

dramatic consequences in important production areas (Brown and Idris 2006; Morales 2006; Polston et al. 1999; Zambrano et al. 2007). Therefore, preventive measures to avoid the traffic and spread of infected plant material or viruliferous whiteflies are crucial as a first barrier in preventing spread of TYLCD epidemics.

It is difficult to completely eradicate virus infections once the disease has become established in a certain area, although a combination of good production practices may help to minimize the impact of the disease. These measures include selecting planting dates to avoid critical periods when high whitefly populations are present. The use of screens in protected crops might also help to exclude the vector and infections (see Antignus Chapter 13). Cropping practices that reduce vector transmission rates and/or virus population densities can also minimize spreading of the infection (Seal et al. 2006a). For example, using plastic covers in protected crops with complete or partial absorption of solar UV radiation alters the behavior of *B. tabaci* and leads to reduced TYLCD impact (Antignus et al. 2001; Monci et al. 2004; Raviv and Antignus 2004; see also Antignus Chapter 13). In other words, practices to reduce virus spread through elimination of primary and secondary virus sources can help minimize the damage. Intensive cropping systems that employ a continuous presence of host plants susceptible to viruses associated with TYLCD increase the prevalence and severity of epidemics year-round, making control more difficult (Sánchez-Campos et al. 1999).

Disruption of the virus infection cycle by introduction of host-free periods can further help to reduce TYLCD damage as shown in the Dominican Republic by Salati et al. (2002). Nevertheless, knowledge about the sources of infection and importance of wild hosts as reservoirs is essential to evaluate the effectiveness of such measures. Wild hosts can be important reservoirs of genetic diversity for TYLCD-associated virus populations (García-Andrés et al. 2006), and may facilitate their adaptation to changing environmental conditions. Therefore, management of reservoirs might help to control TYLCD epidemics. Eradication of *C. acutum* before the beginning of *B. tabaci* migration into the Jordan Valley (Israel) was proposed to reduce the primary spread of TYLCV in that region (Cohen et al. 1988). In Cyprus, knowledge of TYLCD epidemiology, including seasonal patterns, was used to determine the most convenient crop management practices (Ioannou 1987).

In practice, TYLCD control is problematic because it is based mainly upon intensive use of insecticides to manage *B. tabaci* vector populations. However, the use of chemicals is only partially effective in reducing crop losses, and results in the development of pesticide resistance in *B. tabaci* populations, not to mention severe environmental impact and side effects on natural enemies (Cahill et al. 1996a, 1996b; Elbert and Nauen 2000; Picó et al. 1996). Therefore, integrated management methodologies should be sought to prevent begomovirus infections (see also Stansly and Natwick Chapter 17).

Incorporation of virus resistance into the host plants is the most desirable alternative to manage the damage caused by TYLCD-associated viruses and much work has been done in searching for sources of natural resistance to these viruses in tomato (see Nombela and Muñiz Chapter 14 for a review of plant resistance to the vector). Resistance traits have been found that can affect viral replication or local


**Fig. 8.6** Hybridization of tissue blots from petiole cross sections of newly emerged young leaves performed on positively-charged nylon membranes with samples from plants of a *Solanum habrochaites* accession selected for resistance to TYLCD. Plants were inoculated with isolates of the strain "IL" (TYLCSV-IL) or "Mld" (TYLCV-Mld) of *Tomato yellow leaf curl virus*. Blots were hybridized with a digoxigenin (DIG)-labeled DNA probe able to detect both viruses. Plant number is indicated at the top of the figure in each case. Differences were observed in the ability to resist virus infection depending on the type of TYLCD-associated virus inoculated

and systemic movement within plants (Lapidot and Friedman 2002; Morales and Anderson 2001; Picó et al. 1996). Search for resistance in *Solanum lycopersicum* germplasm failed, so wild relatives (*Solanum* section *Lycopersicon*) have been screened intensively (Lapidot and Friedmann 2002; Picó et al. 1996). Breeding programs have been based primarily on introgression of resistance to TYLCV. Variable levels of resistance have been found in wild *Solanum* species used for introgression of resistance into cultivated tomato (Friedman et al. 1998; Rom et al. 1993; Vidavsky and Czosnek 1998; Vidavsky et al. 1998). *S. chilense* has proven to be one of the best sources of resistance (Michelson et al. 1994). The most promising TYLCV resistance, derived from this wild *Solanum* species, is controlled by a major gene named *Ty-1*, and at least two other modifier genes (Zamir et al. 1994). This resistance results in a dramatic reduction in virus accumulation (Michelson et al. 1994; Zamir et al. 1994) and is widely used in commercial hybrids (de Castro et al. 2007). However, under high inoculum pressure, *Ty-1* resistance can be overcome (Michelson et al. 1994; Picó et al. 1996).

Because important differences can exist for resistance traits in their ability to control different TYLCD-associated viruses (see Fig. 8.6 for an example from a *S. habrochaites* accession) (de Castro et al. 2005), it is essential to have information about the genetic diversity of TYLCD-associated viruses present in the geographical region where control is to be applied. It should be taken into account that the generalized use of resistant cultivars can alter the virus population structure by selection for fitter variants (García-Andrés et al. 2009). Therefore, the use of resistant varieties to control TYLCD should be managed carefully to minimize risk of selection for virulent, resistance-breaking virus variants (Seal et al. 2006a, b). Resistance gene rotations could be employed to disrupt selection of virulent virus variants. Also, the combination of virus resistance with vector resistance that directly reduces vector populations would be desirable (Jeger et al. 2004). Marker-assisted conventional selection will facilitate the production of commercial cultivars with improved resistance traits (e.g. de Castro et al. 2007). The potential of genetic engineering to develop commercial cultivars resistant to TYLCD is high. Transgenic plants that incorporate TYLCD resistance have been obtained (Bendahmane and Gronenborn 1997; Brunetti et al. 1997; Lucioli et al. 2003; Kunik et al. 1994; Yang et al. 2004)

following different approaches. However, transgene-mediated resistance still can occur (e.g. Noris et al. 2004). Advances in understanding the basis of virus-vector-host plant interactions can provide novel targets for genetic engineering to interfere with infections caused by TYLCD-associated virus.

## Conclusions

Emergence of TYLCD epidemics has led to increased yield losses in tomato worldwide. The intensive use of insecticides to reduce vector populations has resulted in the development of resistant *B. tabaci* populations, making control difficult. Much knowledge is currently available about TYLCD-associated viruses and their epidemics to help design novel control approaches. Complex epidemiological situations, as well as interactions among cultivated and wild hosts, make single solutions less effective. Therefore, integrated management strategies combining several control practices are recommended. However, more knowledge about the epidemiology and ecology of this complex disease is needed to develop better control strategies. Also, greater understanding of virus diversity and factors driving the evolution of their populations is essential to establish more effective and durable measures to control TYLCD epidemics. Good progress has been made in developing TYLCD resistant tomato cultivars in past years. These developments, and the combination of traditional and marker-assisted breeding techniques, will result in improved cultivars for tomato growers. However, our current lack of knowledge about pathogenesis mechanisms of TYLCD-associated viruses and the precise interactions with *B. tabaci* hinder our ability to develop novel and definitive control strategies.

## Literature Cited

Accotto GP, Navas-Castillo J, Noris E, Moriones E, Louro D. 2000. Typing of *Tomato yellow leaf curl viruses* in Europe. *Eur. J. Plant Pathol.* 106:179–186.

Accotto GP, Noris E. 2007. Detection methods for TYLCV and TYLCSV. In *Tomato Yellow Leaf Curl Virus Disease. Management, Molecular Biology, Breeding for Resistance*, ed. H Czosnek, pp. 241–249. The Netherlands: Springer.

Anfoka GH, Abhary M, Nakhla MK. 2005. Molecular identification of species of the *Tomato yellow leaf curl virus* complex in Jordan. *J. Plant Pathol.* 87:65–70.

Antignus Y, Cohen S. 1994. Complete nucleotide sequence of an infectious clone of a mild isolate of *Tomato yellow leaf curl virus* (TYLCV). *Phytopathology* 84:707–712.

Antignus Y, Nestel D, Cohen S, Lapidot M. 2001. Ultraviolet-deficient greenhouse environment affects whitefly attraction and flight-behavior. *Environ. Entomol.* 30:394–399.

Atzmon G, van Oss H, Czosnek H. 1998. PCR-amplification of *Tomato yellow leaf curl virus* (TYLCV) DNA from squashes of plants and whitefly vectors: application to the study of TYLCV acquisition and transmission. *Eur. J. Plant Pathol.* 104:189–194.

Bedford ID, Kelly A, Banks GK, Briddon RW, Cenis JL, Markham PG. 1998. *Solanum nigrum*: an indigenous weed reservoir for *Tomato yellow leaf curl geminivirus* in southern Spain. *Eur. J. Plant Pathol.* 104:221–222.

Bendahmane M, Gronenborn B. 1997. Engineering resistance against *Tomato yellow leaf curl virus* (TYLCV) using antisense RNA. *Plant Mol. Biol.* 33:351–357.

Bosco D, Mason G, Accotto GP. 2004. TYLCSV DNA, but not infectivity, can be transovarially inherited by the progeny of the whitefly vector *Bemisia tabaci* (Gennadius). *Virology* 323:276–283.

Brown JK, Czosnek H. 2002. Whitefly transmission of plant viruses. *Adv. Bot. Res.* 36:65–100.

Brown JK, Idris AM. 2006. Introduction of the exotic monopartite *Tomato yellow leaf curl virus* into West Coast Mexico. *Plant Dis.* 90:1360.

Brunetti A, Tavazza M, Noris E, Tavazza R, Caciagli P, Ancora G, Crespi S, Accotto GP. 1997. High expression of truncated viral Rep protein confers resistance to *Tomato yellow leaf curl virus* in transgenic tomato plants. *Mol. Plant-Microbe Interact.* 10:571–579.

Caciagli P, Bosco D, Al-Bitar L. 1995. Relationships of the Sardinian isolate of *Tomato yellow leaf curl geminivirus* with its whitefly vector *Bemisia tabaci* Gen. *Eur. J. Plant Pathol.* 101:163–170.

Cahill M, Gorman K, Day S, Denholm I, Albert A, Nauen R. 1996a. Baseline determination and detection of resistance to imidacloprid in *Bemisia tabaci* (Homoptera: Aleyrodidae). *Bull. Entomol. Res.* 86:343–349.

Cahill M, Jarvis W, Gorman K, Denholm I. 1996b. Resolution of baseline responses and documentation of resistance to buprofezin in *Bemisia tabaci* (Homoptera: Aleyrodidae). *Bull. Entomol. Res.* 86:117–122.

Cohen S, Antignus Y. 1994. *Tomato yellow leaf curl virus*, a whitefly-borne geminivirus of tomatoes. *Adv. Dis. Vector Res.* 10:259–288.

Cohen J, Gera A, Ecker R, Ben-Joseph R, Perlsman M, Gokkes M, Lachman O, Antignus Y. 1995. Lisianthus leaf curl – a new disease of lisianthus caused by *Tomato yellow leaf curl virus*. *Plant Dis.* 79:416–420.

Cohen S, Nitzany FE. 1966. Transmission and host range of the *Tomato yellow leaf curl virus*. *Phytopathology* 56:1127–1131.

Cohen S, Kern J, Harpaz I, Ben-Joseph R. 1988. Epidemiological studies of the *Tomato yellow leaf curl virus* (TYLCV) in the Jordan Valley, Israel. *Phytoparasitica* 16:259–270.

Crespi S, Accotto GP, Caciagli P, Gronenborn B. 1991. Use of digoxigenin-labeled probes for detection and host-range studies of *Tomato yellow leaf curl geminivirus*. *Res. Virol.* 142:283–288.

Crespi S, Noris E, Vaira AM, Accotto GP. 1995. Molecular characterization of cloned DNA from a *Tomato yellow leaf curl virus* isolate from Sicily. *Phytopathol. Mediterranea* 34:93–99.

Czosnek H, Ghanim M, Ghanim M. 2002. The circulative pathway of begomoviruses in the whitefly vector *Bemisia tabaci* – insights from studies with *Tomato yellow leaf curl virus*. *Ann. Appl. Biol.* 140:215–231.

Davino S, Napoli C, Davino M, Accotto GP. 2006. Spread of *Tomato yellow leaf curl virus* in Sicily: partial displacement of another geminivirus originally present. *Eur. J. Plant Pathol.* 114:293–299.

Davino S, Napoli C, Dellacroce C, Miozzi L, Noris E, Davino M, Accotto GP. 2009. Two new natural begomovirus recombinants associated with the tomato yellow leaf curl disease co-exist with parental viruses in tomato epidemics in Italy. *Virus Res.* 143:15–23.

Davino M, D'Urso F, Areddia R, Carbone M, Mauromicale G. 1994. Investigations on the epidemiology of *Tomato yellow leaf curl virus* (TYLCV) in Sicily. *Petria* 4:151–160.

de Castro AP, Blanca JM, Díaz MJ, Nuez F. 2007. Identification of a CAPS marker tightly linked to the Tomato yellow leaf curl disease resistance gene Ty-1 in tomato. *Eur. J. Plant Pathol.* 117:347–356.

de Castro AP, Díez MJ, Nuez F. 2005. Evaluation of breeding tomato lines partially resistant to *Tomato yellow leaf curl Sardinia virus* and *Tomato yellow leaf curl virus* derived from *Lycopersicon chilense*. *Can. J. Plant Pathol.* 27:268–275.

Delatte H, Holota H, Naze F, Peterschmitt M, Reynaud B, Lett JM. 2005. The presence of both recombinant and nonrecombinant strains of *Tomato yellow leaf curl virus* on tomato in Réunion island. *Plant Pathol.* 54:262.

Delatte H, Holota H, Moury B, Reynaud B, Lett JM, Peterschmitt M. 2007. Evidence for a founder effect after introduction of *Tomato yellow leaf curl virus*-mild in an insular environment. *J. Mol. Evol.* 65:112–118.

Devereux J, Haeberli P, Smithies O. 1984. A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res.* 12:387–395.

Duffy S, Holmes EC. 2007. Multiple Introductions of the Old World Begomovirus *Tomato yellow leaf curl virus* into the New World. *Appl. Environ. Microbiol.* 73:7114–7117.

Elbert A, Nauen R. 2000. Resistance of *Bemisia tabaci* (Homoptera: Aleyrodidae) to insecticides in southern Spain with special reference to neonicotinoids. *Pest Manag. Sci.* 56:60–64.

Esteban JA, Salas M, Blanco L. 1993. Fidelity of phi-29 DNA-polymerase: comparison between protein-primed initiation and DNA polymerization. *J. Biol. Chem.* 268:2719–2726.

Fauquet CM, Briddon RW, Brown J, Moriones E, Stanley J, Zerbini M, Zhou X. 2008. Geminivirus strain demarcation and nomenclature. *Arch. Virol.* 153:783–821.

Fauquet CM, Sawyer S, Idris AM, Brown JK. 2005. Sequence analysis and classification of apparent recombinant begomoviruses infecting tomato in the Nile and Mediterranean basins. *Phytopathology* 95:549–555.

Font MI, Córdoba C, García A, Santiago R, Jordá, C. 2005. First report of tobacco as a natural host of *Tomato yellow leaf curl virus* in Spain. *Plant Dis.* 89:910.

Font MI, Rubio L, Martínez-Culebras PV, Jorda C. 2007. Genetic structure and evolution of natural populations of viruses causing the tomato yellow leaf curl disease in Spain. *Virus Res.* 128:43–51.

Friedman M, Lapidot M, Cohen S, Pilowsky M. 1998. A novel source of resistance to *Tomato yellow leaf curl virus* exhibiting a symptomless reaction to viral infection. *J. Am. Soc. Hortic. Sci.* 123:1004–1007.

García-Andrés S, Accotto GP, Navas-Castillo J, Moriones E. 2007a. Founder effect, plant host, and recombination shape the emergent population of begomoviruses that cause the tomato yellow leaf curl disease in the Mediterranean basin. *Virology* 359:302–312.

García-Andrés S, Monci F, Navas-Castillo J, Moriones E. 2006. Begomovirus genetic diversity in the native plant reservoir *Solanum nigrum*: evidence for the presence of a new virus species of recombinant nature. *Virology* 350:433–442.

García-Andrés S, Tomás DM, Navas-Castillo J, Moriones E. 2009. Resistance-driven selection of begomoviruses associated with the tomato yellow leaf curl disease. *Virus Res*. 146:66–72.

García-Andrés S, Tomás DM, Sánchez-Campos S, Navas-Castillo J, Moriones E. 2007b. Frequent occurrence of recombinants in mixed infections of tomato yellow leaf curl disease-associated begomoviruses. *Virology* 365:210–219.

Ghanim M, Czosnek H. 2000. *Tomato yellow leaf curl geminivirus* (TYLCV-Is) is transmitted among whiteflies (*Bemisia tabaci*) in a sex-related manner. *J. Virol.* 74:4738–4745.

Ghanim M, Morin S, Zeidan M, Czosnek H. 1998. Evidence for transovarial transmission of *Tomato yellow leaf curl virus* by its vector, the whitefly *Bemisia tabaci*. *Virology* 240:295–303.

Goldman V, Czosnek H. 2002. Whiteflies (*Bemisia tabaci*) issued from eggs bombarded with infectious DNA clones of *Tomato yellow leaf curl virus* from Israel (TYLCV) are able to infect tomato plants. *Arch. Virol.* 147:787–801.

Gámez-Jiménez C, Romero-Romero JL, Santos-Cervantes ME, Leyva-López NE, Méndez-Lozano J. 2009. Tomatillo (*Physalis ixocarpa*) as a natural new host for *Tomato yellow leaf curl virus* in Sinaloa, Mexico. *Plant Dis.* 93:545.

Gorsane F, Gharsallah-Chouchene S, Nakhla MK, Fekih-Hassan I, Maxwell DP, Marrakchi M, Fakhfakh H. 2005. Simultaneous and rapid differentiation of members of the *Tomato yellow leaf curl virus* complex by multiplex PCR. *J. Plant Pathol.* 87:43–48.

Gorsane F, Fekin-Hassen I, Nakhla MK, Fakhfakh H, Maxwell DP, Marrakchi M. 2004. Molecular evidence of *Tomato yellow leaf curl virus*-Sicily spreading on tomato, pepper and bean in Tunisia. *Phytopathol. Mediterranea* 43:177–186.

Gutiérrez C. 1999. Geminivirus DNA replication. *Cell. Mol. Life Sci.* 56:313–329.

Haible D, Kober S, Jeske H. 2006. Rolling circle amplification revolutionizes diagnosis and genomics of geminiviruses. *J. Virol. Methods* 135:9–16.

Hanley-Bowdoin L, Settlage SB, Orozco BM, Nagar S, Robertson D. 2000. Geminiviruses: models for plant DNA replication, transcription, and cell cycle regulation. *Critical Rev. Biochem. Mol. Biol.* 35:105–140.

Harrison BD, Swanson MM, Fargette D. 2002. Begomovirus coat protein: serology, variation and functions. *Physiol. Mol. Plant Pathol.* 60:257–271.

Inoue-Nagata AK, Albuquerque LC, Rocha WB, Nagata T. 2004. A simple method for cloning the complete begomovirus genome using the bacteriophage phi29 DNA polymerase. *J. Virol. Methods* 116:209–211.

Ioannou N. 1987. Cultural management of tomato yellow leaf curl disease in Cyprus. *Plant Pathol.* 36:367–373.

Jeger MJ, Holt HJ, van den Bosch F, Madden LV. 2004. Epidemiology of insect-transmitted plant viruses: modelling disease dynamics and control interventions. *Physiol. Entomol.* 29:291–304.

Jiu M, Zhou XP, Tong L, Xu J, Yang X, Wan FH, Liu SS. 2007. Vector-virus mutualism accelerates population increase of an invasive whitefly. *PLoS One* 2:e182.

Jones DR. 2003. Plant viruses transmitted by whiteflies. *Eur. J. Plant Pathol.* 109:195–519.

Jordá C Font I, Martínez P, Juárez M, Ortega A, Lacasa A. 2001. Current status and new natural hosts of *Tomato yellow leaf curl virus* (TYLCV) in Spain. *Plant Dis.* 85:445.

Kheyr-Pour A, Bendhamane M, Matzeit V, Accotto GP, Crespi S, Gronenborn B. 1991. *Tomato yellow leaf curl virus* from Sardinia is a whitefly-transmitted monopartite geminivirus. *Nucleic Acids Res.* 19:6763–6769.

Kunik T, Salomon R, Zamir D, Navot N, Zeidan M, Michelson I, Gafni Y, Czosnek H. 1994. Transgenic tomato plants expressing the *Tomato yellow leaf curl virus* capsid protein are resistant to the virus. *Biotechnology* 12:500–504.

Lapidot M, Friedmann M. 2002. Breeding for resistance to whitefly-transmitted geminiviruses. *Ann. Appl. Biol.* 140:109–127.

Lucioli A, Noris E, Brunetti A, Tavazza R, Ruzza V, Castillo AG, Bejarano ER, Accotto GP, Tavazza M. 2003. *Tomato yellow leaf curl Sardinia virus* Rep-derived resistance to homologous and heterologous geminiviruses occurs by different mechanisms and is overcome if virus-mediated transgene silencing is activated. *J. Virol.* 77:6785–6798.

Mansoor S, Briddon RW, Zafar Y, Stanley J. 2003. Geminivirus disease complexes: an emerging threat. *Trends Plant Sci.* 8:128–134.

McGrath PF, Harrison BD. 1995. Transmission of *Tomato yellow leaf curl geminivirus* by *Bemisia tabaci*: effects of virus isolate and vector biotype. *Ann. Appl. Biol.* 126:307–316.

Martínez-Zubiaur Y, Fonseca D, Quiñones M, Palenzuela I. 2004. Presence of *Tomato yellow leaf curl virus* infecting squash (*Cucurbita pepo*) in Cuba. *Plant Dis.* 88:572.

Martínez-Zubiaur Y, Quiñones M, Fonseca D, Potter JL, Maxwell DP. 2002. First report of *Tomato yellow leaf curl virus* associated with beans, *Phaseolus vulgaris*, in Cuba. *Plant Dis.* 86:814.

Michelson I, Zamir D, Czosnek H. 1994. Accumulation and translocation of *Tomato yellow leaf curl virus* in a *Lycopersicon esculentum* breeding line containing the *L. chilense* tolerance gene *Ty-1*. *Phytopathology* 84:928–933.

Monci F, Sánchez-Campos S, Navas-Castillo J, Moriones E. 2002. A natural recombinant between the geminiviruses *Tomato yellow leaf curl Sardinia virus* and *Tomato yellow leaf curl virus* exhibits a novel pathogenic phenotype and is becoming prevalent in Spanish populations. *Virology* 303:317–326.

Monci F, García-Andrés S, Sánchez F, Moriones E, Espí E, Salmerón A. 2004. Tomato yellow leaf curl disease control with UV-blocking plastic covers in commercial plastichouses of Southern Spain. *Acta Hortic.* 633:537–542.

Morales FJ. 2006. History and current distribution of begomoviruses in Latin America. *Adv. Virus Res.* 67:127–162.

Morales FJ, Jones PG. 2004. The ecology and epidemiology of whitefly-transmitted viruses in Latin America. *Virus Res.* 100:57–65.

Morales FJ, Anderson PK. 2001. The emergence and dissemination of whitefly-transmitted geminiviruses in Latin America – brief review. *Arch. Virol.* 146:415–441.

Morilla G, Antúnez C, Bejarano ER, Janssen D, Cuadrado IM. 2003. A new *Tomato yellow leaf curl virus* strain in southern Spain. *Plant Dis.* 87:1004.

Morilla G, Janssen D, García-Andrés S, Moriones E, Cuadrado IM, Bejarano ER. 2005. Pepper (*Capsicum annuum*) is a dead-end host for *Tomato yellow leaf curl virus*. *Phytopathology* 95:1089–1097.

Morin S, Ghanim M, Zeidan M, Czosnek H, Verbeek M, van den Heuvel JFJM. 1999. A GroEL homologue from endosymbiotic bacteria of the whitefly *Bemisia tabaci* is implicated in the circulative transmission of *Tomato yellow leaf curl virus*. *Virology* 256:75–84.

Moriones E, García-Andrés S. 2008. Diagnosis of begomoviruses. In *Technique in Diagnosis of Plant Viruses*. ed. GP Rao, RA Valverde, CI Dovas, pp. 165–187. TX, USA: Studium Press, LLC.

Moriones E, Navas-Castillo J. 2000. *Tomato yellow leaf curl virus*, an emerging virus complex causing epidemics worldwide. *Virus Res.* 71:123–134.

Navas-Castillo J, Sánchez-Campos S, Díaz JA, Sáez-Alonso E, Moriones E. 1999. *Tomato yellow leaf curl virus*-Is causes a novel disease of common bean and severe epidemics in tomato in Spain. *Plant Dis.* 83:29–32.

Navas-Castillo J, Díaz JA, Sánchez-Campos S, Moriones E. 1998. Improvement of the print-capture polymerase chain reaction procedure for efficient amplification of DNA virus genomes from plants and insect vectors. *J. Virol. Methods* 75:195–198.

Navas-Castillo J, Sánchez-Campos S, Noris E, Louro D, Accotto GP, Moriones E. 2000. Natural recombination between *Tomato yellow leaf curl virus-Is* and *Tomato leaf curl virus*. *J. General Virol.* 81:2797–2801.

Navot N, Ber R, Czosnek H. 1989. Rapid detection of *Tomato yellow leaf curl virus* in squashes of plants and insect vectors. *Phytopathology* 79:562–568.

Navot N, Pichersky E, Zeidan M, Zamir D, Czosnek H. 1991. *Tomato yellow leaf curl virus*: a whitefly-transmitted geminivirus with a single genomic component. *Virology* 185:151–161.

Noris E, Accotto GP, Luisoni E. 1994. Advances in diagnosing *Tomato yellow leaf curl geminivirus* infection. *Mol. Biotechnol.* 2:219–226.

Noris E, Lucioli A, Tavazza R, Caciagli P, Accotto GP, Tavazza M. 2004. *Tomato yellow leaf curl Sardinia virus* can overcome transgene-mediated RNA silencing of two essential viral genes. *J. General Virol.* 85:1745–1749.

Padidam M, Sawyer S, Fauquet CM. 1999. Possible emergence of new geminiviruses by frequent recombination. *Virology* 265:218–225.

Picó, B, Díez MJ, Nuez F. 1996. Viral diseases causing the greatest economic losses to the tomato crop. 2. The *Tomato yellow leaf curl virus* – a review. *Sci. Hortic.* 67:151–196.

Polston JE, Cohen L, Sherwood TA, Ben-Joseph R, Lapidot M. 2006. *Capsicum* species: symptomless hosts and reservoirs of *Tomato yellow leaf curl virus*. *Phytopathology* 96:447–452.

Polston JE, McGovern RJ, Brown LJ. 1999. Introduction of *Tomato yellow leaf curl virus* in Florida and implications for the spread of this and other geminiviruses of tomato. *Plant Dis.* 83:984–988.

Preiss W, Jeske H. 2003. Multitasking in replication is common among geminiviruses. *J. Virol.* 77:2972–2980.

Quiñones M, Fonseca D, Martínez Y, Accotto GP. 2002. First report of *Tomato yellow leaf curl virus* infecting pepper plants in Cuba. *Plant Dis.* 86:73.

Raviv M, Antignus Y. 2004. UV radiation effects on pathogens and insect pests of greenhouse-grown crops. *Photochem. Photobiol.* 79:219–226.

Reina J, Morilla G, Bejarano ER, Rodríguez MD, Janssen D, Cuadrado IM. 1999. First report of *Capsicum annuum* plants infected by *Tomato yellow leaf curl virus*. *Plant Dis.* 83:1176.

Rom M, Antignus Y, Gidoni D, Pilowsky M, Cohen S. 1993. Accumulation of *Tomato yellow leaf curl virus* DNA in tolerant and susceptible tomato lines. *Plant Dis.* 77:253–257.

Rubinstein G, Czosnek H. 1997. Long-term association of *Tomato yellow leaf curl virus* with its whitefly vector *Bemisia tabaci*: effect on the insect transmission capacity, longevity and fecundity. *J. General Virol.* 78:2683–2689.

Rybicki EP. 1994. A phylogenetic and evolutionary justification for three genera of *Geminiviridae*. *Arch. Virol.* 139:49–77.

Rybicki EP, Pietersen G. 1999. Plant virus problems in the developing world. *Adv. Virus Res.* 53:127–175.

Salati R, Nahkla MK, Rojas MR, Guzmán P, Jaquez J, Maxwell DP, Gilbertson RL. 2002. *Tomato yellow leaf curl virus* in the Dominican Republic: characterization of an infectious clone, virus monitoring in whiteflies, and identification of reservoir hosts. *Phytopathology* 92:487–496.

Sánchez-Campos S, Díaz JA, Monci F, Bejarano ER, Reina J, Navas-Castillo J, Aranda MA, Moriones E. 2002. High genetic stability of the begomovirus *Tomato yellow leaf curl Sardinia virus* in Southern Spain over an 8-year period. *Phytopathology* 92:842–849.

Sánchez-Campos S, Navas-Castillo J, Camero R, Soria C, Díaz JA, Moriones E. 1999. Displacement of *Tomato yellow leaf curl virus* (TYLCV)-Sr by TYLCV-Is in tomato epidemics in Spain. *Phytopathology* 89:1038–1043.

Sánchez-Campos S, Navas-Castillo J, Monci F, Díaz JA, Moriones E. 2000. Mercurialis ambigua and Solanum luteum: two newly discovered natural hosts of tomato yellow leaf curl geminiviruses. *Eur. J. Plant Pathol.* 106:391–394.

Saunders K, Lucy A, Stanley J. 1991. DNA forms of the geminivirus African cassava mosaic virus consistent with a rolling circle mechanism of replication. *Nucleic Acids Res.* 19:2325–2330.

Seal SE, Jeger MJ, van den Bosch F. 2006a. Begomovirus evolution and disease management. In *Advances in Virus Research Plant Virus Epidemiology*, ed. JS Aaron, K Maramorosch, pp. 297–316. New York: Academic Press.

Seal SE, van den Bosch F, Jeger MJ. 2006b. Factors influencing begomovirus evolution and their increasing global significance: implications for sustainable control. *Critical Rev. Plant Sci.* 25:23–46.

Stanley J, Bisaro DM, Briddon RW, Brown JK, Fauquet CM, Harrison BD, Rybicki, EP, Stenger DC. 2005. Family *Geminiviridae*. In *Virus Taxonomy* (Eighth Report of the International Committee on Taxonomy of Viruses), ed. CM Fauque, MA Mayo, J Maniloff, U Desselberger, LA Ball, pp. 301–326. London: Elsevier/Academic Press.

Tahiri A, Sekkat A, Bennani A, Granier M, Delvare G, Peterschmitt M. 2006. Distribution of tomato-infecting begomoviruses and *Bemisia tabaci* biotypes in Morocco. *Ann. Appl. Biol.* 149:175–186.

Ueda S, Kimura T, Onuki M, Hanada K, Iwanami T. 2004. Three distinct groups of isolates of *Tomato yellow leaf curl virus* in Japan and construction of an infectious clone. *J. General Plant Pathol.* 70:232–238.

Varma A, Malathi VG. 2003. Emerging geminivirus problems: a serious threat to crop production. *Ann. Appl. Biol.* 142:145–164.

Vidavsky F, Czosnek H. 1998. Tomato breeding lines resistant and tolerant to *Tomato yellow leaf curl virus* issued from *Lycopersicon esculentum*. *Phytopathology* 88:910–914.

Vidavsky F, Leviatov S, Milo J, Rabinowitch HD, Kedar N, Czosnek H. 1998. Response of tolerant breeding lines of tomato, *Lycopersicon esculentum*, originating from three different sources (*L. peruvianum*, *L. pimpinellifolium*, and *L. chilense*) to early controlled inoculation by *Tomato yellow leaf curl viruses* (TYLCV). *Plant Breeding* 117:165–169.

Yang Y, Sherwood TA, Patte CP, Hiebert E, Polston JE. 2004. Use of *Tomato yellow leaf curl virus* (TYLCV) rep gene sequences to engineer TYLCV resistance in tomato. *Phytopathology* 94:490–496.

Zambrano K, Carballo O, Geraud F, Chirinos D, Fernández C, Marys E. 2007. First report of *Tomato yellow leaf curl virus* in Venezuela. *Plant Dis.* 91:768.

Zamir D, Ekstein-Michelson I, Zakay Y, Navot N, Zeidan M, Sarfatti M, Eshed Y, Harel E, Pleban T, Van-Oss H, Kedar N, Rabinowitch HD, Czosnek H. 1994. Mapping and introgression of a *Tomato yellow leaf curl virus* (TYLCV) tolerance gene, *Ty-1*. *Theor. Appl. Genet.* 88:141–146.

# Chapter 9
# Distribution and Dissemination of Begomoviruses in Latin America and the Caribbean

**Francisco J. Morales**

## Introduction

Latin America and the Caribbean islands have the highest incidence and diversity of begomoviruses in the world transmitted by the whitefly, *Bemisia tabaci* (the term Latin America is used here in a cultural, rather than linguistic manner, and includes all the continental countries from Mexico to Chile). It is not known when *B. tabaci* first appeared in the Americas, but historical records indicate that it was not an important pest or virus vector before the 1930s, in spite of the New World origin of some of its main hosts, including tobacco, cotton, and cassava (Pickersgill 1977).

## Historical Background

### *The Original Begomoviruses*

The history of New World begomoviruses is closely associated with the abundance of wild malvaceous plant species that act as hosts to these viruses in the region. The Malvaceae includes over 1,700 plant species, of which the genera *Abutilon, Malva,* and *Sida* account for some 400 species of neotropical origin (Fryxell 1997). The susceptibility of many of these species to pathogens transmitted by *B. tabaci* was demonstrated early in the study of begomoviruses in Latin America (Bird 1958; Cook 1931; Costa 1955; Silberschmidt 1943; Silberschmidt and Tommasi 1955), where these diseases became collectively known as "infectious chloroses of the Malvaceae" (Orlando and Silberschmidt 1946). Some additional wild plant species suspected of harboring begomoviruses were also described in the Euphorbiaceae, Leguminosae, and Convolvulaceae up to the 1970s (Bird et al. 1975; Costa and Bennett 1950).

F.J. Morales (✉)
Virology Research Unit, International Center for Tropical Agriculture, Km 17,
Recta Cali-Palmira, Valle Cali, Colombia
e-mail: f.morales@cgiar.org

P.A. Stansly, S.E. Naranjo (eds.), *Bemisia*: Bionomics and Management of a Global Pest,
DOI 10.1007/978-90-481-2460-2_9, 

New World cotton (*Gossypium hirsutum* and *G. barbadense*) was the first cultivated, native malvaceous plant in Latin America to known to suffer attack of a whitefly-transmitted virus, probably as a result of introduction and intensive use of insecticides in cotton production in the 1950s throughout Latin America (Costa et al. 1973; Spillari 1994). The causal agent could be readily transmitted from malvaceous weeds – notably *Sida micranta* – to cotton by *B. tabaci*, but not from infected to healthy cotton (Costa 1937). Cotton was also the first cultivated plant affected by a virus transmitted by *B. tabaci* in northwestern Mexico (Brown and Nelson 1987). This crop was suspected to play a major role in the epidemiology of these viruses as early as the 1950s, even though it had not yet become an important host – with the notable exception of *Cotton leaf crumple virus* in northwestern Mexico (Idris and Brown 2004) and Guatemala (Spillari 1994) – of begomoviruses in Latin America.

## *Original Begomovirus Reservoirs*

The important role of weeds as sources of begomoviruses infecting cultivated plant species has been known to Latin American virologists since the 1950s. In Brazil, a *B. tabaci*-transmitted virus found in malvaceous weeds of the genera *Malva* and *Sida* was transmitted to cotton (Costa and Carvalho 1960a). The begomoviruses found in *Jatropha gossypifolia* and *Euphorbia prunifolia* (Euphorbiaceae) in Puerto Rico (Bird 1957; Bird and Maramorosch 1978) and Brazil (Costa and Bennett 1950), respectively, were found to be pathogens of common bean, *Phaseolus vulgaris*, demonstrating the capacity of begomoviruses to infect plant species in different families. The common bean disease described in Brazil as bean mottle dwarf – currently known as bean dwarf mosaic – was shown to be induced by a virus transmitted by *B. tabaci* from malvaceous weeds in the mid 1960s. However, while this virus was common in its wild hosts, it was not an important pathogen of the common bean (Costa 1975). These observations suggest that the original begomoviruses found in Latin America were well adapted to their wild hosts but not to cultivated plant species native to Latin America, such as the common bean or cotton. Nevertheless, the potential role of wild species in the epidemiology of begomoviruses was noted. In the mid-1970s, Costa pointed out that malvaceous species of the genus *Sida* were important reservoirs of viruses transmitted by *B. tabaci* to bean, cotton, okra, soybean, and other crops in Brazil (Costa 1976). Recent examples of malvaceous species acting as reservoirs of different begomoviruses include: okra, *Abelmoschus esculentus*, infected in Mexico by a begomovirus related to a virus originally detected in *Sida* (Torre-Almaraz et al. 2004); a yellow mosaic of lima bean, *Phaseolus lunatus*, in northern Peru, shown in 2006 to be caused by a begomovirus related to *Sida mottle virus* from Brazil; and a begomovirus infecting Passifloraceae in Colombia (FJ Morales and AK Martínez unpublished data).

The diverse nature of the begomoviruses found in wild hosts was also apparent to the early virologists. Wild *E. prunifolia, Leonurus sibiricus, Phaseolus longepedunculatus,* and species of *Sida* were shown to be commonly infected by different viruses transmitted by *B. tabaci* (Costa and Carvalho 1960b). A recent study

including *Aspillia tenella* (Compositae), *Desmodium uncinatum, Macroptilium lathyroides, Rhynchosia minima,* (Leguminosae), *Malva* sp., *Malvastrum* sp., *Sida rhombifolia* (Malvaceae), *E. prunifolia* (Euphorbiaceae), *Melochia villosa* (Sterculiaceae), and *Pavonia* sp. (Boraginaceae) showed all of these species to be sources of different begomoviruses transmitted by *B. tabaci* to common bean. In addition to infecting common bean, the begomoviruses present in *M. lathyroides* and *R. minima* also infected pigeon pea (*Cajanus cajan*), demonstrating the presence of different begomoviruses in these weeds (Morales 2006a).

## *The Vector:* Bemisia tabaci

As discussed above, the origin and time of introduction of the first *B. tabaci* individuals in Latin America is uncertain, although this pest could have been introduced from Africa or Asia during the active slave and commercial trade that took place in colonial times (sixteenth–nineteenth centuries) between the Spanish and Portuguese colonies of Latin America, Asia (India), and West Africa. Today, *B. tabaci* is considered a complex of species or races, exhibiting a variety of behaviors with respect to host preference, fecundity, adaptation, and efficiency of begomovirus transmission (De Barro et al. 2005; Perring 2001; Pickersgill 1977). Phylogenetic analyses of *B. tabac*i's mitochondrial 16S ribosomal subunit and cytochrome oxidase 1 (mtCO1) separates Old World from New World populations of *B. tabaci* (Frolich et al. 1999). Originally, the so-called A biotype predominated in the Americas until the introduction of the B biotype in the late 1980s (Bellows et al. 1994), also of Old World origin. Recently, the *B. tabaci* species complex was differentiated into six races using ITS1 and CO1 nucleotide sequences (De Barro et al. 2005; see also Brown Chapter 2 ). This investigation showed that there was only one major race in the Americas, the "New World" race, until the B – and more recently the Q biotypes, were introduced into the Caribbean region (Brown 2007; Segarra et al. 1990). The molecular characterization of genetic variability present in *B. tabaci* populations – and resulting differentiation into races – is currently the most recommended approach. However, the original concept of races of *B. tabaci* was introduced in Puerto Rico in the late 1950s (Bird and Maramorosch 1978; Bird and Sanchez 1971), based on the existence of *B. tabaci* populations that differed in their ability to feed or breed on specific host plants and ultimately, in their efficiency to transmit different begomoviruses. A similar proposal was advanced in Brazil (Flores and Silberschmidt 1958), although the authors used the term "ecological biotypes" rather than "races" to define *B. tabaci* populations that exhibited marked host preferences.

Despite the knowledge that host-specific whitefly populations may arise naturally in response to the availability of suitable reproductive plant hosts in a particular agroecosystem, it was also known that *B. tabaci* populations could rapidly adapt to non-preferred hosts in the absence of their primary reproductive hosts (Costa 1965, 1975). Furthermore, the arrival of the B biotype of *B. tabaci* in the Americas – characterized by its extraordinary ability to feed on a very broad range of host plants (De Barro 1995) – further minimized the possible role of the "physiological races"

described by Bird in the epidemiology of begomoviruses (Bird and Maramorosch 1978; Bird and Sanchez 1971). This observation would be particularly relevant in such Latin American countries as Guatemala, Panama, Cuba, Dominican Republic, Puerto Rico, Jamaica, Brazil, Bolivia, and Colombia where, to a large extent, the B biotype has displaced the original A biotype of *B. tabaci* (Morales 2006a).

The introduction of the B biotype of *B. tabaci* in the Americas – and its gradual predominance in the agricultural regions originally colonized by the A biotype – posed a logical question regarding comparative ability to transmit the existing New World begomoviruses. Evidence suggesting that the B biotype was not as efficient as the original A biotype of *B. tabaci* as a vector of New World begomoviruses was first provided in the southwestern United States (Duffus et al. 1992). Nevertheless the B biotype caused more direct damage than the A biotype after its introduction from Florida around 1988 (Brown 1994). A recent study conducted at CIAT, Colombia (ME Cuellar and FJ Morales unpublished data), showed that although the invasion of B biotype resulted in an epidemic of begomovirus and collapse of the snap bean industry in the Cauca Valley of Colombia – the original A biotype is a more efficient vector of the virus. In this study, single insect transmission efficiency averaged 4.9% for the B biotype compared to 26.6% for A biotype. However, the higher populations of B biotype – over 1,000 nymphs per bean leaf in the Cauca Valley – made this difference in transmission efficiency irrelevant in epidemiological terms. In this investigation, the efficiency of transmission of the B biotype increased significantly during the 3 years of the study, suggesting a gradual adaptation of the virus to the new whitefly biotype. The recent introduction of the Q biotype of *B. tabaci* into Mexico and Guatemala (Brown 2007) poses a new challenge to Latin American farmers.

## *The Origin of Begomovirus Epidemics in Latin America*

It has been mentioned that cotton was the first crop reportedly affected by *B. tabaci* and begomoviruses in Latin America. A high demand for cotton in the international market of the 1940s substantially increased the area planted in Mexico, Central America, and Brazil. For instance, the Pacific lowlands of Central America saw an increase in the area planted with cotton in the 1940s from a few thousand hectares to over 300,000 ha in the 1970s (Spillari 1994). In Brazil, large *B. tabaci* populations developed on cotton in the northern zones of the state of Paraná and in Ourinhos, São Paulo, in 1968 (Costa et al. 1973). Cotton pests have been controlled with different insecticides, such as DDT, methyl parathion, toxaphene and malathion, at an ultra low volume (Gilliand et al. 1971), a practice which probably accelerated the resistance in *B. tabaci* and the elimination of its biological control agents.

The critical role played by extensive cultivation of a suitable reproductive host of the whitefly *B. tabaci* in the epidemiology of begomoviruses is best illustrated by the emergence of *Bean golden mosaic virus* (BGMV), the type species for the genus *Begomovirus*. In 1965, Costa (1965) described three whitefly-transmitted viral

diseases of common bean in Brazil including bean golden mosaic. This disease had been observed in the state of São Paulo since 1961, but was not considered "of sufficient economic importance." A decade later, Costa (1975) reported the presence of bean golden mosaic in Paraná, another important bean-growing state of Brazil. He attributed the rapid dissemination of this disease to an exponential increase in soybean plantings, which increased from 1.3 million ha in the early 1970s, to over 6 million ha by 1976 (FAO 1994). By 1990, BGMV had already reached all of the main bean-growing states of Brazil (Morales and Anderson 2001). It is also probable that the use of broad-spectrum insecticides to control insect pests in soybeans – such as stink bugs and caterpillars (Gazzoni et al. 1999) – may also have contributed to the elimination of the natural enemies of *B. tabaci* in Brazilian soybean. By 1975, there were over 10 million ha planted to soybeans in Brazil and Argentina, coinciding with the onset of noticeable outbreaks of BGMV and other begomoviruses of common bean, such as *Bean dwarf mosaic virus* (BDMV). This begomovirus would later (1978–1981) cause the destruction of over 100,000 ha of highly prized common bean varieties in northwestern Argentina (Morales and Anderson 2001). Although the situation with respect to cultivar susceptibility did not change drastically with the arrival of the B biotype to the Americas, this event did result in isolated but severe episodes of direct damage caused by the B biotype of *B. tabaci* to soybean plantings in northwestern Mexico (INIFAP 1995), coastal Ecuador (Mendoza 1996), and Brazil (Lima and Lara 2004).

## Main Crops Affected by Begomoviruses in Latin America

### *Common Bean*

Common bean (*Phaseolus vulgaris* L.) was domesticated in different regions of Latin America leading to various geographical "races" distributed from Mexico to Chile (Singh 1988). Latin America is still the main producer and consumer of common bean in the world, with over 10 million ha grown throughout the region (http://faostat.fao.org). Common bean has been the second most important food staple, after maize, in Latin America since pre-Columbian times. The first outbreaks of begomoviruses in Latin America changed the concept of viral diseases of common bean due to the significant and often complete yield losses that compromised food security in the region.

#### South America

Bean golden mosaic was the first disease that showed the epidemiological potential of viruses transmitted by *B. tabaci* in Latin America. From 1961 on, this disease had been observed at a low incidence in the state of São Paulo. Nevertheless, Costa's (1965) predictions regarding the epidemiological potential of this disease were fulfilled the following decade when bean golden mosaic was disseminated into the

main common bean-producing states of Brazil: Paraná, Minas Gerais, Goiania, and Bahia (Costa 1975). For the first time, over 2 million ha of common bean were at risk of significant, and often total, yield losses, as the virus spread to many other bean-growing areas of Brazil (Ferreira et al. 2002). Bean golden mosaic continued to spread west and north [northwest], as soybean cultivation was extended – currently over 21 million ha – into all traditional common bean production areas of Brazil (http://faostat.fao.org). The dissemination of bean golden mosaic in northern Brazil was finally arrested by the eastern boundaries of the Amazon forest, an area characterized by its abundant rainfall averaging over 3,000 mm per year, – its unsuitability for extensive production of annual crops, and thus, development of significant *B. tabaci* populations. Bean golden mosaic occasionally crossed the southern boundary of the tropical zone, into the southernmost state of Brazil, Rio Grande do Sul. However, the main hotspot of bean golden mosaic in Brazil was located within the tropical zone near the so-called "Minas Gerais Triangle" (about 20°S–50°W). To the southwest lies the Gran Chaco, a hot and dry alluvial plain that covers parts of Brazil, Argentina, Paraguay, and Bolivia traditionally devoted to cotton production, and known as a major whitefly hotspot, although there are no formal reports in the literature. Although there are no reports that cotton was affected by begomoviruses in this zone, the Gran Chaco has probably played an important role as a bridge for BGMV between southern Brazil and northwestern Argentina (Morales and Anderson 2001) where bean golden mosaic emerged around 1983. The ensuing epidemics of BGMV in northwest Argentina were also closely associated with the expansion of soybean production there, from over 400,000 ha in 1975 to three million ha in 1985 (Morales and Anderson 2001). In the late 1970s, prior to the emergence of BGMV in northwest Argentina, a major outbreak of a disease – associated with the emergence of *B. tabaci* as a new pest – completely destroyed over 60,000 ha of common beans. In 1981, the author characterized this disease as bean dwarf mosaic after a similar syndrome originally described by Costa (Costa 1975) in Brazil caused by a begomovirus transmitted from malvaceous species of *Sida* to common bean. The bean dwarf mosaic disease of common bean in northwest Argentina was shown to be induced by one or more begomoviruses associated with *Sida* spp. (Morales 2006a), the most abundant weeds found around common bean plantings in this region. This observation supports the putative importance of *Sida* species as reservoirs of economically important begomoviruses in Latin America (Frischmuth et al. 1997; Höfer et al. 1997; Jovel et al. 2004; Lima et al. 2002a; Rampersad and Umaharan 2003).

As mentioned above, the emergence of *B. tabaci* as a new pest and virus vector in northwest Argentina in the late 1970s was associated with the introduction of extensive soybean production in the region – particularly in the provinces of Santiago del Estero, Tucuman, and Salta – where approximately 150,000 ha of common bean were planted soon after the harvest of a similar area planted with soybeans. In the absence of other suitable hosts, the whitefly populations that bred on soybeans were forced to move onto the newly planted common bean fields.

The soybean boom in South America did not stop until it covered some 35 million ha in Brazil, Argentina, and the tropical plains of the Bolivian provinces of

Santa Cruz de la Sierra and Tarija (http://faostat.fao.org). In the provinces east of the Bolivian Andes, the area planted with soybeans increased from less than 6,000 has in 1973 to over 400,000 has in 1995 (Morales and Anderson 2001). BGMV emerged in this region of Bolivia around 1992, and molecular tests showed that the Argentine, Bolivian and Brazilian begomoviruses associated with this disease in the three countries were the same species originally characterized in Brazil (Morales and Anderson 2001). The genetic stability of BGMV was also confirmed in Brazil (Faria and Maxwell 1999) despite the introduction in 1991 of the B biotype of *B. tabaci* in this country (Lima et al. 2002).

The distribution of BGMV in Brazil is closely linked to that of *B. tabaci* in this region. The vector is limited by the high precipitation and scant agriculture of the Amazon forest to the north, low winter temperatures of the temperate zone south of the Tropic of Capricorn, low temperatures of the Andean highlands to the west, and the Atlantic Ocean to the east. Ecologically, this region is categorized as tropical savanna: grasslands with scattered trees and/or shrubs in regions with alternating wet and prolonged dry seasons. Rainfall in these ecosystems is moderate at best with an average annual precipitation ranging from 400 to 800 mm in the drier areas and 1,100–1,600 mm in the central plateau (Archibold 1995; Cole 1986). The dry season (May–September) corresponds to winter in the southern hemisphere, although with average temperatures (12–18°C) still favorable for the development of *B. tabaci*, albeit at a lower reproductive rate (Zalom et al. 1985). Relative humidity in the 30% range may be a limiting factor for the reproduction of *B. tabaci* during wintertime, but dew often falls at night. Rainfall may take place in July and August in some regions. The normal summer rains between October to April provide moisture during the warmer periods of the year which are favorable for crop production, and *B. tabaci* populations are then seen to increase on newly planted hosts. Sometimes the warmer temperatures occur in September before the rainy season starts.

The Minas Gerais Triangle mentioned above as a hotspot in Brazil corresponds to the main area of savanna woodlands and grasslands – or "cerrados" – in the states of Minas Gerais, Goias and Mato Grosso. Some outliers – "campos cerrados" – of characteristic savanna vegetation occur in the states of Bahia, Sergipe, Alagoas, and Pernambuco where begomoviruses have also become important pathogens of several crops in the last couple of decades. These are the low tree and shrub savannas of the Brazilian "sertão" or "caatinga." (Cole 1986). A probability model clustered 5 climates in the BGMV region (Morales and Jones 2004), which includes northwestern Argentina and the southwestern lowlands of Bolivia, all of which have well-defined dry seasons lasting at least 4 months during which rainfall averaged under 80 mm/month, typical of savanna regions.

### Central America

In Central America, the golden mosaic of common bean had been observed since the late 1960s from Guatemala to Panama (Gámez 1970). In the Caribbean Region, bean golden mosaic was first observed in the Dominican Republic (Schieber 1970),

and later in Puerto Rico (Bird et al. 1973), Jamaica (Pierre 1975), and Cuba (Blanco and Bencomo 1978). Isolates could be mechanically transmitted while the Brazilian BGMV isolates were only transmitted by *B. tabaci* (Costa 1976). The Puerto Rican isolate of a begomovirus from lima bean (*P. lunatus*) became the first whitefly-transmitted virus to be cloned and sequenced in Latin America (Howarth et al. 1985; Morinaga et al. 1987). Eventually this virus, named *Bean golden yellow mosaic virus* (BGYMV), became the type species of the genus *Begomovirus*, and the main species that infects common bean from southern Mexico to Colombia in northern South America (Bird et al. 1973; Faria et al. 1994; Gilbertson et al. 1993; Howarth and Vandemark 1989; Morales 2006a; Morales and Jones 2004; Rybicki 1994; Rybicki et al. 2000). Thus, BGMV and BGYMV remain distinct begomovirus species separated by the Andean mountain range and the Amazon region.

In Central America, the Caribbean and southern Mexico, BGYMV and *B. tabaci* generally affect common bean in the lowlands and mid-altitude valleys under 1,000 m of altitude (Morales 2006a; Morales and Anderson 2001; Morales and Jones 2004). Most of the lowlands in this region are also classified as savanna ecosystems; for 55% of the common bean-producing regions of Latin America the affected areas belong to the tropical wet/dry (Aw) climate classification of Koeppen (Morales and Jones 2004). In the Caribbean, most of the islands and agricultural regions have a wet dry climate (Aw), with some arid (BS) areas in irrigation districts. In this region, rainfall is well distributed during the year, with a distinct winter season (December–April) characterized by low to moderate precipitation (>50 mm/month). Common bean plantings can be heavily affected by BGYMV during the winter season due to the lower rainfall and higher whitefly populations that enjoy temperatures up to 23.5°C in January (Morales et al. 2005a, b). In southern Mexico, the main areas affected by BGYMV since 1977 are the Gulf Coast, – Veracruz, Tamaulipas, and Chiapas along the Gulf of Tehuantepec. The dry season in this region lasts from January through April and also coincides with the winter season of the northern hemisphere. However, average low temperatures for the dry season seldom drop below 20°C and thus remain above the lower developmental threshold for *B. tabaci* (Zalom et al. 1985). The zone of Soconusco and Tapachula in Chiapas have been historically planted with cotton and tobacco which are both suitable plant hosts of *B. tabaci.* In Central America, the main common bean production areas and *B. tabaci* populations are found along the Pacific region. Due to the prolonged dry, warm season that lasts from November through April, this region is conducive to the rapid development of *B. tabaci* populations. Cotton – and to a lesser extent tobacco – are still important crops in the Pacific region; both are known hosts of *B. tabaci*. The Caribbean region, on the contrary, receives abundant rainfall 9–11 months of the year.

### Northern Mexico

In 1974, a "yellow mosaic" of common bean was observed in the Valley of Culiacan, Sinaloa, in northwestern Mexico (Lopez 1974). The disease rapidly spread in the region affecting common bean plantings in Baja California Sur; Los Mochis,

Sinaloa; Sonora; and Nayarit south of Sinaloa. Until 1988, the intense yellowing was believed to be caused by BGMV, when a virus isolate from Sonora was shown to be a different begomovirus (Brown et al. 1999). This virus, named *Bean calico mosaic virus* (BCaMV), was shown (Brown et al. 1999; Loniello et al. 1992) to be related to *Squash leaf curl virus* (SLCV), which was first observed in 1977 affecting summer and winter squash in southwestern United States (Flock and Mayhew 1981). A survey conducted in common bean plantings by the author and collaborating Mexican scientists in Los Mochis, Culiacan (Sinaloa), and Etchojoa (Sonora) demonstrated the presence of three different strains related to BCaMV and SLCV (Morales et al. 2005c). Northwestern Mexico is considered a hot, arid region with little rain between the months of October through June, and particularly during March-May. However, this is one of the most dynamic agricultural areas of Mexico. The large volume of the agricultural commodities exported to the United States during the winter time – December to May – are produced thanks to its extensive irrigation districts fed by the Fuerte, Sinaloa, Culiacan, and San Lorenzo rivers. Average low temperatures may occasionally drop below the 10°C degree developmental threshold for *B. tabaci* (Zalom et al. 1985) near the United States border, but average low temperatures in the Valley of Culiacan usually remain above 18°C during the winter season. Melon, soybean, and cotton are the main reproductive hosts of *B. tabaci* in northwestern Mexico (Morales et al. 2005c).

## *Tomato*

Tomato (*Solanum lycopersicum* Mill.) is also native to Latin America, specifically to the western region of central South America from Ecuador to Chile. However, tomato seemed to have been domesticated mainly in Mexico, where the common name "tomatl" – in Nahuatl – comes from. Tomato has been a rather neglected crop in Latin America in terms of crop improvement, area of cultivation, and total production. It is only in the last two decades that tomato has been re-discovered as a high value export crop in Latin America. Unfortunately, different begomoviruses rapidly emerged and have subsequently become the most limiting factor to tomato production in the region.

### South America

The first documented outbreaks of whitefly-transmitted viruses in tomato occured in the 1950s when an "infectious chlorosis" was observed in Brazil (Flores and Silberschmidt 1967; Flores et al. 1960). This disease was apparently related to begomoviruses transmitted from wild malvaceous plants in tomato fields. By 1975, other viral diseases of tomato associated with *B. tabaci* were reported in Brazil (Costa 1974; Costa et al. 1975), including *Tomato golden mosaic virus* (TGMV), the first begomovirus characterized in the Americas (Matys et al. 1975). Currently, there are at least eight begomoviruses affecting tomato production in seven states of eastern Brazil (Andrade et al. 2006; Lima et al. 2000; Ribeiro et al. 2003), some of which

are related to TGMV, while others show a distant relationship to viruses that infect legumes. For example, a begomovirus previously described in Brazil as *Tomato yellow vein streak virus* (ToYVSV) showed 85% identity to BGMV (Faria et al. 1997). Ribeiro and his colleagues (2003) proposed the names *Tomato chlorotic mottle virus* and *Tomato rugose mosaic virus* for two of the tomato begomoviruses detected, and concluded that they were indigenous to Brazil. However, ToYVSV is, in fact, Potato deforming mosaic virus (PDMV), a begomovirus first described in 1962 from the southeastern potato-growing regions of the province of Buenos Aires, Argentina (Calderoni et al. 1962; Delhey et al. 1981). PDMV was also isolated in 1995 in northwestern Argentina from severely affected common bean plants before it was detected in Brazil infecting tomatoes (Morales and Anderson 2001). This observation shows that begomoviruses of common bean and tomato can infect species of the same or different families, and move within the tropical and subtropical agricultural regions formed by eastern Brazil, northern Argentina, and southeastern Bolivia, probably using the Gran Chaco zone of Paraguay and the above-mentioned countries as a natural bridge. Ribeiro and co-authors (2003) also linked the emergence of new begomoviruses in tomato plantings in Brazil to the introduction of B biotype *B. tabaci* in the early 1990s. This aggressive and prolific biotype – unlike the original A biotype – readily colonizes tomato in Brazil (Lima et al. 2002b). Ribeiro et al. (2006) recognized that ToYVSV and PDMV are the same begomovirus, but conclude that "potato deforming mosaic disease" is caused by an isolate of ToYVSV. The correct consideration in these cases is that PDMV was described 35 years before it was inadvertently misidentified in Brazil as a new tomato virus. Therefore, the original name should take precedent regardless of which virus was sequenced first. This is important in epidemiological and disease control terms to avoid conclusions such as the one expressed by these researchers who believed that all the begomoviruses detected "are indigenous to Brazil." More recently, two other tomato begomoviruses have been detected in Brazil: *Tomato yellow spot virus* – shown to be closely related to begomoviruses infecting *Sida* species – and Tomato crinkle leaf yellows virus. These begomoviruses are apparently closely related as they were shown to form pseudorecombinants (Andrade et al. 2006). The historical role of wild malvaceous species in the emergence of new begomoviruses affecting cultivated plant species in this region is reinforced by this report. The other begomovirus of tomatoes in Brazil is *Tomato severe rugose virus* (ToSRV), which seems to be more adapted to species of *Capsicum* than tomato (Bezerra-Agasie et al. 2006; Nozaki et al. 2006).

The second disease of tomato in Latin America was first described in Venezuela in 1963, as a "yellowish mosaic" (Debrot et al. 1963). By 1975, this disease, already named tomato yellow mosaic, had disseminated into the main tomato-producing states of Venezuela: Aragua, Carabobo, Guarico, and Lara (Lastra and Gil 1981; Lastra and Uzcategui 1975). In 1981 and 1985, *Tomato yellow mosaic virus* (ToYMV) was described as an occasional pathogen of potato (*S. tuberosum*) in the state of Aragua, Venezuela. These reports, presented to the scientific community both in English (Debrot 1981) and Spanish (Debrot and Centeno 1985), were apparently unknown by Roberts, Buck, and Coutts (1986) when they obtained

samples of potato plants infected with ToYMV from Venezuela and proceeded to publish a report on "Potato yellow mosaic, a new geminivirus infecting potatoes." Unfortunately, ToYMV is the most important neotropical begomovirus of tomatoes in the Caribbean Basin, where it affects tomato production in Guadeloupe, Martinique, Trinidad and Tobago, Puerto Rico and the Dominican Republic in the Antilles (Polston et al. 1998; Urbino et al. 2004), Panama (Engel et al. 1998), Colombia (Morales 2006a), Venezuela (Nava et al. 2006), and other Latin American regions considered part of the Caribbean Basin. Furthermore, potatoes are primarily grown in the highlands of Latin America and are not commonly found in the agricultural areas affected by ToYMV. In order to correct this misnomer, an investigation was conducted in 2000 to characterize an original isolate of ToYMV preserved in Venezuela since the early 1980s. The molecular characterization of this original ToYMV isolate clearly showed that it had over 95% nucleotide and amino acid sequence identities with the so-called "*Potato yellow mosaic virus*" isolate from Venezuela (Morales et al. 2001).

Tomato yellow mosaic has been spreading in the Caribbean Basin from Venezuela through the Lesser Antilles (Trinidad & Tobago, Martinique, and Guadeloupe) and eventually reaching Puerto Rico and the Dominican Republic in the Greater Antilles. This chain of islands is close enough to act as a natural bridge for whiteflies, particularly in a region affected every year by tropical storms and hurricanes suspected of aiding the dissemination of whiteflies and begomoviruses in the Caribbean Basin. However, one cannot discard the possibility of illegal transport of infected seedlings or plant material infested with viruliferous *B. tabaci* individuals in this region. The latter is probably the way ToYMV reached Panama without affecting the tomato plantings found in the northern coast of Colombia. However, ToYMV has finally disseminated into Colombia – probably down the Magdalena Valley – transported either by whiteflies or by farmers. ToYMV has also overcome the natural barrier of the central range of the Andes to reach the Cauca Valley department in western Colombia, a nascent tomato production region (Morales et al. 2002). In Colombia, there is evidence that ToYMV and the B biotype of *B. tabaci* are being transported on commercial tomato seedlings from warm, mid-altitude valleys (under 1,000 m) to tomato-producing areas in the central highlands of Colombia (above 1,500 m), including the departments of Cundinamarca and Tolima, where these pests were not present in the past (AR Corrales and FJ Morales unpublished data).

In Venezuela, ToYMV has continued to disseminate from the original hotspot in the state of Aragua to neighboring states to the east, south, and predominantly west into the Andean states of Trujillo, Merida and Tachira (Nava et al. 2006). This epidemiological tendency may be associated with the drier and warmer climates found in northwestern Venezuela and northeastern Colombia. The entry point for ToYMV into Colombia was probably the neighboring department of Northern Santander, as suggested by the detection of ToYMV in this region of Colombia – not far from the central highlands – where ToYMV has emerged as well (unpublished data). The rapid dissemination of ToYMV is closely associated with the presence and higher activity of the B biotype of *B. tabaci* in most of the agricultural areas of Colombia

(Rodriguez et al. 2005) and Venezuela (Tropical Whitefly IPM Project unpublished data). In 2001, a survey conducted in tomato growing areas of the state of Lara, Venezuela, resulted in detection of a new begomovirus species related to *Merremia mosaic virus* (Morales 2006a).

Begomoviruses are just beginning to emerge in other tomato-producing areas of South America – namely in Peru (Murayama et al. 2005) – and in temperate countries of the Southern Cone such as Uruguay (D. Maeso, INIA-Uruguay, personal communication). As tomato production increases due to the development of promising tomato industries in countries such as Chile and Peru, one should expect the emergence of new tomato begomoviruses further south of the current geographic boundary (about 30°S).

## Mexico

Northwestern Mexico has been considered one of the main hotspots for whitefly-transmitted viruses in Latin America, due to its intensive irrigated agriculture and dry and hot agroecosystems. In 1971, growers in Culiacan, Sinaloa, noticed a foliar malformation of their tomato plants, which they described as "enchinamiento" (curling) (Gallegos 1978). The causal virus was eventually characterized (Brown and Nelson 1988) as a begomovirus (*Chino del tomate virus*) that has now disseminated into several tomato-growing states of Mexico, including: Jalisco, San Luis Potosi, Guanajuato, Michoacan, Tamaulipas, Morelos, Chiapas, and Baja California Sur (Garzon-Tiznado et al. 2002; Hernandez 1972; Holguin-Peña et al. 2003, 2005; Montes-Belmont et al. 1995; Torres-Pacheco et al. 1996). Another begomovirus originally detected in northwestern Mexico in the early 1990s was named *Tomato leaf curl Sinaloa virus* (Brown et al. 1993). However, this name is confusing because *Tomato leaf curl virus* is a begomovirus that only exists in the Old World (Fauquet et al. 2005). The Sinaloa virus probably originated in pepper fields in Texas, United States, where it has been observed since 1988 (Idris and Brown 2004); it has now disseminated down the Pacific coast of Mexico and Central America infecting tomato plantings in Nicaragua (Rojas et al. 2000) and Costa Rica (Idris et al. 1999). The Huasteca region of Mexico – formed by southern part of the northeastern state of Tamaulipas, eastern San Luis Potosi, and northern Veracruz – witnessed the emergence of Pepper Huasteco virus in 1988, which was later re-named *Pepper Huasteco yellow vein virus* (PHYVV). This begomovirus, whose correct name should be Pepper yellow vein Huasteco virus (PYVHV), is an important pathogen of tomato in the central Mexican states of Jalisco, Morelos, and Hidalgo (Morales et al. 2005c).

*Pepper golden mosaic virus* (PepGMV), formerly Texas pepper virus, was first reported from Texas in 1987 (Stenger et al. 1990), and currently affects tomato production in Baja California Sur, Nayarit, Hidalgo, and Oaxaca. Tomato plants doubly infected with PYVHV and PepGMV, are commonly observed in this region (Holguin-Peña et al. 2004; Morales 2006a). The dissemination of these begomoviruses into central Mexico is somewhat unexpected given the mountainous nature of the central region of Mexico and the existence of mountain ranges between the northwestern coast of Mexico and the central highlands. However, this region

has numerous natural depressions between 500 and 1,000 m, where agriculture takes place and *B. tabaci* can thrive. Moreover, these regions share a similar ecosystem characterized by arid climates and prolonged dry seasons as well as a cotton-based agriculture suitable for the development of *B. tabaci* (Cardenas et al. 1996).

## Central America

The Central American region has been the point of origin of several begomoviruses that infect tomato, though some of these begomoviruses probably originated in either Mexico or southern United States (Polston and Anderson 1997) and spread to this region due to the rapid expansion of non-traditional export crops (Morales and Anderson 2001). PepGMV was isolated in Guatemala from affected tomato plants (Mejia et al. 1998; Morales and Anderson 2001), and *Tomato severe leaf curl virus* (ToSLCV) was later described from Guatemala, Honduras (Nakhla et al. 1994), and Nicaragua (Rojas 2005; Rojas et al. 2000). Curiously, this begomovirus was detected last year in Central Mexico – San Luis Potosi and Morelos – causing severe stunting and leaf curling symptoms in tomato fields invaded by high populations of *B. tabaci* (Mauricio-Castillo et al. 2006). Another begomovirus, Tomato mild mottle virus – originally described from Central America (Maxwell et al. 2002; Rojas et al. 2000) – has also been detected in Baja California Sur, Mexico (Holguin-Peña et al. 2005). The name of this virus should be changed considering that it already belongs to a different plant virus in Africa (Walkey et al. 1994).

In El Salvador, begomoviruses have practically brought tomato cultivation to a complete halt in many of the traditional tomato-producing areas of this country. El Salvador was selected as the regional base of the Tropical Whitefly IPM Project (TWFP); consequently, the project had been monitoring the incidence of begomoviruses in this country since 1997 – particularly in common bean, tomato and peppers (Morales 2006a).

The predominant begomovirus affecting tomato in El Salvador seems to be *Tomato mottle virus* (ToMoV), a begomovirus originally described from Florida, United States (Abouzid et al. 1992b). ToSLCV and *Tomato mosaic Havana virus* (ToMHV) were both present. The latter virus was previously identified in Cuba (Martinez-Zubiaur et al. 1998). Tomato in El Salvador is also infected by a virus originally isolated by the TWFP in 1998 from *Cucurbita argyrosperma* (pipián), which is a popular food staple in this country (Morales 2006a). This begomovirus named *Squash yellow mild mottle virus* (SYMMoV) (Karkashian et al. 2002) was later found in Costa Rica infecting squash. Interestingly, this virus was also found on in Nicaragua, where it infects both cucurbits (Ala-Poikela et al. 2005) and tomatoes (Rojas 2005; Rojas et al. 2000).

Honduras has also witnessed the destruction of its tomato fields by begomoviruses, particularly in the Valley of Comayagua. In a recent survey of various tomato-producing regions of central Honduras, the TWFP and scientists from the Pan-American Agricultural School (Zamorano) detected the presence of begomoviruses in tomatoes and peppers. These virus isolates proved to be a single begomovirus species showing 98 and 97% identities at the amino acid level with

fragments of the replication (AC1) and capsid (CP) proteins respectively, and of ToMHV, the Cuban tomato mosaic begomovirus also described above from El Salvador (FJ Morales and MM Roca unpublished data). In 2004, a sample from an infected sweet potato plant showing severe virus-like symptoms was assayed by PCR; the fragment amplified confirmed the presence of a begomovirus closely related (95%) to a *Sweet potato leaf curl virus* (SPLCV) isolate from the United States (Lotrakul et al. 2002).

A few begomoviruses apparently related to SPLCV have been isolated from sweet potatoes in Latin America including Brazil, Peru, Costa Rica, Mexico, and Puerto Rico (Fuentes and Salazar 2003; Lotrakul et al. 2000), but there is no sequence data for these viruses. The genomic organization of SPLCV is similar to that of monopartite begomoviruses. The fact that sweet potato leaf curl was first observed in Japan and Taiwan may suggest that SPLCV could be the second Old World begomovirus introduced in the Americas. However, sweet potato leaf curl may be associated with different begomoviruses in Asia (Loebenstein et al. 2003) and probably in the Americas.

In Nicaragua, different begomoviruses isolated from diseased tomato plants have been identified as the so-called "Tomato leaf curl Sinaloa virus", Euphorbia mosaic virus; SYMMoV – a begomovirus previously detected in the neighboring country of Costa Rica infecting cucurbits, and PepGMV (Karkashian et al. 2002; Rojas 2005; Rojas et al 2000). A recent survey conducted by the TWFP in collaboration with the National Agricultural University (UNA) detected a begomovirus associated with severe foliar malformation of tomato plants in the locality of Tisma located not far from the capital Managua. This begomovirus had a partial sequence similarity over 90% when compared to the corresponding region of *Chino del Tomate virus* from Mexico. Other tomato samples gave identities of 88% with *Sida yellow vein virus* and *Okra yellow mosaic virus* from Mexico. ToSLCV was once again detected in tomatoes during this survey, respectively (FJ Morales and E Jimenez unpublished data).

Costa Rica also increased the area planted with tomato, particularly in the central valley. The first begomovirus of tomato identified in Costa Rica was *Tomato yellow mottle virus*, originally referred to – and misidentified – as "Tomato yellow mosaic." (Castillo 1997). The second begomovirus isolated from tomatoes in this country was again the so-called Tomato leaf curl Sinaloa virus (Idris et al. 1999). Finally, PepGMV was detected in Costa Rica (Karkashian et al. 1998; Lotrakul et al. 2000), demonstrating that some begomoviruses can be widely disseminated in a large geographic area spanning the southwestern United States, Mexico and five countries in Central America.

Tomato production is an important activity in the dry region of Azuero, Panama. The first begomovirus affecting tomato production in this region was described in 1998 (Engel et al. 1998) as Tomato leaf curl virus, but was later recognized as a strain of ToYMV. This virus is currently listed as a distinct species, Potato yellow mosaic Panama virus, but it is yet another pathogenic variant of ToYMV. A recent survey of the Azuero region confirmed the endemic nature of this begomovirus in tomato plantings.

### Caribbean Region

Tomato production is a very important agricultural and industrial activity in the Caribbean region (Morales 2006b; Morales and Anderson 2001; Morales et al. 2005a, b). The importance of begomoviruses affecting tomato production in the Caribbean region is closely associated with the careless introduction of the most important begomovirus of tomato in the Old World – *Tomato yellow leaf curl* (TYLCV) – in this region and the Americas (Polston et al. 1996). This virus rapidly spread on Hispaniola, paralyzing the tomato paste industry in the Dominican Republic and Haiti (Morales 2006a; Morales and Anderson 2001). TYLCV continued to disseminate in the Caribbean, reaching Cuba, Puerto Rico, Guadeloupe, and Jamaica (Bird et al. 2001; Gonzales and Valdes 1995; McGlashan et al. 1994; Urbino and Tassius 1999). In the late 1990s, TYLCV was detected in Yucatan, Mexico (Bellows et al. 1994). This virus is not only a major pathogen of tomatoes, but can also infect other cultivated species such as peppers, tobacco, common bean, and cucurbits (Dalmon and Marchoux 2000; Martinez-Zubiaur et al. 2002, 2003, 2004). We have already described the dissemination path of the most important neotropical begomovirus in the Caribbean – ToYMV – from its center of origin in Venezuela. Most of the remaining tropical American begomoviruses reported to affect tomatoes in the Caribbean have been detected in Cuba. In 1997, *Tomato mottle Taino virus* (ToMoTV) and ToMHV were identified on this island (Martinez-Zubiaur 1998; Ramos et al. 1997, 2003). ToMoTV is very similar to ToYMV; these two Caribbean begomoviruses can, for example, form pseudorecombinants (Ramos et al. 1997). In Puerto Rico, four begomoviruses were known to infect tomatoes: TYLCV, ToYMV, ToMoV, and a begomovirus isolated from the weed *Merremia* sp. (Idris et al. 1998). A begomovirus related to the latter virus was found by the author to be infecting tomatoes in Venezuela. In Jamaica, TYLCV has been present since the early 1990s (McGlashan et al. 1994), in addition to a new begomovirus named Tomato dwarf leaf curl virus (Roye et al. 1999).

While the Yucatan Peninsula is part of Mexico, it is eco-geographically integrated into the Caribbean region. Yucatan is classified as an ecosystem belonging to the "Dry Tropics" although the annual precipitation ranges from 600 to 1,500 mm. Peak *B. tabaci* populations in this zone occur in May and June, after 5 months (December–April) of dry weather (0–18 mm/month). Tomato production has been affected in the states of Yucatan, Campeche and Quintana Roo since 1991 by begomoviruses that include TYLCV, ToMoV, PepGMV and PYVHV (Diaz-Plaza et al. 1996; Garrido-Ramirez and Gilbertson 1998; Morales 2006a).

## *Sweet and Hot Peppers*

Peppers (*Capsicum* spp.) have been severely affected by begomoviruses in Latin America, particularly in mixed cropping systems where *B. tabaci* is present. As mentioned above, some of the begomoviruses that affect tomato were originally characterized as pathogens of peppers – for instance PYVHV and PepGMV. PYVHV was first reported in the 1980s as a disease of Serrano pepper (*Capsicum*

*annuum*) in the Huasteca plateau (Leal and Quintero 1989). A survey conducted by Dr. Rafael Rivera-Bustamante for the TWFP in Mexico detected PYVHV in the states of Campeche, Colima, Nayarit, Guanajuato, Veracruz, Morelos, Hidalgo, Queretaro, and San Luis Potosi (Morales et al. 2005c). This virus has also been reported to infect peppers in the northwestern state of Sonora (Ramirez et al. 1998) and is a common virus in the Yucatan Peninsula (Diaz-Plaza et al. 1996; Morales 2006a). PepGMV is an important hot pepper production constraint in the states of Colima, Nayarit, Veracruz, Oaxaca, Aguascalientes, Morelos, and San Luis Potosi (Morales et al. 2005c) and also affects sweet peppers in the state of Coahuila (Bravo-Luna et al. 2000). PepGMV has been isolated from Tabasco peppers (*C. frutescens*) and Habanero peppers (*C. chinense*) in Costa Rica (Lotrakul et al. 2000), and most recently in Nicaragua affecting sweet peppers (FJ Morales and AK Martinez unpublished data). On the other hand, some tomato begomoviruses – such as Tomato leaf curl Sinaloa virus – were originally isolated from peppers in the state of Sinaloa, northwestern Mexico (Brown et al. 1993), Nicaragua, and Costa Rica. In the Caribbean, Tomato dwarf leaf curl virus also affects peppers in Jamaica (Roye et al. 1999) and it is commonly found in mixed infections with TYLCV. The latter virus has been isolated from peppers in Cuba (Quiñones et al. 2001) and Yucatan, Mexico (Ascencio-Ibañez et al. 1999). Recently, a begomovirus closely related to *Cabbage leaf curl virus* – originally described in Florida – was isolated in Cuba from *C. annuum* plants showing stunting and severe foliar malformation (Abouzid et al. 1992a; Martinez-Zubiaur et al. 2006). In Trinidad & Tobago, begomoviruses related to PYVHV and ToYMV have been isolated from *C. annuum* and *C. frutescens* (Umaharan et al. 1998). Evidence for the infection of peppers by ToYMV was also provided by a recent investigation conducted in Colombia (Morales 2006a). In South America, sweet peppers have been infected by begomoviruses in northeastern Brazil (Lima et al. 2001) and Colombia (Morales 2006a).

Recently, a yellow mosaic and foliar distortion of chilli peppers (*C. baccatum*) observed in the states of Goias and São Paulo, Brazil [was shown to be induced] by ToSRV (Bezerra-Agasie et al. 2006; Nozaki et al. 2006).

## *Cucurbits*

The cultivated Cucurbitaceae include two genera of American origin: *Cucurbita* and *Sechium* (Saade 1995). The first epidemics of begomoviruses in cucurbits were observed in the southwestern United States and northwestern Mexico in 1976 (Flock and Mayhew 1981) and 1981 (Dodds et al. 1984; McCreight and Kishaba 1991). The main disease induced by a begomovirus – referred to as "squash leaf curl" – affected squash (*Cucurbita maxima*) and other cucurbit species (*C. argyrosperma, C. pepo,* and *Cucumis melo*), causing severe foliar malformations. Squash leaf curl was shown to be caused by SLCV, a bipartite begomovirus (Lazarowitz and Lazdins 1991). SLCV has been detected in Arizona and later in Texas, where it was first observed (Isakeit and Robertson 1994) infecting watermelon (*Citrullus lanatus*). In 1990, SLCV was detected in the state of Sonora,

northwestern Mexico, and farther south in 1992, affecting "calabacita" (*Cucurbita pepo*) in the state of Sinaloa (Ramirez et al. 1995). SLCV-like symptoms had already been observed in experimental cucurbit plots in Los Mochis and Culiacan, state of Sinaloa, in 1988 (Silva et al. 1994).

Surveys conducted in 1999 by the TWFP in Central America revealed the presence of the begomovirus in melon in the Valley of Zacapa, Guatemala, and in "pipian" (*C. argyrosperma*) and "ayote" (*C. moschata*) in El Salvador. The begomoviruses isolated from melon and pipian had partial sequence identities of 85% between them, and sequence identities above 80% when compared to SLCV (Morales 2006a). The begomovirus detected in melon in 1999 was later re-isolated by other scientists from the same location and named *Melon chlorotic leaf curl virus* (MCLCuV) (Brown et al. 2001). The begomovirus isolated from *C. argyrosperma* in El Salvador was also found later on in Costa Rica infecting squash, and was named "*Squash yellow mild mottle*" (Karkashian et al. 2002). This virus was recently detected in Nicaragua infecting cucurbits (Ala-Poikela et al. 2005), where it was also known to infect tomato (Rojas 2005; Rojas et al. 2000). However, the name given to this virus in Costa Rica appears in the GenBank as a synonym of MCLCuV. In 1998, a begomovirus was isolated from melon showing chlorosis and leaf rugosing in the Caribbean region of Colombia Department of Atlantico. This disease, referred to as "melon chlorosis" (Morales et al. 2000), was associated with the introduction of biotype B of *B. tabaci* in northern Colombia. A short sequence of a begomovirus was obtained by PCR that had 94% identity with a portion of the coat protein of SLCV and MCLCuV (unpublished data). A similar begomovirus named Melon chlorotic mosaic virus was recently reported from Venezuela. This begomovirus is also closely related (>80%) to SLCV (Ramirez et al. 2004). TYLCV was also recently isloated from squash (*Cucurbita pepo*) in Cuba (Martinez-Zubiaur et al. 2004).

The proliferation of species names for American begomoviruses is closely associated with the practice of species demarcation based on a nucleotide identity threshold of <90% as set by the *Geminiviridae* Study Group of the International Committee on Taxonomy of Viruses (Fauquet et al. 2005). The capacity of SLCV to generate new pathogenic variants – currently considered as distinct species – will likely continue, as suggested from the numerous begomoviruses of cucurbits described in past years.

## *Potato*

The first disease of potato – *S. tuberosum* – caused by a begomovirus described from in Latin America was termed "Potato deforming mosaic" (Calderoni 1965; Calderoni et al. 1962; Calderoni and Malamud 1965). This disease was first described in the early 1960s from the southeastern potato-growing region of the province of Buenos Aires, Argentina (Delhey et al. 1981). Two decades later "potato deforming mosaic" was observed to occur in the states of Rio Grande do Sul

(Daniels and Castro 1985) and São Paulo, Brazil. Researchers in the latter state concluded that PDMV was different from the begomovirus (ToYMV) that infects potato in Venezuela based on sequence data obtained in Brazil (Vega et al. 1992). However, a recent report from Brazil (Ribeiro et al. 2006) claims that "Potato deforming mosaic" is caused by ToYVSV described in 1997, again based on comparative genomic analyses of these two begomoviruses (Faria et al. 1997). In fact, the authors should have concluded that ToYVSV is a misnomer of PDMV considering that this potato begomovirus was described 35 years before the report from tomato in Brazil. Curiously, the author had isolated PDMV from common bean in Northwestern Argentina in 1995 before the isolation from tomatoes in Brazil (Morales and Anderson 2001).

The second begomovirus shown to infect potato in Latin America was ToYMV (Debrot 1981; Debrot and Centeno 1985). This virus was also erroneously re-named *Potato yellow mosaic virus* later on (Roberta et al. 1986). This misnomer persists despite molecular evidence confirming the identity of ToYMV as a major viral pathogen of tomato – and occasionally of potato – in Venezuela (Morales and Anderson 2001). ToMoTV was detected in 1998 infecting potatoes in Cuba (FJ Morales and G Gonzalez unpublished data and later confirmed in Cuba (Cordero et al. 2003). ToMoTV is closely related ToYMV, and these begomoviruses have been shown to form pseudorecombinants (Ramos et al. 1997).

## *Tobacco*

Tobacco (*Nicotiana tabacum*.) is another plant species native of the Americas. As the name suggests, it is supposed to be the main host of the whitefly *B. tabaci*. However, tobacco is not a preferred host of *B. tabaci* or the begomoviruses transmitted by this insect vector in Latin America. Research conducted in the early 1950s in Brazil (Silberschmidt and Tommasi 1955) showed that the begomoviruses associated with the "infectious chlorosis of *Malvaceae*" did not readily infect tobacco. Nevertheless, the original A biotype of *B. tabaci* occasionally colonized tobacco in Latin America – albeit in relatively low numbers (Morales 2006a) – and transmitted opportunistic begomoviruses to tobacco in Brazil, Venezuela, Puerto Rico, Dominican Republic, Mexico, Guatemala, and Colombia (Bird and Maramorosch 1978; Costa and Forster 1939; Morales and Anderson 2001; Morales et al. 2000; Paximadis et al. 1999; Wolf et al. 1949). Symptoms induced by neotropical begomoviruses in tobacco are usually of the "leaf curl" type (Costa 1976). Susceptible tobacco plants may also show dwarfing and different types of variegation, and disease incidence may be significant (> 30%) in some tobacco plantings (Morales et al. 2000).

The introduction of the B biotype of *B. tabaci* in Latin America has apparently changed the privileged isolation of tobacco from emerging begomoviruses. Two begomoviruses isolated in the state of Chiapas, southern Mexico (Paximadis et al. 1999) were shown to be closely related to *Cabbage leaf curl virus* (CaLCuV) and PepGMV. The begomovirus found that infected tobacco in the Magdalena

valley of Colombia was closely related to a begomovirus of *Merremia* sp. from Puerto Rico (Brown et al. 1995). The Magdalena valley of Colombia is still under from attack B biotype and begomoviruses currently in the process of characterization. Coincidentally, the Puerto Rican begomovirus from *M. quinquefolia* had been observed to infect tobacco (Bird et al. 1975), and as already mentioned, these begomoviruses can also infect tomato. A begomovirus isolated in Cuba – tentatively named Tobacco leaf rugose was related to *Jatropha mosaic virus* from Puerto Rico (Dominguez et al. 2002). Recently, another begomovirus inducing foliar rugosity of tobacco was reported from Cuba. The virus was considered a new species named tobacco leaf curl Cuba virus (Moran et al. 2006). Again, this is a misnomer considering that Tobacco leaf curl begomoviruses only exist in the Old World (Fauquet et al. 2005). Tobacco plantations in northwest Argentina (Jujuy) are currently suffering heavy whitefly infestations apparently related to the recent invasion of this region by biotype B of *B. tabaci*. However, this report must be confirmed because *Trialeurodes vaporariorum* was also present in the region. These reports demonstrate that begomoviruses may also become important pathogens of tobacco in Latin America.

## *Soybean*

Soybean (*Glycine max*) is the most extensively cultivated reproductive host of *B. tabaci* in Latin America (*c.* 39 million ha) although there are other reproductive hosts that generate higher whitefly populations per plant (Anderson et al. 2005; Morales and Anderson 2001).

The transmission of begomoviruses to soybean by *B. tabaci* was first observed in the early 1970s in the state of São Paulo, Brazil. Susceptible soybean plants showed leaf crinkling and plant stunting associated with the presence of large populations of *B. tabaci* (Costa et al. 1973). Reports on the occasional detection of begomoviruses infecting soybean in Brazil continue to appear in the literature (Moreira et al. 2005) but their incidence and economic importance remains low (Faria et al. 2000). Other begomoviruses capable of infecting soybean elsewhere in Latin America have been reported from Venezuela (Debrot and Ordoisgotti 1975) and Mexico, where a begomovirus related to PepGMV and CaLCuV has been implicated in occasional disease outbreaks in soybean plantings in the state of Sinaloa (Mendez-Lozano et al. 2006b). Curiously, a begomovirus isolated from a few symptomatic soybean plants in Colombia was also related to CaLCuV (Morales et al. 2000). Recently, a begomovirus related to *Rhynchosia golden mosaic virus* was isolated from stunted and chlorotic soybean plants in Sinaloa, Mexico (Mendez-Lozano et al. 2006a). The presence of begomoviruses affecting soybeans in northwestern Argentina has also been reported in the provinces of Salta and Tucuman (Laguna et al. 2005; Pardina et al. 1998) with incidences of up to 45% in some fields. A recent survey of several soybean fields conducted by the author in northwestern Argentina showed average virus incidences to be under 5%. Attempts to recover a begomovirus from symptomatic soybean plants yielded negative results.

It thus appears that soybeans are a better whitefly than begomovirus host. Whether this situation will remain the same, or begomoviruses might become a serious constraint to soybean production in the future, is not known. The presence of the B biotype of *B. tabaci* might change this situation for the worse, given the potential damage that high populations of this biotype can inflict on soybean.

## *Fruit Crops*

Fruit crops have not escaped infection by begomoviruses, particularly species in the Passifloraceae. Passionfruit (*Passiflora edulis*) has been infected by begomoviruses in Puerto Rico (Brown et al. 1993) and Brazil (Novaes et al. 2003), expressing mottling and little leaf mosaic, respectively. In the northern coast of Colombia, a plant and fruit malformation disease of giant granadilla (*Passiflora quadrangularis*) was shown in 2000 to be caused by a begomovirus (Morales 2006a). Recently, passionfruit and giant granadilla have been attacked in the Cauca Valley of Colombia by an apparently new begomovirus related (84–88%) to begomoviruses infecting weeds, namely *Sida* and *Wissadula* species (FJ Morales and AK Martinez unpublished data). In central Mexico, tomatillo (*Physalis ixocarpa*) was shown to be a host of *Pepper yellow vein Huasteco virus* (Torre-Almaraz et al. 2002). The current popularity of the neglected tropical fruit species of Latin America, will probably cause an increase in the number of these species attacked by begomoviruses in the near future.

## *Integrated Whitefly and Begomovirus Management*

Epidemiology has been defined as the "science of disease in populations" (Vanderplank 1963), and plant disease is often considered the outcome of the interaction between populations of plants, pathogens, and their environment. Farmers also play a major role in disease epidemiology, as they affect the interaction between the three variables mentioned above – often referred to as the "disease triangle." Obviously, this is an oversimplification of the complex dynamics of plant disease in some pathosystems – such as those associated with whitefly-transmitted viruses – in which the insect vector plays an important, if not the most important role in the epidemiology of begomoviruses. It is often accepted that "an understanding of the epidemiology of pathogens is necessary for the development of reliable and effective disease management systems" (Jeger 2004; Royle and Ostry 1995). This review and previous publications (Morales and Anderson 2001; Morales and Jones 2004; Morales 2006a,b; Morales et al. 2002, 2000) analyze the different biotic and abiotic factors that influence begomovirus outbreaks in Latin America, but within the realm of circumstantial, etiological, and biological epidemiology (MacDonald 1957). Circumstantial epidemiology describes the disease and the circumstances under which it occurs. Etiological epidemiology deals with the causal agents, their plant hosts, and mode of transmission. The characterization of begomoviruses has been greatly facilitated by the advent and implementation of molecular techniques

currently available to scientists in developing nations. However, virologists have been more preoccupied with the detection, characterization – and mainly – discovery of "new" begomovirus species than with the true concept of "etiological" and "evolutionary" epidemiology. Currently, we have an ever-increasing list of "related-but-nevertheless-distinct" begomovirus species that only serve to confuse agricultural professionals and farmers alike; what is needed is a rational picture of begomovirus evolution and adaptation to different hosts and environments. If we expect to make sense of the extreme genetic diversity displayed by begomoviruses due to mutation, pseudo-recombination – or recombination (Gutierrez 1999) – and its implication in begomovirus evolution (Van den Bosch et al. 2006), we cannot continue to enlarge the list of begomovirus species at the current rate; 117 and 54 begomoviruses are currently listed as accepted or tentative species, respectively, by the Geminiviridae Study Group of the International Committee on Taxonomy of Viruses. A recent proposal (Seal et al. 2006), based on a previous review on plant virus evolution (Roossinck 1997), considers begomovirus variants as "quasispecies" in order to study their diversity at the population rather than at the molecular level. For instance, certain agro-ecosystems may place considerable selection pressure on certain begomoviruses, such as *Squash leaf curl virus* (SLCV), in order to adapt to variable and intensive mixed cropping systems that include different species of cucurbits, such as squash (*C. maxima*), pipian (*C. argyrosperma*), ayote (*C. moschata*), chayote (*Sechium edule*), cucumber (*Cucumis sativus*), melon (*C. melo*), watermelon (*C. lanatus*), and even species in different families, such as common bean (Leguminosae). Within the existing population of SLCV variants – currently considered different species – there must be "fitness" sequences responsible for their broad adaptation to changing selection pressures (Roosinck 1997; Seal et al. 2006). On the other hand, begomoviruses such as *Bean golden mosaic virus* (BGMV), which could potentially infect 4 million ha of common bean monoculture, may not have the necessary selection pressure to generate variants; this may result in reduced fitness or deleterious mutations as it has been observed for some virus variants (Garcia-Arenal et al. 2003).

Finally, "biological" epidemiology generates basic knowledge on the pathogens, hosts, and vectors that comprise a pathosystem. This branch has been re-named "ecological epidemiology" (Anderson 1994) to include not only the organisms that make up the pathosystem but the relationships between them. Anderson (1994) further proposes that this branch should shift the emphasis from the causal agent to etiological pathways – basically the role of human activity in the determination of diseases. However, in this chapter, I use the term "ecological" epidemiology to describe the relation of the organisms associated with the pathosystem, namely begomoviruses and *B. tabaci*, to their physical surroundings or ecosystems.

In Latin America, we have circumstantial evidence suggesting that specific cultivated plant species, and cultural practices associated with these crops, were responsible for the emergence of begomoviruses and *B. tabaci* biotypes as major pathogens and pests of food and industrial crops. The first crop associated with the emergence of *B. tabaci* as a pest was cotton. The main factor suspected for the initial outbreaks of this whitefly pest was the introduction and intensive use of insecticides

to control other pests such as the cotton boll weevil. These practices eliminated biological control organisms that maintained *B. tabaci* populations below a damage threshold (Morales 2006a; Morales and Anderson 2001; Spillari 1994). From 1919 to 1947, cotton growers dusted this pest with calcium arsenate, but the Second World War motivated the development of chemicals that eventually became insecticides – such as DDT – developed in Switzerland in 1939 – and other chlorinated hydrocarbons. At that time, German scientists developed organophosphates as potential war gasses and eventually as insecticides. The carbamates were developed by Swiss scientists in the 1940s (Philip and Rozeboom 1973). As a result of the intensive use of pesticides in cotton in the 1960s, and perhaps other factors, *B tabaci* emerged as a serious pest of cotton in the Pacific lowlands of Central America. The development of the new insecticide metamidophos in 1969 made cotton production possible once more in this region until 1975 when resistance to this insecticide was apparent in the existing *B. tabaci* populations. The use of biological control agents temporarily reversed the situation until 1977, but the release and abuse of new insecticides created new epidemics of whiteflies and whitefly-borne viruses that eventually reduced by 90% the area planted to cotton between 1975 and 1990 (Spillari 1994). Cotton was also the first crop to be attacked by high populations of *B. tabaci* in the state of Paraná, Brazil, in 1967 (Costa 1975). Although the pests of cotton in Brazil did not include the cotton boll weevil at that time – it was first reported in Brazil in the 1980s – other pests of cotton must have been the target of the widespread use of insecticides in cotton in the western hemisphere since the 1950s. However, the main factor driving the development of high populations of *B. tabaci* in Brazil was apparently the exponential increase in the area planted with soybean, a suitable reproductive host for *B. tabaci*. Whether or not insecticides played a role in the case of soybeans is not known, although *B. tabaci* was initially considered a *bona fide* pest of soybean in the 1970s, particularly in the states of Paraná and São Paulo (Costa 1975); insecticides were used in soybean against other pests in the 1970s.

Thus, prior to the 1960s, *B. tabaci* populations may have been under control by biological control agents, even in the presence of suitable reproductive hosts of *B. tabaci* grown commercially in Latin America, such as cotton. The introduction and widespread use of insecticides in Latin America eliminated the biological control of *B. tabaci,* and led to the emergence of insecticide-resistant populations of this whitefly species on suitable reproductive hosts grown extensively. Epidemiological models suggest that high populations of vectors can favor the evolution of higher virulence in virus populations (Escriu et al. 2003; Seal et al. 2006). These models might explain the clear association between the emergence of different begomovirus-induced diseases previously associated with wild malvaceous hosts – specifically Bean golden mosaic in common bean – and the exponential increase of *B. tabaci* populations in soybean plantings as a result of the increasing area planted and the extended sowing period of this crop in Brazil (September–January). These cultural practices permit the emergence of several generations of *B. tabaci* per cropping season until the soybean crop is harvested around January. At this time, common bean plantings are being established during the dry and warm season and provide a continuous supply of food and a breeding host for *B. tabaci* (Costa 1975).

The move towards crop diversification in Latin America since the 1980s, and the introduction of biotype B of *B. tabaci* in the 1990s, further complicated the situation as a more aggressive and polyphagous biotype (B) extended the plant host range of *B. tabaci* and brought different begomoviruses in contact with a larger number of potential hosts (Morales 2006a). At the same time, the year-round availability of a larger number of feeding and reproductive hosts in the tropics increases whitefly populations and the chances of begomovirus evolution and adaptation to new plant species. For instance, tomato has been one of the most rapidly expanding crops in Latin America since the 1980s. Consequently, the number of begomoviruses infecting tomato in Latin America has more than quadrupled in the last two decades (Morales 2006a; Polston and Anderson 1997). As discussed, many of these tomato begomoviruses can infect not only various solanaceous plant species, but also others such as hot and sweet peppers, legumes, and common and lima beans. The ever-changing maelstrom of epidemiological factors tends to confuse plant pathologists and other agricultural scientists responsible for the management of plant diseases caused by begomoviruses, particularly within the realm of etiological epidemiology (MacDonald 1957). However, if we extend this purely biological – causal organism focus to the concept of etiological "pathways," as suggested by Anderson (1994), we can understand how human activity and intervention lead to the emergence of begomovirus and whitefly variants. This point brings us back to the subject of epidemiology and plant disease management. As Zadoks and Schein (1979) stated, "plant disease management, the applied side of epidemiology, is considered as a part of crop management within the framework of existing agro-ecosystems."

The concept of Integrated Pest Management (IPM) was initially conceptualized to reduce dependence on pesticides and their effect on the environment; IPM had been built into virus control strategies from the beginning of plant virology (Bos 1999) perhaps because of the natural in vivo insensitivity of viruses to chemical agents. Unfortunately, the association of begomoviruses with insect vectors – whiteflies, that are also *bona fide* pests on their own, has caused extreme cases of insecticide abuse. Consequently, farmers are witnessing the extraordinary ability of *B. tabaci* to develop resistance to insecticides (see Castle et al. Chapter 16), and the inefficiency of most insecticides to prevent plant infection by begomoviruses in situations of high virus and vector incidence. The selection and development of virus-resistant cultivars has therefore been a major endeavor of plant virologists and breeders since the beginning of plant virology (Morales 2001; Morales and Bos 1988; Thresh 1980).

The search for sources of resistance to neotropical begomoviruses has often been a time-consuming and rather frustrating experience, mainly because of the rare occurrence of cases of immunity to begomoviruses in food and industrial crops. After 30 years of screening over 30,000 accessions of common bean for resistance to BGMV and BGYMV, not a single common bean genotype has been identified as immune to these begomoviruses. However, some common bean genotypes possessing immunity or high levels of resistance have been identified and used to control an economically important common bean begomovirus – *Bean dwarf mosaic virus* (BDMV) in Latin America (Morales 2001; Morales et al. 1990).

Considerable progress in breeding for resistance to BGYMV and BGMV has also been achieved in the past three decades by pyramiding resistance genes from different races of common bean (Morales 2001; Morales and Singh 1993). Some of the most recent common bean cultivars produced and released in Central America by the Pan American School in Zamorano, Honduras possess such high levels of BGYMV resistance that they can be grown under high begomovirus and *B. tabaci* incidence without chemical protection. However, it is known that the deployment of virus-resistant plant genotypes exerts considerable selection pressure on plant viruses and begomoviruses are no exception (Seal et al. 2006; Van den Bosch et al. 2006). Most of the early BGYMV-resistant common bean genotypes released in the 1980s and 1990s have already shown increased susceptibility to this begomovirus within the first decade of cultivation. Both BGMV and BGYMV, however, have shown to be highly stable from the pathogenic point of view, perhaps because only two or three sources of resistance to common bean begomoviruses have been widely used throughout Latin America (Morales et al. 1990). To date, only some minor changes have been detected in the antigenic properties of most geographical isolates of BGYMV in Central America and the Caribbean (Morales 2006a), which coincided with the arrival of the new biotype (B) of *B. tabaci* in the Americas. The behavior of common bean begomoviruses contrasts with that of other begomoviruses of tomatoes, peppers, and cucurbits in Latin America that have shown considerable pathogenic variability. This situation may be changing, however, as observed in the case of a new begomovirus that practically annihilated snap bean production in the Cauca Valley of Colombia. The emergence of this begomovirus was associated with the invasion of the valley by the B biotype of *B. tabaci*; more importantly, the begomovirus was shown to be a recombinant between the A component of BGYMV and the B component of a local tomato begomovirus related to ToYMV (FJ Morales and AK Martinez unpublished data). This is probably a new adaptation strategy for begomoviruses that do not have a wide host range such as the common bean begomoviruses in Latin America. The cucurbit, tomato, and pepper begomoviruses, on the contrary, have a much wider host range in the Cucurbitaceae and Solanaceae.

Unlike the progress made in the development of begomovirus-resistant common bean cultivars, breeding for resistance to tomato or *Capsicum* begomoviruses has, with few exceptions, been largely neglected in Latin America. In fact, most of the crop improvement for these Latin American plant species has been carried out mainly in temperate countries of the industrialized world. Thus, most of the improved tomato or pepper varieties grown in Latin America are not adapted to the tropics, let alone to the different begomoviruses that exist in tropical America. The introduction of TYLCV into the Americas created an opportunity for the major tomato seed companies to market tomato hybrids and varieties bred for resistance to this virus in Israel, Europe, and the United States. The dissemination of TYLCV in the southern United States, and Mediterranean region of Europe, resulted in the emergence of several active tomato breeding programs engaged in introducing different TYLCV resistance genes derived from various wild species of *Lycopersicon,*

such as *L. peruvianum, L. chilense, L. pimpinellifolium, L. cheesmanii,* and *L. hirsutum* (Lapidot and Polston 2006). The early TYLCV-resistant materials introduced in Caribbean countries in the late 1990s were evaluated for disease resistance with mixed results, suggesting that the genes for resistance to TYLCV were not as effective against the local begomoviruses. Also, some of the first improved materials introduced did not have the required market or industrial properties. However, as breeding efforts intensified – by using different sources of virus resistance – a number of the most recent commercial materials have shown acceptable levels of field resistance to neotropical tomato begomoviruses. These materials may be less likely to induce begomoviruses to mutate as the resistance genes involved are probably not pathogen-specific. The effectiveness and consequences of deploying genetically-modified plants to control begomoviruses remain to be seen.

The close association of highly aggressive, polyphagous and fecund virus vector populations with the emergence of new begomoviruses agrees with studies on plant virus evolution that support the use of control measures to reduce vector/virus populations. High vector populations increase transmission rates and consequently the possibility of mixed virus infections (Seal et al. 2006). Therefore, one of the complementary IPM measures recommended for the control of begomoviruses in the case of BGYMV- and BGMV-resistant common bean cultivars is the use of the new generation of systemic insecticides – e.g., imidacloprid and thiametoxam – at sowing time, which can provide protection at least until initial stages of pod/fruit formation. The effectiveness of systemic insecticides to control begomoviruses has been demonstrated for ToMoV in Florida, USA.

Cultural practices can make a significant contribution to begomovirus control, and they do not exert any selection pressure on plant viruses. However, begomoviruses are transmitted by an insect vector that has an extraordinary capacity to adapt to new environments. A traditional strategy to avoid begomoviruses in Latin America has been the cultivation of susceptible crops at elevations above 900 m, where the original *B. tabaci* biotypes do not thrive. The arrival, and subsequent dissemination of the B biotype of *B. tabaci*, has greatly reduced the effectiveness of this strategy because this biotype managed to adapt to agricultural areas above 1,500 m. Crop rotation is another cultural practice recommended to reduce *B. tabaci* populations. In the past, this whitefly species was rather selective, breeding on just a few crops or plant species. This selective behavior gave rise to the concept of "races" of *B. tabaci* (Bird and Sanchez 1971). However, even the moderately aggressive A biotype of *B. tabaci* was known to colonize non-host plant species when there were no preferred plant hosts in the vicinity. The introduction of the highly polyphagous B biotype of *B. tabaci* further complicated this situation due to its extended host range, which is probably the reason why begomoviruses are increasingly jumping to different plant species and even families. For instance, PDMV was first detected in potatoes, then in common beans, and lastly in tomatoes. ToYMV is known to infect tomatoes, potatoes, peppers, and common beans. Furthermore, SLCV jumped from cucurbits to legumes causing major outbreaks in common bean and different cucurbit species, as mentioned above.

Farmers in general know that the rainy season is the best time to escape heavy yield losses caused by whitefly-borne begomoviruses. Rain causes physical damage to whiteflies, and humidity favors the activity of entomopathogens, which results in increased mortality rates in whitefly populations (e.g., Naranjo et al. 2004, see also Arnó et al. Chapter 15). However, the dry season in Mesoamerica and some Andean countries may last over 5 consecutive months. This represents a significant economic loss for farmers who have chosen not to grow crops during the dry season due to the expected high whitefly populations and virus incidence. Furthermore, developing countries in Latin America have invested considerable resources, particularly in the construction of irrigation districts in regions characterized by a prolonged dry season. Unfortunately, the crop microclimate created by the availability of irrigation water in dry areas is extremely favorable for the development of large whitefly populations. Crop rotation should be a viable alternative in these regions, but the few crops that are not attacked by *B. tabaci*, such as sugarcane, sorghum or maize, are not as profitable as the high-value horticultural crops – e.g., tomatoes, chilies, sweet peppers – attacked by whitefly-borne viruses. In these cases, the enforcement of susceptible crop-free periods has been a highly unpopular but effective whitefly and begomovirus control measure. However, most countries in Latin America are reluctant to enforce such legal measures. The rationalization of cropping systems would be another effective strategy to reduce the incidence of these pests, but the erratic behavior of markets in Latin America has taught farmers to plant their most valuable crops at different times of the year – often in successive plantings – in the hope of capturing peak prices for their produce at any time. Farmers are well aware of the fact that planting crops during a well-defined season usually results in lower prices due to over supply.

In view of the complexity of the current cropping systems in Latin America, the insufficient agricultural research and technical assistance in the region, the favorable conditions for *B. tabaci*, and the emergence of new begomoviruses, there is an understandable interest in the practice of "protected agriculture." The physical exclusion of whitefly and other insect vectors is particularly important for crops that are transplanted, such as tomato or sweet and hot peppers (see Stansly and Natwick Chapter 17). The nurseries of these high-value crops must be completely protected from viruliferous vectors in order to avoid the early infection of seedlings which would result in very high yield losses. However, one objective of "protected agriculture" is to prevent the infection of begomovirus-susceptible plants during the most critical crop stage – from transplanting to fruit set. Thus, virus-free seedlings are treated with a new generation systemic insecticide before transplanting, and are then covered with a suitable insect-proof material until flowering time. Considering that the TWFP was conceptualized to help resource-limited farmers, the project first promoted the use of micro-tunnels which were about 70 cm high over the row. However, different problems associated with the lack of the recommended protective materials to cover susceptible plants – e.g., the right width of the material to allow plants to develop inside tunnels with the necessary height to keep plants protected until flowering time – led to the need to uncover susceptible plants at an earlier stage than desirable to avoid significant yield losses in plants exposed to viruliferous whiteflies

before flowering time. Consequently, many farmers are increasingly interested in the use of "macro-tunnels" at least 2 m high and about six rows wide. The advantage of these large tunnels is that they can be used without the end or side walls during the rainy season using systemic insecticides only. They can then be completely covered during the dry season when high humidity is less likely to induce fungal and bacterial problems. However, high temperatures must be avoided in macro-tunnels by providing suitable ventilation outlets. Physical protection drastically reduces the use of pesticides and creates suitable conditions for the emergence and use of biocontrol agents. Furthermore, physical exclusion of whiteflies and whitefly-borne viruses does not induce any selection pressure on begomoviruses.

The first outbreaks of whitefly-borne viruses were likely the consequence of pesticide abuse at a time when the world was not concerned with environmental pollution or food quality issues as much as today. The elimination of biological control agents in nature, and the creation of pesticide-resistant whitefly populations, are the main factors driving begomovirus epidemics. The strict food quality requirements of markets and the availability of more selective insecticides in developing countries could gradually reduce pesticide abuse. Recovery of the biological stability of the affected agro-ecosystems is expected. Only then can we expect IPM practices to work efficiently and, hopefully, help us manage *B. tabaci* and begomovirus populations in Latin America.

## Literature Cited

Abouzid AM, Hiebert E, Strandberg J. 1992a. Cloning, identification and partial sequencing of the genome components of a geminivirus infecting the *Brassicaceae*. *Phytopathology* 82:1070.

Abouzid AM, Polston JE, Hiebert E. 1992b. The nucleotide sequence of tomato mottle virus, a new geminivirus isolated from tomato in Florida. *J. Gen Virol.* 73:3225–3229.

Ala-Poikela M, Svensson E, Rojas A, Horko T, Paulin T, Valkonen JP, Kvarnheden A. 2005. Genetic diversity and mixed infections of begomoviruses infecting tomato, pepper and cucurbit crops in Nicaragua. *Plant Pathol.* 54:448–459.

Anderson PK. 1994. A conceptual framework of integrated epidemiology for application to emerging diseases. In *Disease in Evolution: Global Changes and Emergence of Infectious Diseases*, ed. ME Wilson, R Levins, A Spielman, *Ann. NY Acad. Sci.* 740:439–444.

Anderson PK, Hamon A, Hernandez P, Martin J. 2005. Reproductive crop hosts of *Bemisia tabaci* (Gennadius) in Latin America and the Caribbean. In *Whiteflyand Whitefly-Borne Viruses in the Tropics: Building a Knowledge Base*, ed. PK Anderson, FJ Morales, pp. 243–250. Palmira, Colombia: CIAT. Publication No. 341.

Andrade EC, Manhani GG, Alfenas PF, Calegario R, Fontes EPB, Zerbini FM. 2006. Tomato yellow spot virus, a tomato-infecting begomovirus from Brazil with a closer relationship to viruses from *Sida* sp., forms pseudorecombinants with begomoviruses from tomato but not from *Sida*. *J. Gen. Virol.* 87:3687–3696.

Archibold OW. 1995. *Ecology of World Vegetation*. New York: Chapman & Hall Publishers.

Ascencio-Ibañez JT, Diaz-Plaza R, Mendez-Lozano J, Monsalve-Fonnegra ZI, Arguello-Astorga GR, Rivera-Bustamante RF. 1999. First report of tomato yellow leaf curl geminivirus in Yucatan, Mexico. *Plant Dis.* 83:1178.

Bellows TS, Perring TM, Gill RJ, Headrick DH. 1994. Description of a species of *Bemisia* (Homoptera: Aleyrodidae) infesting North American agriculture. *Ann. Entomol. Soc. Am.* 87:195–206.

Bezerra-Agasie IC, Ferreira GB, Avila AC, Inoue-Nagata AK. 2006. First report of tomato severe rugose virus in chili pepper in Brazil. *Plant Dis.* 90:114.

Bird J. 1957. A whitefly-transmitted mosaic of *Jatropha gossypifolia*. *Agric. Exp. Sta. Univ. P. R.*, Tech Paper 22, 35pp.

Bird J. 1958. Infectious chlorosis of *Sida carpinifolia* in Puerto Rico. *Agr. Exp. Sta. Univ. P. R.*, Tech. Paper No. 26, 23pp.

Bird J, Idris AM, Rogan D, Brown JK. 2001. Introduction of the exotic *Tomato yellow leaf curl virus*-Israel in tomato to Puerto Rico. *Plant Dis.* 85:1028.

Bird J, Maramorosch K. 1978. Viruses and virus diseases associated with whiteflies. *Adv. Virus Res.* 22:55–110.

Bird J, Sanchez J. 1971. Whitefly-transmitted viruses in Puerto Rico. *J. Agric. Univ. P. R.* 55:461–467.

Bird J, Sánchez J, Rodríguez RL, Juliá, FJ. 1975. Rugaceous (whitefly-transmitted) viruses in Puerto Rico. In *Tropical Diseases of Legumes*, ed. J Bird, K Maramorosch, pp. 3–26. New York: Academic Press.

Bird J, Sanchez J, Vakili NG. 1973. Golden yellow mosaic of beans (*Phaseolus vulgaris*) in Puerto Rico. *Phytopathology* 63:1435.

Blanco N, Bencomo I. 1978. Afluencia de la mosca blanca (*Bemisia tabaci*) vector del virus del mosaico dorado en plantaciones de frijol. *Cienc. Agric.* 2:39–46.

Bos L. 1999. *Plant Viruses, Unique and Intriguing Pathogens*. Leiden: Backhuys Publishers, 358pp.

Bravo-Luna L, Frias-Treviño GA, Sánchez-Valdés V, Garzon-Tiznado JA. 2000. Sources of inoculum and vectors of Texas pepper geminivirus of chilli (*Capsicum annuum*) in Ramos Arizpe, Coahuila, Mexico. *Rev. Mex. Fitopatol.* 18:97–102.

Brown JK. 1994. Current status of *Bemisia tabaci* as a plant pest and virus vector in agroecosystems worldwide. *FAO Plant Prot. Bull.* 42:3–32.

Brown JK. 2007. The *Bemisia tabaci* complex: genetic and phenotypic variability drives begomovirus spread and virus diversification. APSnetvFeature. www.apsnet.org/online/feature/btabaci/

Brown JK, Bird J, Banks G, Sosa M, Kiesler CI, Fornaris G. 1995. First report of an epidemic in tomato caused by two whitefly-transmitted geminiviruses in Puerto Rico. *Plant Dis.* 79:1250.

Brown JK, Bird J, Fletcher DC. 1993. First report of passiflora leaf mottle disease caused by a whitefly-transmitted geminivirus in Puerto Rico. *Plant Dis.* 77:1264.

Brown JK, Idris AM, Rogan D, Hussein MH, Palmieri M. 2001. Melon chlorotic leaf curl virus, a new begomovirus associated with *Bemisia tabaci* infestations in Guatemala. *Plant Dis.* 85:1027.

Brown JK, Nelson MR. 1987. Host range and vector and vector relationships of cotton leaf crumple virus. *Plant Dis.* 71:522–524.

Brown JK, Nelson MR. 1988. Transmission, host range, and virus-vector relationships of chino del tomate virus, a whitefly-transmitted geminivirus from Sinaloa, Mexico. *Plant Dis.* 72:866–869.

Brown JK, Ostrow KM, Idris AM, Stenger DC. 1999. Biotic, molecular, and phylogenetic characterization of Bean calico mosaic virus, a distinct begomovirus species with affiliation in the Squash leaf curl virus cluster. *Phytopathology* 89:273–280.

Calderoni AV. 1965. An unidentified virus of deforming mosaic type in potato varieties in Argentina. *Am. Potato J.* 42:257.

Calderoni AV, Garay OA, Pasquale DR, Induni C. 1962. Los virus de la papa en la región sudeste de la provincia de Buenos Aires. *IDIA* 10(Suppl.):389–390.

Calderoni AV, Malamud O. 1965. Enfermedades de la papa. *Tech. Bull*. No. 49, INTA, Balcarce, R. Argentina.

Cardenas JA, Perez F, Nieves F. 1996. Campaña contra la mosquita blanca en México. *Resumen del Taller de Moscas Blancas*, Acapulco, Mexico, pp. 167–169.

Castillo J. 1997. *Movimiento diario de Bemisia tabaci en parcelas de tomate, diseminación local del mosaico amarillo y fuentes de inóculo del ToYMV-CR en Guayabo, C.R.* MSc Thesis, CATIE, Turrialba, Costa Rica.

Cole MM. 1986. *The Savannas: Biogeography and Geobotany*. Academic Press: New York.
Cook MT. 1931. New virus diseases of plants in Porto Rico. *J. Dep. Agric.* 15:193–195.
Cordero M, Ramos PL, Hernandez L, Fernández AI, Echemendia AL, Peral R, Gonzalez G, Garcia D, Valdes S, Estevez A, Hernadez K. 2003. Identification of *Tomato mottle Taino virus* strains in Cuban potato fields. *Phytoparasitica* 31:478–489.
Costa AS. 1937. Nota sobre o mosaico do algodoeiro. *Rev. Agric. Piracicaba Bras.* 12:453–470.
Costa AS. 1955. Studies on *Abutilon* mosaic in Brazil. *Phytopathol. Z.* 24:97–112.
Costa AS. 1965. Three whitefly-transmitted diseases of beans in the state of São Paulo, Brazil. *FAO Plant Prot. Bull.* 13:121–130.
Costa AS. 1974. Molestias do tomateiro no Brasil transmitidas pela mosca branca *Bemisia tabaci*. In *VII Congresso Anual da Sociedade. Brasileira de Fitopatologia*, Brasilia, DF.
Costa AS. 1975. Increase in the populational density of *Bemisia tabaci*, a threat of widespread virus infection of legume crops in Brazil. In *Tropical Diseases of Legumes*, ed. J Bird, K Maramorosch, pp. 27–49. New York: Academic Press.
Costa AS. 1976. Whitefly-transmitted plant diseases. *Annu. Rev. Phytopath.* 14:429–449.
Costa AS, Bennett CW. 1950. Whitefly-transmitted mosaic of *Euphorbia prunifolia*. *Phytopathology* 40:266–283.
Costa AS, Carvalho AM. 1960a. Mechanical transmission and properties of the *Abutilon* mosaic virus. *Phytopathol. Z.* 37:250–272.
Costa AS, Carvalho AM. 1960b. Comparative studies between the *Abutilon* and *Euphorbia* mosaic virus. *Phytopathol. Z.* 38:129–152.
Costa AS, Costa CL, Sauer HF. 1973. Outbreak of whiteflies on crops in Paraná and São Paulo. *An. Soc. Entom. Bras.* 2:20–30.
Costa AS, Forster R. 1939. Uma suspeita de virus do fumo (*Nicotiana tabacum* L.) semelhante a 'leaf curl' presente no estado de São Paulo. *J. Agron. Piracicaba* 2:295–302.
Costa AS, Oliveira AR, Silva DM. 1975. Transmisão mecânica do agente causal do mosaico dourado do tomateiro. In *VIII Congr. An. Soc. Bras. Fitopatol*., Mossoró, Brasil.
Dalmon A, Marchoux G. 2000. Which plants host *Tomato yellow leaf curl virus*? *Phytoma* 527:14–17.
Daniels J, Castro LA. 1985. Ocorrencia do virus do mosaico deformante da batata no Rio Grande do Sul. *Fitopatol. Bras.* 10:306.
De Barro PJ. 1995. *Bemisia tabaci* biotype B: a review of its biology, distribution and control. C*SIRO Australia Div.f Entomol*. Tech. Paper No. 36, 58pp.
De Barro PJ, Trueman WH, Frohlich DR. 2005. *Bemisia argentifolii* is a race of *B. tabaci* (Hemiptera: Aleyrodidae): the molecular genetic differentiation of *B. tabaci* populations around the world. *Bull. Entomol. Res.* 95:193–203.
Debrot EA. 1981. Natural infection of potatoes in Venezuela with the whitefly-transmitted mosaico amarillo del tomate virus. In *Proceedings International Workshop*, p. 17. Keeble College, Oxford, UK.
Debrot EA, Centeno F. 1985. Infección natural de la papa en Venezuela con el mosaico amarillo del tomate, un geminivirus transmitido por moscas blancas. *Agron. Trop.* 35:125–138.
Debrot E, Herold F, Dao F. 1963. Nota preliminar sobre un "mosaico amarillento deltomate" en Venezuela. *Agron. Trop.* 13:33–41.
Debrot E, Ordoisgotti A. 1975. Estudios sobre un mosaico amarillo de la soya en Venezuela. *Agron. Trop.* 25:435–450.
Delhey R, Kiehr-Delhey M, Heinze K, Calderoni AV. 1981. Symptoms and transmission of potato deforming mosaic in Argentina. *Potato Res.* 24:123–133.
Diaz-Plaza R, Aviles-Baeza W, Torres-Pacheco I, Rivera-Bustamante R. 1996. Geminivirus en la region horticola de Yucatan, Mexico. In *Resumen Taller Moscas Blancas*, Acapulco, Mexico.
Dodds JA, Lee JG, Nameth ST, Laemmlen FF. 1984. Aphid- and whitefly-transmitted cucurbit viruses in Imperial County, California. *Phytopathology* 74:221–225.
Dominguez M, Ramos PL, Echemendia AL, Peral R, Crespo J, Andino V. 2002. Molecular characterisation of tobacco leaf rugose virus, a new begomovirus infecting tobacco in Cuba. *Plant Dis.* 86:1050.

Duffus JE, Cohen S, Liu HI. 1992. The sweet potato whitefly in western USA: biotypes, plant interactions and virus epidemiology. In *Recent Advances in Vegetable Virus Research*, pp. 76–77. Seventh Conference ISHS Vegetable Virus Workshop Group, Athens, Greece.

Engel M, Fernandez O, Jeske H, Frischmuth T. 1998. Molecular characterization of a new whitefly-transmissible bipartite geminivirus infecting tomato in Panama. *J. Gen. Virol.* 79:2313–2317.

Escriu F, Fraile A, Garcia-Arenal F. 2003. The evolution of virulence in a plant virus. *Evolution* 57:755–765.

FAO. 1994. *Anuario FAO de Producción*. Roma: FAO.

Faria JC, Bezerra IC, Zerbini FM, Ribeiro SG, Lima MF. 2000. Current status of geminiviruses in Brazil. *Fitopatol. Bras.* 25:125–137.

Faria JC, Gilbertson RL, Hanson SF, Morales FJ, Ahlquist P, Loniello O, Maxwell DP. 1994. Bean golden mosaic geminivirus type II isolates from the Dominican Republic and Guatemala: nucleotide sequences, infectious pseudorecombinants, and phylogenetic relationships. *Phytopathology* 84:321–329.

Faria JC, Maxwell DP. 1999. Variability in geminivirus isolates associated with *Phaseolus* spp. in Brazil. *Phytopathology* 89:262–268.

Faria JC, Souza-Dias JAC, Slack SA, Maxwell DP. 1997. A new geminivirus associated with tomato in the state of São Paulo, Brazil. *Plant Dis.* 81:423.

Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA. 2005. *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*. London: Elsevier, Academic Press.

Ferreira CM, Peloso MJ, Faria LC. 2002. Feijão na economia nacional. *EMBRAPA* Doc. No. 135, 47pp.

Flock RA, Mayhew DE. 1981. Squash leaf curl, a new disease of cucurbits in California. *Plant Dis.* 65:75–76.

Flores E, Silberschmidt K. 1958. Relations between insect and host plant in transmission experiments with 'infectious chlorosis' of *Malvaceae*. *An. Acad. Bras. Cien.* 30:535–560.

Flores E, Silberschmidt K. 1967. Contribution to the problem of insect and mechanical transmission of infectious clorosis of *Malvaceae* and the disease displayed by *Abutilon thompsonii*. *Phytopath. Z.* 60:181–195.

Flores E, Silberschmidt K, Kramer M. 1960. Observacões da clorose infecciosa das malváceas em tomateiros do campo. *Biológico* 26:65–69.

Frischmuth T, Engel M, Lauster S, Jeske H. 1997. Nucleotide sequence evidence to the occurrence of three distinct whitefly-transmitted, *Sida*-infecting bipartite geminiviruses in Central America. *J. Gen. Virol.* 78:2675–2682.

Frohlich DR, Torres-Jerez I, Bedford ID, Markham PG, Brown JK. 1999. A phylogeographical analysis of *Bemisia tabaci* species complex based on mitochondrial DNA markers. *Mol. Ecol.* 8:1683–1691.

Fryxell PA. 1997. The American genera of Malvaceae-II. *Brittonia* 49:204–269.

Fuentes S, Salazar LF. 2003. First report of sweet potato leaf curl virus in Peru. *Plant Dis.* 87:98.

Gallegos HML. 1978. Enchinamiento del tomate. In *Enfermedades de Cultivos del Estado de Sinaloa*, ed. HML Gallegos, pp. 13–17. Sinaloa, Mexico: Secr. Rec Hidr.

Gámez R. 1970. Los virus del frijol en Centroamérica. I. Transmisión por moscasblancas (*Bemisia tabaci* Genn.) y plantas hospedantes del virus del mosaico dorado. *Turrialba* 21: 22–27.

Garcia-Arenal F, Fraile A, Malpica JM. 2003. Variability and genetic structure of plant virus populations. *Int. Microbiol.* 6:225–232.

Garrido-Ramirez ER, Gilbertson RL. 1998. First report of tomato mottle geminivirus infecting tomatoes in Yucatan, Mexico. *Plant Dis.* 82:592.

Garzón-Tiznado JA, Acosta-García G, Torres-Pacheco I, Gonzales-Chavira M, Rivera-Bustamante, RF, Maya-Hernandez V, Guevara-Gonzalez RG. 2002. Presence of the geminiviruses *Pepper Huasteco virus* (PHV), Texas pepper virus-Tamaulipas, and Chino del tomate virus (CdTV) in the states of Guanajuato, Jalisco, and San Luis Potosí, Mexico. *Rev. Mex. Fitopatol*. 20: 45–52.

Gazzoni DL, Corso IC, Miguel M. 1999. Effect of insecticides on predators and parasitoids of soybean pests. *Pesqui. Agropecu. Gaucha* 5:255–264.

Gilbertson R, Faria JC, Ahlquist P, Maxwell DP. 1993. Genetic diversity in geminiviruses causing bean golden mosaic disease: the nucleotide sequence of the infectious cloned DNA components of a Brazilian isolate of bean golden mosaic virus. *Phytopathology* 83:709–715.

Gilliand FR, Dumas WT, Arant FS, Ivey HW. 1971. Cotton insect control with ULV-applied insecticides. *Bull. Agric. Exp. Sta*., Auburn University, No. 414, 23pp.

Gonzales AG, Valdes RS. 1995. Virus del encrespamiento amarillo de las hojas del tomate (TYLCV) en Cuba. *CEIBA* 36:103.

Gutierrez C. 1999. Geminivirus DNA replication. *Cellul. Mol. Life Sci.* 56:313–329.

Hernandez RF. 1972. Estudio sobre la mosca blanca en el estado de Morelos. *Agric Tec. Mex.* 3:165–170.

Höfer P, Engel M, Jeske H, Frischmuth T. 1997. Sequence of a new bipartite geminivirus isolated from the common weed *Sida rhombifolia* in Costa Rica. *J. Gen. Virol.* 78:1785–1790.

Holguin-Peña RJ, Vazquez-Juarez R, Rivera-Bustamante R. 2003. First report of a geminivirus associated with leaf curl in Baja California Peninsula tomato fields. *Plant Dis.* 87:1397.

Holguin-Peña RJ, Vazquez-Juarez R, Rivera-Bustamante RF. 2004. Host range, incidence and phylogeny of *Pepper golden mosaic virus* (PepGMV) in Baja California Sur, Mexico. *Rev. Mex. Fitopatol.* 22:206–215.

Holguin-Peña RJ, Vazquez-Juarez R, Rivera-Bustamante RF. 2005. A new begomovirus causes tomato leaf curl disease in Baja California Sur, Mexico. *Plant Dis.* 89:341.

Howarth AJ, Caton J, Bossert M, Goodman RM. 1985. Nucleotide sequence of bean golden mosaic virus and a model for gene regulation in geminiviruses. *Proc. Matl. Acad. Sci. USA* 82:3572–3576.

Howarth AJ, Vandemark GJ. 1989. Phylogeny of geminiviruses. *J. Gen. Virol.* 70:2717–2727.

Idris AM, Brown JK. 2004. *Cotton leaf crumple virus* is a distinct western hemisphere begomovirus species with complex evolutionary relationships indicative of recombination and reassortment. *Phytopathology* 94:1068–1074.

Idris AM, Lee SH, Lewis EA, Bird J, Brown JK. 1998. Three tomato-infecting begomoviruses from Puerto Rico. *Phytopathology* 88:42.

Idris AM, Rivas-Platero G, Torres-Jerez I, Brown JK. 1999. First report of Sinaloa tomato leaf curl geminivirus in Costa Rica. *Plant Dis.* 83:303.

INIFAP. 1995. Mosquita blanca en el noroeste de Mexico. *Memoria Cientifica* 3:73.

Isakeit T, Robertson NL. 1994. First report of *Squash leaf curl virus* on watermelon in Texas. *Plant Dis.* 78:1010.

Jeger MJ. 2004. Analysis of disease progress as a basis for evaluating disease management practices. *Annu. Rev. Phytopathol.* 42:61–82.

Jovel J, Reski G, Rothenstein D, Ringel M, Frischmuth T, Jeske H. 2004. *Sida micrantha* mosaic is associated with a complex infection of begomoviruses different from *Abutilon mosaic virus*. *Arch. Virol.* 149:829–841.

Karkashian JP, Maxwell DP, Ramirez P. 2002. Squash yellow mottle geminivirus: a new cucurbit-infecting geminivirus from Costa Rica. *Phytopathology* 92:125.

Karkashian JP, Nakhla MK, Maxwell DP, Ramirez P. 1998. Molecular characterization of tomato-infecting geminiviruses in Costa Rica. In *International Workshop on Bemisia and Geminiviruses*, p. 45. San Juan, Puerto Rico.

Laguna IG, Fiorona MA, Ploper LD, Galvez MR, Rodriguez PE. 2005. Prospección de enfermedades virales del cultivo de soja en distintas areas de producción de Argentina. In *XIII Congreso Latinoamericano de Fitopatologia*, p. 564. Córdoba, Argentina.

Lapidot M, Polston JE. 2006. Resistance to Tomato yellow leaf curl virus in tomato. In *Natural Resistance Mechanisms of Plants to Viruses*, ed. G Lobenstein, JP Carr, pp. 513–520. The Netherlands: Springer.

Lastra JR, Gil F. 1981. Ultrastructural host cell changes associated with tomato yellow mosaic. *Phytopathology* 71:524–528.

Lastra JR, Uzcátegui RC. 1975. Viruses affecting tomatoes in Venezuela. *Phytopath. Z.* 84:253–258.

Lazarowitz SG, Lazdins IB. 1991. Infectivity and complete nucleotide sequence of the cloned genomic components of a bipartite squash leaf curl geminivirus with a broad host range phenotype. *Virology* 180:58–69.

Leal RA, Quintero S. 1989. Caracterización de una virosis del chile transmisible por mosquita blanca en la planicie Huasteca. *Rev. Mex. Fitopatol.* 7:147–149.

Lima A, Lara F. 2004. Resistencia de genotipos de soja a mosca branca *B. tabaci* (Genn.) Biotipo B (Hemiptera: Aleyrodidae). *Neotrop. Entomol.* 33:71–75.

Lima GS, Assunção IP, Resende LV, Ferreira MA, Viana TH, Gallindo FA, Freitas NS. 2002a. Detection of a begomovirus associated to weeds in the state of Pernambuco and partial molecular characterization of an isolate from *Sida rhombifolia*. *Summa Phytopathol.* 28:353–356.

Lima JA, Goncalves MF, Oliveira VB, Torres FJ, Miranda AC. 2000. Serological and PCR detection of a begomovirus infecting tomato fields in Ibiapaba Mountain, Ceara. *Fitopatol. Bras.* 25:104–108.

Lima LHC, Campos L, Moretzsohn MC, Navia D, Oliveira MRV. 2002b. Genetic diversity of *Bemisia tabaci* (Genn.) populations in Brazil revealed by RAPD markers. *Genet. Mol. Biol.* 25:217–223.

Lima MF, Bezerra IC, Ribeiro SG, Avila AC. 2001. Distribution of geminiviruses in tomato and sweet pepper crops in twelve counties of the lower basin of the San Francisco Valley. *Fitopatol. Bras.* 26:81–85.

Loebenstein G, Fuentes S, Cohen J, Salazar LF. 2003. Sweet potato. In *Virusand Virus-like Diseases of Major Crops in Developing Countries*, ed. G Loebenstein, G Thottappilly, pp. 223–248. London: Kluwer Academic Publishers.

Loniello AO, Martinez RT, Rojas MR, Gilbertson RL, Brown JK, Maxwell DP. 1992. Molecular characterization of bean calico mosaic geminivirus. *Phytopathology* 82:1149.

Lopez GH. 1974. Aumente sus rendimientos en frijol en el Valle de Culiacán. *CIAS-INIA-SARH* Circular No. 12.

Lotrakul P, Valverde RA, Clark CA, Hurtt S, Hoy MW. 2002. Sweet potato leaf curl virus and related geminiviruses in sweet potato. *Acta Hortic.* 583:135–141.

Lotrakul P, Valverde RA, De La Torre R, Jeonggu S, Gomez A. 2000. Occurrence of a strain of Texas pepper virus in Tabasco and Habanero pepper in Costa Rica. *Plant Dis.* 84: 168–172.

MacDonald G. 1957. *The Epidemiology and Control of Malaria*. Oxford University Press: London.

Martinez-Zubiaur Y. 1998. *Contribución al conocimiento de geminivirus que afectan el cultivo del tomate* (Lycopersicon esculentum Mill) *en Cuba*. Doctoral Thesis. Instituto Superior de Ciencias Agropecuarias de la Habana, 39pp.

Martinez-Zubiaur Y, Blas C, Quiñones M, Castellanos C, Peralta E, Romero J. 1998. Havana tomato virus, a new bipartite geminivirus infecting tomatoes in Cuba. *Arch. Virol.* 143:1757–1772.

Martinez-Zubiaur Y, Fonseca D, Quiñónez M, Palenzuela I. 2004. Presence of tomato yellow leaf curl virus infecting squash (*Cucurbita pepo*) in Cuba. *Plant Dis.* 88:572.

Martinez-Zubiaur Y, Muñiz-Martin Y, Quiñones-Pantoja M. 2006. New begomovirus infecting pepper plants in Cuba. *Plant Pathol.* 55:817.

Martinez-Zubiaur Y, Quiñónez M, Fonseca D, Miranda I. 2003. National survey of begomoviruses in tomato crops in Cuba. *Rev. Prot. Veg.* 18:168–175.

Martinez-Zubiaur Y, Quiñónez M, Fonseca D, Potter J, Maxwell DP. 2002. First report of *Tomato yellow leaf curl virus* associated with beans, *Phaseolus vulgaris*, in Cuba. *Plant Dis.* 86:814.

Matys JC, Silva DM, Oliveira AR, Costa AS. 1975. Purificacão e morfologia do virus do mosaico dourado do tomateiro. *Summa Phytopathol.* 1:267–274.

Mauricio-Castillo JA, Arguello-Astorga GR, Alpuche-Solis AG, Monreal-Vargas CT, Dias-Gomez O, Torre-Almaraz R. 2006. First report of Tomato severe leaf curl virus in Mexico. *Plant Dis.* 90:1116.

Maxwell DP, Nakhla MK, Maxwell MD, Ramirez P, Karkashian JP, Roca MM, Roye M, Faria JC. 2002. Diversity of begomoviruses and their management in Latin America. *Phytopathology* 92:127.

McCreight JD, Kishaba AN. 1991. Reaction of cucurbit species to squash leaf curl virus and sweetpotato whitefly. *J. Am. Soc. Hortic. Sci.* 116:137–141.

McGlashan D, Polston JE, Bois D. 1994. Tomato yellow leaf curl geminivirus in Jamaica. *Plant Dis.* 78:1219.

Mejia L, Teni RE, Nakhla MK, Maxwell DP. 1998. Tomato-infecting, whitefly-transmitted geminiviruses in Guatemala. In *International Workshop on Bemisia and Geminiviruses*. San Juan, Puerto Rico.

Mendez-Lozano J, Perea-Araujo LL, Ruelas-Ayala RD, Leyva-Lopez NE, Mauricio-Castillo JA, Arguello-Astorga GR. 2006a. A begomovirus isolated from stunted and chlorotic soybean plants in Mexico is a new strain of *Rhynchosia golden mosaic virus*. *Plant Dis.* 90: 972.

Mendez-Lozano J, Quintero-Zamora E, Barbosa-Jasso MP, Leyva-Lopez NE. 2006b. A begomovirus associated with leaf curling and chlorosis of soybean in Sinaloa, Mexico, is related to *Pepper golden mosaic virus*. *Plant Dis.* 90:109.

Mendoza J. 1996. Que esta pasando con la mosca blanca en Ecuador? *Rev*. *INIAP* 8:8–10.

Montes-Belmont R, Espino-García S, Sosa-Hernandez A, Torres-Pacheco R. 1995. Evaluación de extractos vegetales para el control de la virosis "chino del tomate" en dos regiones agroecologicas de Mexico. *Rev. Mex. Fitopatol.* 13:111–116.

Morales FJ. 2001. Conventional breeding for resistance to *Bemisia tabaci*-transmitted geminiviruses. *Crop Prot.* 20:825–834.

Morales FJ. 2006a. History and current distribution of begomoviruses in Latin America. *Adv. Virus Res.* 67:127–162.

Morales FJ. 2006b. Tropical whitefly IPM project. *Adv. Virus Res.* 69:249–311.

Morales FJ, Anderson PK. 2001. The emergence and dissemination of whitefly-transmitted geminiviruses in Latin America. *Arch. Virol.* 146:415–441.

Morales FJ, Bos L. 1988. Bean common mosaic virus. *AAB Descriptions of Plant Viruses*, No. 337, 6pp.

Morales FJ, Gomez E, Villar A, Nin J. 2005a. Dominican Republic. In *Whitefly and Whitefly-Borne Viruses in the Tropics: Building a Knowledge Base*, ed. PK Anderson, FJ Morales, pp. 237–242. Palmira, Colombia: CIAT. Publication No. 341.

Morales FJ, Gonzales G, Murguido C, Echemendia A, Martinez Y, Hernández Y, Faure B, Chailloux M. 2005b. Cuba. In *Whitefly and Whitefly-Borne Viruses in the Tropics: Building a Knowledge Base*, ed. PK Anderson, FJ Morales, pp. 230–236. Palmira, Colombia: CIAT. Publication No. 341.

Morales FJ, Rivera-Bustamante R, Salinas R, Torres-Pacheco I, Diaz-Plaza R, Aviles W, Ramirez G. 2005c. Mexico. In *Whitefly and Whitefly-Borne Viruses in the Tropics: Building a Knowledge Base*, ed. PK Anderson, FJ Morales, pp. 177–187. Palmira, Colombia: CIAT. Publication No. 341.

Morales FJ, Jones PG. 2004. The ecology and epidemiology of whitefly-transmitted viruses in Latin America. *Virus Res.* 100:57–65.

Morales FJ, Lastra R, Uzcategui RC, Calvert L. 2001. Potato yellow mosaic virus: a synonym of *Tomato yellow mosaic virus*. *Arch. Virol.* 146:2249–2253.

Morales FJ, Martinez AK, Velasco AC. 2002. Nuevos brotes de begomovirus en Colombia. *Fitopatol. Colomb.* 26:75–79.

Morales FJ, Muñoz C, Castaño M, Velasco AC. 2000. Geminivirus transmitidos por mosca blanca en Colombia. *Fitopatol. Colomb.* 24:95–98.

Morales F, Niessen A, Ramirez B, Castaño M. 1990. Isolation and partial characterization of a geminivirus causing bean dwarf mosaic. *Phytopathology* 80:96–101.

Morales FJ, Singh SP. 1993. Breeding for resistance to bean golden mosaic virus in an interracial population of *Phaseolus vulgaris* L. *Euphytica* 67:59–63.

Moran YM, Ramos PL, Dominguez M, Fuentes AD, Sanchez I, Crespo JA. 2006. Tobacco leaf curl Cuba virus, a new begomovirus infecting tobacco (*Nicotiana tabacum*) in Cuba. *Plant Pathol.* 55:570.

Moreira AG, Pereira CO, Andrade EC, Zerbini FM. 2005. Caracterización molecular de dos begomovirus que afectan a la soja (*Glycine max*) y malezas asociadas, en Brasil. In *Resúmenes XIII Congreso Latinoamericano de Fitopatologia*, p. 602. Cordoba, Argentina.

Morinaga T, Ikegami M, Miura K. 1987. Molecular cloning of full-length of bean golden mosaic virus DNA. *Ann. Phytopathol. Soc. Jpn.* 53:549–553.

Murayama A, Aragon L, Fernández-Northcote E. 2005. Nuevo begomovirus del grupo del Nuevo Mundo asociado al encrespamiento de la hoja del tomate en la costa del Peru. In *Resúmenes XIII Congreso Latinoamericano de Fitopatologia*, Cordoba, Argentina, p. 603.

Nakhla MK, Maxwell MD, Hidayat SH, Lange DR, Loniello AO, Rojas MR, Maxwell DP, Kitajima EW, Rojas A, Anderson P, Gilbertson RL. 1994. Two geminiviruses associated with tomatoes in Central America. *Phytopathology* 84:1155.

Naranjo SE, Cañas L, Ellsworth PC. 2004. Mortality factors affecting populations of sweet potato whitefly, *Bemisia tabaci*, in a multi-crop system. *Hortic. Int.* 2:14–21.

Nava AR, Patte CP, Hiebert E, Polston JE. 2006. Detection and variability of begomoviruses in tomato from the Andean states of Venezuela. *Plant Dis.* 90:61–66.

Novaes QS, Freitas-Astua J, Auki VA, Kitajima EW, Camargo LE, Rezende JA. 2003. Partial characterization of a bipartite begomovirus infecting yellow passion flower in Brazil. *Plant Pathol.* 52:648–654.

Nozaki DN, Krause-Sakate R, Hasegawa JM, Cezar MA, Dziuba PH, Pavan MA. 2006. First report of Tomato severe rugose virus infecting pepper plants in Brazil. *Fitopatol. Bras.* 31:321.

Orlando A, Silberschmidt K. 1946. Estudos sobre a disseminacão natural do virus da clorose infecciosa das malvaceas (Abutilon virus 1 Baur) e a sua relacão com o inseto-vetor *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae). *Arq. Inst. Biol. São Paulo* 17:136.

Pardina PE, Ploper LD, Truol GA, Hanada K, Platero GR, Ramirez P, Herrera PS, Laguna IG. 1998. Detection of a geminivirus in soybean crops in northwestern Argentina. *Rev. Ind. Agric. Tucuman* 75:51–56.

Paximadis M, Idris AM, Torres-Jerez I, Villarreal A, Rey MEC, Brown JK. 1999. Characterisation of tobacco geminiviruses in the Old and New World. *Arch. Virol.* 144:703–717.

Perring TM. 2001. The *Bemisia tabaci* species complex. *Crop Prot.* 20:725–737.

Philip CB, Rozeboom LE. 1973. Medico-veterinary entomology: a generation of progress. In *History of Entomology*, ed. RF Smith, TE Mittler, CN Smith, pp. 333–360. California, USA: Ann. Rev. Inc.

Pickersgill B. 1977. Taxonomy and the origin and evolution of cultivated plants in the New World. *Nature* 268:591–595.

Pierre RE. 1975. Observations on the golden mosaic of bean (*Phaseolus vulgaris* L.). In *Tropical Diseases of Legumes*, ed. J Bird, K Maramorosch, pp. 55–60. New York: Academic Press.

Polston JE, Anderson PK. 1997. The emergence of whitefly-transmitted geminiviruses in tomato in the Western Hemisphere. *Plant Dis.* 81:1358–1369.

Polston JE, Bois D, Ano G, Poliakoff N, Urbino C. 1998. Occurrence of a new strain of potato yellow mosaic geminivirus infecting tomato in the eastern Caribbean. *Plant Dis.* 82: 126.

Polston JE, Bois D, Serra CA, Concepción S. 1996. First report of a tomato yellow leaf curl-like geminivirus from tomato in the western hemisphere. *Plant Dis.* 78:831.

Quiñones M, Fonseca D, Accotto GP, Martinez Y. 2001. Viral infections associated with the presence of begomovirus in pepper plants in Cuba. *Rev. Prot. Veg.* 16: 147–151.

Ramirez JA, Armenta I, Delgadillo F, Rivera-Bustamante RF. 1995. Geminivirus transmitidos por mosquita blanca (*Bemisia tabaci* Gennn.) en los cultivos de chile y calabacita en el Valle de Mayo, Sonora, México. *Rev. Mex. Fitopatol.* 13:100–105.

Ramirez JA, Cardenas IA, Sánchez FD, Garzon-Tiznado JA. 1998. Virus transmitted by whitefly (*Bemisia tabaci* Gennadius) in pepper and zucchini squash in the Mayo Valley, Sonora, Mexico. *Agric. Tec. Mex.* 24:37–43.

Ramirez P, Chicas M, Salas J, Maxwell D, Karkashian J. 2004. Identificacion de un nuevo begomovirus en melon (*Cucumis melo* L.) en Lara, Venezuela. *Rev. MIP Agroecol.* 72:22–30.

Ramos PL, Guerra O, Peral R, Oramas P, Guevara RG, Rivera-Bustamante R. 1997. Taino tomato mottle virus, a new bipartite geminivirus from Cuba. *Plant Dis.* 81:1095.

Ramos PL, Guevara RG, Peral R, Ascencio-Ibañez JT, Polston JE, Arguello GR, Vega JC, Rivera-Bustamante RF. 2003. Tomato mottle Taino virus pseudorecombines with PYMV but not with ToMoV: implications for the delimitation of cis- and trans-acting replication specificity determinants. *Arch. Virol.* 148:1697–1712.

Rampersad SN, Umaharan P. 2003. Detection of two bipartite geminiviruses infecting dicotyledonous weeds in Trinidad. *Plant Dis.* 87:602.

Ribeiro SG, Ambrocevicius LP, Avila AC, Bezerra IC, Calegario RF, Fernandes JJ, Lima MF, Mello RN, Rocha H, Zerbini FM. 2003. Distribution and genetic diversity of tomato-infecting begomoviruses in Brazil. *Arch. Virol.* 148:281–295.

Ribeiro SG, Inoue-Nagata AK, Daniels J, Avila AC. 2006. Potato deforming mosaic disease is caused by an isolate of Tomato yellow vein streak virus. *Plant Pathol.* 55:569.

Roberts EJ, Buck KW, Coutts R. 1986. A new geminivirus infecting potatoes in Venezuela. *Plant Dis.* 70:603.

Rodriguez I, Morales H, Bueno JM, Cardona C. 2005. El biotipo B de *Bemisia tabaci* adquiere mayor importancia en el Valle del Cauca. *Rev. Col. Entomol.* 31:21–28.

Rojas A. 2005. A complex of begomoviruses affecting tomato crops in Nicaragua. In *Proceedings IX International Plant Virus Epidemiology Symposium*, p. 72. Lima, Peru.

Rojas A, Kvarnheden A, Valkonen JPT. 2000. Geminiviruses infecting tomato crops in Nicaragua. *Plant Dis.* 84:843–846.

Roossinck MJ. 1997. Mechanisms of plant virus evolution. *Annu. Rev. Phytopathol.* 35:191–209.

Roye ME, Wernecke ME, McLaughlin WA, Nakhla MK, Maxwell DP. 1999. *Tomato dwarf leaf curl virus*, a new bipartite geminivirus associated with tomatoes and peppers in Jamaica and mixed infection with *Tomato yellow leaf curl virus*. *Plant Pathol.* 48:370–378.

Royle DJ, Ostry ME. 1995. Disease and pest control in the bioenergy crops poplar and willow. *Biomass Bioenerg.* 9:69–75.

Rybicki EP. 1994. A phylogenetic and evolutionary justification for three genera of Geminiviridae. *Arch. Virol.* 139:49–77.

Rybicki EP, Briddon RW, Fauquet CM, Maxwell DP, Stanley J, Harrison BD, Brown JK. 2000. Geminiviridae. In *Virus Taxonomy: Seventh Report of the International Committee on Taxonomy of Viruses*, ed. MHV Regenmortel, CM Fauquet, DHL Bishop, EB Carsten, MK Estes, SM Lemon, J Maniloff, MA Mayo, DJ McGeoch, CR Pringle, RB Wickner, pp. 285–297. San Diego: Academic Press.

Saade RL. 1995. Estudios taxonómicos y ecogeográficos de las *Cucurbitaceae* latinoamericanas de importancia económica. *Inst. Int. Recur. Fitogen. (IPGRI)* 28:1.

Schieber E. 1970. Enfermedades del frijol (*Phaseolus vulgaris*) en la República Dominicana. *Turrialba* 20:20–23.

Seal S, VandenBosch F, Jeger MJ. 2006. Factors influencing begomovirus evolution and their increasing global significance: implications for sustainable control. *Crit. Rev. Plant Sci.* 25:23–46.

Segarra AE, Bird J, Escudero J. 1990. Silvering of *Cucurbita moschata* (Duchesne) associated with *Bemisia tabaci* Genn (Homoptera: Aleyrodidae) in Puerto Rico. *J. Agric. Univ. P.R.* 74:477–478.

Silberschmidt K. 1943. Estudos sobre a transmissão experimental da "cloroseinfecciosa" das malvaceas. *Arq. Inst. Biol. São Paulo* 14:105–156.

Silberschmidt K, Tommasi CR. 1955. Observações e estudos sobre especies de plantas suscetíveis à clorose infecciosa das malváceas. *An. Acad. Bras. Cienc.* 27:195–214.

Silva S, Rodriguez R, Garzon-Tiznado JA, Delgadillo F, Cardenas E. 1994. Efecto de una variante del virus enrollamiento de la hoja de la calabaza (VEHC, Squash leaf curl virus, SqLCV) en genotipos de cucurbitáceas. *Rev. Mex. Fitopatol.* 12:15–20.

Singh SP. 1988. Patterns of variation in cultivated common bean (*Phaseolus vulgaris*, Fabaceae). *Econ. Bot.* 43:39–57.

Spillari AG. 1994. Problemática del complejo mosca blanca-virus en algodón en Centroamerica. In *Memorias III Taller Centroamericano y del Caribe sobre Mosca Blanca*, pp. 23–38. Antigua Guatemala, Guatemala.

Stenger DC, Duffus JE, Villalon B. 1990. Biological and genomic properties of a geminivirus isolated from pepper. *Phytopathology* 80:704–709.

Thresh JM. 1980. The origins and epidemiology of some important plant virus diseases. *Appl. Biol.* 5:1–65.

Torre-Almaraz R, Monsalvo-Reyes AC, Mendez-Lozano J, Rivera-Bustamante RF. 2004. Characterization of a new geminivirus associated with okra (*Abelmoschus esculentus*) yellow mottle symptoms in Mexico. *Agrociencia* 38:227–238.

Torre-Almaraz R, Valverde R, Mendez J, Ibáñez JT, Rivera-Bustamante RF. 2002. Preliminary characterization of a geminivirus in tomatillo (*Physalis ixocarpa* B.) in the central region of Mexico. *Agrociencia* 36:471–481.

Torres-Pacheco I, Garzón-Tiznado JA, Brown JK, Rivera-Bustamante RF. 1996. Detection and distribution of geminiviruses in Mexico and the southern United States. *Phytopathology* 86:1186–1192.

Umaharan P, Padidam M, Phelps RH, Beachy RN, Fauquet CM. 1998. Distribution and diversity of geminiviruses in Trinidad Tobago. *Phytopathology* 88:1262–1268.

Urbino C, Polston JE, Patte CP, Caruana ML. 2004. Characterization and genetic diversity of *Potato yellow mosaic virus* from the Caribbean. *Arch. Virol.* 149:417–424.

Urbino C, Tassius K. 1999. First report of *Tomato yellow leaf curl virus* in tomato in Guadeloupe. *Plant Dis.* 35:46–53.

Van den Bosch F, Akudibilah G, Seal S, Jeger M. 2006. Host resistance and the evolutionary response of plant viruses. *J. App. Eco.* 43:506–516.

Vanderplank JE. 1963. *Plant Diseases: Epidemics and Control*. New York: Academic Press.

Vega J, Winter S, Pural A, Hamilton RI. 1992. Partial characterization of a whitefly-transmitted geminivirus from potato in Brazil. *Fitopatol. Bras.* 17:167.

Walkey DGA, Spence NJ, Clay CM, Miller A. 1994. A potyvirus isolated from solanaceous hosts. *Plant Pathol.* 43:931–937.

Wolf FA, Whitecomb WH, Mooney WC. 1949. Leaf curl of tobacco in Venezuela. *J. Elisha Mitchel Sci. Soc.* 65:38–47.

Zadoks JC, Schein RD. 1979. *Epidemiology and Plant Disease Management*. Oxford, UK: Oxford University Press, 427pp.

Zalom FG, Natwick ET, Toscano NC. 1985. Temperature regulation of *Bemisia tabaci* (Homoptera: Aleyrodidae) populations in Imperial Valley cotton. *J. Econ. Entomol.* 78:61–64.

# Chapter 10
# Transmission Efficiency and Epidemiology of Criniviruses

**William M. Wintermantel**

## Introduction

An emergence of numerous whitefly-transmitted criniviruses has followed the increase in whitefly populations over the last decade throughout the world (Wintermantel 2004a). The impact of these criniviruses on vegetable and fruit production has created a critical need for better understanding of host range and vector relationships in order to develop effective and efficient management practices. Criniviruses have large bipartite RNA genomes encoding several open reading frames (ORFs). RNA1 encodes functions involved in virus replication, while RNA2 encodes up to 7 ORFs involved in virion assembly and vector transmission in addition to several other functions, many of which remain to be determined (Karasev 2000). Virions are encapsidated into long flexuous rods averaging between 650 and 900 nm in length.

Criniviruses are an emerging genus worldwide, with numerous new species having been identified within the past several years (Celix et al. 1996; Cohen et al. 1992; Duffus et al. 1996a, b; Salazar et al. 2000; Tzanetakis et al. 2006; Winter et al. 1992; Wisler et al. 1998b). Criniviruses often cause symptoms that are readily mistaken for physiological or nutritional disorders, or pesticide phytotoxicity. Depending on the host plant affected, these symptoms include interveinal yellowing of leaves, an associated loss of photosynthetic capability, leaf brittleness, reduced plant vigor, yield reductions, and early senescence. Symptoms typically appear on the middle and older parts of plants, with new growth appearing normal (Fig. 10.1) and symptoms progressing outward over time. Criniviruses remain confined to cells associated with host plant phloem. Symptoms are thought to result from plugging the phloem with large viral inclusion bodies that interfere with normal vascular transport in infected plants (Wisler and Duffus 2001).

Correlation of virus-like symptoms, with prior or continuing incidence of significant whitefly populations, is an excellent indicator that the symptoms may be

W.M. Wintermantel (✉)
USDA-ARS, 1636 East Alisal Street, Salinas, CA 93905-3018, USA
e-mail: bill.wintermantel@ars.usda.gov

P.A. Stansly, S.E. Naranjo (eds.), *Bemisia*: Bionomics and Management of a Global Pest,
DOI 10.1007/978-90-481-2460-2_10, 

**Fig. 10.1** Symptoms of CYSDV on melon illustrating the typical crinivirus symptom pattern of interveinal chlorosis on older tissues (*yellowing* near center of plant), with newer growth remaining *green* (*green* tissues at vine ends). Chlorotic symptoms generally appear first on tissues near the crown, and progress outward or upward over time, depending on the type of plant infected

the result of virus infection. Confirmation, however, requires testing of plant material for the presence of virus particles or viral RNA. This can be done using either serological or molecular methods. Serological methods – including enzyme-linked immunosorbant assay (ELISA) as well as Western blot – can effectively identify specific criniviruses from infected leaf tissue (Li et al. 1998). Although these methods can be effective, antiserum is not always readily available, and many crinivirus antisera are ineffective for reliable detection using standard methods such as ELISA. In addition, some cross reactivity has been seen between related criniviruses (Li et al. 1998). More reliable detection involves reverse transcriptase-polymerase chain reaction (RT-PCR). Total nucleic acid – or double-stranded RNA (dsRNA) – can be extracted from symptomatic leaves of plants suspected of crinivirus infection. Virus-specific primers can be obtained or designed from sequences deposited in genomic databases, as well as from numerous publications listing information on sequence information and/or virus detection. Primers specific to sections of individual crinivirus genes can be used to selectively amplify crinivirus sequences from nucleic acid extracts of numerous and diverse plant species. RT-PCR offers the advantage of being able to screen the same sample for numerous criniviruses in a relatively short period of time. In addition to RT-PCR, molecular probes designed to match specific sections of crinivirus genomes can be used to identify and differentiate criniviruses from one another by using dot blot hybridization. This

technique offers the advantage of screening large numbers of samples simultaneously. Both RT-PCR and hybridization are highly efficient methods for detection and differentiation of criniviruses; each offers unique advantages.

## Crinivirus Epidemiology Is Influenced by Vector Whitefly Population Shifts

Unlike whitefly-borne begomoviruses – which are exclusively transmitted by *Bemisia tabaci* – criniviruses can be transmitted by multiple species of both *Bemisia* and *Trialeurodes*. The prevalence of one whitefly species, or biotype, can have profound effects on the diversity and prevalence of crinivirus diseases. Changes in the whitefly species common to a region, or increases in populations of a vector, can dramatically influence disease prevalence and crop losses. One of the earliest well-characterized criniviruses, LIYV is transmitted with high efficiency only by the A biotype of *B. tabaci*. This biotype was common in the American Southwest in the 1980s when the virus caused severe losses for a wide range of crops including melon, lettuce, and sugarbeet (Wisler et al. 1998a). In spite of great efforts toward control, LIYV remained a serious threat to crop production until the early 1990s, when the B biotype rapidly displaced the A biotype over a period of several months. The rapid disappearance of LIYV that resulted from the elimination of its vector, *B. tabaci* biotype A, led to the discovery of a second, somewhat milder crinivirus in the same region. *Lettuce chlorosis virus* (LCV) can be transmitted with relatively equal efficiency by both A and B biotypes (Wisler et al. 1998a). LCV symptoms on lettuce are essentially identical to those of LIYV, but the virus does not affect the same range of hosts as LIYV (Duffus et al. 1996b). LCV is less aggressive than LIYV; unless it infects young lettuce plants early, it does not lead to the economic losses associated with LIYV infection. It is suspected that LCV was present at the same time as LIYV, but due to the severity of LIYV, and similar virion structure, it was not identified prior to the decline of LIYV (Wisler et al. 1998a). Another possibility is that LCV was less competitive in host plant species than LIYV, and, therefore, may not have accumulated efficiently during co-infections. While competitiveness has not been tested for these viruses, recent studies comparing co-infection of *Tomato infectious chlorosis virus* (TICV) and *Tomato chlorosis virus* (ToCV) suggest competitiveness may vary among host plant species.

The prevalence of vectors plays a significant role in the incidence and severity of crinivirus outbreaks. Although *Beet pseudo yellows virus* (BPYV) had been known to exist in coastal regions of California since the 1960s, it was only identified as a problem in pumpkin and strawberry in the early 2000s during a period of tremendous population growth by its vector, *Trialeurodes vaporariorum* (Tzanetakis et al. 2003; Wintermantel 2004b). The emergence of *Cucurbit yellow stunting disorder virus* (CYSDV) in 2006 in California and Arizona, USA, and Sonora, Mexico, resulted in the infection of nearly 100% of the melon crop in the region (Kuo et al. 2007; Brown et al. 2007). This was most likely due to persistence of the virus in the

most common vector in the region, *B. tabaci* biotype B (Celix et al. 1996; Wisler et al. 1998a), and the tremendous vector populations that occur in the region each summer and fall.

*Sweet potato chlorotic stunt virus* (SPCSV) is the crinivirus component of a crinivirus-potyvirus synergism responsible for sweet potato virus disease. In most areas of the world, SPCSV is transmitted primarily by *B. tabaci* biotype B; however, *Bemisia afer sens. lat.*, an old world whitefly well established in coastal regions of Peru (Anderson et al. 2001), was recently shown through experimental transmission studies, to efficiently transmit this virus (H. Gamarra per. comm.). *B. afer* populations peak during the winter months when populations of *B. tabaci* biotype B are in decline. This cyclic pattern of population increase and decline, involving two different *Bemisia* vector species, may prove an important component of the epidemiology of SPCSV and sweet potato virus disease in South America.

## Unique Vector Transmission Characteristics of Tomato Criniviruses

In the mid-1990s, two new criniviruses emerged as threats to tomato production in North America. TICV and ToCV cause identical symptoms on tomato, including interveinal yellowing and thickening of leaves (Wisler et al. 1996; 1998b). Although no obvious symptoms occur on the fruit, production is affected through decreased fruit size and number, as well as early senescence. Both tomato-infecting criniviruses are transmitted by the greenhouse whitefly (*T. vaporariorum*). ToCV is unique, however, in that it is also transmitted by the bandedwinged whitefly *T. abutiloneus* (Haldeman) and *B. tabaci* biotypes A and B. Both viruses have now been found in widespread areas of North America and Europe in both field and greenhouse environments. They are also becoming increasingly identified in other subtropical as well as temperate areas of the world where vectors are present. TICV is abundant in tomato production fields along the west coast of North America, both in Mexico and California (Wintermantel 2004a). ToCV is common in the southeastern USA and also has been found in Puerto Rico (Wintermantel et al. 2001), Mexico (Alvarez-Ruiz et al. 2007), Europe (Accotto et al. 2001; Dalmon et al. 2005; Dovas et al. 2002; Louro et al. 2000; Navas-Castillo et al. 2000), Morocco (Hanafi 2002), Taiwan (Tsai et al. 2004) and the Middle East (Abou-Jawdah et al. 2006; Segev et al. 2004). TICV was first identified in 1993 in Orange County, CA, where growers experienced serious losses as a result of TICV infection (Wisler et al. 1998a). Fields in coastal regions of southern California and western Mexico continue to experience high levels of TICV infection annually, and greenhouse producers have experienced significant economic losses from tomato criniviruses. Enclosed greenhouse production centers can lead to accumulation of high *T. vaporariorum* populations, which in turn facilitate viral spread throughout the facility. This is particularly a problem with organic or reduced chemical production operations, where insecticide use is either limited or impossible.

Both tomato criniviruses have relatively long latent periods in infected host plants, often not inducing symptoms until 3–4 weeks after infection. Symptoms are identical on tomato, the most important cultivated host. If nursery plants are exposed to vector populations at an early age, it is possible for these viruses to be spread to new areas through movement of transplants prior to symptom development. Weed hosts near production areas represent another potential virus reservoir. For example, TICV can infect bristly oxtongue (*Picris echioides* L.), tree tobacco (*Nicotiana glauca* Graham), and wild artichoke (*Cynara cardunculus* L.) (Duffus et al. 1996a). Black nightshade (*Solanum nigrum* L.) was found to be infected with ToCV in Alicante and Murcia, Spain (Font et al. 2004). Similarly, some ornamental plants can serve as reservoirs for virus infection (Wisler et al. 1996, 1998a). Reservoir hosts near field or greenhouse production areas may also serve as sources for whitefly feeding, resulting in movement of the virus into surrounding fields. Criniviruses are not transmitted mechanically, and are therefore dependent on whiteflies for spreading the virus from plant-to-plant.

The increased incidence of criniviruses, and their whitefly vectors in field and greenhouse production systems highlights the need for additional efforts toward resistance and management of whitefly-transmitted viruses. Efforts to elucidate factors contributing to the emergence and prevalence of criniviruses are important in understanding virus epidemiology as well as in developing effective management strategies for virus control. The relationship between criniviruses, their host plants, and their *Bemisia* and *Trialeurodes* vectors is central to crinivirus epidemiology.

## Factors Influencing Crinivirus Transmission Efficiency

ToCV is unique in its transmission properties as well as its ability to be transmitted by four different whitefly vectors in two genera (Wisler et al. 1998b). This is highly unusual for any whitefly-transmitted virus, most of which are transmitted by a single genus or species of whitefly. Among criniviruses, only ToCV is known to be transmitted by species within both genera. Although this has been known since the initial characterization of this virus, the efficiency of transmission by the different vector species was only recently demonstrated (Wintermantel and Wisler 2006). ToCV can be transmitted equally well and with high efficiency by both *B. tabaci* biotype B and *T. abuliloneus*, even with individual whiteflies of either species (Fig. 10.2; Wintermantel and Wisler 2006). Transmission by other members of these genera was much less efficient. Both *B. tabaci* biotype A and *T. vaporariorum* can transmit ToCV, but single insect transmission was not observed with either of the latter two vectors (Wintermantel and Wisler 2006).

A comparison of virus persistence in the two more efficient vectors, *B. tabaci* biotype B and *T. abuliloneus*, further illustrates their unique relationship with the virus. (Wintermantel and Wisler 2006). Although overall transmission efficiency is comparable between the two species (Fig. 10.2), persistence of ToCV in *T. abutiloneus* far exceeds that in *B. tabaci* biotype B (Fig. 10.3). Clearly, both of these

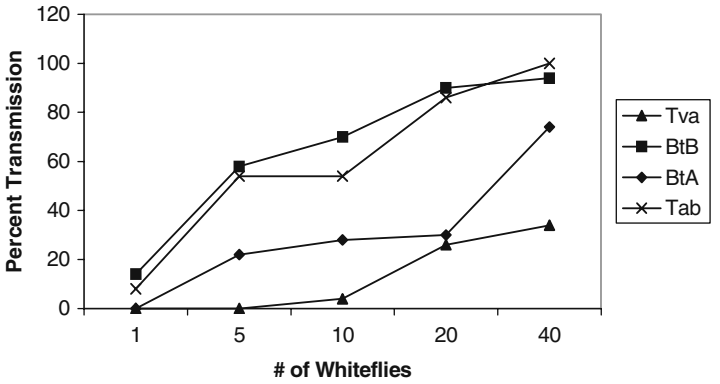



**Fig. 10.2** Efficiency of ToCV transmission differed among each of four different whitefly vectors ($p < 0.0001$). Percent transmission by each vector (48 h inoculation access period) is compared by number of whiteflies fed on *P. wrightii* test plants, following 24 h acquisition access period on ToCV-infected *P. wrighii*. Transmissions involved 5 independent replications of 10 plants each, per whitefly species, per number of whiteflies. Tva, *T. vaporariorum*; BtA, *B. tabaci* biotype A; BtB, *B. tabaci* biotype B; Tab, *T. abutiloneus* (Wintermantel and Wisler 2006)

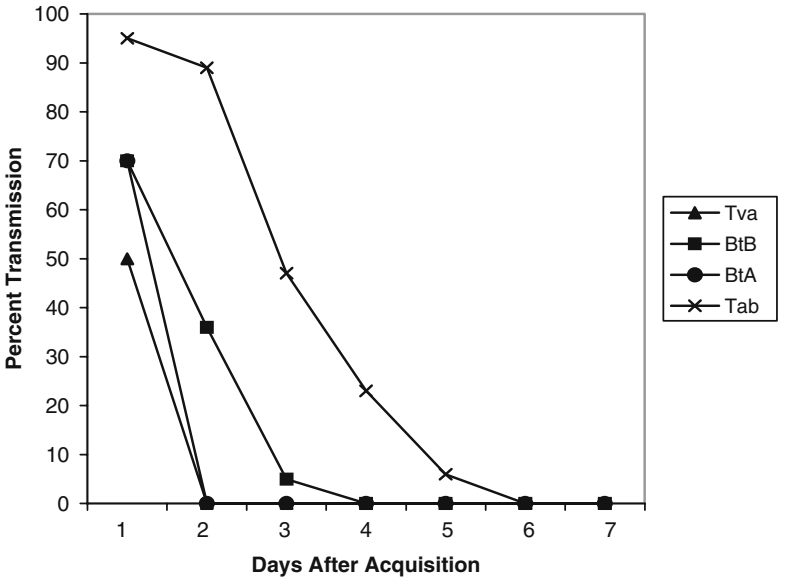



**Fig. 10.3** Persistence of ToCV in four different whitefly vectors. Percent transmission by each vector is compared by the number of days after virus acquisition during serial passage. Acquisition access periods consisted of 24 h mass feeding on ToCV-infected *P. wrightii*. Transmissions involved 30 whiteflies per plant in clip cages, moved to new plants each day for 7 days, with approximately 24 h inoculation access periods per transfer. Experiments with the most efficient vectors, Tab* and BtB, involved 7 replications of 10 plants each per whitefly species. Experiments with the less efficient vectors, BtA and Tva, involved 2 replications of 10 plants each. Tab, *T. abutiloneus*; BtB, *B. tabaci* biotype B; BtA, *B. tabaci* biotype A; Tva, *T. vaporariorum*. *One replication with Tab consisted of only 5 plants per date rather than 10 (Wintermantel and Wisler 2006)

more efficient vectors retain virus in a transmissible form longer than either of the less efficient vectors. However, *T. abutiloneus* was able to transmit ToCV at a low level 5 days after feeding on an infected source, while *B. tabaci* biotype B lost its ability to transmit after 3 days. ToCV persisted in *B. tabaci* biotype A and *T. vaporariorum* 24 h or less (Fig. 10.3) (Wintermantel and Wisler 2006). The poor retention observed in the less efficient vectors was not surprising, but the variation in persistence among efficient vectors was unexpected.

A number of factors' are likely to contribute to crinivirus transmission efficiency and virus persistence in whitefly vectors. Recent studies with another crinivirus, LIYV, demonstrated that transmission by *B. tabaci* biotype A is influenced not only by the number of whiteflies used for transmission, but also by the amount of virus available for transmission (Ng et al. 2004). Studies on transmission efficiency of ToCV and the related tomato crinivirus, TICV, confirmed the correlation of crinivirus transmission efficiency and number of vectors. The data also demonstrated that both efficiency of transmission and persistence can vary by vector species (Wintermantel and Wisler 2006). ToCV may represent an evolutionary link between *Trialeurodes*-transmitted criniviruses and those transmitted exclusively by *Bemisia* species. This was supported by a phylogenic examination of crinivirus-encoded proteins (Wintermantel et al. 2005). Although little is known about the role of crinivirus-encoded proteins in whitefly transmission, evidence based on deletion mutants suggests that the minor coat protein (CPm) of LIYV may be involved in its transmission by *B. tabaci* biotype A (Tian et al. 1999). Interestingly, the CPm of ToCV is phylogenically situated between similar proteins of the both *Trialeurodes*-transmitted and *Bemisia*-transmitted criniviruses (Wintermantel et al. 2005). A comparison of protein similarity found comparable levels of relatedness for the ToCV CPm with the CPm of *T. vaporariorum*-transmitted BPYV (49%) and *Potato yellow vein virus* (PYVV) (44%), as well as with the *B. tabaci* biotype B-transmitted SPCSV (51%) and CYSDV (48%) (Wintermantel et al. 2005). It should be noted that other viral proteins may be involved in vector transmission as well, but to date only the CPm has been implicated.

## *Does the Host Plant Influence Crinivirus Transmission by Whitefly Vectors?*

As a follow-up to studies on vector transmission efficiency of criniviruses, an experiment was conducted to examine competitiveness of ToCV and TICV in different hosts, in addition to how host-specific accumulation influences vector transmission (Wintermantel et al. 2008). The viruses share numerous hosts, although there are differences as well. Research focused on two hosts common to a broad array of criniviruses, *Physalis wrighii* and *Nicotiana benthamiana*. Plants of each host were inoculated with TICV and ToCV individually by viruliferous *T. vaporariorum* (for TICV) and *T. abutiloneus* or *B. tabaci* biotype B (for ToCV). As illustrated in Figs. 10.2 and 10.3, these are the most efficient vectors of ToCV. *T. vaporariorum* is the only known vector of TICV (Duffus et al. 1996a) and cannot be transmitted by

*B. tabaci* or *T. abutiloneus*. In addition, plants of each host were established with mixed infections of both TICV and ToCV. Infections of host plants were confirmed by hybridization using molecular probes for detection of each virus and/or RT-PCR. Singly-infected and co-infected plants were subsequently tested by quantitative RT-PCR to identify plants with comparable levels of virus for use in transmission studies. Once identified, individual leaves from the selected plants were used for virus acquisition, separately, by both *T. abutiloneus*, and *T. vaporariorum.* Following 48 h virus acquisition periods, whiteflies were transferred to new plants of the same species from which they originated for 48 h after which they were killed with insecticide. Four weeks post-transmission, virus titer was again checked using qRT-PCR to determine the level of virus accumulation in single and mixed infections in each host. Results demonstrated that each host differed in its ability to accumulate each virus. Data also showed that when both viruses co-infected a host plant, there were differences in accumulation, suggesting fitness of each virus differed by host.

During single infections, TICV accumulated to higher concentrations in *P. wrightii* than ToCV by approximately 3:2 (Fig. 10.4). During co-infection, however, ToCV was clearly the dominant virus, accumulating to levels over three times higher than TICV. Not surprisingly, both viruses accumulated less during mixed infections than in single infections. This may be a result of competition for host factors since the two viruses are closely related to one another. It is interesting that ToCV, although less aggressive in this host than TICV during single infections, seems to be better at competing for necessary replication factors than TICV when the two viruses co-infected *P. wrightii*. In contrast, there was no significant difference in virus accumulation by TICV or ToCV during single infections of *N. benthamiana* by each virus. During mixed infections, however, TICV accumulated to levels 2.5 times higher than ToCV. Accumulation of both viruses was

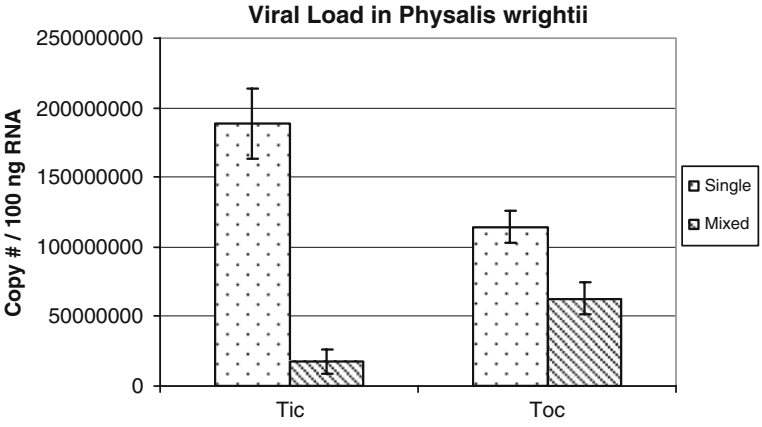



**Fig. 10.4** Mean differences in amount of TICV and ToCV present in single infections of each virus in *Physalis wrightii*, compared with mixed infections containing both viruses. $p = 0.0008$ for difference in amount of TICV between single and mixed infections. $P = 0.0075$ for difference in amount of ToCV between single and mixed infections

significantly reduced during mixed infection just as it was in *P. wrightii*, again suggesting that competition for host factors may limit accumulation of each virus during co-infection. TICV accumulation was reduced by over half during mixed infection compared with single infection, while ToCV accumulation was reduced approximately six fold (data not shown).

These results suggest that the ability of both viruses to accumulate in a host is altered during co-infection in a host-specific manner (Wintermantel et al. 2008). Although both *P. wrightii* and *N. benthamiana* are excellent experimental hosts for the study and maintenance of both viruses, these results demonstrate that when two viruses compete with one another for the ability to replicate, there are clear differences in fitness or competitiveness among host plants. This difference in accumulation during mixed infection also translates into differences in transmissibility of each virus during mixed infection. When vector whitefly species feed on *N. benthamiana,* or *P. wrightii* plants singly-infected with either virus, transmission efficiency is excellent. It should be noted that whitefly feeding on *N. benthamiana* is not as aggressive as in *P. wrightii*, but this does not impact crinivirus transmission (unpubl. data). When plants containing mixed infection of TICV and ToCV are used as source plants for transmission by vector whiteflies, the rate of transmission – or percent of infected plants – reflects the titer of each virus in the source, although transmission efficiency of the vector whitefly is also a contributing factor.

Transmission of ToCV by *T. vaporariorum* has been demonstrated to be much less efficient than transmission by *T. abutiloneus* or *B. tabaci* biotype B, (Wintermantel and Wisler 2006; Figs. 10.2 and 10.3). *T. vaporariorum* is capable of relatively efficient transmission of ToCV during the first 24 h following virus acquisition, although still much less efficiently than by the other two vectors under identical conditions. In transmission experiments conducted immediately after a 48-h virus acquisition period on infected *P. wrightii*, TICV and ToCV were transmitted with equal efficiency from single infections – 93 and 95%, respectively – to new *P. wrightii* plants by *T. vaporariorum* (Table 10.1). When *P. wrightii* source plants were infected with both viruses, however, ToCV was transmitted much better to new *P. wrightii* than was TICV (Table 10.1), even though *T. vaporariorum* is more efficient at transmission of TICV than ToCV during single infections (Duffus et al. 1996a; Wintermantel and Wisler 2006). Although TICV transmission from single infections was effective, it was difficult to obtain transmission of TICV by its native vector, *T. vaporariorum,* from *P. wrightii* plants co-infected with ToCV. Transmission from mixed infections containing approximately equivalent levels of ToCV and TICV resulted in only eight of 53 new plants developing TICV infections; six of these contained mixed infections of TICV and ToCV together (Table 10.1). In contrast, 39 of 53 plants developed infection with ToCV. ToCV was also transmitted well from mixed infections by *T. abutiloneus*, its most efficient vector. ToCV was transmitted with a 100% infection rate from single infections. TICV was not transmitted from single infections by *T. abutiloneus*, which has been demonstrated to be a non-vector of TICV. Transmission of ToCV by *T. abutiloneus* from *P. wrightii* co-infected with TICV and ToCV resulted in 90% ToCV infection of new *P. wrightii* (Table 10.1).

**Table 10.1** Percent transmission of TICV and ToCV from single and mixed virus infections in two hosts by *Trialeurodes vaporariorum* and *T. abutiloneus*

| | Transmission from single infections | | Transmission from mixed infections | | |
|---|---|---|---|---|---|
| | TICV[1] | ToCV[1] | TICV | ToCV | TICV + ToCV |
| *P. wrightii* × TVA[1] | 13/14 (93) [2] | 19/20 (95) | 8/53 (15) | 39/53 (74) | 6/53 (11) |
| *P. wrightii* × TAB[1] | 0/15 (0) | 16/16 (100) | 1/58 (2) | 52/58 (90) | 1/58 (2) |
| *N. benthamiana* × TVA | 8/16 (50) | 11/16 (69) | 19/22 (86) | 11/22 (50) | 11/22 (50) |
| *N. benthamiana* × TAB | 0/14 (0) | 23/28 (82) | 1/45 (2) | 35/45 (78) | 1/45 (2) |

[1]TICV, tomato infectious chlorosis virus; ToCV, tomato chlorosis virus; TVA, trialeurodes vaporariorum; TAB, trialeurodes abutiloneus.
[2]Number of plants infected/number tested; parentheses indicate percent infection.

Transmission rates for both viruses by *T. vaporariorum* – from singly infected *N. benthamiana* to new *N. benthamiana* plants – were statistically identical, although the actual number of ToCV infected plants resulting from transmissions were slightly higher than for TICV, and fewer plants were involved than in *P. wrightii* experiments (Table 10.1). In contrast to results with *P. wrightii*, however, *T. vaporariorum* transmission from mixed infections containing approximately equivalent levels of TICV and ToCV resulted in higher rates of TICV transmission than ToCV, with 19 of 22 plants becoming infected with TICV following transmission from co-infected source plants, compared with 11 of 22 infected with ToCV (Table 10.1). It should be noted that all plants infected with ToCV were also infected with TICV. When *T. abutiloneus* was used as the vector, ToCV was transmitted from single and mixed infections at approximately equal rates, but in both cases transmission rates were higher than when ToCV was transmitted to this host by *T. vaporariorum* (Table 10.1), a less efficient vector than *T. abutiloneus*. TICV was not transmitted from single infections by *T. abutiloneus*.

Although *T. abutiloneus* is not a vector of TICV, transmission from mixed infections containing approximately equivalent levels of TICV and ToCV resulted in transmission of TICV to individual plants of both *P. wrightii* and *N. benthamiana*. One plant of 53 *P. wrightii* and one of 45 *N. benthamiana* plants tested in transmission experiments became infected with both TICV and ToCV following transmission from mixed infections by *T. abutiloneus* using nucleic acid hybridization (Table 10.1). Two later experiments were conducted in an attempt to repeat this transmission of TICV by a non-vector from mixed infections in both hosts, but without success. In order to have a significant level of confidence in this non-vector transmission of a crinivirus, from plants co-infected with a virus transmissible by the vector, it will be necessary to repeat these results. However, the results at this early stage seem to indicate a potential for mixed infections to facilitate transmission of a virus by a vector that would not normally be expected to do so. Transmission of

TICV by a non-vector whitefly species from plants co-infected, with a related virus that can be readily transmitted by that whitefly species, suggests that criniviruses can be complemented for transmission by a non-vector.

It is now clear that numerous factors contribute toward crinivirus epidemiology, virus emergence and dominance, including whitefly transmission efficiency of a virus, virus titer in a host, and competition between viruses co-infecting a plant. The focus was on experimental hosts in the studies described above, but this data has relevance to field infections as well. The results have far-reaching implications. The clear differences in virus accumulation between single and mixed infections in two hosts demonstrates the importance of both the host, and the fitness of the virus in that host, in determining the ability of the virus to compete during a mixed infection. Furthermore, the combined effect of these factors clearly has the ability to influence which virus is transmitted most frequently in areas where co-infection is already prevalent, in addition to where it can potentially have a profound impact on the emergence and dominance of a virus introduced to a new region. Although the frequency of transmission of TICV by a non-vector whitefly was quite low, the mere occurrence of this event indicates that mixed infections of related viruses may influence virus transmission. It also suggests potential for viable recombinants to form among crinivirus species infecting the same host. The occurrence of mixed crinivirus infections is not uncommon in the field; it has, in fact, been documented for a number of virus-vector combinations in several hosts (Wisler et al. 1998a; Tsai et al. 2004; Tzanetakis et al. 2006).

Once a crinivirus is introduced to a new area, its unique vector transmissibility and host range determine its ability to establish and recur in the region. The potential for virus movement increases as whitefly species and biotypes are inadvertently moved throughout the world on plants and produce; the potential for co-infection by related viruses that infect similar hosts is also amplified. Viruses that are transmitted exclusively by a single vector species, however, will not become established in an area where that vector is not present, even if inadvertently introduced. If criniviruses complement one another for vector transmission, or potentially recombine with one another – resulting in a new virus with different vectoring potential – the unexpected could occur. Only through further study of these complex relationships among vectors, hosts, and viruses can we be prepared to manage virus epidemiology in modern agriculture where these viruses and vectors are continuously being introduced to new areas through transportation, trade, and environmental factors.

## Literature Cited

Abou-Jawdah Y, El Mohtar C, Atamian H, Sobh H. 2006. First report of *Tomato chlorosis virus* in Lebanon. *Plant Dis.* 90:378.

Accotto GP, Vaira AM, Vecchiati M, Finetti Sialer MM, Gallitelli D, Davino M. 2001. First report of *Tomato chlorosis virus* in Italy. *Plant Dis.* 85:1208.

Alvarez-Ruiz P, Gamez Jimenez C, Leyva-Lopez NE, Mendez-Lozano J. 2007. First report of *Tomato chlorosis virus* infecting tomato crops in Sinaloa, Mexico. *New Dis. Rep.* http://www.bspp.org.uk/publications/new-disease-reports/ndr.php?id=014040

Anderson PK, Martin JH, Hernandez P, Lagnaui A. 2001. *Bemisia afer sens. lat*. (Homoptera: Aleyrodidae) outbreak in the Americas. *Fla. Entomol.* 84:316–317.

Brown JK, Guerrero JC, Matheron M, Olson M, Idris AM. 2007. Widespread outbreak of *Cucurbit yellow stunting disorder virus* in melon, squash, and watermelon crops in the Sonoran Desert of Arizona and Sonora, Mexico. *Plant Dis.* 91:773.

Celix A, Lopez-Sese A, Almarza N, Gomez-Guillamon ML, Rodriguez-Cerezo E. 1996. Characterization of *Cucurbit yellow stunting disorder virus*, a *Bemisia tabaci*-transmitted closterovirus. *Phytopathology* 86:1370–1376.

Cohen J, Frank A, Vetten HJ, Lesemann DE, Loebenstein G. 1992. Purification and properties of closeteovirus-like particles associated with a whitefly transmitted disease of sweet potato. *Ann. Appl. Biol.* 121:257–268.

Dalmon A, Bouyer S, Cailly M, Girard M, Lecoq H, Desbiez C, Jacquemond M. 2005. First report of *Tomato chlorosis virus* and *Tomato infectious chlorosis virus* in tomato crops in France. *Plant Dis.* 89:1243.

Dovas CI, Katis NI, Avgelis AD. 2002. Multiplex detection of criniviruses associated with epidemics of a yellowing disease of tomato in Greece. *Plant Dis.* 86:1345–1349.

Duffus JE, Liu H-Y, Wisler GC. 1996a. *Tomato infectious chlorosis virus*-A new clostero-like virus transmitted by *Trialeurodes vaporariorum*. *Eur. J. Plant Pathol.* 102:219–226.

Duffus JE, Liu H-Y, Wisler GC, Li RH. 1996b. *Lettuce chlorosis virus*-A new whitefly-transmitted closterovirus. *Eur. J. Plant Pathol.* 102:591–596.

Font MI, Juarez M, Martinez O, Jorda C. 2004. Current status and newly discovered natural hosts of *Tomato infectious chlorosis virus* and *Tomato chlorosis virus* in Spain. *Plant Dis.* 88:82.

Hanafi A. 2002. Invasive species: a real challenge to IPM in the Mediterranean region? EWSN Newlett., no. 13, May 2002, ed. ID Bedford, DJ Oliver, p. 4.

Karasev AV. 2000. Genetic diversity and evolution of Closteroviruses. *Annu. Rev. Phytopathol.* 38:293–324.

Kuo Y-W, Rojas MR, Gilbertson RL, Wintermantel WM. 2007. First report of *Cucurbit yellow stunting disorder virus* in California and Arizona, in association with *Cucurbit leaf crumple virus* and *Squash leaf curl virus*. *Plant Dis.* 91:330.

Li RH, Wisler GC, Liu H-Y, Duffus JE. 1998. Comparison of diagnostic techniques for detecting *Tomato infectious chlorosis virus*. *Plant Dis.* 82:84–88.

Louro D, Accotto GP, Vaira AM. 2000. Occurrence and diagnosis of *Tomato chlorosis virus* in Portugal. *Eur. J. Plant Pathol.* 106:589–592.

Navas-Castillo J, Camero R, Bueno M, Moriones E. 2000. Severe yellowing outbreaks in tomato in Spain associated with infections of *Tomato chlorosis virus*. *Plant Dis.* 84:835–837.

Ng JCK, Tian T, Falk BW. 2004. Quantitative parameters determining whitefly (*Bemisia tabaci*) transmission of *Lettuce infectious yellows virus* and an engineered defective RNA. *J. Gen. Virol.* 85:2697–2707.

Salazar LF, Muller G, Querci M, Zapata JL, Owens RA. 2000. *Potato yellow vein virus*: its host range, distribution in South America, and identification as a crinivirus transmitted by *Trialeurodes vaporariorum*. *Ann. Appl. Biol.* 137:7–19.

Segev L, Wintermantel WM, Polston JE, Lapidot M. 2004. First report of *Tomato chlorosis virus* in Israel. *Plant Dis.* 88:1160.

Tian TY, Rubio L, Yeh HH, Crawford B, Falk BW. 1999. *Lettuce infectious yellows virus*: in vitro acquisition analysis using partially purified virions and the whitefly *Bemisia tabaci*. *J. Gen. Virol.* 80:1111–1117.

Tsai WS, Shih SL, Green SK, Hanson P, Liu HY. 2004. First report of the occurrence of *Tomato chlorosis virus* and *Tomato infectious chlorosis virus* in Taiwan. *Plant Dis.* 88:311.

Tzanetakis IE, Wintermantel WM, Cortez AA, Barnes JE, Barrett SM, Bolda MP, Martin RR. 2006. Epidemiology of *Strawberry pallidosis-associated virus* and occurrence of pallidosis disease in North America. *Plant Dis.* 90:1343–1346.

Tzanetakis IE, Wintermantel WM, Martin RR. 2003. First report of *Beet pseudo yellows virus* in strawberry: a second crinivirus able to cause pallidosis disease. *Plant Dis.* 87:1398.

Winter S, Purac A, Leggett F, Frison EA, Rossel HW, Hamilton RI. 1992. Partial characterization and molecular cloning of a closterovirus from sweet potato infected with the sweet potato virus disease complex from Nigeria. *Phytopathology* 82:869–875.

Wintermantel WM. 2004a. Emergence of greenhouse whitefly (*Trialeurodes vaporariorum*) transmitted criniviruses as threats to vegetable and fruit production in North America. APSnet Feature (www.apsnet.org/online/feature/whitefly/).

Wintermantel WM. 2004b. Pumpkin (*Cucurbita maxima and C. pepo*), a new host of *Beet pseudo yellows virus* in California. *Plant Dis.* 88:82.

Wintermantel WM, Polston JE, Escudero J, Paoli ER. 2001. First report of *Tomato chlorosis virus* in Puerto Rico. *Plant Dis.* 85:228.

Wintermantel WM, Wisler GC. 2006. Vector specificity, host range, and genetic diversity of *Tomato chlorosis virus*. *Plant Dis.* 90:814–819.

Wintermantel WM, Wisler GC, Anchieta AG, Liu H-Y, Karasev AV, Tzanetakis IE. 2005. The complete nucleotide sequence and genome organization of *Tomato chlorosis virus*. *Arch. Virol.* 150:2287–2298.

Wintermantel WM, Cortez AA, Anchieta AG, Gulati Sakhuja AN, Hladky LL. 2008. Co-infection by two criniviruses alters accumulation of each virus in a host-specific manner and influences efficiency of virus transmission. *Phytopathology* 98:1340–1345.

Wisler GC, Duffus JE. 2001. Transmission properties of whitefly-borne criniviruses and their impact on virus epidemiology. In *Virus-Insect-Plant Interactions*, ed. KF Harris, OP Smith, JE Duffus, pp. 293–308. San Diego, CA: Academic.

Wisler GC, Duffus JE, Liu H-Y, Li RH. 1998a. Ecology and epidemiology of whitefly-transmitted closteroviruses. *Plant Dis.* 82:270–280.

Wisler GC, Li RH, Liu H-Y, Lowry D, Duffus JE. 1998b. *Tomato chlorosis virus*: a new whitefly-transmitted, phloem-limited bipartite closterovirus of tomato. *Phytopathology* 88:402–409.

Wisler GC, Liu H-Y, Klaassen VA, Duffus JE, Falk BW. 1996. *Tomato infectious chlorosis virus* has a bipartite genome and induces phloem-limited inclusions characteristic of the closteroviruses. *Phytopathology* 86:622–626.

# Chapter 11
# A Review of *Ipomoviruses* and Watermelon Vine Decline in Florida

**Scott Adkins, Susan E. Webb, Pamela D. Roberts, Chandrasekar S. Kousik, Philip A. Stansly, Benny D. Bruton, Diann Achor, Rosa M. Muchovej, and Carlye A. Baker**

## Ipomoviruses

The genus *Ipomovirus* is a small group of whitefly-transmitted viruses within the family *Potyviridae*, the largest group of RNA plant viruses, which are mostly aphid transmitted (Berger et al. 2005). Under current taxonomic guidelines there are three accepted members [*Cucumber vein yellowing virus* (CVYV), *Cassava brown streak virus* (CBSV) and *Sweet potato mild mottle virus* (SPMMV)] and one tentative member [Sweet potato yellow dwarf virus (SPYDV)] in the genus *Ipomovirus* (Berger et al. 2005; Colinet et al. 1996, 1998; Janssen et al. 2005; Lecoq et al. 2000). CVYV has been an economic problem in cucurbits in the Middle East for many years (Cohen and Nitzany 1960; Harpaz and Cohen 1965) and has recently become widespread throughout the Mediterranean region (Al-Musa et al. 1985; Cuadrado et al. 2001; Louro et al. 2003; Yilmaz et al. 1989). CBSV and SPMMV have similarly been reported for many years in cassava and sweet potatoes, respectively, in Africa (Alicai et al. 2007; Hollings et al. 1976).

These viruses are distinct from the whitefly-transmitted begomoviruses and criniviruses commonly found in many regions of the world, and are relatively unusual members of the family *Potyviridae* in that they are transmitted by whiteflies (Cohen and Nitzany 1960; Harpaz and Cohen 1965; Mansour and Al-Musa 1993; Maruthi et al. 2005). Whitefly transmission is relatively inefficient for CVYV (Mansour and Al-Musa 1993) and CBSV (Maruthi et al. 2005) although it is apparently sufficient to lead to economically damaging levels of virus-infected plants. Difficulty in replication of the original SPMMV whitefly transmission has been noted (Tairo et al. 2005).

CVYV and CBSV induce veinal chlorosis (or yellowing) in leaves of cucurbits and cassava, respectively (Alicai et al. 2007; Cohen and Nitzany 1960). CBSV also induces necrotic lesions on cassava stems and necrosis within cassava roots

S. Adkins (✉)
USDA-ARS, US Horticultural Research Laboratory, 2001 South Rock Road, Fort Pierce, FL 34945, USA
e-mail: scott.adkins@ars.usda.gov

P.A. Stansly, S.E. Naranjo (eds.), *Bemisia*: Bionomics and Management of a Global Pest,
DOI 10.1007/978-90-481-2460-2_11, 

(Alicai et al. 2007). SPMMV induces a mild mottle of sweet potato leaves (Hollings et al. 1976).

CBSV and SPMMV can experimentally infect plants unrelated to cassava and sweet potato, respectively (Monger et al. 2001; Hollings et al. 1976). CVYV was originally reported to be restricted to cucurbits (Cohen and Nitzany 1960; Mansour and Al-Musa 1993) and remains an important pathogen only in cucurbits. Recently, however, CVYV was demonstrated to also infect non-cucurbit species naturally (Janssen et al. 2002) and experimentally (Morris et al. 2006).

Full genome sequences have only been determined for CVYV (Janssen et al. 2005) and SPMMV (Colinet et al. 1998). CVYV is unique among recognized members of the *Potyviridae* in that it has two P1 proteins and lacks HC-Pro (Janssen et al. 2005; Valli et al. 2006, 2007). This duplication of P1 and absence of HC-Pro have apparently occurred during selective evolution, and one of the CVYV P1 proteins was recently shown to function as a suppressor of gene silencing (Valli et al. 2006, 2007). In contrast, SPMMV encodes a typical set of potyviral proteins including a single P1 protein and HC-Pro (Colinet et al. 1998). Full genome sequences of CBSV and tentative ipomoviruses are needed to determine a definitive ipomovirus genome organization.

## Watermelon Vine Decline in Florida

A severe decline of watermelon vines has been observed as crops approach harvest or soon after the first harvest during spring and/or fall growing seasons in southwest and west central Florida since approximately 2002. Symptoms include yellowing, scorched or brown leaves, defoliation and wilting of the vines, and a rapid collapse of mature vines. Progress of the disease is rapid with decline incidence increasing from 10 to 80% within a week in some fields. Frequently, the interior of the fruit rind appears discolored and/or necrotic, rendering the fruit unmarketable. Grower losses in the 2003–2004 seasons ranged from 50 to 100%. The disease has continued to appear in Florida watermelons through the current (fall 2009) growing season.

Numerous field samples of watermelons with vine decline were evaluated for the presence of abiotic and/or biotic causes. No abiotic cause (such as fertilization, pesticide usage or irrigation) was identified. Fungal and bacterial pathogens isolated, including six *Fusarium oxysporum* isolates (Race 1 and 0), did not reproduce the characteristic symptoms seen with this disease. However, watermelon plants inoculated with crude extracts of plant sap from declining watermelons that were filtered to remove fungi and bacteria developed symptoms similar to those observed in the field. These results indicated the presence of viruses or virus-like agents.

## Squash Vein Yellowing Virus

In fall 2004, an unknown virus, often in mixed infections with an aphid-borne potyvirus, *Papaya ringspot virus* watermelon strain (PRSV-W), was detected in declining watermelon plants. While characterization of this unknown virus was in

progress, it was also detected in a squash sample exhibiting vein yellowing symptoms collected in west central Florida in fall 2003. Serological and molecular tests [double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA), nucleic acid hybridization and/or reverse transcription-polymerase chain reaction (RT-PCR)] for 16 viruses, and four groups of viruses known to infect cucurbits in Florida and elsewhere, were negative (Whidden and Webb 2004). Initial light microscopic examination for inclusion bodies and electron microscopy of leaf dips revealed no known virus-induced structures or virions, and dsRNA analysis revealed no viral-associated dsRNA.

Subsequent isolation, characterization and sequence analysis of this unknown virus, for which the name Squash vein yellowing virus (SqVYV) was proposed, showed that it was likely to be a novel ipomovirus within the family *Potyviridae* (Adkins et al. 2007). Isolated virions had a morphology consistent with those of other members of the family *Potyviridae*, and were subsequently used to infect squash and watermelon resulting in the originally observed symptoms. Pinwheel-like and cylindrical inclusions found in phloem tissue of SqVYV-infected tissue have previously been associated with infections by other members of the family *Potyviridae* (Edwardson and Christie 1996; Shukla et al. 1991). Similar inclusions have also been observed in tissue infected by other ipomoviruses including CVYV (Lecoq et al. 2000). SqVYV was transmitted to squash and watermelon by whiteflies (like recognized ipomoviruses) but was not transmitted by aphids (unlike most of the common cucurbit-infecting members of the family *Potyviridae*).

The only sequences producing significant alignments with the deduced SqVYV coat protein (CP) sequence were the CPs of members of the family *Potyviridae*, and in particular, members of the genus *Ipomovirus*. SqVYV is most closely related to, but distinct from, CVYV, with nucleotide identities well below the levels recently proposed for demarcating species within the family *Potyviridae* (Adams et al. 2004). In phylogenetic analyses, SqVYV always clustered with the genus *Ipomovirus* (Adkins et al. 2007). Although sequence data clearly place SqVYV within the genus *Ipomovirus* in the family *Potyviridae*, definitive designation of SqVYV as a novel species awaits completion and analysis of the SqVYV genome. However, the biological properties of SqVYV (e.g., differences in symptoms caused by SqVYV and CVYV on several key cucurbit species) already provide evidence for it being a distinct species (Adkins et al. 2007).

Isolation of SqVYV virions permitted testing of the role of SqVYV in watermelon vine decline. Seedlings (approximately transplant size) of all tested watermelon cultivars developed systemic wilt and necrosis resulting in plant death within 7–10 days post-inoculation. More mature, greenhouse-grown plants developed systemic wilt within 14 days post-inoculation and also fruit symptoms including rind necrosis and discoloration similar to those observed in the field. SqVYV was re-isolated from these dying plants, demonstrating that it is sufficient to induce vine decline and fruit rind necrosis and discoloration, thereby suggesting SqVYV is the likely cause of this serious watermelon disease. Furthermore, watermelons grown in field plots in proximity to squash mechanically inoculated with SqVYV also developed typical symptoms of vine decline.

SqVYV is now widely distributed in southwest and west central Florida and has also recently been reported from southern Indiana (Adkins et al. 2007; Egel and Adkins 2007). At the present time, the host range of SqVYV appears to be limited to cucurbits. Wild watermelon germplasm and watermelon grafted onto gourd rootstocks are currently being evaluated for resistance to SqVYV (Kousik et al. 2009). Use of insecticides and aluminized reflective polyethylene mulch are showing promise for management of whiteflies and thus SqVYV (Kousik et al. 2007).

## Literature Cited

Adams MJ, Antoniw JF, Fauquet CM. 2004. Molecular criteria for genus and species discrimination within the family *Potyvirida*e. *Arch. Virol.* 150:459–479.

Adkins S, Webb SE, Achor D, Roberts PD, Baker CA. 2007. Identification and characterization of a novel whitefly-transmitted member of the family *Potyviridae* isolated from cucurbits in Florida. *Phytopathology* 97:145–154.

Al-Musa AM, Qusus SJ, Mansour AN. 1985. Cucumber vein yellowing virus on cucumber in Jordan. *Plant Dis.* 69:361.

Alicai T, Omongo CA, Maruthi MN, Hillocks RJ, Baguma Y, Kawuki R, Bua A, Otim-Nape GW, Colvin J. 2007. Re-emergence of cassava brown streak disease in Uganda. *Plant Dis.* 91:24–29.

Berger PH, Adams MJ, Barnett OW, Brunt AA, Hammond J, Hill JH, Jordan RL, Kashiwazaki S, Rybicki E, Spence N, Stenger DC, Ohki ST, Uyeda I, van Zaayen A, Valkonen J, Vetten HJ. 2005. *Virus Taxonomy – Classification and Nomenclature of Viruses*, 8th Report of the ICTV, ed. CM Fauquet, MA Mayo, J Maniloff, U Desselberger, LA Ball, pp. 819–841. San Diego: Elsevier Academic Press.

Cohen S, Nitzany FE. 1960. A whitefly-transmitted virus of cucurbits in Israel. *Phytopathol. Mediter.* 1:44–46.

Colinet D, Kummert J, Lepoivre P. 1996. Molecular evidence that the whitefly-transmitted sweetpotato mild mottle virus belongs to a distinct genus of the Potyviridae. *Arch. Virol.* 141:125–135.

Colinet D, Kummert J, Lepoivre P. 1998. The nucleotide sequence and genome organization of the whitefly transmitted sweetpotato mild mottle virus: a close relationship with members of the family *Potyviridae*. *Vir. Res.* 53:187–196.

Cuadrado IM, Janssen D, Velasco L, Ruiz L, Segundo E. 2001. First report of Cucumber vein yellowing virus in Spain. *Plant Dis.* 85:336.

Edwardson JR, Christie RG. 1996. Cylindrical inclusions. *University of Florida, Institute of Food and Agricultural Sciences*. Bulletin 894.

Egel DS, Adkins S. 2007. Squash vein yellowing virus identified in watermelon (*Citrullus lanatus*) in Indiana. *Plant Dis.* 91:1056.

Harpaz I, Cohen S. 1965. Semipersistent relationship between cucumber vein yellowing virus (CVYV) and its vector, the tobacco whitefly (*Bemisia tabaci* Gennadius). *Phytopathol. Z.* 54:240–248.

Hollings M, Stone OM, Bock KR. 1976. Purification and properties of sweet potato mild mottle, a whitefly borne virus from sweet potato (*Ipomoea batatas*) in East Africa. *Ann. Appl. Biol.* 82:511–528.

Janssen D, Martín G, Velasco L, Gómez P, Segundo E, Ruiz L, Cuadrado IM. 2005. Absence of a coding region for the helper component-proteinase in the genome of the cucumber vein yellowing virus, a whitefly-transmitted member of the *Potyviridae*. *Arch. Virol.* 150:1439–1447.

Janssen D, Ruiz L, Velasco L, Segundo E, Cuadradro IM. 2002. Non-cucurbitaceous weed species shown to be natural hosts of *Cucumber vein yellowing virus* in south-eastern Spain. *Plant Pathol.* 51:797.

Kousik CS, Adkins ST, Turechek WW, Roberts PD. 2007. Use of reflective plastic mulch and insecticide sprays to manage viral watermelon vine decline in Florida, 2007. *Plant Dis. Management Rep.* 2:V169.

Kousik CS, Adkins S, Turechek WW, Roberts PD. 2009. Sources of resistance in U.S. plant introductions to watermelon vine decline caused by Squash vein yellowing virus. *HortScience* 44:256–262.

Lecoq H, Desbiez C, Delécolle B, Cohen S, Mansour A. 2000. Cytological and molecular evidence that the whitefly-transmitted Cucumber vein yellowing virus is a tentative member of the family Potyviridae. *J. Gen. Virol.* 81:2289–2293.

Louro D, Quinot A, Neto E, Fernandes JE, Marian D, Vecchiati M, Caciagli P, Vaira AM. 2003. Occurrence of *Cucumber vein yellowing virus* in cucurbitaceous species in southern Portugal. *Plant Pathol.* 53:241.

Mansour A, Al-Musa A. 1993. Cucumber vein yellowing virus; host range and virus vector relationships. *J. Phytopathol.* 137:73–78.

Maruthi MN, Hillocks RJ, Mtunda K, Raya MD, Muhanna M, Kiozia H, Rekha AR, Colvin J, Thresh JM. 2005. Transmission of *Cassava brown streak virus* by *Bemisia tabaci* (Gennadius). *J. Phytopathol.* 153:307–312.

Monger WA, Seal S, Cotton S, Foster GD. 2001. Identification of different isolates of *Cassava brown streak virus* and development of a diagnostic test. *Plant Pathol.* 50:768–775.

Morris J, Steel E, Smith P, Boonham N, Spence N, Barker I. 2006. Host range studies for *Tomato chlorosis virus*, and *Cucumber vein yellowing virus* transmitted by *Bemisia tabaci* (Gennadius). *Eur. J. Plant Pathol.* 114:265–273.

Shukla DD, Frenkel MJ, Ward CW. 1991. Structure and function of the potyvirus genome with special reference to the coat protein coding region. *Can. J. Plant Pathol.* 13:178–191.

Tairo F, Mukasa SB, Jones RAC, Kullaya A, Rubaihayo PR, Valkonen JPT. 2005. Unravelling the genetic diversity of the three main viruses involved in sweet potato virus disease (SPVD), and its practical implications. *Mol. Plant Pathol.* 6:199–211.

Valli A, López-Moya JJ, García JA. 2007. Recombination and gene duplication in the evolutionary diversification of P1 proteins in the family Potyviridae. *J. Gen. Virol.* 88:1016–1028.

Valli A, Martín-Hernández AM, López-Moya JJ, García JA. 2006. RNA silencing suppression by a second copy of the P1 serine protease of Cucumber vein yellowing ipomovirus, a member of the family Potyviridae that lacks the cysteine protease HCPro. *J. Virol.* 80:10055–10063.

Whidden A, Webb S. 2004. Virus in yellow squash in Hillsborough County. *Vegetarian* 4 (1). Horticultural Sciences Dept., Vegetable Crops Extension Newsletter, University of Florida, Gainesville. http://www.hos.ufl.edu/vegetarian/04/January/Jan04.htm

Yilmaz MA, Ozaslan M, Ozaslan D. 1989. Cucumber vein yellowing virus in Cucurbitaceae in Turkey. *Plant Dis.* 73:610.

# Chapter 12
# Transovarial Transmission of Begomoviruses in *Bemisia tabaci*

**Gian Paolo Accotto and Luca Sardo**

## Introduction

Transovarial transmission of viral nucleic acid in insect vectors, or more precisely of viral infectivity, can be very important in maintaining a source of infection and therefore has great epidemiological relevance. The first clear case of transovarial transmission of an arbovirus demonstrated the ability of sand fly and black fly vector populations to maintain *Vesicular stomatitis virus* without a vertebrate host (Tesh et al. 1972). This phenomenon is also known in the plant world and continues to receive attention from virologists. Transovarial passage of plant viruses in their vectors can have dramatic consequences on epidemiology, particularly if the virus has a limited range of host plants. It allows the vector to maintain the source of inoculum in the absence of host plant, and can facilitate spread over long distances.

The first case of transovarial transmission in plants was reported by Fukushi (1933). He showed that *Rice dwarf virus* – a phytoreovirus – was transmitted for several generations through the egg of the leafhopper vector *Nephotettix apicalis*. Other cases have been described involving leafhopper, planthopper and aphid vectors, but transovarial transmission by insects remains uncommon among plant viruses (Grylls 1954; Black 1953; Sylvester 1969; Conti 1980).

Recently two virus groups, geminiviruses (genus *Begomovirus*) and tospoviruses, have emerged as major plant pathogens worldwide (Prins and Goldbach 1998; Moffat 1999; Varma and Malathi 2003; Boulton 2003). Originally from warm areas of the world, both virus types are now present in subtropical and temperate regions, causing dramatic crop losses, mainly among vegetables and ornamentals. The resulting epidemics have awakened interest in studying the possible transovarial transmission of these viruses, although not much has been published. Among tospoviruses, studies on *Tomato spotted wilt virus* (Wijkamp et al. 1996) showed that while the virus unequivocally replicated in its insect vector and the salivary glands were a major site of multiplication, no transovarial transmission to the

G.P. Accotto (✉)
Istituto di Virologia Vegetale, Consiglio Nazionale delle Ricerche, Strada delle Cacce 73, Torino 10135, Italy
e-mail: g.accotto@ivv.cnr.it

P.A. Stansly, S.E. Naranjo (eds.), *Bemisia*: Bionomics and Management of a Global Pest,
DOI 10.1007/978-90-481-2460-2_12, 

progeny of the vector thrips took place. This multiplication was not accompanied by pathological effects on the vector, and no effect of virus infection was found on development time, reproduction rate and survival. Results for geminiviruses are more controversial, and thus bear detailed analysis here.

## Are Begomoviruses Transmitted Transovarially?

Interest in transovarial transmission began to attract the attention of geminivirologists in the period 1960–1980, when many new viruses were discovered or characterized. Transmission studies were part of the story, although only a few reports were published. Cohen and Nitzany (1966) found that infectivity of *Tomato yellow leaf curl virus* (TYLCV) in Israel could not be transmitted to the progeny of the whitefly vector *Bemisia tabaci*. They allowed viruliferous whiteflies to lay eggs on a non-host plant (cotton). Adults derived from those eggs were moved to host plants (*Datura stramonium*) for 48 h. Out of 360 female offspring tested, none transmitted the virus. Similar experiments, conducted by Ioannou (1985) on TYLCV in Cyprus, also had negative results.

An Indian isolate of *Tomato leaf curl virus* (TLCV), a virus similar to TYLCV, was tested for its transmissibility through the eggs of *B. tabaci* (Butter and Rataul 1977). Whiteflies fed on diseased tomato plants for 24 h were kept on cotton for several days to lay eggs. New adults were transferred to tomato test plants, in groups of 5–50. Although the experiment was repeated seven times – with a total of about 3,750 whiteflies and almost 200 test plants – no transovarial transmission was demonstrated.

Geminiviruses of legumes have also been analysed for transmission through the egg. Viruses causing yellow mosaic on mungbean – probably strains of *Mungbean yellow mosaic virus* (MYMV) – were not transmitted transovarially (Ahmad and Harwood 1973; Rathi and Nene 1974; Murugesan and Chelliah 1977). The same negative result was obtained for *African cassava mosaic virus* (ACMV), the causal agent of a devastating disease of cassava in Africa (Dubern 1979, 1994).

Molecular techniques commonly used today – such as Western and Southern blotting or PCR – were not available when most of these reports on different begomoviruses were published. Therefore certain aspects – like the path of the virus following ingestion, the accumulation of viral DNA and coat protein, or the location of the virus in the insect body – could not be studied. When these methods became available to virologists and entomologists, a number of interesting aspects of the virus/vector relationship could be addressed more directly. Particularly useful was the ability to detect viral DNA in single whiteflies at any stage (including eggs) using PCR. The circulative pathway of begomoviruses in the vector has been reviewed in Czosnek et al. (2002).

In 1997, Rubinstein and Czosnek reported for the first time the detection by PCR of TYLCV DNA in eggs of *B. tabaci*. One year later, Ghanim et al. (1998)

analysed in detail the transmission of both viral DNA and virus disease from a viruliferous female to its progeny through the eggs. PCR and Southern analyses were applied in an experimental design which did not differ much from that used three decades before by Cohen and Nitzany (1966): whiteflies acquire TYLCV from infected tomato, then females are moved to non-host plants (cotton or eggplant) to lay eggs; these eggs, and the following stages – including adults of the first and second generation – are analysed. In the first generation TYLCV DNA was detected by PCR/Southern blot hybridization in 81% of the eggs, 37% of the crawlers and 57% of the adults. Moreover, 10% of these first generation adults were reported to infect tomato plants. Viral DNA was also detected in the second generation and 8% of resulting adults were infective. Viral DNA was also located in ovaries and maturing eggs dissected from viruliferous adults. The authors concluded that the disease could be transmitted through the egg for at least two generations and therefore, whiteflies could serve as reservoirs of the virus between growing seasons in the absence of a plant host.

Contrary results were soon obtained by Ghanim and Czosnek (2000) while analysing TYLCV transmission between males and females. They found that, although viral DNA was present in eggs laid by females that had acquired TYLCV from males, adults developed from those eggs caged on tomato test plants (40 plants, five insects per plant) were not infective. Therefore in this case, in spite of inheritance of viral DNA, no transmission was demonstrated. In another study (Polston et al. 2001) viral DNAs of two begomoviruses (TYLCV and *Tomato mottle virus*, ToMoV, a bipartite begomovirus) were analysed in the progeny of viruliferous whiteflies: TYLCV DNA was detected in eggs and larval stages but not in adults, while ToMoV DNA could not be found in any stage of the progeny. In neither case was the virus transmitted to the progeny.

The question of transovarial transmission was again addressed by Bosco et al. (2004). Two whitefly biotypes, B and Q, and two virus species, TYLCV and *Tomato yellow leaf curl Sardinia virus* (TYLCSV), were studied. Different life stages of the progeny of viruliferous female whiteflies were analysed by PCR detection of viral DNA and by infectivity tests. With the B biotype, TYLCSV DNA could be detected in eggs (10/110) and nymphs (32/110) and to a lesser extent in adults (5/250) of the first-generation progeny, but not in adults of second and third generation. On the contrary, no viral DNA could be detected in eggs (100), nymphs (100), and adults (125) when TYLCV was studied.

Even more interesting were the results of infectivity tests: altogether, 1,840 adults and 368 tomato test plants were analysed, with the four combinations of the two virus species and the two *B. tabaci* biotypes. Nevertheless, the ability of these whiteflies derived from viruliferous females to infect tomato plants could not be demonstrated. The authors concluded that, because the inherited viral DNA is unable to give rise to infections, the transovarial passage of TYLCSV DNA appears to have no epidemiological relevance. In an attempt to summarize the results on begomoviruses (Table 12.1), one could say that transmission of viral DNA through the vector egg is relatively common, but transovarial transmission of the

**Table 12.1** A comparison of the results of different studies on the detection of begomoviral DNA in *Bemisia tabaci* and the ability of the whiteflies to transmit the begomovirus over two generations of whiteflies

| | | Viral DNA | | | | | | Infectivity | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1st generation | | | 2nd generation | | | 1st generation | | 2nd generation | |
| Virus[a] | References | Eggs[b] | Nymphs[b] | Adults[b] | Eggs | Nymphs | Adults | Plants[c] | Wf[d] | Plants | Wf |
| TYLCV | Cohen and Nitzany (1966) | /[e] | / | / | / | / | / | 0% | 360 | / | / |
| TYLCV (B biotype) | Ghanim et al. (1998) | 81% (46/57) | 37% (25/68) | 57% (46/81) | 38% (26/68) | 71% (35/49) | 79% (41/52) | 10% (5/50) | 50 | 8% (4/49) | 49 |
| TYLCV (B biotype) | Ghanim and Czosnek (2000) | / | / | 2/6[f] | / | / | / | 0% (0/40) | 200 | / | / |
| TYLCV-[PT] (B and Q biotypes) | Bosco et al. (2004) | 0% (100) | 0% (100) | 0% (125) | / | / | 0% (0/60) | 0% (0/106) | 530 | 0% (0/36) | 180 |
| TYLCSV (B and Q biotypes) | Bosco et al. (2004) | 9% (10/110) | 29% (32/110) | 2% (5/250) | / | / | 0% (0/50) | 0% (0/118) | 590 | 0% (0/36) | 180 |
| TLCV | Butter and Rataul (1977) | / | / | / | / | / | / | 0% (0/195) | 3,750 | / | / |
| ACMV | Dubern (1979) | / | / | / | / | / | / | 0% (0/45) | 77 | / | / |
| MYMV | Ahmad and Harwood (1973) | / | / | / | / | / | / | 0% (0/15) | / | / | / |
| MYMV | Rathi and Nene (1974) | / | / | / | / | / | / | 0% (0/70) | 1,400 | / | / |

[a] vector biotype is indicated where known
[b] positive/tested
[c] infected/tested
[d] total number of adult whiteflies used
[e] not tested
[f] tested in groups of 5 insects

infectious agent appears to be at best a rare event, reported only once, and not verified later.

Apart from the genus *Begomovirus,* the phenomenon was studied in *Beet mild curly top virus* (BMCTV), a member of the genus *Curtovirus* in the family *Geminiviridae*, transmitted by leafhoppers rather than whiteflies. This virus is also transmitted in a persistent circulative manner and persists for up to 30 days in leafhoppers maintained on maize, a non-host for BMCTV. However, transovarial transmission of infectivity was not detected (Soto and Gilbertson 2003). It is thus likely that viral DNA remains (and is detectable in some cases) in the progeny but without forming virions and is therefore unable to infect plants. It would be interesting to analyse the salivary glands of the adult progeny, to look for viral DNA and virions.

## Conclusions

Plant viruses for which transovarial transmission has been demonstrated have a persistent propagative mode of transmission (reviewed in Hull 2002), and have been shown to replicate in its vector. This is the case for some members of the *Phytoreovirus* genus, transmitted by Cicadellidae and the *Tenuivirus* genus, transmitted by Delphacidae, as well as in some rhabdoviruses. Geminiviruses have a persistent circulative mode of transmission, and in the case of TYLCV, there is some evidence of a limited replication of viral DNA and of transcriptional activity in the vector. There is evidence that the amount of TYLCSV and TYLCV DNAs does not increase in whiteflies after they are transferred to a non-host plant (Caciagli and Bosco 1997; Sinisterra et al. 2005), but feeding whiteflies on radiolabelled artificial medium caused the appearance of a labelled band comigrating with the TYLCV genomic ssDNA, suggesting de novo synthesis (Czosnek et al. 2001). Using RT-Real Time PCR, Sinisterra et al. (2005) showed that TYLCV transcripts increased after transfer of whiteflies to cotton, indicating active TYLCV transcription. However, when ToMoV was studied in the same experimental conditions, viral DNA level rapidly decreased and viral transcripts became undetectable. It is worth considering that TYLCV has several negative effects on the whitefly vector, such as reduced fecundity and lifespan (Rubinstein and Czosnek 1997), while ToMoV does not (McKenzie 2002). Therefore it appears that different begomoviruses can behave quite differently, and may have different levels of adaptation to the vector, being either neutral or damaging in such a way that, as with TYLCV, the virus can be seen also as a pathogen for the insect (Czosnek et al. 2001). These observations – together with the very rapid evolution that is occurring in these viruses (Seal et al. 2006) – may leave space for transovarial transmission by some viruses, or even strains, and not others. Future work on this very interesting virus/vector relationship will improve our knowledge of the phenomenon and help to devise better control strategies.

## Literature Cited

Ahmad M, Harwood RF. 1973. Studies on a whitefly-transmitted yellow mosaic of urd bean (*Phaseolus mungo*). *Plant Dis. Rep.* 57:800–802.

Black LM. 1953. Occasional transmission of some plant viruses through the eggs of their insect vectors. *Phytopathology* 43:9–10.

Bosco D, Mason G, Accotto GP. 2004. TYLCSV DNA, but not infectivity, can be transovarially inherited by the progeny of the whitefly vector *Bemisia tabaci* (Gennadius). *Virology* 323:276–283.

Boulton MI. 2003. Geminiviruses: major threats to world agriculture. *Ann. Appl. Biol.* 142:143.

Butter NS, Rataul HS. 1977. The virus-vector relationship of the *Tomato leafcurl virus* (TLCV) and its vector, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae). *Phytoparasitica* 5:173–186.

Caciagli P, Bosco D. 1997. Quantitation over time of *Tomato yellow leaf curl geminivirus* DNA in its whitefly vector. *Phytopathology* 87:610–613.

Cohen S, Nitzany FE. 1966. Transmission and host range of the *Tomato yellow leaf curl virus*. *Phytopathology* 56:1127–1131.

Conti M. 1980. Vector relationships and other characteristics of barley yellow striate mosaic virus (BYSMV). *Ann. Appl. Biol.* 95:83–92.

Czosnek H, Ghanim M, Morin S, Rubinstein G, Friedman V, Zeidan M. 2001. Whiteflies: vectors, and victims (?), of geminiviruses. *Adv. Virus Res.* 57:291–322.

Czosnek H, Ghanim M, Ghanim M. 2002. The circulative pathway of begomoviruses in the whitefly vector *Bemisia tabaci* – insights from studies with *Tomato yellow leaf curl virus*. *Ann. Appl. Biol.* 140:215–231.

Dubern J. 1979. Quelques propriétés de la Mosaique Africaine du Manioc. *Phytopath. Z.* 96:25–39.

Dubern J. 1994. Transmission of *African cassava mosaic geminivirus* by the whitefly (*Bemisia tabaci*). *Trop. Sci.* 34:82–91.

Fukushi T. 1933. Transmission of the virus through the eggs of an insect vector. *Proc. Imp. Acad. (Tokyo)* 9:457–460.

Ghanim M, Czosnek H. 2000. *Tomato yellow leaf curl geminivirus* (TYLCV-Is) is transmitted among whiteflies (*Bemisia tabaci*) in a sex-related manner. *J. Virol.* 74:4738–4745.

Ghanim M, Morin S, Zeidan M, Czosnek H. 1998. Evidence for transovarial transmission of *Tomato yellow leaf curl virus* by its vector, the whitefly *Bemisia tabaci*. *Virology* 240:295–303.

Grylls NE. 1954. Rugose leaf curl – a new virus disease transovarially transmitted by the leafhopper *Austroagallia torrida*. *Aust. J. Biol. Sci.* 7:47–58.

Hull R. 2002. *Matthews' Plant Virology*, 4th edition New York: Academic Press.

Ioannou N. 1985. Yellow leaf curl and other virus diseases of tomato in Cyprus. *Plant Pathol.* 34:428–434.

McKenzie CL. 2002. Effect of *Tomato mottle virus* (ToMoV) on *Bemisia tabaci* biotype B (Homoptera: Aleyrodidae) oviposition and adult survivorship on healthy tomato. *Fla. Entomol.* 85:367–368.

Moffat A. 1999. Geminiviruses emerge as serious crop threat. *Science* 286:1835.

Murugesan S, Chelliah S. 1977. Transmission of greengram yellow mosaic virus by the white fly, *Bemisia tabaci* (Genn.). *Madras Agric. J.* 64:437–441.

Polston JE, Sherwood T, Rosell R, Nava A. 2001. Detection of *Tomato yellow leaf curl* and *Tomato mottle virus* in developmental stages of the whitefly vector, *Bemisia tabaci*. In *Third International Geminivirus Symposium*, John Innes Centre, Norwich, UK, July 24–28, 2001, Abstract 81.

Prins M, Goldbach R. 1998. The emerging problem of *Tospovirus* infection and nonconventional methods of control. *Trends Microbiol.* 6:31–35.

Rathi YPS, Nene YL. 1974. Some aspects of the relationship between *Mung bean yellow mosaic virus* and its vector *Bemisia tabaci*. *Indian Phytopathol.* 27:459–462.

Rubinstein G, Czosnek H. 1997. Long-term association of *Tomato yellow leaf curl virus* with its whitefly vector *Bemisia tabaci*: effect on the insect transmission capacity, longevity and fecundity. *J. General Virol.* 78:2683.

Seal SE, van den Bosch F, Jeger MJ. 2006. Factors influencing begomovirus evolution and their increasing global significance: implications for sustainable control. *Crit. Rev. Plant Sci.* 25:23–46.

Sinisterra XH, McKenzie CL, Hunter WB, Powell CA, Shatters RG, Jr. 2005. Differential transcriptional activity of plant-pathogenic begomoviruses in their whitefly vector (*Bemisia tabaci*, Gennadius: Hemiptera Aleyrodidae). *J. Gen. Virol.* 86:1525–1532.

Soto MJ, Gilbertson RL. 2003. Distribution and rate of movement of the *Curtovirus Beet mild curly top virus* (Family *Geminiviridae*) in the beet leafhopper. *Phytopathology* 93:478–484.

Sylvester ES. 1969. Evidence of transovarial passage of the *Sowthistle yellow vein virus* in the aphid *Hyperomyzus lactucae*. *Virology* 38:440–446.

Tesh RB, Chaniotis BN, Johnson KM. 1972. *Vesicular stomatitis virus* (Indiana serotype): transovarial transmission by phlebotomine sandlies. *Science* 175:1477–1479.

Varma A, Malathi VG. 2003. Emerging geminivirus problems: a serious threat to crop production. *Ann. Appl. Biol.* 142:145–164.

Wijkamp I, Goldbach R, Peters D. 1996. Propagation of *Tomato spotted wilt virus* in *Frankliniella occidentalis* does neither result in pathological effects nor in transovarial passage of the virus. *Entomol. Exp. Appl.* 81:285–292.

# Section IV
# Management of *Bemisia* in Diverse Cropping Systems

**Dan Gerling and A. Rami Horowitz**

## Introduction

Pest management practices evolve in response to the demands of the grower community, consumer perception, governmental regulation and availability of technology to meet these demands. The grower looking for immediate solutions to pest problems, offered mainly by insecticides, often puts himself at odds with consumers and regulators concerned with food safety and environmental protection. Frequently, the deciding factor in this societal conflict that leads to interest and deployment in alternative control measures is the development of insecticide resistance by the evolving pest complex. Consequently, there is general agreement that effective non-chemical control measures will have many beneficial effects, including reduced selection for insecticide resistance.

In response to these forces, Integrated Pest Management (IPM) has been the main paradigm in modern pest control. IPM relies on the use of a combination of tactics including biological control by natural enemies, the judicious use of insecticides based on sampling and economic thresholds, cultural control methods such as mechanical screening and resistant plant varieties to control pests in an economically efficient and environmentally sound manner. The following chapters attempt to provide an overview of these tactics that can be integrated into IPM strategies for *Bemisia tabaci*, namely biological control, insecticides, mechanical and cultural control measures, and host plant resistance.

Antignus (Chapter 13) provides us with the latest information on optical manipulations to block the spread of *B. tabaci* and its vectored viruses. Nombela and Muñiz (Chapter 14) review progress in host plant resistance for management of *B. tabaci* in tomatoes and other crop systems. During the past decade, new sources of host plant resistance against *B. tabaci* have been identified, mainly the unique cloning of the *Mi-1* gene in tomato. The combination of classical genetic breeding programs along with characterization of available plant resistance (both innate and induced) are discussed and should assist the development of resistance in many crops.

---

Dan Gerling and A. Rami Horowitz (Section Editors)

The last few years have witnessed many developments in the study of natural enemies as reviewed in Chapter (15) by Arnó et al., who also provide a guide for future research in this growing area. The list of potentially available natural enemies of *B. tabaci* has increased by 19 parasitoid and 41 predator species during the last few years, and continues to grow. However, only a few of these have made their way into commercial production and release for *B. tabaci* management. Available parasitoid species for augmentation are still limited to *Eretmocerus eremicus*, *Eretmocerus mundus* and *Encarsia formosa,* although the addition of one predator species, *Amblysieus swirskii,* has provided an invaluable tool for controlling *B. tabaci* and other pests on some greenhouse crops and even in the open field. Laboratory and preliminary field studies evaluating the latter species provide a valuable example since they outline methodology and point the way toward successful development of other predator species. This leads also to the conclusion that future effective biological control agents might be obtained from local fauna where they evolved as enemies *B. tabaci* or even other species. These findings emphasize the need to enhance programs for evaluating known whitefly natural enemies to determine which could be employed under particular conditions.

Research on insecticides includes studies on modes of action, resistance mechanism studies, and examination of the ecological characteristics of *B. tabaci* that influence the evolution of resistance in various agricultural systems. These, in conjunction with appropriate chemical investigations, have led to the development of new insecticides and to the improvement of application methods in general. The preservation of the neonicotinoid-group of insecticides is of major concern, as it has proved essential for continued effective *B. tabaci* control in many IPM systems. Thus, stringent guidelines for conservation of this crucial group have been suggested and successful implementation of IRM (insecticide resistance management) strategies against *B. tabaci* has been achieved. These IRM strategies are exemplified by programs implemented in Israel and Arizona. Programs in these countries also exemplify how a better understanding of the means by which biorational insecticides help to conserve natural enemies in the field. These subjects and more are covered by Castle et al. in Chapter 16, Ecological Determinants of *B. tabaci* Resistance to Insecticides.

Agricultural specialization and innovations in plant cultivation and protection technologies open the way for the use of all these tactics in traditional and novel ways within the framework of an IPM program. The final chapter (17) by Stansly and Natwick pulls this and other disparate information together into integrated systems for managing *B. tabaci* in protected and open field cropping systems. The greenhouse provides a conducive environment for augmentative biological control that is being fully exploited in some regions. The adaption of these strategies to the open field represents one of the great challenges for future research and development. Thus, our hope is to provide guideposts for present day IPM practitioners in addition to pointing the way toward future developments.

# Chapter 13
# Optical Manipulation for Control of *Bemisia tabaci* and Its Vectored Viruses in the Greenhouse and Open Field

**Yehezkel Antignus**

## Introduction

During the past 20 years, *Bemisia tabaci* (Gennadius) has become a major pest of crops throughout numerous tropical and subtropical zones in the world. In addition to damage caused by direct feeding pressure, *B. tabaci* transmits 111 virus species, including the notorious *Tomato yellow leaf curl virus* (TYLCV) and a wide range of other viral pathogens assigned to the *Begomovirus*, *Crinivirus*, *Closterovirus*, *Ipomovirus* and *Carlavirus* genera (Duffus 1987; Markham et al. 1994; Brown et al. 1995; Jones 2003).

The limits of chemical control (Perring et al. 1999) stimulated studies directed to ways of preventing contact between insect vectors of viral pathogens and the crop thus avoiding epiphytotics of virus diseases. Cultural control practices are important elements of integrated pest management (IPM) (Hilje et al. 2001) in both manipulating the environment and interfering with the ability of virus vectors to contact the crop.

Whiteflies lack an olfactory reaction, relying instead on their vision for navigation and orientation (Mound 1962). The spectral sensitivities of the insects to both the UV and visible ranges of the spectrum have been extensively investigated (Lubbock 1882; Bertholf 1931, 1934; Mound 1962; Vaishampayan et al. 1975a, b; Menzel 1979; Coombe 1981, 1982; Scherer and Kolb 1987; Goldsmith 1994). Previous studies on the visual sensitivity of whiteflies found that these insects are strongly attracted to certain UV wavelengths (Mound 1962), indicating that interference with the UV-vision may lead to interruption of orientation and dispersal processes. Mound (1962) correlated this response to migratory behavior, showing that yellow wavelengths induced settling behavior that may be a part of the natural host selection mechanism. Coombe (1981, 1982) found that the greenhouse whitefly took off more readily and walked faster – when exposed to spectral quantum flux at 400 nm compared to wavelengths above 500 nm. He confirmed Mound's

Y. Antignus (✉)
Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, Israel
e-mail: antignus@volcani.agri.gov.il

P.A. Stansly, S.E. Naranjo (eds.), *Bemisia*: Bionomics and Management of a Global Pest,
DOI 10.1007/978-90-481-2460-2_13, 

suggestion that the two types of radiation were complementary, with an apparent balance between migratory behavior induced by UV wavelengths and the landing reaction controlled by yellow wavelengths. These results were later supported by the findings of Antignus et al. (1996, 1998) who showed that the filtration of UV light in the range of 280–380 hindered the ability of whiteflies to disperse inside tunnels covered with UV-absorbing films or screens.

This review is focused on cultural methods of forming an optical barrier to manipulate the sunlight spectrum in the UV and visible ranges in ways that interfere with the whitefly's ability to orient and ultimately locate the plant.

## Light Manipulation in Protected Crops

### *UV-Absorbing Films Protect Greenhouses from Insect Invasion and Spread of Virus Diseases*

In vegetable crops that were grown in "walk in" tunnels or greenhouses, use of either UV-absorbing polyethylene films or UV-absorbing 50-mesh nets acted to filter and eliminate the majority of the UV portion of the light spectrum between 280 and 380 nm. This light filtration significantly reduced the infestation of crops by a variety of insect pests, including whiteflies, aphids, thrips, and leaf miners (Antignus et al. 1996, 1998; Antignus 2001). TYLCV and *Cucumber yellowing stunting disorder virus* (CYSDV) were also greatly reduced on tomato and cucumber crops, respectively, under these UV absorbing greenhouse covers (Antignus et al. 1996; Monci et al. 2002; Kumar and Phoeling 2006). These results indicated that the elimination of the UV portion of the light spectrum interferred with the "UV vision" of insects, affecting their ability to orient themselves to the crop (Antignus 2001; Antignus et al. 2000, 2001a, b; Antignus and Ben Yakir 2004; Raviv and Antignus 2004). These results have been confirmed by reports from different geographical zones around the world (Costa and Robb 1999; Costa et al. 2002; Doukas 2002; Doukas and Payne 2007a, b; Kumar and Poehling 2006; Monci et al. 2002; Mutwiwa et al. 2005; Rapisarda and Tropea-Garzia 2002).

### *The Effect of the Chemical Attributes of UV-Blocking Films on Their Protection Capacity*

The introduction of UV-blocking compounds into polyethylene films determines their UV-absorbing capacity. The activity of UV-blockers is time limited due to chemical degradation. Polyvinyl chloride (PVC) films on the other hand act as efficient UV filters due to the chemical characteristics of the PVC molecules and their UV-absorbing capacity is more stable (Antignus et al. 1996).

The degree of UV-blocking by plastic film determines its protective efficiency (Doukas 2002). Polyvinyl chloride (PVC) films, which are highly efficient UV

blockers, gave significantly better protection against insect pests, such as whiteflies, thrips, and aphids, than standard UV-absorbing polyethylene films that are affected by the relatively low stability of the UV-blocking additive (Antignus et al. 1996).

## *Effect of UV Absorbing Films on Natural Enemies*

Kajita (1986) found that parasitism of whiteflies by *Encarsia formosa* Gahan was the same under both standard and UV-blocking films. In choice experiments, significantly more (two to three times) *E. formosa* individuals were trapped under standard rather than under UV-blocking films. It seemed that the parasitoids – like their hosts – oriented more toward an environment with high UV radiation. However, when they had no other choice, they performed well in a UV-deficient environment (Doukas 2002; Doukas and Payne 2007b).

The effect of UV-absorbing plastic sheets on the host location ability of three commercially available parasitoids – *Aphidius colemani* Viereck, *Diglyphus isaea* Walker, and *Eretmocerus mundus* Mercet was tested in the laboratory as well as in field trials. The parasitoids preference for natural versus UV-filtered light was tested under laboratory conditions using Y-tube system. Approximately 90% of the tested insects, regardless of species, chose natural light.

The parasitoid's ability to locate a host-infested plant from a distance (approximately 10 m) was also tested in field trials (Chiel et al. 2006). Host location by *A. colemani* and *D. isaea* as indicated by parasitization rates was not affected by greenhouse covering plastic type whether standard or UV-absorbing plastic. *E. mundus*, on the other hand, was unable to locate the host-infested plant when the latter was placed in the center of the UV-absorbing plastic covered greenhouses. Also, parasitization rates were lower under UV-absorbing plastic than under regular plastic when the host-infested plants were located in the corners of the greenhouse and the wasps were released in the center. Therefore, it was recommended that the number of release points be increased to facilitate host location when releasing *E. mundus* in greenhouses covered with UV-absorbing plastic, whereas no modification was necessary for *D. isaea* and *A. colemani* (Chiel et al. 2006).

## *Effect of UV-Absorbing Films on Pollinators*

Efficient pollination of the tomato flower requires agitation of the anther cones to remove the pollen. In greenhouse crops where wind is lacking, the necessary agitation can be provided by foraging bumblebees *Bombus* spp. (Kevan et al. 1991) that can be purchased commercially.

Studies carried out under laboratory conditions showed that foraging bumblebees responded to the addition or removal of ultraviolet radiation but quickly adjusted and continue to recognize a particular nectar source (Dyer and Chittka 2004).

Nevertheless, lack of UV caused a significant reduction in bumblebee (*B. impatiens*) activity and reduction of the colony's growth rate in tomato greenhouses, as well as increased escape through gutter ventilation systems (Morandin et al. 2001). Usually a 4–9 day delay of bumble bee (*Bombus terrestris*) activity occurred in tomato greenhouses that were covered with UV-absorbing plastic sheets (Steinberg et al. 1997).

The problem was mitigated by placing hives near the greenhouse walls providing exposure to unfiltered light (unpublished data). No significant differences were found in bumblebee activity or in the numbers of flowers visited in a greenhouse study comparing standard with UV-blocking films (Antignus and Ben Yakir 2004). Further studies demonstrated that the biomass and size of hives were not significantly different between commercial tomato greenhouses covered with standard or UV-blocking films (Antignus and Ben Yakir 2004; Hefez et al. 1999; Seker 1999).

### *Putative Mechanism of UV-Blocking Films*

Flight activity of many insects in the environment is governed by UV radiation (Coombe 1981; Kevan et al. 1991). Observations carried out in commercial greenhouses, where roofs were alternately covered by regular and UV-absorbing films, showed that insect and virus epidemics were confined to plants growing under roof arches covered with non-absorbing films. This unique phenomenon was designated as the "two compartment effect" (unpublished data). Insects given the option moved toward an environment with a normal level of UV radiation in preference to a UV deficient environment. A greenhouse with a UV-absorbing roof forms a UV-deficient compartment while the space outside the greenhouse is a UV rich compartment. Insects that approach the greenhouse wall from the external environment are exhibiting a positive UV phototaxic behavior and are diverted from their original course, away from the UV deficient greenhouse walls. The protective effect of UV-absorbing films was also associated with reduced flight activity in the UV deficient environment (Antignus et al. 2001a, b; Antignus and Ben Yakir 2004; Chyzik et al. 2003). Under these conditions the efficiency of virus transmission was reduced.

## Light Manipulation in the Open Field

### *Soil Mulches Protect Crops in the Open Field*

The use of soil mulch to protect tomato plants from infestation by whiteflies was reported by Avidov (1956) who used sawdust or whitewash spray to mulch the crop seedbeds. Similar results were obtained from straw mulches that not only markedly reduced whitefly population but also delayed the spread of cucumber vein yellowing virus (CVYV) and TYLCV vectored by whiteflies (Cohen 1982).

Cohen and Melamed-Madjar (1978) tested yellow, aluminized, and blue polyethylene film, demonstrating the high efficiency of the yellow polyethylene in delaying infection of tomatoes by TYLCV. Similar protection against whiteflies, aphids and their vectored viruses were reported later by others (Suwwan et al. 1988; Csizinszky et al. 1995, 1997; Summers et al. 2005).

## *The Putative Mechanism of Action of Colored Soil Mulches*

The protective effect of yellow mulches was explained by a combination of attractiveness to whiteflies and heat emission from the plastic surface that kills the insects (Cohen 1982).

Yellow, silver, and metalized polyethylene mulches were tested for their ability to protect zucchini plants from the spread of *Squash leaf curl virus* (SLCV). Two weeks after planting, disease incidence was lowest (10–20%) in plants grown over yellow and silver mulches, respectively, compared to 50% disease incidence in the unmulched plots. The landing rate of whiteflies on plants grown over silver and yellow soil mulches was 5–7 fold lower than the landing rate on plants that grew over bare soil (Antignus et al. 2004; Antignus et al. 2005).

These results demonstrate the interference effect of yellow mulches with whiteflies landing on plants grown on these mulches. These data seem to be contradictory to the attraction effects attributed to yellow sticky cards routinely used to monitor whiteflies populations. However, we were able to show in field experiments that yellow sticky plates (20 × 15 cm) placed horizontally over bare soil trapped whiteflies normally; the same traps could not catch a single whitefly when placed near by on a large yellow surface (100 × 80 cm) made of the same material (Antignus et al. 2005). The failure of large yellow surfaces to attract whiteflies was formerly reported by Cohen (1982) who demonstrated that large (50 × 200 cm) sticky polyethylene sheets located vertically, 70 cm above ground, failed to trap whiteflies. Therefore, yellow plates have a trapping activity only when they are small enough for the insect eye to perceive the yellow color against a background formed by the soil. In cases where the insect eye perceived a high level of yellow wavelength reflection in the absence of a low reflecting background, a repelling effect resulted in place of attraction (Antignus et al. 2005).

Insight into the protection mechanism by polyethylene soil mulches was provided by spectrophotometric analysis comparing light reflection from yellow and silver polyethylene mulches, soil surfaces, and the plant canopy (Antignus et al. 2005). The soil surface reflected light at a uniformly low level between 300–700 nm, while foliage had a distinct reflection peak at 550 nm. Under these circumstances, the contrast between the soil background and the plant canopy was maximal, enabling insects to detect the crop for landing. In cases where the background of the plant was formed by yellow or silver mulches, the amount of the reflected light in the visible range was considerably higher than the reflection of the soil and plant canopy. The resulting poor contrast that formed because of the plastic's reflection interfered

with the ability of the insect to detect the plant image and perceive a landing signal (Antignus et al. 2004, 2005).

## Conclusions

The incurable nature of viral diseases and the drawbacks of insecticide use are incentives to look for other control approaches. Optical barriers described here have proven to be powerful IPM tools that hinder the epiphytotics of insect pests and virus diseases in greenhouses and the open field. They reinforce efforts being made to develop a sustainable agriculture compatible with environmental and public health objectives.

Much remains to be learned regarding role of light radiation from different parts of the spectrum in orientation, navigation and insect-plant communication. Close cooperation among virologists, entomologists, photobiologists, and physicists may lead to a breakthrough in understanding visual behavior governing insect interaction with light in their natural habitat. A better understanding of the mechanisms behind the protective effects of optical barriers will enable the production of plastics with tailor-made spectral characteristics that are able to enhance the growth and survival of a particular crop, adapt to a wide range of climatic conditions, and are effective in preventing or reducing the incidence of plant diseases and insect pests.

## Literature Cited

Antignus Y. 2001. Manipulation of wavelength-dependent behavior of insects: an IPM tool to impede insects and restrict epidemics of insect-borne viruses. *Virus Res.* 71:213–220.

Antignus Y, Ben-Yakir D. 2004. Greenhouse photoselective cladding materials serve as an IPM tool to control the spread of insect pests and their vectored viruses. In *Insect Pest Management*, ed. AR Horowitz, I Ishaaya, pp. 319–330. Berlin: Springer.

Antignus Y, Lachman O, Pearlsman M. 2005. Light manipulation by soil mulches protects crops from the spread of Begomoviruses. Abstracts of the *IX International Plant Virus Epidemiology Symposium*, Lima, Peru.

Antignus Y, Lachman O, Pearlsman M, Koren A, Matan E, Tregerman M, Ucko O, Messika Y, Omer S, Unis H. 2004. Development of an IPM system to reduce the damage of squash leaf curl begomovirus in zucchini squash crops. Abstract Compendium, In *2nd European Whitefly Symposium*, Cavtat, Croatia.

Antignus Y, Lapidot M, Cohen S. 2000. Interference with UV vision of insects: an IPM tool to impede epidemics of insect pests and insect associated virus diseases. In *Virus-Insect-Plant Interactions*, ed. KF Harris, JE Duffus, OP Smith, pp. 331–347. San Diego: Academic Press.

Antignus Y, Lapidot M, Cohen S. 2001b. Interference with Ultraviolet vision of insects to impede insect pests and insect-borne plant viruses. In *Virus-Insect-Plant Interactions*, ed. KS Harris, pp. 331–350. USA: Academic Press.

Antignus Y, Lapidot M, Hadar D, Messika Y, Cohen S. 1998. UV absorbing screens serve as optical barriers to protect vegetable crops from virus diseases and insect pests. *J. Econ. Entomol.* 91:1401–1405.

Antignus Y, Mor N, Ben-Joseph R, Lapidot M, Cohen S. 1996. UV-absorbing plastic sheets protect crops from insect pests and from virus diseases vectored by insects. *Environ. Entomol.* 25:919–924.

Antignus Y, Neste D, Cohen S, Lapidot M. 2001a. Ultraviolet-deficient greenhouse environment affects whitefly attraction and flight behavior. *Environ. Entomol.* 30:394–399.

Avidov Z. 1956. Bionomics of the tobacco whitefly (*Bemisia tabaci* Gennad.) in Israel. *Ktavim* 7:25–41.

Bertholf LM. 1931. The distribution of stimulative efficiency in the ultraviolet spectrum for the honeybee. *J. Agric. Res.* 43:703–713.

Bertholf LM. 1934. The extent of the spectrum for *Drosophila* and the distribution of stimulative efficiency in it. *Z. Val. Physiol.* 18:32–64.

Brown JK, Frohlich DR, Rosell RC. 1995. The sweetpotato or silverleaf whiteflies: biotypes of *Bemisia tabaci* or a species complex? *Annu. Rev. Entomol.* 40:511–534.

Chiel E, Messika Y, Steinberg S, Antignus Y. 2006. The effect of UV-absorbing plastic sheet on the attraction and host location ability of three parasitoids: *Aphidius colemani*, *Diglyphus isaea* and *Eretmocerus mundus*. *Biocontrol* 51:65–78.

Chyzik R, Dobrinin S, Antignus Y. 2003. The Effect of a UV-deficient environment on the biology and flight activity of *Myzus persicae* and its Hymenopterous parasite *Aphidius matricariae*. *Phytoparasitica* 31:467–477.

Cohen S. 1982. Control of whitefly vectors of viruses by color mulches. In *Pathogens, Vectors and Plant Diseases, Approaches to Control*, ed. KF Harris, K Maramorosch, pp. 45–56. New York: Academic Press.

Cohen S, Melamed-Madjar V. 1978. Prevention by soil mulching of spread of tomato yellow leaf curl virus transmitted by *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) in Israel. *Bull. Entomol. Res.* 68:465–470.

Coombe PE. 1981. Wavelength behavior of the whitefly *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). *J. Comp. Physiol.* 144:83–90.

Coombe PE. 1982. Visual behavior of the greenhouse whitefly, *Trialeurodes vaporariorum*. *Physiol. Entomol.* 7:243–251.

Costa HS, Robb KL. 1999. Effects of ultraviolet – absorbing greenhouse plastic films on flight behavior of *Bemisia argentifolii* (Homoptera: Aleyrodidae) and *Frankliniella occidentalis* (Thysanoptera: Thripidae). *J. Econ. Entomol.* 92:557–562.

Costa HS, Robb KL, Wilen CA. 2002. Field trials measuring the effects of ultraviolet-absorbing greenhouse plastic films on insect populations. *J. Econ. Entomol.* 95:113–120.

Csizinszky AA, Schuster DJ, Kring JB. 1995. Color mulches influence yield and insect pest populations in tomatoes. *J. Am. Soc. Hortic. Sci.* 120:778–784.

Csizinszky AA, Schuster DJ, Kring JB. 1997. Evaluation of color mulches and oil sprays for yield and for the control of silverleaf whitefly, *Bemisia argentifolii* (Bellows and Perring) on tomatoes. *Crop Prot.* 16:475–481.

Doukas D. 2002. Impact of spectral cladding materials on the behaviour of glasshouse whitefly *Trialeurodes vaporariorum* and *Encarsia formosa*, its hymenopteran parasitoid. In *British Crop Protection Conference, Pests and Diseases 2002*, Brighton, pp. 773–776.

Doukas D, Payne C. 2007a. The use of ultraviolet-blocking films in insect pest management in the UK: effects on naturally occuring arthropod pest and natural enemy populations in a protected cucumber crop. *Ann. Appl. Biol.* 151:221–231.

Doukas D, Payne C. 2007b. Greenhouse whitefly (Homoptera: Aleyrodidae) dispersal under different UV-light environments. *J. Econ. Entomol.* 100:380–397.

Duffus JE. 1987. Whitefly transmission of plant viruses. *Curr. Top. Vector Res.* 4:73–91.

Dyer AG, Chittka L. 2004. Bumblebee search time without ultraviolet light. *J. Exp. Biol.* 207:1683–1688.

Goldsmith TH. 1994. Ultraviolet receptors and color vision: evolutionary implications and dissonance of paradigms. *Vis. Res.* 34:1479–1487.

Hefez A, Izikovitch D, Dag A. 1999. Effects of UV-absorbing films on the activity of pollinators (honey bees and bumble bees). A report to the chief scientist. Israeli Ministry of Agriculture, Bet-Dagan ID code 891-0117-96.

Hilje L, Costa HS, Stansly PA. 2001. Cultural practices for managing *Bemisia tabaci* and associated viral diseases. *Crop Prot.* 20:801–812.

Jones DR. 2003. Plant viruses transmitted by whiteflies. *Eur. J. Plant Pathol.* 109:195–219.

Kajita H. 1986. Parasitism of the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleurodidae) by *Encarsia formosa* (Hymenoptera: Aphelinidae) in a greenhouse covered with near-ultraviolet absorbing vinyl film. *Proc. Plant Protect. Kyushu* 32:155–157.

Kevan PG, Straver WA, Offer O, Laverty TM. 1991. Pollination of greenhouse tomatoes by bumblebees in Ontario. *Proc. Entomol. Soc. Ontario.* 122:15–19.

Kumar P, Poehling HM. 2006. UV-blocking plastic films and nets influence vectors and virus transmission on greenhouse tomatoes in the humid tropics. *Environ. Entomol.* 35:1069–1082.

Lubbock J. 1882. *Ants, Bees and Wasps: A Record of Observations on the Habits of the Social Hymenoptera* (new edition, based on the 17th 1929). London: Kegan Paul, Trench, Trubner.

Markham PG, Bedford ID, Liu S, Pinner MS. 1994. The transmission of geminiviruses by *Bemisia tabaci*. *Pestic. Sci.* 42:123–128.

Menzel R. 1979. Spectral sensitivity and colour vision in invertebrates. In *Handbook of Sensory Physiology*, *VII 6A*, ed. H Autrum, pp. 503–580. Heidelberg: Springer.

Monci F, Garcia-Andres S, Sanchez F, Moriones E. 2002. Tomato yellow leaf curl disease control with UV-blocking plastic covers in commercial plastichouses of southern Spain. In the *XXVIth International Horticultural Congress & Exhibition*, Toronto Canada, August 11–17, 2002.

Morandin LA, Laverty TM, Kevan PG, Khosla S, Shipp L. 2001. Bumble bee (Hymenoptera: Apidae) activity and loss in commercial tomato greenhouses. *Can. Entomol.* 133:883–893.

Mound LA. 1962. Studies on the olfaction and colour sensitivity of *Bemisia tabaci* (Genn.) (Homoptera, Aleurodidae). *Entomol. Exp. Appl.* 5:99–104.

Mutwiwa UN, Brogemeister C, Von Elsner B, Tantu H. 2005. Effects of UV-absorbing plastic films on greenhouse whitefly (Homoptera: Aleyrodidae). *J. Econ. Entomol.* 98:1221–1228.

Perring TM, Gruenhagen NM, Farrar CH. 1999. Management of plant viral diseases through chemical control of insect vectors. *Annu. Rev. Entomol.* 44:457–481.

Rapisarda C, Tropea-Garzia G. 2002. Tomato yellow leaf curl Sardinia virus and its vector *Bemisia tabaci* in Sicilia (Italy): present status and control. *OEPP/EPPO Bull.* 32:25–29.

Raviv M, Antignus Y. 2004. UV radiation effects on pathogens and insect pests of greenhouse-grown crops. *Photochem. Photobiol.* 79:219–226.

Scherer C, Kolb G. 1987. Behavioral experiments on the visual processing of color stimuli in *Pieris brassicae* L. (Lepidoptera). *J. Comp. Physiol.* 160:647–656.

Seker I. 1999. The use of UV-blocking films to reduce insect pests damage in tomato greenhouses and the effect of UV-filtration on the pollination activity of bumble bees. *Gan Sadeh VaMeshek* 12:55–59. (in Hebrew).

Steinberg S, Prag H, Gouldman D, Antignus Y, Pressman E, Asenheim D, Moreno Y, Schnitzer M. 1997. The effect of ultraviolet-absorbing plastic sheets on pollination of greenhouse tomatoes by bumblebees. In *Proc. Int. Congress Plastics Agric*. (CIPA), Tel-Aviv, Israel.

Summers CG, Mitchell JP, Stapleton JJ. 2005. Mulches reduce aphid-borne viruses and whiteflies in cantaloupe. *California Agric.* 59:90–94.

Suwwan MA, Akkawi M, Al-Musa MA, Mansour A. 1988. Tomato performance and incidence of tomato yellow leaf curl (TYLC) virus as affected by type of mulch. *Scientia Hortic.* 37:39–45.

Vaishampayan SM, Kogan M, Waldbauer GP, Woolley JT. 1975a. Spectral specific responses in the visual behaviour of the greenhouse whitefly, *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). *Entomol. Exp. Appl.* 18:344–356.

Vaishampayan SM, Waldbauer GP, Kogan M. 1975b. Visual and olfactory responses in orientation to plants by the greenhouse whitefly *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). *Entomol. Exp. Appl.* 18:412–422.

# Chapter 14
# Host Plant Resistance for the Management of *Bemisia tabaci*: A Multi-crop Survey with Emphasis on Tomato

**Gloria Nombela and Mariano Muñiz**

## Introduction

Control measures commonly used against insect pests in horticultural crops rely mainly on the use of pesticides, but these products are often toxic to the environment and to non-target species. Moreover, control of insects is difficult because many are polyphagous and develop insecticide resistance quickly. Also, the use of these compounds favors the development of resistant populations, rendering their application counter-productive in the long term. Consequently, there is a general opinion that the best way to solve the pest problem is by Integrated Pest Management (IPM), based on the rational and coordinated application of appropriate selective, economical and environmentally friendly techniques. As an example, prevention and management of virus-transmitting insects (specifically whiteflies) on horticultural crops was declared officially in 2004 as a public benefit issue in Spain. In this context, the use of more adequate control measures in place of conventional chemical control should be recommended when feasible for virus vector control.

Host plant resistance is one of the main basic components of IPM, and the utilization of resistant plants has long been considered as one of the most effective components of insect control (Russell 1978). As a consequence, an increase in research aiming to favor the use of varieties resistant to pest organisms has been observed during recent years in many countries. This has produced an important advance in the available knowledge of the defensive plant response against attack by certain organisms. The phenomenon of plant resistance can be described as the relative reduction of pest population size compared with the standard varieties due to genetic characteristics of the host plant (Ponti et al. 1990). The "gene-to-gene" hypothesis (Flor 1971) was for many years the main concept supporting the whole theory of plant-pathogen interactions. However, this concept is being reformulated in the context of recent advances in understanding the molecular structure of plant resistance genes. Earlier data suggested that resistance usually results from

G. Nombela (✉)
Department of Plant Protection, Institute for Agricultural Sciences, Spanish National Research Council (CSIC), Serrano 115 Dpdo., Madrid 28006, Spain
e-mail: gnombela@ccma.csic.es

P.A. Stansly, S.E. Naranjo (eds.), *Bemisia*: Bionomics and Management of a Global Pest,
DOI 10.1007/978-90-481-2460-2_14, 

a biochemical pathway wherein pathogen specificity is controlled only by a few components (Beynon 1997). More recent investigations reveal that plant resistance has evolved by an integration of biochemical processes in a general network of responses induced by parasite attack.

The resistance process can be summarized as follows: When the parasite contacts the plant, plant cells recognize a parasite-produced compound, generating an initial plant signal. This signal is transduced intracellularly, producing substances that also cause synthesis of compounds either directly involved in defence or in transducting the initial signal. Finally, these transductors translocate to the action site. This process is very complex and involves radical changes in cell metabolism and gene expression, either by gene activation or silencing. As a consequence, these changes lead finally to the appearance of different defence mechanisms which can be classified in three main categories: (a) reinforcement of physical barriers by an increase of cell wall consistency and a decrease of cell wall porosity, (b) alteration of phytohormone balance, and (c) increase in the production of toxic substances. The sequence of all these processes involved in plant defense and resistance has been widely reviewed, especially in angiosperms (Dixon et al. 1994; Hutcheson 1998).

Plant resistance can be classified in two categories: natural (also referred to as innate or congenital) and induced (also referred to as acquired). Regarding natural resistance, there are plant species as well as multiple germplasm within a species which posses a high level of resistance that has been selected through the evolutionary process. In many cases this resistance applies also to other physical or chemical conditions that are adverse to the plants. However, induced resistance is acquired in plants after being attacked in such a way that in many instances if a plant survives an attack, it defends itself much better from a later attack from the same organism or even other organisms. Inducible responses are thought to be a means of avoiding costs associated with constitutively expressed resistance mechanism (Stout et al. 2002).

## Innate Resistance in Tomato: The *MI-1* Gene

Tomato, *Solanum lycopersicum* L. (Solanaceae), is widely distributed, being one of the economically most important crops in the Mediterranean area and elsewhere. One of the limiting factors affecting the success of this crop is the existence of whiteflies. Among them, the whitefly *Bemisia tabaci* (Gennadius) is one of the most significant. Damage caused by this insect to commercial tomato may be directly through phloem feeding, or indirectly by the transmission of plant viruses such as *Tomato Yellow Leaf Curl Virus* (TYLCV) (Mehta et al. 1994; Moriones and Navas-Castillo 2000).

Resistance to whiteflies and other insects found on the wild species of tomato *Solanum pennellii* (Corr.) D'Arcy were mostly attributed for the past to the presence of sugar esters in the glandular exudate of type IV trichomes (Gentile et al. 1968; Juvik et al. 1994; Heinz and Zalom 1995). It was therefore concluded that selection for sugar ester accumulation should be an efficient technique in selecting

for general insect resistance in *S. pennellii* (Goffreda et al. 1990) and other tomato plants (Kisha 1981; Berlinger 1986). Choice and non-choice assays revealed a correlation between resistance to *B. tabaci* (reported as *B. argentifolii*) and type IV trichome densities of six wild accessions of *Solanum habrochaites* (reported as *L. hirsutum*); the accessions included LA1777 (Snyder et al. 1998). More recently, multigenic resistance to *B. tabaci* in LA1777 was further demonstrated to be correlated with the presence of type IV trichomes (Momotaz et al. 2005). Similarly, leaf-trichome densities and presence of acyl sugars in the exudate of glandular trichomes as well as type of trichomes were reported to be important factors affecting whitefly-tomato relationships (Williams et al. 1980; Kisha 1981; Berlinger 1986; Kishaba et al. 1992; Simmons 1994; Freitas et al. 2002; Baldin et al. 2005; Srinivasan and Uthamasamy 2005; Simmons and Gurr 2005; Sanchez-Pena et al. 2006). However, the actual effect of these, and other factors, on tomato resistance has been broadly questioned (Goffreda and Mutschler 1989; Steffens and Walters 1991; Liedl et al. 1995; Toscano et al. 2002a, b; Muigai et al. 2003). Field resistance to *B. tabaci* was reported in different lines of the wild species *L. peruvianum* (L.) Mill (Hassan et al. 1982; Channarayappa et al. 1992), a close relative of commercial tomato, *S. lycopersicum*. This resistance was shown to be due to factors other than the presence of trichomes and their exudates, because Va and Vb non-glandular trichomes were predominant and VIc glandular trichomes were absent (Channarayappa et al. 1992). These authors also reported that commercial cultivars of tomato mostly exhibit non-glandular type III and V trichomes and four-lobed glandular VIa trichomes which are not considered to be important in whitefly control mechanisms (Ponti et al. 1990). So, differential host response to insects in these commercial cultivars might be due to alternative mechanisms of resistance regulated by genes that were previously unknown.

In a study carried out in our laboratory (Nombela et al. 2000), two commercial cultivars of tomato, 'Alta' and 'Peto 95', the accession LA716 of *S. pennellii* and lines 94GH-006 and 94GH-033 (backcrosses between 'Peto 95' and LA716), with different leaf acyl sugar contents were screened for resistance to the Spanish B-biotype of *B. tabaci* in greenhouse and field no-choice experiments. There was no oviposition on LA716 (with the highest acyl sugar content) while the greatest fecundity and fertility values were observed on the cultivar Alta (no acyl sugar content). However, no clear relationship was found between the low acyl sugar content in the other tomato cultivars tested and whitefly reproduction. Resistance to *B. tabaci* appears to be independent of acyl sugar content below a threshold level of 37.8 $\mu g\ cm^{-2}$ leaf (Fig. 14.1). Moreover, feeding similarities between piercing-sucking insects, such as aphids and whiteflies, and root-knot nematodes (Byrne and Bellows 1991; Walker and Perring 1994; Kaloshian et al. 1995) suggested that a specific gene-for-gene defence response (Flor 1955) could be highly effective in regulating the prolonged interaction of the stylets of these insects and the plant cell contents (Fernandes 1990).

There are many examples of single resistance genes conferring gene-for-gene resistance to piercing-sucking insects and nematodes. The *Mi-1* gene, present in many varieties of cultivated tomato (*S. lycopersicum*) and introduced into this plant

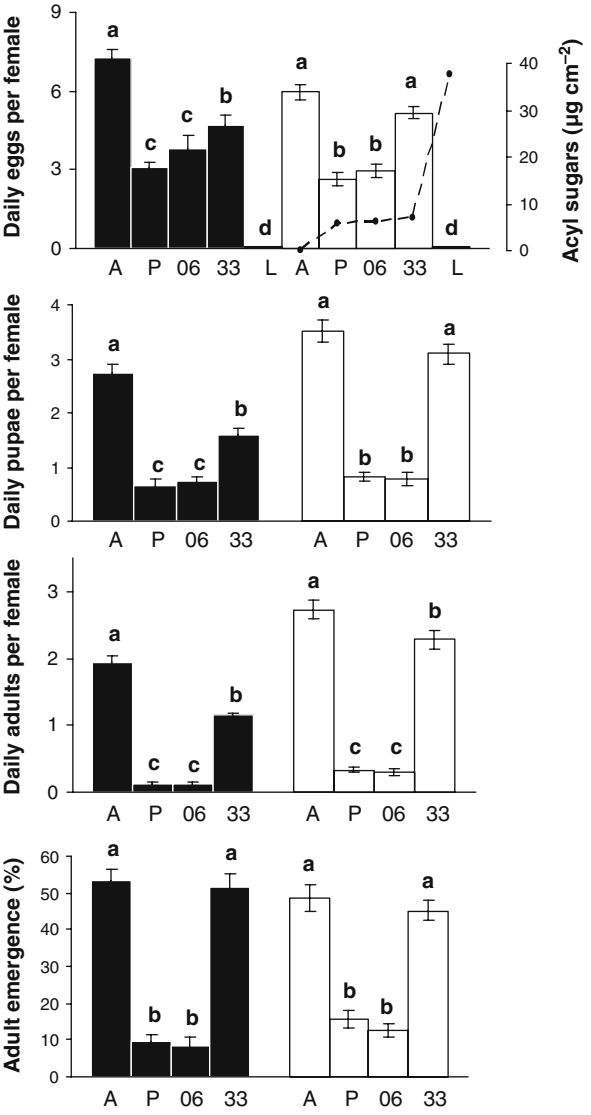



**Fig. 14.1** Mean daily egg, pupa and adult production per female and adult emergence of *Bemisia tabaci* on tomato, represented as bars scaled on the Y1 axis, in greenhouse (*black*) and field (*white*) no-choice experiments. *Error bars* indicate the SEM. *Different letters* on *bars* from the same experiment indicate that corresponding means differ significantly ($p<0.05$) by Fisher's Protected LSD. *Black points* connected by *dashed lines* in the first graph represent the Total Acyl sugar Content (TAC = glucose + sucrose) of each cultivar, backcrossing line or wild species accession, scaled on the Y2 axis. A = Alta, P = Peto 95, 06 = 94GH-006, 33 = 94GH-033, L = LA716

from its wild relative, *S. peruvianum* (Smith 1944), confers resistance characterized by a hypersensitive reaction to three species of root-knot nematodes *Meloidogyne* spp. (Roberts and Thomason 1986). It was since demonstrated that *Mi-1* in tomato also regulates resistance to insects such as the potato aphid *Macrosiphum euphorbiae* (Thomas) (Kaloshian et al. 1995; Rossi et al. 1998).

In a greenhouse choice assay (Nombela et al. 2000), *B. tabaci* exhibited reduced host preference and reproduction on three commercial tomato cultivars with the *Mi-1* gene ('Motelle', 'VFN8' and 'Ronita') compared with three *Mi-1*-negative

cultivars ('Moneymaker', 'Rio Fuego' and 'Roma'). When data of *Mi-1*-positive tomato plants were pooled, the mean values for daily infestation and pupal production of *B. tabaci* were significantly lower than those of *Mi-1*-negative plants (Table 14.1).

Similarly, three tomato varieties ('Motelle', 'Ronita', and 'VFN8') bearing the *Mi-1* gene, and three varieties not bearing this gene ('Moneymaker', 'Roma', and 'Río Fuego'), were compared by choice and no-choice assays for host preference using the Q-biotype of *B. tabaci* (Nombela et al. 2001). The most preferred hosts, determined by infestation levels and numbers of feeding adults were 'Moneymaker', 'Río Fuego' and 'Roma', all of which were not carrying the *Mi-1* gene. 'Ronita' and 'Motelle', both of which bore the *Mi-1* gene, were the least preferred hosts. In a no-choice assay, *B. tabaci* females laid significantly fewer eggs on varieties that carried the *Mi-1* gene than on those lacking the gene. Detectable differences were more dramatic when plants carrying the *Mi-1* gene were pooled together and compared with pooled plants without this gene. Significantly greater values were obtained for the *Mi-1*-negative group for all parameters tested (Fig. 14.2). Comparing these results with those from our previous study on the B-biotype of *B. tabaci* (Nombela et al. 2000), Q-biotype was found to produce higher daily infestation rates on most of the tomato varieties. When results from plants carrying *Mi-1* were pooled, they showed lower infestation levels of Q-biotype than B-biotype. Q-biotype infested fewer *Mi-1* plants and more non-*Mi-1* plants than B-biotype. Q-biotype females produced significantly fewer pupae than B-biotype females on both groups of plants. These results suggested the existence of an antixenosis and antibiosis-based resistance to the Q-biotype of *B. tabaci* in *Mi*-bearing commercial tomato varieties, which is greater than that previously reported for the B-biotype. More importantly, these results indicated that *Mi-1*, or another closely linked gene, might be implicated in a partial resistance which was not associated with either the presence of glandular trichomes or their exudates. These findings supported the general hypothesis for the existence of similarities among the resistance mechanisms to whiteflies, aphids and nematodes in commercial tomato plants.

The *Mi-1* gene was cloned and this locus in chromosome six of tomato was found to contain two transcribed genes, *Mi-1.1* and *Mi-1.2*, with 91% homology (Milligan et al. 1998). *Mi-1.2*, but not *Mi-1.1*, is sufficient to confer resistance to root-knot nematodes (Milligan et al. 1998) and aphids (Rossi et al. 1998), and we refer to this gene as *Mi-1*. Cloning of *Mi-1* allowed us to finally demonstrate that this gene mediates resistance to both B- and Q-biotypes of *B. tabaci* in 2-month-old tomato plants (Nombela et al. 2003). The response of whiteflies to tomato plants carrying the cloned *Mi-1* was compared to that of the isogenic untransformed tomato line 'Moneymaker' in both free-choice and no-choice assays. The transgenic lines used in that work differed from susceptible 'Moneymaker' only in the presence of a 14.7 kb DNA insert containing *Mi-1.2*. The daily infestation rates of both B- and Q-biotypes during the free-choice assays were lower on the transgenic line 143-11-16-36 than on 'Moneymaker' at all time points examined. Mean values of the percentage of adults (Fig. 14.3A) as well as the final numbers of pupae per plant (Fig. 14.3B) or per leaf (Fig. 14.3C) were also significantly reduced on the

**Table 14.1** Daily infestation rate and pupal production (Mean ± SEM) of *Bemisia tabaci* (B-biotype) on six cultivars of tomato of varying trichome density, with and without *Mi* gene, under greenhouse conditions

| | | | Daily infestation | | | | | Pupal production | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cultivars | n | Trichomes/cm$^2$ | n | Plants infested by females (%) | Plants infested by adults (%) | Females on plants (%) | Adults on plants (%) | n | Pupae per plant | Pupae per leaflet | Leaflets infested by pupae (%) |
| *Separated* | | | | | | | | | | | |
| Moneymaker (–*Mi*) | 20 | 68 ± 7.0 ab | 25 | 74 ± 3.8 a | 82 ± 3.5 a | 24 ± 0.9 a | 23 ± 0.8 a | 10 | 372 ± 73.1 ac | 3 ± 0.6 b | 26 ± 2.6 ab |
| Río Fuego (–*Mi*) | 20 | 56 ± 4.9 b | 25 | 70 ± 3.5 a | 82 ± 2.9 a | 23 ± 1.7 a | 25 ± 1.8 a | 10 | 435 ± 68.4 a | 5 ± 0.9 a | 31 ± 3.1 a |
| Roma (–*Mi*) | 20 | 75 ± 5.0 a | 25 | 66 ± 3.9 ab | 76 ± 3.4 ab | 14 ± 0.6 b | 15 ± 0.6 b | 10 | 369 ± 69.6 ac | 3 ± 0.6 ab | 26 ± 2.7 ab |
| Motelle (+*Mi*) | 20 | 42 ± 4.8 c | 25 | 60 ± 4.7 bc | 68 ± 3.9 bc | 14 ± 1.0 b | 13 ± 0.7 bc | 10 | 230 ± 62.5 b | 2 ± 0.5 b | 19 ± 2.9 b |
| VFN8 (+*Mi*) | 20 | 35 ± 3.9 c | 25 | 56 ± 4.5 c | 62 ± 4.4 c | 14 ± 0.9 b | 13 ± 0.8 bc | 10 | 191 ± 45.3 bc | 2 ± 0.3 b | 21 ± 2.0 b |
| Ronita (+*Mi*) | 20 | 68 ± 4.6 ab | 25 | 54 ± 4.3 c | 70 ± 3.6 bc | 11 ± 0.7 c | 12 ± 0.6 c | 10 | 283 ± 75.6 ab | 3 ± 0.7 b | 22 ± 2.4 b |
| *Pooled* | | | | | | | | | | | |
| Without *Mi* | | | 25 | 70 ± 18.6 a | 80 ± 16.4 a | 61 ± 1.7 a | 62 ± 1.4 a | 30 | 392 ± 39.6 a | 4 ± 0.4 a | 28 ± 1.6 a |
| With *Mi* | | | 25 | 57 ± 22.3 b | 67 ± 19.9 b | 39 ± 1.7 b | 38 ± 1.4 b | 30 | 235 ± 35.5 b | 2 ± 0.3 b | 20 ± 1.4 b |

Means followed by the same letter in the same columns are not significantly different ($p<0.05$) by Fisher's Protected LSD.

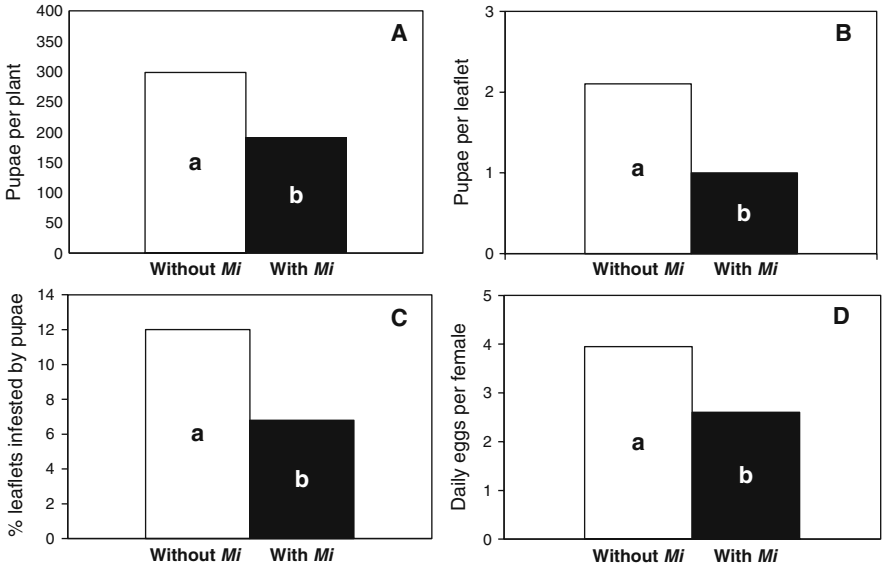



**Fig. 14.2** Pupal production and fecundity of *B. tabaci* (Q-biotype) on pooled tomato plants with and without *Mi* gene. (**A**) number of pupae per plant. (**B**) number of pupae per leaflet. (**C**) percentage of leaflets infested by pupae. (**D**) daily number of eggs per female

transgenic line 143-11-16-36. At the end of the no-choice assays, the total number of insects from the B-biotype (Fig. 14.3D) and Q-biotype (Fig. 14.3E) were significantly lower on the transgenic line than on 'Moneymaker'. In summary, our results indicated that *Mi-1.2* is responsible for the resistance in tomato plants to both B- and Q-biotypes when tomato plants were 2-months old. We had previously observed that the development of *B. tabaci* on younger tomato plants (2–4 true leaf stage) did not differ in relation to the presence/absence of the *Mi* gene (Pascual et al. 2000), suggesting that the activity of this gene against *B. tabaci* could be developmentally regulated. Similarly, *Mi-1.2* plants were resistant to aphids only at 4–5 weeks of age (Kaloshian et al. 1995).

The *Mi-1* gene belongs to the NB-LRR class of R genes as it encodes a protein with a nucleotide binding site and leucine rich repeat (NB-LRR) motifs. Proteins of this motif structure make up the largest class of cloned plant resistance genes with specificity against diverse pathogens (including viruses, bacteria and fungi), nematodes and insects (Dangl and Jones 2001). Recent findings have shown that resistance genes of the NB-LRR type could confer resistance to insects. So far, *Mi-1* gene is the only cloned resistance gene that has been demonstrated to mediate resistance to insects (Kaloshian and Walling 2005).

Probing and feeding behaviour of *B. tabaci* B-biotype on the near-isogenic tomato lines 'Moneymaker' (without *Mi-1*) and 'Motelle' (with *Mi-1*) was studied using a DC Electrical Penetration Graph (EPG) to characterize *Mi-1*-mediated resistance (Jiang et al. 2001). Significant differences between tomato lines were found

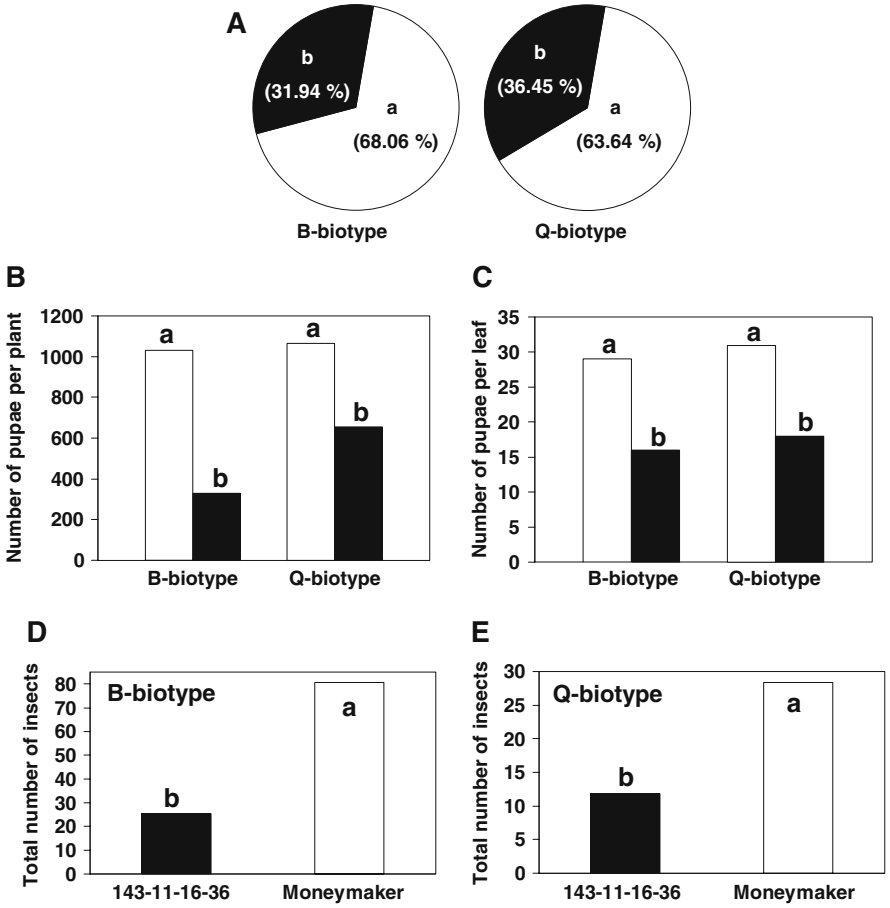



**Fig. 14.3** Reproductive activity of B- and Q-biotypes *B. tabaci* on tomato transgenic line 143-11-16-36 ($Mi^+$) in *black* and cv. Moneymaker ($Mi^-$) in *white*. *Different letters* for the same biotype indicate significant differences by ANOVA ($p < 0.05$). (**A**) percentages of adults present during the free-choice assays under greenhouse conditions. (**B**) average number of pupae per plant at the end of the greenhouse free-choice experiments. (**C**) average number of pupae per leaf at the end of the greenhouse free-choice experiments. (**D**) total number of B-biotype individuals (N3, pupae and new adults) at the end of the no-choice assay. (E) total number of Q-biotype individuals (N3, pupae and new adults) at the end of the no-choice assay ($p = 0.073$)

in EPG parameters related to epidermis and/or mesophyll tissues (Table 14.2). On 'Motelle', a lower percentage of whiteflies achieved phloem phase and more probes were made before first phloem phase was attained. Also, a higher ratio (number of probes before first phloem phase)/(total number of probes) was observed as were more non-probing events, and longer times to achieve first intracellular puncture and first phloem phase. In contrast, most of the parameters related to phloem phase were found not to differ significantly between these near-isogenic lines. The behavioural

**Table 14.2** Electrical Penetration Graph (EPG) parameters, possible relations to resistance factors in specific plant tissue and comparison of EPG parameters of B-biotype of *B. tabaci* on two isogenic tomato lines during 6-h recordings

| EPG parameters | Tissue(s)/factors involved | Moneymaker mean ± SE (n) | Motelle mean ± SE (n) |
|---|---|---|---|
| *Non-phloem parameters* | | | |
| Time to the first probe | Surface factors | 39.09 ± 14.94 (25) | 61.68 ± 25.76 (26) NS |
| Duration of 1st probe | Surface factors + epidermis/mesophyll | 89.48 ± 24.96 (25) | 99.78 ± 31.11 (26) NS |
| Total number of probes | Surface factors + all plant tissues | 44.36 ± 5.05 (25) | 57.23 ± 8.38 (26) NS |
| Number of probes made before attaining the first phloem phase (1st E(pd)) | Epidermis, mesophyll and other parenchyma | 27.08 ± 3.65 (25) | 53.00 ± 8.87 (26)* |
| Ratio of probes before 1st E(pd)/total number of probes | Epidermis, mesophyll and other parenchyma | 0.65 ± 0.06 (25) | 0.86 ± 0.05 (26)** |
| Total duration of non-probing time | All plant tissues except phloem | 65.81 ± 7.95 (25) | 100.39 ± 11.50 (26)* |
| Total duration of F | All plant tissues except phloem | 21.12 ± 4.00 (12) | 32.54 ± 6.65 (19) NS |
| Total duration of G | Xylem factors | 20.31 ± 3.25 (9) | 42.88 ± 15.62 (14) NS |
| Time whitefly took to make its first intracellular puncture (1st pd) from the start of the 6 h exper. (min) | All plant tissues except phloem | 94.31 ± 12.44 (24) | 154.38 ± 20.32 (25)* |
| Time whitefly took to the 1st E(pd) from the start | All plants tissues except phloem | 163.25 ± 20.01(25) | 272.16 ± 22.82 (26)** |
| Time whitefly took to 1st E(pd) within probe | All plants tissues except phloem | 21.19 ± 2.28 (22) | 38.97 ± 7.15 (13) NS |
| Percentage of whitefly reaching the phloem phase | Epidermis, mesophyll and other parenchyma | 21.19 ± 2.28 (22)<br>88.00 | 38.97 ± 7.15 (13) NS<br>50.00** |
| *Phloem parameters* | | | |
| Duration between time to the 1st E(pd) and time to the first sustained ingestion (1st SI) (E(pd)2>15 min) | Vascular tissue and sieve elements (initial factors) | 35.99 ± 17.79 (19) | 11.34 ± 9.72 (11) NS |
| Time whitefly took to 1st SI from start | All plant tissues | 188.62 ± 21.12 (25) | 277.79 ± 21.45 (26)** |
| Percentage of time whitefly spent in E(pd)2 after the 1st phloem phase | Phloem factors | 51.96 ± 28.31 (21) | 62.4 ± 35.35(13) NS |

**Table 14.2** (continued)

| EPG parameters | Tissue(s)/factors involved | Moneymaker mean ± SE (n) | Motelle mean ± SE (n) |
|---|---|---|---|
| Duration of E(pd)1 preceding the first E(pd)2 >15 min | Phloem factors | 3.17 ± 0.72 (22) | 1.63 ± 0.43 (13) NS |
| Duration of E(pd)2 after the 1st SI | Phloem factors | 29.35 ± 9.18 (22) | 13.39 ± 7.73 (13) NS |
| Total duration of E(pd)1 | Phloem factors | 5.85 ± 1.09 (22) | 3.05 ± 0.87 (13) NS |
| Mean duration of E(pd)1 | Phloem factors | 2.73 ± 0.48 (22) | 1.67 ± 0.44 (13) NS |
| No. of E(pd)1 | Phloem factors | 2.09 ± 0.24 (22) | 2.08 ± 0.50 (13) NS |
| Total duration of E(pd)2 | Phloem factors | 106.44 ± 17.55 (21) | 105.10 ± 24.00 (13) NS |
| Mean duration of E(pd)2 | Phloem factors | 64.88 ± 14.63 (21) | 84.41 ± 25.14 (13) NS |
| No. of E(pd)2 | Phloem factors | 1.90 ± 0.25 (21) | 1.69 ± 0.33 (13) NS |
| Percentage of whiteflies showing sustained ingestion (E(pd)>15 min | All plant tissues | 76.00 (19/25) | 42.30 (11/26)* |

*$p < 0.05$

**$p < 0.01$.

Significant differences were compared at the 0.05 and 0.01 level according to the Mann-Whitney U test (Statview, Abacus Concepts, 1987). In the case of percentage or ratio, a chi-square test was used at 0.05 and 0.01 levels. NS, no significant differences ($p$>0.05).

data strongly suggest that tomato resistance mediated by *Mi-1* is due to factors in the epidermis and/or mesophyll that inhibit whiteflies from reaching phloem sieve elements. However, once the stylets reach a sieve element, whitefly behaviour did not differ between the two varieties. Thus, phloem sap of the two varieties appears to be equally acceptable to the whiteflies.

Avirulence effectors that interact with *Mi-1* protein have not yet been identified in any root-knot nematodes, aphids or whiteflies. Apparently, *Mi-1* either recognizes more than one distinct avirulence product or recognizes perturbations in a host protein conveyed by these three organisms (Dangl and Jones 2001; Mackey et al. 2002). Using a genetic screen to identify suppressors of *Mi-1*, a tomato mutant, *rme1* (resistant to *Meloidogyne*), compromised in resistance to the root-knot nematode *M. javanica,* and to the potato aphid *M. euphorbiae*, was identified (Martínez de Ilaarduya et al. 2001). Subsequently, our group demonstrated that *rme1* mutant plants were also compromised in resistance to *B. tabaci* and to two other 2 species of root-knot nematode (*M. incognita* and *M. arenaria*), indicating that the *Rme1* gene is required for *Mi-1* resistance (Martínez de Ilaarduya et al. 2004). In a free-choice assay, the mean values of the number of adults per plant per day on the *rme1* mutant were similar to those on susceptible 'Moneymaker' and significantly greater than on resistant 'Motelle' (Fig. 14.4A). Moreover, the average number of eggs

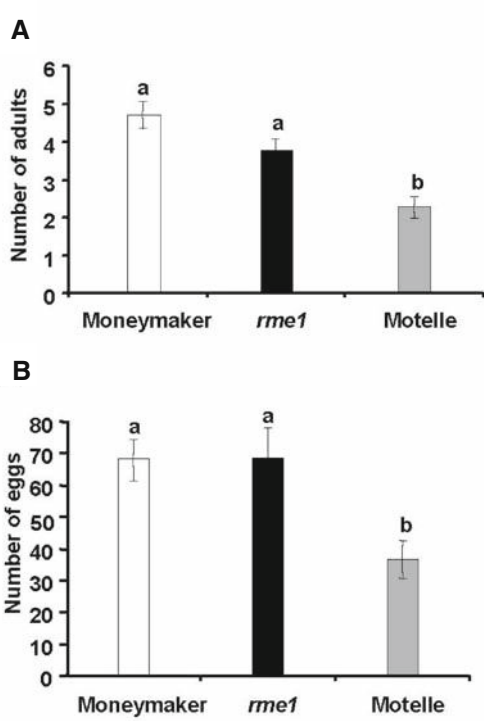



**Fig. 14.4** Infestation of *B. tabaci* B-biotype on wild-type 'Motelle' (*Mi–1/Mi–1*), *rme1* mutant (*Mi–1/Mi–1*), and 'Moneymaker' (*mi–1/mi–1*) tomato plants. (**A**) Average number of adult whiteflies per sampling time on each plant genotype in the free-choice assay. Sampling was done every other day. Values represent the mean and standard error of 10 plant replicates. (**B**) Average number of eggs produced by *B. tabaci* B-biotype on each plant genotype during the no choice assay. Five adult female whiteflies were confined to a single leaflet per plant for 6 days. Each *bar* represents the mean and standard error of 11 replicates. Significantly different means are indicated by *different letters* ($p < 0.05$)

observed on the *rme1* mutant plants 6 days after infestation (no-choice experiment) was similar to that on 'Moneymaker' and significantly greater than that observed on 'Motelle' (Fig. 14.4B). This work also provided evidence that *Rme1* acts early in the *Mi-1*-pathway, either at the same step as the *Mi-1* product or earlier in the response cascade.

In addition to resistance mediated by *Mi-1* gene, other studies are in progress to identify new sources of tomato resistance to *B. tabaci*, with interesting results during the last few years. From a screening of 25 wild and cultivated tomato genotypes, *S. habrochaites* LA1777 was determined to be resistant to both *B. tabaci* and the transmitted virus ToLCBV-[Ban4] (Maruthi et al. 2003). Ninety-four recombinant inbred lines were developed from wild accession LA1777 of *S. habrochaites*, and were tested for whitefly resistance together with the resistant parent (LA1777), susceptible parent (E6203), and three interspecific $F_1$ hybrids (Momotaz et al. 2005). No resistance was detected in any recombinant inbred lines. From an interspecific F2 population, 11 resistant and 10 susceptible plants were selected to locate resistance genes by testing them with 400 molecular markers. So far, markers in five regions on four different chromosomes appear to be associated with resistance and subsequent research is in progress to identify the resistant loci (Momotaz et al. 2006). Other authors investigated eight wild populations of *S. lycopersicum* var. *cerasiforme* and one population of *S. habrochaites* (C-360), and found lower numbers of *B. tabaci* than on the cultivated variety 'Rio Grande'; the lowest whitefly incidence was on *S. habrochaites* (Sanchez-Pena et al. 2006).

## Induced Resistance in Tomato

In addition to innate resistance, plants can activate protective mechanisms upon contact by invaders; this is termed induced or acquired resistance (Sticher et al. 1997). Induced resistance is a long known phenomenon. However, only a few laboratories around the world had studied it until the last decade. Since then, induced resistance has received growing attention as a new and environmentally friendly control method, as well as a model to study genes involved in defence and control signaling. It was initially observed that plants inoculated with attenuated micro-organisms were protected against later infections by the same or another pathogen, but no relationship was established with the activation of host defence mechanisms (reviewed in Hammerschmidt and Kuc 1995). The activation of true resistance mechanisms was demonstrated when it was observed that a localized infection could lead to resistance against later infections by very different pathogens (Ross 1966). Induced resistance in plants can be compared as a whole with immunization in humans and other animals, although underlying resistant mechanisms are different in animals. Acquired or induced resistance can be expressed locally, at the site of primary inoculation (localized acquired resistance or LAR). It may also be expressed systemically in tissues far away from the initial inoculation site, and this is termed systemic acquired resistance or SAR (Agrawal et al. 1999).

Some biological agents such as certain bacteria, fungi or viruses can induce plant resistance to other pathogens (Agrawal et al. 1999; Hammerschmidt et al. 2001; Siddiqui and Shaukat 2004). Similarly, positive and negative associations as a result of cross-talk between insect- and pathogen-induced defence pathways have been widely reported (reviewed by Hunter 2000), including the induction of plant resistance to insects due to a previous attack by the same or another organism. Available information on induced resistance to arthropods mostly refers to chewing herbivores, which usually cause extensive leaf damage to infested plants. A less studied phenomenon is the induction of plant resistance to or by phloem-feeding insects, such as whiteflies, which maintain a longer interaction with their host plant but causing only limited direct damage to the plant tissues with their stylets (Walling 2000). Little is known to date about plant responses to whiteflies induced after previous attacks by arthropods or by other inducer organisms (Agrawal et al. 2000; Inbar et al. 1999; Mayer et al. 2002; Murugan and Dhandapani 2007).

Several different bioassays have been carried out recently under controlled conditions in our laboratory to determine if resistance against *B. tabaci* could be induced in susceptible tomato plants (lacking the *Mi-1* gene) after a previous infestation by the same or another organism. To date, we found that 3 days of contact with 20 apterous adults of the potato aphid *M. euphorbiae* were enough for the plants to acquire resistance to *B. tabaci* (Nombela et al. 2004). This resistance was transient because whitefly numbers were significantly reduced when aphid attack occurred between 1 and 18 h prior to *B. tabaci* infestation, whereas the reduction was less detectable when 4 days passed between aphid and whitefly infestations. The resistance observed when *B. tabaci* infested the plants 18 h after aphid contact on a single leaflet was both locally (LAR) and systemically (SAR) induced (Nombela et al. 2009). However, our results indicate that the tomato responses induced by whitefly feeding depended on the aphid clone.

In addition to biological inducers, various chemicals have been discovered that seem to mimic all or part of the biological activation of resistance, but only a few have reached commercialization (Oostendorp et al. 2001). Benzo[1,2,3]thiadiazole-7-carbothioic acid-*S*-methyl ester (BTH) is the active ingredient of the Syngenta plant activator Bion® or Actigard®. Benzo[1,2,3]thiadiazole derivatives have been shown to mimic the biological activation of systemic acquired resistance by necrogenic pathogens (Kunz et al. 1997). A number of reports exist on the efficacy of Bion® as an inducer of resistance in different cultivated plants against a broad spectrum of fungal, bacterial, and viral diseases (Oostendorp et al. 2001; Smith-Becker et al. 2003). Treatment with this product in combination with other crop management strategies has been tested for protection against TYLCV and its vector *B. tabaci*, resulting in increased fruit production (Monci et al. 2003). However, little is known about the actual effect of BTH on insect pest populations and the available information on the effect of BTH treatment on whitefly densities is even more limited.

Recently, a reduction of whitefly densities was reported on cotton due to BTH treatment, although the authors considered the effect negligible (Inbar et al. 2001). In tomato, a trend toward reduced densities of whiteflies, although not significant,

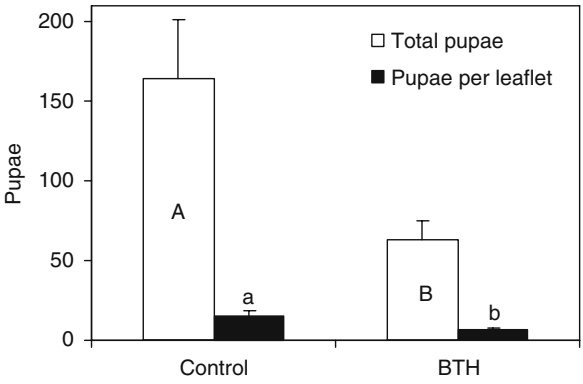



**Fig. 14.5** Mean (±SE) numbers of total pupae and pupae per tomato leaflet of *B. tabaci* (B-biotype) observed on BTH and control plants at 29 days after treatment in the greenhouse free-choice assay. *Different letters* indicate significant differences between treatments: $p = 0.005$ for total pupae (capital letters) and $p = 0.006$ for pupae per leaflet (*lower case letters*)

was observed on BTH-treated plants (Inbar et al. 1998). More recently, we carried out a study to test the possible induction of resistance to the B- and Q-biotypes of *B. tabaci* by BTH in susceptible tomato plants of cv. Marmande (Nombela et al. 2005). The BTH treatment affected host preference of adults from both biotypes on plants sprayed with Bion® at 0.2 and 0.4 g/L during early free-choice assays. Consequently, a decrease in the total number of eggs the final number of pupae and empty pupal cases was observed (Fig. 14.5). The test for an effect produced by BTH applied at 0.1 g/L Bion® was not significant. In no-choice assays, a reduction in number of first-stage nymphs and total individuals, and a delay in insect development were observed when the local treatment was restricted to one leaflet per plant 5 days before *B. tabaci* (biotype B) infestation (Table 14.3). This acquired resistance induced by BTH was locally expressed because of the differences between treated and untreated leaflets on the same plants (Table 14.4), whereas no differences in untreated leaflets were observed between BTH-treated and control plants (Nombela et al. 2005).

## Plant Resistance to *B. tabaci* in Other Crops

Although other genes for resistance against *B. tabaci* have not been cloned to date, a number of studies on the resistance to this pest in plants other than tomato have been carried out during the last decade. Diverse research has been carried out to compare the biotic potential of whiteflies in different cultivated plants such as sesame, beans, cucumber, cantaloupe, zucchini, cassava, corn, poinsettia, cabbage and tomato (Morales and Cermeli 2001; Villas-Bôas et al. 2002). However, most studies focused on testing different cultivars or accessions as potential sources of resistance to *B. tabaci* on a single crop.

There have been many studies searching for host plant resistance on cotton because of the severity of whitefly problems in this crop, mostly investigating the relative resistance of different cotton genotypes (Meagher et al. 1997; Wahla et al.

**Table 14.3** Mean (± SE) numbers of *B. tabaci* (B-biotype) eggs, N1, N2 and N3 observed on treated and untreated contiguous tomato leaflets, comparing treatments with BTH and water (control) at the end of the first (RT, n=10) and the second (CC, n=12) no-choice assays

| Treated leaflets | | | | | No-treated (contiguous) leaflets | | | |
|---|---|---|---|---|---|---|---|---|
| Life stage | BTH plants | Control plants | *t* | *P* | BTH plants | Control plants | *t* | *P* |
| *RT assay* | | | | | | | | |
| Eggs | 0.4 ± 0.3 a | 0.8 ± 0.3 a | 0.92 | 0.37 | 0.2 ± 0.1 a | 2.3 ± 1.8 a | 1.21 | 0.24 |
| N1 | 3.1 ± 1.3 b | 5.3 ± 1.0 a | 2.07 | 0.05 | 6.9 ± 1.4 a | 7.8 ± 2.5 a | 0.04 | 0.97 |
| N2 | 0.0 ± 0.0 a | 0.8 ± 0.5 a | 1.61 | 0.13 | 0.1 ± 0.1 a | 1.1 ± 0.7 a | 1.27 | 0.22 |
| Total | 3.5 ± 1.3 b | 6.9 ± 0.7 a | 3.17 | 0.01 | 7.2 ± 1.4 a | 11.1 ± 4.1 a | 0.77 | 0.45 |
| *CC assay* | | | | | | | | |
| Eggs | 3.0 ± 1.0 a | 3.6 ± 0.7 a | 0.95 | 0.35 | 3.8 ± 0.8 a | 3.7 ± 1.0 a | 0.03 | 0.98 |
| N1 | 4.3 ± 1.4 b | 11.7 ± 2.8 a | 3.04 | 0.01 | 11.7 ± 3.5 a | 14.3 ± 3.1 a | 1.13 | 0.27 |
| N2 | 0.0 ± 0.0 b | 5.8 ± 1.0 a | 14.97 | 0.00 | 3.0 ± 1.1 a | 4.2 ± 0.8 a | 1.66 | 0.11 |
| N3 | 0.0 ± 0.0 a | 1.0 ± 0.7 a | 1.66 | 0.11 | 0.3 ± 0.3 a | 0.0 ± 0.0 a | 0.96 | 0.35 |
| Total | 7.3 ± 1.6 b | 22.0 ± 3.5 a | 4.09 | 0.00 | 18.8 ± 4.0 a | 22.2 ± 2.6 a | 1.30 | 0.21 |

RT: room temperature; CC: controlled conditions = 24°C, 16:8(L:D) h. Means in within a column followed by the same letter do not differ significantly ($P \leq 0.05$) by the Student's *t* test. N1, N2 and N3 are 1st, 2nd and 3rd instar, respectively.

**Table 14.4** Mean (± SE) numbers per leaflet of *Bemisia tabaci* (B-biotype) eggs, N1, N2 and N3 observed on tomato plants treated with BTH or water (control), comparing treated and untreated leaflets from the same plants at the end of the first (RT, n=10) and the second (CC, n=12) no-choice assays

| BTH plants | | | | | Control plants | | | |
|---|---|---|---|---|---|---|---|---|
| Life stage | Treated leaflet | No-treated leaflet | *t* | *P* | Treated leaflet | No-treated leaflet | *t* | *P* |
| *RT assay* | | | | | | | | |
| Eggs | 0.4 ± 0.3 a | 0.2 ± 0.1 a | 0.39 | 0.70 | 0.8 ± 0.3 a | 2.3 ± 1.8 a | 0.34 | 0.74 |
| N1 | 3.1 ± 1.3 b | 6.9 ± 1.4 a | 2.71 | 0.02 | 5.3 ± 1.0 a | 7.8 ± 2.5 a | 0.75 | 0.46 |
| N2 | 0.0 ± 0.0 a | 0.1 ± 0.1 a | 0.94 | 0.36 | 0.8 ± 0.5 a | 1.1 ± 0.7 a | 0.21 | 0.84 |
| Total | 3.5 ± 1.3 b | 7.2 ± 1.4 a | 2.52 | 0.02 | 6.9 ± 0.7 a | 11.1 ± 4.1 a | 0.75 | 0.47 |
| *CC assay* | | | | | | | | |
| Eggs | 3.0 ± 1.0 a | 3.8 ± 0.8 a | 0.66 | 0.52 | 3.6 ± 0.7 a | 3.7 ± 1.0 a | 0.23 | 0.82 |
| N1 | 4.3 ± 1.4 b | 11.7 ± 3.5 a | 2.07 | 0.05 | 11.7 ± 2.8 a | 14.3 ± 3.1 a | 0.71 | 0.48 |
| N2 | 0.0 ± 0.0 b | 3.0 ± 1.1 a | 3.15 | 0.00 | 5.8 ± 1.0 a | 4.2 ± 0.8 a | 1.44 | 0.17 |
| N3 | 0.0 ± 0.0 a | 0.3 ± 0.3 a | 1.00 | 0.33 | 1.0 ± 0.7 a | 0.0 ± 0.0 a | 1.58 | 0.13 |
| Total | 7.3 ± 1.6 b | 18.8 ± 4.0 a | 3.02 | 0.01 | 22.0 ± 3.5 a | 22.2 ± 2.6 a | 0.27 | 0.79 |

RT: room temperature; CC: controlled conditions = 24°C, 16:8(L:D) h. Means in a column followed by the same letter do not differ significantly ($P \leq 0.05$) by the Student's *t* test. N1, N2 and N3 are 1st, 2nd and 3rd instar, respectively.

1998; Chu et al. 2002; Syed et al. 2003; Ripple 2004). The structure of cotton leaves was suggested to have potential for breeding whitefly-resistant upland cotton cultivars (Chu et al. 1999). However, as occurred with tomato, the actual effect of this factor on *B. tabaci* populations has been broadly questioned. For example, whitefly adults and nymphs showed positive correlations with hair density and length of hair on leaf lamina, midrib and vein (Raza et al. 2000; Bashir et al. 2001; Aslam et al. 2004). However, while hair density on midrib and gossypol glands on veins was positive and highly significantly correlated to whitefly population, the length of hair on leaf lamina was negatively and highly significantly correlated (Sial et al. 2003). Research carried out in Arizona, USA (Chu et al. 1998, 1999, 2002) to identify cotton plant characteristics related to *B. tabaci* colonization agreed with previous results (Ellsworth et al. 1993; Norman and Sparks 1996, 1997) that hairy leaf cotton cultivars harbor higher populations compared with glabrous cultivars. More recently, Chu et al. (2000, 2001) reported that other factors, including leaf color, morphology and leaf-age related effects on lysigenous glands and leaf trichome densities, may affect *B. tabaci* biotype B oviposition and nymphal densities. In another screening for cotton resistance against B-biotype of *B. tabaci*, adults, eggs and nymphs were significantly corelated to leaf hairiness, with seasonal variability due to leaf color, shape, and hairiness types (Alexander et al. 2004). A brief review by Walker and Natwick (2006) pointed out the mixed and sometimes contradictory results presented in these and other previous studies which associate the two different traits in cotton (smooth-leaf and okra-leaf) with reduced whitefly susceptibility, while in other studies, a slight effect, no effect, or even the opposite effect occurred. All these results together indicate that many morphological plant traits cumulatively contribute to whitefly population fluctuation (Sial et al. 2003).

Other studies have focused on the relationship of biochemical constituents of cotton leaves with whitefly populations. Significant negative correlation for total phenols, *o*-dihydroxy phenols, gossypol and tannins with egg, nymph and adult was established. Reducing sugars showed a significant positive correlation for egg and nymph, but not adults. Total and nonreducing sugars did not show any significant correlation with insect population (Raghuraman et al. 2004).

Recent field studies in California's Imperial Valley revealed consistent very high levels of resistance against the silverleaf whitefly in *Gossypium thurberi* Todaro, a wild cotton species native to Mexico and parts of the southwestern USA (Walker and Natwick 2006). However, the mechanisms of this resistance remains an enigma because both choice and no-choice experiments comparing oviposition and nymphal survival among *G. thurberi* and commercial cotton cultivars did not detect antibiosis or antixenosis (Walker and Natwick 2008).

Research on host plant resistance to *B. tabaci* in melon (*Cucumis melo* L.) is relatively recent, with most of the results obtained during the last decade. Simmons and McCreight (1996) developed a method in a 2-week open-choice greenhouse test to screen germplasm of 31 selected melon entries based on whitefly immature density and changes in plant biomass and tolerance. Another screening of 8 cultivars of export cantaloupes by counts of eggs, live nymphs and pupae, showed

that 'Amarelo' and 'Concorde' were the most resistant cultivars, with no correlation between whitefly densities and leaf pubescence (Morales 1997). This contradicted previous and later results which suggested the presence of the glabrous character of leaves as a resistance factor (Riley and Palumbo 1995a, b; Riley et al. 2001). Soria et al. (1999) reported the existence of genetic resistance to *B. tabaci* in both genotypes, *C. melo* variety *agrestis* 87 and *C. melo* TGR-1551. In the French West Indies, field trials have been conducted since 1997 to test a number of genotypes from the germplasm collection of INRA-Avignon, France. Results indicated that 10 genotypes had potential partial resistance against the B-biotype of *B. tabaci* and that this resistance would be independent from resistance to *Aphis gossypii* (Boissot and Pavis 1999). Later assays indicated that three Indian accessions, PI 414723, PI 164723, and 90625, and one Korean accession, PI 161375, had partial resistance to *B. tabaci*, although higher levels of resistance are needed for a genetic analysis (Boissot et al. 2000, 2003). After introgression of the *Vat* gene (responsible of *A. gossypii* resistance) into melon breeding lines from both the Korean and Indian germplasm sources, the ineffectiveness of *Vat* against *B. tabaci* was demonstrated, and a strategy to breed lines that express resistance to aphids and whiteflies in the short term was proposed (Sauvion et al. 2005).

The information on host resistance to *B. tabaci* in watermelon (*Citrullus lanatus*) is much more limited. Although current levels of resistance in commercial watermelon is quite inadequate, some useful sources of germplasm have been identified that can be used for the improvement of this crop and to incorporate resistance from wild species such as *C. colocynthis* (L.), into advanced breeding lines (Simmons and Levi 2002; Simmons et al. 2006).

Different breeding lines and varieties of *Cucurbita pepo* L. (zucchini and yellow crookneck squash) and accessions of two wild species, *C. ecuadorensis* Cutler and Whitaker and *C. martinezii* Bailey, were evaluated for resistance to *B. argentifolii* and for severity of silvering symptoms, but no clear relationship was found between both factors (McAuslane et al. 1996). Other screenings have been conducted to compare whitefly resistance in different genotypes of *C. moschata* and *C. maxima* (Wessel-Beaver 1997a; Baldin et al. 2000). Several sources of silverleaf resistance have been identified in *C. moschata* controlled by a single recessive gene (Wessel-Beaver 1997b; Wessel-Beaver and Paris 2000; Gonzalez-Roman and Wessel-Beaver 2002). In a more recent study under greenhouse conditions, the main squash cultivars available in the Brazilian market were compared for resistance to the B-biotype of *B. tabaci* (Alves et al. 2005).

Whitefly resistance in soybean (*Glycine max* L.) has been carried out during recent years mostly in the USA, Brazil, Pakistan and India. Significant differences in *B. argentifolii* densities were observed among 14 soybean genotypes in Georgia (USA), with Perrin, Cook and N88-91 harboring the lowest mean numbers of whiteflies (McPherson 1996; Lambert et al. 1997). Low abundance, parasitism and oviposition rates of *B. argentifolii* on certain soybean isolines in Florida (USA) were related to low trichome density (McAuslane et al. 1995a; McAuslane, 1996). Different field and controlled condition trials were conducted in Brazil to evaluate oviposition, non-preference and antibiosis of *B. tabaci* biotype B on different

soybean genotypes (Lima et al. 2002; Lima and Lara 2004). The obtained results strongly suggest that the resistance observed in some of these genotypes was stable (Do Valle and Lourencao, 2002). In Pakistan, 23 varieties of soybean were tested; G-9956 and AGS-344 were the most resistant against *B. tabaci* (Khaliq et al. 2000). In India, soybean line DS 1016 was consistently found to be a promising source of resistance to *B. tabaci* (Sridhar and Siddiqui 2001; Sridhar et al. 2003).

Elite germplasm from the peanut breeding program at the University of Florida (USA) and several commercial cultivars were evaluated for resistance to *B. argentifolii*, but only two genotypes supported fewer whiteflies (although not significantly) than the cultivar "Southern Runner" and no resistance was found in the peanut germplasm tested (McAuslane et al. 1995b). Another study was carried out to determine whether soybean could be used as a trap crop to reduce whitefly infestation in peanut and whiteflies preferred soybean (McAuslane et al. 1995a).

Research to evaluate whitefly resistance in mungbean (*Vigna radiata* L.) has been conducted in the past, especially in Pakistan. To highlight the results obtained during the past decade, out of 23 mungbean accessions, VC2755A was least susceptible while VCA 82 was the most susceptible accession (Arutkani and Ayyanathan 1999; Fargali et al. 1996). Moreover, NM-92 and NM-98 showed significantly lower mean whitefly population/leaf as compared with three other tested varieties (Khattak et al. 2004).

The resistance level of 19 common bean (*Phaseolus vulgaris* L.) genotypes to whiteflies was studied for 2 years in Brazil, with variable results depending on the plant age and the rainy or dry season (Boiça et al. 2008).

Wide screening assays were conducted in India on many genotypes of *Mentha arvensis, M. piperita, M. cardiaca* (*M. gracilis*), *M. citrata (M. piperita* var. *citrata*) and *M. spicata*, and their resistance potential against *B. tabaci* was compared (Singh and Singh 2004; Singh et al. 2004).

Thirty-eight plants of alfalfa (*Medicago sativa* L.) were evaluated for resistance to *B. argentifolii* in California, USA; 17 of them displayed low whitefly infestation and were categorized as potentially resistant. The plants were propagated vegetatively so that replicated measurements of whitefly performance could be made on each genotype. After different greenhouse and field assays, four genotypes demonstrated high whitefly resistance and three demonstrated moderate resistance (Teuber et al. 1999; Jiang et al. 2003).

Nine sweet-pepper (*Capsicum annuum* L.) genotypes obtained from the Asian Vegetable Research and Development Centre (Taiwan) and local cultivars were screened at the University of Agricultural Sciences, Bangalore (India); none were suitable for the development of *B. tabaci* (Maruthi et al. 2003).

Much of the research on whitefly resistance in cassava (*Manihot esculenta* Crantz) in recent years has been carried out at the International Center for Tropical Agriculture, in Colombia, and several cultivars were identified with high levels of resistance to the cassava whitefly, *Aleurotrachelus socialis* Bondar (Bellotti and Arias 2001). However, research on cassava resistance to *B. tabaci* continues to be limited. Nevertheless, a range of new cassava elite clones were assessed in experimental fields of the International Institute of Tropical Agriculture, in Ibadan,

Nigeria, and the researchers found that cassava genotypes 96/1089A and TMS 30572 supported the lowest whitefly infestation in all locations (Ariyo et al. 2005).

Three genotypes (PN 2KS, SH 3322 and IBD-2KS) of sunflower (*Helianthus annuus* L.) were found to be resistant against *B. tabaci* among nine genotypes tested in Pakistan, with a negative correlation between pest population and yield of genotypes (Aslam and Misbah-ul-Haq 2003).

Glossy-leaf phenotypes ('SC Glaze', 'SC Landrace', 'Green Glaze') of collard (*Brassica oleraceae* L.) were found to be the most resistant to *B. tabaci* as compared with normal non-glossy cultivars and hybrids (Jackson et al. 2000). The glossy-leaf characteristic results from phenotypes which express a reduced amount of leaf surface wax, and this gives the leaf a glossy appearance. Nonpreference appears to be the primary mode of resistance in certain collards.

Studies on the relative resistance or susceptibility of four sesame cultivars (*Sesamum indicum* L.) were conducted in Pakistan, and 'PR-14-2' was found to be the least susceptible *to B. tabaci* (Wadhero et al. 1998). Additional relevant research was conducted in Venezuela to characterize six sesame genotypes in relation to foliar acidity and the presence or absence of certain secondary metabolites, by an analysis of principal components based on these features. This analysis separated two sesame genotypes with greater foliar acidity values, which harbored less eggs and nymphs of whiteflies (nearly 10 and 50% of the total incidence in the other genotypes), relating foliar acidity with resistance of sesame to whiteflies (Laurentin et al. 2003).

## Final Remarks

The past decade has been fruitful for the identification of new sources of host plant resistance against *B. tabaci*. The unique to date cloning of *Mi-1* gene in tomato underscores the necessity of isolating and cloning other resistance genes with direct application in IPM programs for other crops. This will be possible by a combination of classical genetic breeding programs with characterization of the already available plant resistance (innate and induced). It is currently indispensable to have a better understanding of how resistance genes work to increase the capacity of producing stable and long-lasting resistances to this pest. This can be achieved in the near future with the help of the tools of genetics, genomics, proteomics, and biochemistry (Kaloshian and Walling 2005). Very recent insights obtained from research on model organisms, such as *Arabidopsis* (Kempema et al. 2007; Zarate et al. 2007) will facilitate the advances in the knowledge of the plant-*Bemisia* interactions in cultivated plants.

In the case of tomato, research with mutant plants is being carried out in our laboratory to determine the main defense signaling pathways activated in the *Mi-1*-mediated resistance against *B. tabaci*. Moreover, high throughput technologies such as microarray analysis have been recently used to detect changes in the gene expression of tomato plants in response to whitefly feeding (McKenzie et al. 2005). Our group is currently starting to use this technology as well as the virus induced

gene silencing or VIGS, to select and characterize genes differentially up- or down-regulated in the innate and induced resistant responses of tomato against *B. tabaci*, in combination with plant bioassays to detect possible changes in resistance levels. This may also allow detection of possible overlaps in genetic profiles of the innate and induced resistant responses.

## Literature Cited

Agrawal A, Karban R, Colfer RG. 2000. How leaf domatia and induced plant resistance affect herbivores, natural enemies and plant performance. *Oikos* 89:70–80.

Agrawal A, Tuzun S, Bent E. 1999. *Induced Plant Defenses Against Pathogens and Herbivores*. St. Paul, MI: APS Press.

Alexander PJ, Forlow-Jech L, Henneberry TJ. 2004. Preliminary screening of different cottons for resistance to sweetpotato whitefly infestations. Cotton, A College of Agriculture and Life Sciences Report, Series P-138. University of Arizona, Cotton, College of Agriculture and Life Sciences, Tucson, pp. 209–212.

Alves AC, Louerncao AL, De Melo AMT. 2005. Resistance of squash genotypes to *Bemisia tabaci* (Germ.) biotype B (Hemiptera: Aleyrodidae). *Neotrop. Entomol.* 34:973–979.

Ariyo OA, Dixon AGO, Atiri GI. 2005. Whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae) infestation on cassava genotypes grown at different ecozones in Nigeria. *J. Econ. Entomol.* 98:611–617.

Arutkani KE, Ayyanathan CA. 1999. Evaluation of green-gram genotypes for resistance to yellow mosaic disease. *Madras Agric. J.* 86:527–528.

Aslam M, Misbah-ul-Haq M. 2003. Resistance of different genotypes of *Helianthus annuus* Linnaeus against *Bemisia tabaci* and *Empoasca* spp. and their correlation with yield. *Asian J. Plant Sci. (Pakistan)* 2:220–223.

Aslam M, Saeed NA, Naveed M, Razaq M. 2004. Comparative resistance of different cotton genotypes against sucking insect pest complex of cotton. *Sarhad J. Agric.* 20:441–445.

Baldin ELL, Toscano LC, Lima ACS, Lara FM, Boiça AL, Jr. 2000. Preferencia para oviposición de *Bemisia tabaci* biótipo "B" por genótipos de *Cucurbita moschata* y *Cucurbita maxima*. *Bol. Sanidad Vegetal. Plagas* 26:409–413.

Baldin ELL, Vendramim JD, Lourencao AL. 2005. Resistance of tomato genotypes to the whitefly *Bemisia tabaci* (Gennadius) biotype B (Hemiptera: Aleyrodidae). *Neotrop. Entomol.* 34:435–444.

Bashir MH, Afzal M, Sabri MA, Raza ABM. 2001. Relationship between sucking insect pests and physico-morphic plant characters towards resistance/susceptibility in some new genotypes of cotton. *Pakistan Entomol.* 23:75–78.

Bellotti AC, Arias B. 2001. Host plant resistance to whiteflies with emphasis on cassava as a case study. In *Special Issue: Challenges and Opportunities for Pest Management of Bemisia tabaci in the New Century*, ed. SE Naranjo, PC Ellsworth. *Crop Prot*. 20:813–823.

Berlinger MJ. 1986. Host plant resistance to *Bemisia tabaci*. *Agric. Ecosyst. Environ.* 17:69–82.

Beynon JL. 1997. Molecular genetics of disease resistance: an end to the 'gene-for-gene' concept? In *The Gene-for-Gene Relationship in Plant-Parasite Interactions*, ed. IR Crute, EB Holub, JJ Burdon, pp. 359–378. New York: CABI.

Boissot N, Lafortune D, Pavis C, Sauvion N. 2003. Field resistance to *Bemisia tabaci* in *Cucumis melo*. *HortScience* 38:77–80.

Boissot N, Pavis C. 1999. Resistance against *Bemisia tabaci* B biotype in melon [French West Indies]. In *Silverleaf Whitefly, National Research, Action, and Technology Transfer Plan, 1997–2001: Second Annual Review of the Second 5-Year Plan*. US Dept. Agric., Agric. Res. Serv. 1999–01, p. 97.

Boissot N, Pavis C, Guillaume R, Lafortune D, Sauvion N. 2000. Insect resistance in *Cucumis melo* accession 90625. *Acta Hortic.* 510:297–304.

Boiça AL, Jr, Gonçalves de Jesus F, Pitta RM, Salvador MC, Stein CP. 2008. Resistance of common bean genotypes to *Bemisia tabaci* (Genn. 1889) Biotype B (Hemiptera: Aleyrodidae) in two growing seasons. In *4th International Bemisia Workshop and International Whitefly Genomics Workshop*, ed. PA Stansly CL McKenzie. Florida, USA, http://www.insectscience.org/8.04/ref/abstract11.html.

Byrne D, Bellows TS. 1991. Whitefly biology. *Annu. Rev. Entomol.* 36:431–457.

Channarayappa C, Shivashankar G, Muniyappa V, Frist R. 1992. Resistance of *Lycopersicon* species to *Bemisia tabaci*, a tomato leaf curl virus vector. *Can. J. Bot.* 70:2184–2192.

Chu CC, Cohen AC, Natwick ET, Simmons GS, Henneberry TH. 1999. *Bemisia tabaci* (Hemiptera: Aleyrodidae) biotype B colonisation and leaf morphology relationships in upland cotton cultivars. *Aust. J. Entomol.* 38:127–131.

Chu CC, Freeman TP, Buckner JS, Henneberry TJ, Nelson DR, Natwick ET. 2001. Susceptibility of upland cotton cultivars to *Bemisia tabaci* biotype B (Homoptera: Aleyrodidae) in relation to leaf age and trichome density. *Ann. Entomol. Soc. Am.* 94:743–749.

Chu CC, Freeman T, Natwick ET, Buckner JS, Nelson DR, Henneberry TJ. 2000. *Bemisia argentifolii* adult, nymph and egg densities and egg distribution on selected upland cottons. *J. Entomol. Sci.* 35:39–47.

Chu CC, Natwick ET, Henneberry TJ. 2002. *Bemisia tabaci* (Homoptera: Aleyrodidae) Biotype B colonization on okra- and normal-leaf upland cotton strains and cultivars. *J. Econ. Entomol.* 95:733–738.

Chu CC, Natwick ET, Perkins HH, Brushwood DE, Henneberry TJ, Castle SJ, Cohen AC, Boykin MA. 1998. Upland cotton susceptibility to *Bemisia argentifolii* (Homoptera: Aleyrodidae) infestations. *J. Cotton Sci.* 2:1–9.

Dangl JL, Jones JDG. 2001. Plant pathogens and integrated defence responses to infection. *Nature* 411:826–833.

Dixon RA, Harrison MJ, Lamb CJ. 1994. Early events in the activation of plant defense responses. *Annu. Rev. Phytopathol.* 32:479–501.

Do Valle GE, Lourencao AL. 2002. Resistencia de genotipos de soja a *Bemisia tabaci* (Genn.) Biotipo B (Hemiptera: Aleyrodidae). [Resistance of soybean genotypes to *Bemisia tabaci* (Genn.) Biotype B (Hemiptera: Aleyrodidae)]. *Neotrop. Entomol.* 31:285–295.

Ellsworth PC, Diehl JW, Meade DL. 1993. Cotton insect management in Arizona as impacted by whiteflies. In *Proceedings Beltwide Cotton Conferences. National Cotton Council*, ed. DJ Herber, DA Richter, pp. 966–968. Memphis, TN.

Fargali MA, Ali AG, Hussein HA. 1996. Susceptibility of some mungbean cultivars to whitefly [*Bemisia tabaci* (Genn.)] and mites (*Tetranychus urticae* Koch) with reference to pod setting and yield. *Assiut J. Agric. Sci.* 27:125–134.

Fernandes GW. 1990. Hypersensitivity: a neglected plant resistance mechanism against insect herbivores. *Environ. Entomol.* 19:1173–1182.

Flor HH. 1955. Host-parasite interaction in flax rust. Its genetics and other implications. *Phytopathology* 45:680–685.

Flor HH. 1971. Current status of the gene-for-gene concept. *Annu. Rev. Phytopathol.* 9:275–296.

Freitas JA, Nonato MFB, Souza VS, Maluf WR, Ciociola AI, Jr, Leite GLD. 2002. Relationship between acylsugars, glandular trichome and resistance of tomato plants to the silverleaf whitefly. *Acta Sci.* 24:1313–1316.

Gentile AG, Webb RE, Stoner AK. 1968. Resistance in *Lycopersicon* and *Solanum* to greenhouse whiteflies. *J. Econ. Entomol.* 61:1355–1357.

Goffreda JC, Mutschler MA. 1989. Inheritance of potato aphid resistance in hybrids between *Lycopersicon esculentum* and *L. pennellii*. *Theor. Appl. Gen.* 78:210–216.

Goffreda JC, Steffens JC, Mutschler MA. 1990. Association of epicuticular sugars with aphid resistance in hybrids with wild tomato. *J. Am. Soc. Hort. Sci.* 115:161–165.

Gonzalez-Roman M, Wessel-Beaver L. 2002. Resistance to silverleaf disorder is controlled by a single recessive gene in *Cucurbita moschata* Duchesne. *Cucurbit Genet. Coop. Rep.* 25:49–50.

Hammerschmidt R, Kuc J (eds.). 1995. *Induced Resistance to Disease in Plants*. Dordrecht, Netherlands: Kluwer Academic Publishers.

Hammerschmidt R, Métraux JP, van Loon LC. 2001. Inducing resistance: a summary of papers presented at the First International Symposium on Induced Resistance to Plant Diseases, Corfu, May 2000. *Eur. J. Plant Pathol.* 107:1–6.

Hassan AA, Mayzard HM, Moustafa SE, Nakhla MK. 1982. Assessment of tomato yellow leaf curl-virus disease resistance in the genus *Lycopersicon*. *Egyptian J. Hortic.* 9:103–116.

Heinz KM, Zalom FG. 1995. Variation in trichome-based resistance to *Bemisia argentifolii* (Homoptera: Aleyrodidae) oviposition on tomato. *J. Econ. Entomol.* 88:1494–1502.

Hunter MD. 2000. Mixed signals and cross-talk: interactions between plants. *Agric. For. Entomol.* 2:155–160.

Hutcheson SW. 1998. Current concepts of active defense in plants. *Annu. Rev. Phytopathol.* 36:59–90.

Inbar M, Doostdar H, Gerling D, Mayer RT. 2001. Induction of systemic acquired resistance in cotton by BTH has a negligible effect on phytophagous insects. *Entomol. Exp. Appl.* 99:65–70.

Inbar M, Doostdar H, Leibee GL, Mayer RT. 1999. The role of plant rapidly induced responses in asymmetric interspecific interactions among insect herbivores. *J. Chem. Ecol.* 25:1961–1979.

Inbar M, Doostdar H, Sonoda RM, Leibee GL, Mayer RT. 1998. Elicitors of plant defensive systems reduce insect densities and disease incidence. *J. Chem. Ecol.* 24:135–149.

Jackson DM, Farnham MW, Simmons AM, Van Giessen WA, Elsey KD. 2000. Effects of planting pattern of collards on resistance to whiteflies (Homoptera: Aleyrodidae) and on parasitoid abundance. *J. Econ. Entomol.* 93:1227–1236.

Jiang YX, Nombela G, Muñiz M. 2001. Analysis by DC – EPG of the resistance to *Bemisia tabaci* (Hemiptera: Aleyrodidae) on an *Mi*-tomato line. *Entomol. Exp. Appl.* 99:295–302.

Jiang YX, Zareh N, Walker GP, Teuber LR. 2003. Characterization of alfalfa germplasm expressing resistance to silverleaf whitefly, *Bemisia argentifolii*. *J. Appl. Entomol.* 127:447–457.

Juvik JA, Shapiro JA, Young TE, Mutschler MA. 1994. Acylglucoses from wild tomatoes alter behaviour and reduce growth and survival of *Helicoverpa zea* and *Spodoptera exigua* (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 87:482–492.

Kaloshian I, Lange WH, Williamson VM. 1995. An aphid-resistance locus is tightly linked to the nematode-resistance gene, *Mi*, in tomato. *Proc. Natl. Acad. Sci. USA* 92:622–625.

Kaloshian I, Walling L. 2005. Hemipterans as plant pathogens. *Annu. Rev. Phytopathol.* 43:491–521.

Kempema LA, Cui X, Holzer FM, Walling LL. 2007. *Arabidopsis* transcriptome changes in response to phloem-feeding silverleaf whitefly nymphs. Similarities and distinctions in responses to aphids. *Plant Physiol.* 143:849–865.

Khaliq A, Akbar W, Amjad M. 2000. Studies on the relative resistance of some soybean varieties against whitefly and its population fluctuations during the crop season. *Pakistan J. Biol. Sci.* 3:1658–1661.

Khattak MK, Shafqat A, Chishti JI. 2004. Varietal resistance of mung bean (*Vigna radiata* L.) against whitefly (*Bemisia tabaci* Genn.), jassid (*Amrasca devastans* Dist.), and thrips (*Thrips tabaci* Lind). *Pakistan Entomol.* 26:9–12.

Kisha JS. 1981. Observation on the trapping of the whitefly *Bemisia tabaci* by glandular hairs on tomato leaves. *Ann. Appl. Biol.* 97:123–127.

Kishaba AN, Castle S, McCreight JD, Desjardins PR. 1992. Resistance of white-flowered gourd to sweetpotato whitefly. *HortScience* 27:1217–1222.

Kunz W, Schurter R, Maetzke T. 1997. The chemistry of benzothiadiazole plant activators. *Pestic. Sci.* 50:275–282.

Lambert AL, McPherson RM, Herzog GA. 1997. Field evaluation of fourteen soybean genotypes for resistance to whitefly (Homoptera: Aleyrodidae) infestations. *J. Econ. Entomol.* 90:658–662.

Laurentin H, Pereira C, Sanabria M. 2003. Phytochemical characterization of six sesame (*Sesamum indicum* L.) genotypes and their relationships with resistance against the sweetpotato whitefly *Bemisia tabaci* Gennadius. *Agron. J.* 95:1577–1582.

Liedl BE, Lawson DE, White KK, Shapiro JA, Cohen DE, Carson WG, Trumble JT, Mutschler MA. 1995. Acylsugars of wild tomato *Lycopersicon pennellii* alters settling and reduces oviposition of *Bemisia argentifolii* (Homoptera: Aleyrodidae). *J. Econ. Entomol.* 88:743–748.

Lima ACS, Lara FM. 2004. Resistencia de genotipos de soja a mosca branca *Bemisia tabaci* (Genn.) Biotipo B (Hemiptera Aleyrodidae) [Resistance of soybean genotypes to the silverleaf whitefly *Bemisia tabaci* (Genn.) biotype B (Hemiptera: Aleyrodidae)]. *Neotrop. Entomol.* 33:71–75.

Lima ACS, Lara FM, Barbosa JC. 2002. Preferência para oviposição de *Bemisia tabaci* biótipo B (Hemíptera: Aleyrodidae) em genótipos de soja, sob condições de campo. *Neotrop. Entomol.* 31:297–303.

Mackey D, Holt BF, III, Wiig A, Dangl JL. 2002. RIN4 interacts with *Pseudomonas syringae* Type III effector molecules and is required for RPM1-mediated disease resistance in *Arabidopsis. Cell* 108:743–754.

Martínez de Ilarduya O, Moore AE, Kaloshian I. 2001. The Tomato Rme1 locus is required for *Mi-1*-mediated resistance to root-knot nematodes and the potato aphid. *Plant J.* 27:417–425.

Martínez de Ilarduya O, Nombela G, Hwanq CF, Williamson VM, Muñiz M, Kaloshian I. 2004. Rme1 is necessary for Mi-1-mediated resistance and acts early in the resistance pathway. *Mol. Plant-Microbe Interact.* 17:55–61.

Maruthi MN, Muniyappa V, Green SK, Colvin J, Hanson P. 2003. Resistance of tomato and sweet-pepper genotypes to Tomato leaf curl Bangalore virus and its vector *Bemisia tabaci*. *Int. J. Pest Manage.* 49:297–303.

Mayer RT, Inbar M, Mckenzie CL, Shatters R, Borowicz V, Albrecht U, Powell CA, Doostdar H. 2002. Multitrophic interactions of the silverleaf whitefly, host plants, competing herbivores, and pathogens. *Arch. Insect Biochem. Physiol.* 51:151–169.

McAuslane HJ. 1996. Influence of leaf pubescence on ovipositional preference of *Bemisia argentifolii* (Homoptera: Aleyrodidae) on soybean. *Environ. Entomol.* 25: 834–841.

McAuslane HJ, Johnson FA, Colvin DL, Sojack B. 1995a. Influence of foliar puberscence on abundance and parasitism of *Bemisia argentifolii* (Homoptera: Aleyrodidae) on soybean and peanut. *Environ. Entomol.* 24: 1135–1143.

McAuslane HJ, Knauft DA, Johnson FA. 1995b. Evaluation of peanut breeding lines for resistance to silverleaf whitefly (Homoptera: Aleyrodidae). *Fla. Entomol.* 24: 1135–1143.

McAuslane HJ, Webb SE, Elmstrom GW. 1996. Resistance in germplasm of *Cucurbita pepo* to silverleaf, a disorder associated with *Bemisia argentifolii* (Homoptera: Aleyrodidae). *Fla. Entomol.* 79:206–221.

McKenzie CL, Sisisterra XH, Powell CA, Bausher M, Albano JP, Shatters RG, Jr. 2005. Deciphering changes in plant physiological response to whitefly feeding using microarray technology. *Acta Hortic.* 695:347–352.

McPherson RM. 1996. Rating soybean germplasm for whitefly (Homoptera: Aleyrodidae) infestations. *J. Agric. Entomol.* 13:65–71.

Meagher RL, Smith CW, Smith WJ. 1997. Preference of *Gossypium* genotypes to *Bemisia argentifolii* (Homoptera: Aleyrodidae). *J. Econ. Entomol.* 90:1046–1052.

Mehta P, Wyman JA, Nakhla MK, Maxwell DP. 1994. Transmission of tomato yellow leaf curl geminivirus by *Bemisia tabaci* (Homoptera: Aleyrodidae). *J. Econ. Entomol.* 87:1291-1297.

Milligan SB, Bodeau J, Yaghoobi J, Kaloshian I, Zabel P, Williamson VM. 1998. The root-knot nematode gene *Mi* from tomato is a member of the leucine zipper-nucleotide binding leucine-rich repeat family of plant genes. *Plant Cell* 10:1307–1320.

Momotaz A, Scott JW, Schuster DJ. 2005. Searching for silverleaf whitefly and begomovirus resistance genes from *Lycopersicon hirsutum* Accession LA1777. In *Proc. 1st International Symposium on Tomato Diseases*, ed. MT Momol, P Ji, JB Jones. *ISHS Acta Horticulturae.* 695:417–422.

Momotaz A, Scott JW, Schuster DJ. 2006. Identification of *Bemisia argentifolii* resistant loci from *Solanum habrochaite*s Accession LA1777. *Abstracts of the 4th International Bemisia Workshop and International Whitefly Genomics Workshop.* Florida, USA, p. 67.

Monci F, García-Andrés S, Sánchez F, Díaz J, Soria C, Acebes A, López A, Espi E, Moriones E 2003. UV-blocking plastic covers and induction of systemic resistance in plants to control

*Bemisia tabaci* and geminivirus infections in tomato in commercial plastic houses of southern Spain. In *3rd International Bemisia Workshop*. Barcelona, Spain, p. 100.

Morales P. 1997. Resistencia al ataque de *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) en ocho cultivares de melon en Paraguana, Estado Falcon, Venezuela. *Bol. Entomol. Venez.* 12:63-72.

Morales P, Cermeli M. 2001. Evaluación de la preferencia de la mosca blanca *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) en cinco cultivos agrícolas. *Entomotropica* 16:73–78.

Moriones E, Navas-Castillo J. 2000. Tomato yellow leaf curl virus, an emerging virus complex causing epidemics worldwide. *Virus Res.* 71:123–134.

Muigai SG, Bassett MJ, Schuster DJ, Scott JW. 2003. Greenhouse and field screening of wild *Lycopersicon* germplasm for resistance to the whitefly *Bemisia argentifolii*. *Phytoparasitica* 31:27–38.

Murugan M, Dhandapani N. 2007. Induced systemic resistance activates defense responses to interspecific insect infestations on tomato. *J. Vegetable Sci.* 28:43–62.

Nombela G, Beitia F, Muñiz M. 2000. Variation in tomato host response to *Bemisia tabaci* (Hemiptera: Aleyrodidae) in relation to acylsugar content and presence of the nematode and potato aphid resistance gene *Mi*. *Bull. Entomol. Res.* 90:161–167.

Nombela G, Beitia F, Muñiz M. 2001. A differential interaction study of *Bemisia tabaci* Q-biotype on commercial tomato varieties with or without the *Mi* resistance gene, and comparative host response with the B-biotype. *Entomol. Exp. Appl.* 98:339–344.

Nombela G, Garzo EI, Duque M, Fereres A, Muñiz M. 2004. Resistance to *Bemisia tabaci* is induced in tomato plants after aphid infestation. In *Abstract Compendium of the 2nd European Whitefly Symposium. IWSN-Institute for Adriatic Crops & Karst Reclamation*. Cavtat, pp. 63–64.

Nombela G, Garzo EI, Duque M, Muñiz M. 2009. Preinfestations of tomato plants by whiteflies (*Bemisia tabaci*) or aphids (*Macrosiphum euphorbiae*) induce variable resistance or susceptibility responses. *Bull. Entomol. Res*. 99:183–191.

Nombela G, Pascual S, Avilés M, Guillard E, Muñiz M. 2005. Benzothiadiazole induces local resistance to *Bemisia tabaci* (Hemiptera: Aleyrodidae) in tomato plants. *J. Econ. Entomol.* 98:2266–2271.

Nombela G, Williamson VM, Muñiz M. 2003. The root-knot nematode resistance gene *Mi-1.2* of tomato is responsible for resistance against the whitefly *Bemisia tabaci*. *Mol. Plant-Microbe Interact.* 16:645–649.

Norman JW, Sparks AN. 1996. Silverleaf whiteflies and cotton leaf hairs, lower Rio Grande Valley, 1994–1995. In *Proc. Beltwide Cotton Conference*, ed. R Dugger, DA Richter, pp. 1026–1027. Memphis, TN: National Cotton Council.

Norman JW, Jr, Sparks AN, Jr. 1997. Cotton leaf hairs and silverleaf whiteflies in the lower Rio Grande Valley of Texas. In *Proc. Beltwide Cotton Conference*, ed. R Dugger, DA Richter, pp. 1063–1064. Memphis, TN: National Cotton Council.

Oostendorp M, Kunz W, Dietrich B, Staub T. 2001. Induced resistance in plants by chemicals. *Eur. J. Plant Pathol.* 107:19–28.

Pascual S, Aviles M, Nombela G, Muñiz M, Beitia F. 2000. Development of *Bemisia tabaci* (Biotype Q) on tomato cultivars with/without the *Mi* gene. *Mededelingen Faculteit Landbouwkundige & Toegepaste Biologische Wetenschappen Universiteit Gent.* 65:291–292.

Ponti De OMB, Romanow LR, Berlinger MJ. 1990. Whitefly plant-relationships: plant resistance. In *Whiteflies: their bionomics, pest status and management*, ed. D Gerling, pp. 91–106. Andover, Hants: Intercept Ltd.

Raghuraman M, Gupta GP, Singh RP. 2004. Biochemical constituents imparting resistance in upland cotton (*Gossypium hirsutum*) to whitefly, (*Bemisia tabaci*). *Indian J. Agric. Sci.* 74:505–507.

Raza ABM, Afzal M, Sarfraz M, Bashir MH, Gogi MD, Sarwar MS. 2000. Physico-morphic plant characters in relation to resistance against sucking insect pests in some new cotton genotypes. *Pakistan Entomol.* 22:73–77.

Riley D, Batal D, Wolff D. 2001. Resistance in glabrous-type *Cucumis melo* L. to whiteflies (Homoptera: Aleyrodidae). *J. Entomol. Sci.* 36:46–56.

Riley DG, Palumbo JC. 1995a. Action thresholds for *Bemisia argentifolii* (Homoptera: Aleyrodidae) in cantaloupe. *J. Econ. Entomol.* 88:1733–1738.

Riley DG, Palumbo JC. 1995b. Interaction of silverleaf whitefly (Homoptera: Aleyrodidae) with cantaloupe yield. *J. Econ. Entomol.* 88:1726–1732.

Ripple BW. 2004. Host plant resistance to whiteflies, *Bemisia tabaci* (Gennadius), Biotype B, (Homoptera: Aleyrodidae) in cotton race stocks for breeding improved cotton cultivars. Master's thesis, Texas A&M University. Available electronically from http://handle.tamu.edu/1969.1/239

Roberts PA, Thomason IJ. 1986. Variability in reproduction of isolates of *Meloidogyne incognita* and *M. javanica* on resistant tomato genotypes. *Plant Dis.* 70:547–551.

Ross AF. 1966. Systemic effects of local lesion formation. In *Viruses of Plants*, ed. ABR Beemster, J Dijkstra, pp. 127–150. Amsterdam: North-Holland.

Rossi M, Goggin FL, Milligan SB, Kaloshian I, Ullman DE, Williamson VM. 1998. The nematode resistance gene *Mi* of tomato confers resistance against the potato aphid. *Proc. Natl. Acad. Sci. USA* 95:9750-9754.

Russell GE. 1978. *Plant Breeding for Pest and Disease Resistance*. London: Butterworth.

Sanchez-Pena P, Oyama K, Nunez-Farfan J, Fornoni J, Hernandez-Verdugo S, Marquez-Guzman J, Garzon-Tiznado J. 2006. Sources of resistance to whitefly (*Bemisia* spp.) in wild populations of *Solanum lycopersicum* var. cerasiforme (Dunal) spooner G.J. Anderson and R.K. Jansen in northwestern Mexico. *Gen. Resource. Crop Evol.* 53:711–719.

Sauvion N, Mauriello V, Renard B, Boissot N. 2005. Impact of melon accessions resistant to aphids on the demographic potential of silverleaf whitefly. *J. Econ. Entomol.* 98:557–567.

Sial IA, Arif MJ, Gogi MD, Sial AA. 2003. Cataloguing of cotton genotypes for morphological traits relating to resistance against whitefly, *Bemisia tabaci* (Gen. *Pakistan Entomol.* 25:149–153.

Siddiqui IA, Shaukat SS. 2004. Systemic resistance in tomato induced by biocontrol bacteria against the root-knot nematode, *Meloidogyne javanica* is independent of salicylic acid production. *J. Phytopathol.* 152:48–54.

Simmons AM. 1994. Oviposition on vegetables by *Bemisia tabaci* (Homoptera: Aleyrodidae): temporal and leaf surface factors. *Environ. Entomol.* 23:381–389.

Simmons AM, Gurr GM. 2005. Trichomes of *Lycopersicon* species and their hybrids: effects on pests and natural enemies. *Agric. For. Entomol.* 7:265–276.

Simmons AM, Levi A. 2002. Sources of whitefly (Homoptera: Aleyrodidae) resistance in *Citrullus* for the improvement of cultivated watermelon. *Hortscience* 37:581–584.

Simmons AM, Levi A, Davis A, Ling K-S, Lopez R, Jackson DM, Kousik CH, Shepard BM, Thies J. 2006. Wild germplasm: plant resistance for watermelon. In *4th International Bemisia Workshop and International Whitefly Genomics Workshop.* Florida USA, p. 130.

Simmons AM, McCreight JD. 1996. Evaluation of melon for resistance of *Bemisia argentifolii* (Homoptera: Aleyrodidae. *J. Econ. Entomol.* 89:1663–1668.

Singh D, Singh DK. 2004. Varietal resistance of *Mentha arvensis* Linn. to sweet potato whitefly, *Bemisia tabaci* Genn. *J. Appl. Zool. Res.* 15:22–24.

Singh D, Singh DK, Bahl JR, Tiwari RK. 2004. Evaluation of different genotypes of *Mentha* species for resistance potential to sweetpotato whitefly (*Bemisia tabaci*). *Indian J. Genet. Plant Breed.* 64:253–254.

Smith PG. 1944. Embryo culture of a tomato species hybrid. *Proc. Am. Soc. Hort. Sci.* 44:413–416.

Smith-Becker J, Keen NT, Becker JO. 2003. Acibenzolar-S-metyl induces resistance to *Colletotrichum lagenarium* and cucumber mosaic virus in cantaloupe. *Crop Prot.* 22:769–774.

Snyder JC, Simmons AM, Thacker RR. 1998. Attractancy and ovipositional response of adult *Bemisia argentifolii* (Homoptera: Aleyrodidae) to type IV trichomes density on leaves of *Lycopersicon hirsutum* grown in three day-length regimes. *J. Entomol. Sci.* 33:270–281.

Soria C, Lopez-Sese AI, Gomez-Guillamon ML. 1999. Resistance of *Cucumis melo* against *Bemisia tabaci* (Homoptera: Aleyrodidae). *Environ. Entomol.* 28:31–835.

Sridhar Y, Siddiqui KH. 2001. Morphological basis of resistance to whitefly, *Bemisia tabaci* Genn. in soybean. *Shashpa* 8:167–174.

Sridhar Y, Siddiqui KH, Trimohan. 2003. Field evaluation of soybean germplasm for identifying resistance to stemfly, *Melanagromyza sojae* and whitefly, *Bemisia tabaci*. *Indian J. Entomol.* 65:222–227.

Srinivasan R, Uthamasamy S. 2005. Trichome density and antibiosis affect resistance of tomato to fruitborer and whitefly under laboratory conditions. *J. Vegetable Sci.* 11:3–17.

Steffens JC, Walters DS. 1991. Biochemical aspects of glandular trichome-mediated insect resistance in the Solanaceae. In *Naturally Occurring Pest Bioregulators*. *ACS Symposium Series 449*, ed. PA Hedin, pp. 136–149. Washington, DC: American Chemical Society.

Sticher LB, Maunch-Mani B, Métraux JP. 1997. Systemic acquired resistance. *Annu. Rev. Phytopathol.* 35:235–270.

Stout MJ, Zehnder GW, Baur ME. 2002. Potential for the use of elicitors of plant resistance in arthropod management programs. *Arch. Insect Biochem. Physiol.* 51:222–235.

Syed TS, Abro GH, Khuhro RD, Dhauroo MH. 2003. Relative resistance of cotton varieties against sucking pests. *Pakistan J. Biol. Sci.* 6:1232–1233.

Teuber LR, Gibbs LK, Taggard KL. 1999. Progress in breeding alfalfa for resistance to the silverleaf whitefly. In *Silverleaf Whitefly, National Research, Action, and Technology Transfer Plan, 1997–2001: Second Annual Review of the Second 5-Year Plan*. US Dept. Agric., Agric. Res. Serv. 1999–01, p. 3.

Toscano LC, Boiça AL, Jr, Maruyama WI. 2002a. Factors acting on *Bemisia tabaci* (Genn.) B biotype (Hemiptera: Aleyrodidae) oviposition in tomato. *Neotrop. Entomol.* 31:631–634.

Toscano LC, Boiça AL, Jr, Maruyama WI. 2002b. Non-preference of whitefly for oviposition in tomato genotypes. *Sci. Agric.* 59:677–681.

Villas-Bôas GL, França FH, Macedo N. 2002. Potencial biótico da mosca-branca *Bemisia argentifolii* a diferentes plantas hospedeiras. *Hort. Brasileira* 20:71–79.

Wadhero HB, Hussain T, Talpur MA, Rustamani MA, Qureshi KH. 1998. Relative resistance of sesame varieties to whitefly and red pumpkin beetle. *Pakistan Entomol.* 20:98–100.

Wahla MA, Tufail M, Afzal M, Tariq MN. 1998. The comparative resistance of some recent releases of cotton cultivars to the insect-pest complex. *Pakistan Entomol.* 20:92–94.

Walker GP, Natwick ET. 2006. Resistance to silverleaf whitefly, *Bemisia argentifolii* (Hem., Aleyrodidae), in *Gossypium thurberi*, a wild cotton species. *J. Appl. Entomol.* 130:429–436.

Walker GP, Natwick ET 2008. Resistance to silverleaf whitefly, *Bemisia argentifolii* (Hemiptera: Aleyrodidae) in *Gossypium thurberi*, a wild cotton species. In *Abstracts of the 4th International Bemisia Workshop and International Whitefly Genomics Workshop* ed. PA Stansly CL McKenzie. Florida, USA, http://www.insectscience.org/8.04/ref/abstract98.html.

Walker GP, Perring TM. 1994. Feeding and oviposition behaviour of whiteflies (Homoptera: Aleyrodidae) interpreted from AC electronic feeding monitor waveforms. *Ann. Entomol. Soc. Am.* 87:363–374.

Walling L. 2000. The myriad plant responses to herbivores. *J. Plant Growth Regul.* 19:195–216.

Wessel-Beaver L. 1997a. Resistance in *Cucurbita* spp. to silverleaf and sweetpotato whitefly. *Hortscience* 32:450.

Wessel-Beaver L. 1997b. Screening for silverleaf resistance in *Cucurbita* accessions. *Cucurbit Gen. Coop. Rep.* 20:54–56.

Wessel-Beaver L, Paris HS. 2000. Inheritance of silverleaf resistance in *Cucurbita moschata*. *Acta Hort.* 510:289–295.

Williams SJ, Kennedy GG, Yamamoto RT, Thacker JD, Bordner J. 1980. 2-Tridecanone: a naturally occurring insecticide from the wild tomato *Lycopersicon hirsutum f. glabratum*. *Science* 207:888–889.

Zarate SI, Kempema LA, Walling LL. 2007. Silverleaf whitefly induces salicylic acid defenses and suppresses effectual jasmonic acid defenses. *Plant Physiol.* 143:866–875.

# Chapter 15
# Natural Enemies of *Bemisia tabaci*: Predators and Parasitoids

**Judit Arnó, Rosa Gabarra, Tong-Xian Liu, Alvin M. Simmons, and Dan Gerling**

## Introduction

Arthropod parasitoids and predators are ubiquitous and operate continuously on all life stages of the whitefly, functioning as control factors in the process. The goal of biological control is to better exploit this behavior in order to more effectively manage pests and reduce insecticide use. Biological control of whiteflies and other pests has been pursued through observation and utilization of natural enemy activity (e.g., Albajes et al. 2003), through search for and introduction of natural enemies (e.g., Gould et al. 2008; Nomikou et al. 2001), or through analysis of existing agroecosystems, indicating which key factors control the pest (e.g., Albajes and Alomar 1999; Naranjo and Ellsworth 2005; Naranjo et al. Chapter 6). Implementation has resulted from habitat manipulation to favor or conserve existing species, introduction of new species, and mass rearing and release of both (Albajes et al. 2003; Gould et al. 2008; Nomikou et al. 2001; Naranjo et al. 2004b).

The choice of which natural enemy or combination to use, and whether to only conserve the existing complex or augment numbers or species is often complex. Decisions should be made only after analysis of the efficacy of present pest-enemy interactions, including specific observations and life-table analytical studies. For example, Naranjo et al. (2004a, 2009) have shown, using life table analysis, a marked influence of factors such as plant species upon effectiveness of the natural enemy complex in managing *B. tabaci*.

The driving force behind conducting biological studies on *B. tabaci* enemies is, in addition to overall scientific interest, the desire to improve pest control. This is reflected in both the organisms studied and the kinds of studies conducted. Predators that have proven to be readily exploitable for mass culture – and manipulation in greenhouse agriculture – have been used most often in mass rearing and behavior investigations following host plant and compatibility studies. Studies on parasitoids, of which only three species have been commercially employed so far, incorporate

J. Arnó (✉)
IRTA Entomology, Ctra. Cabrils km 2, E-08348, Cabrils (Barcelona), Spain
e-mail: judit.arno@irta.es

P.A. Stansly, S.E. Naranjo (eds.), *Bemisia*: Bionomics and Management of a Global Pest,
DOI 10.1007/978-90-481-2460-2_15, 

several additional well-known and "hopeful" species. These studies include additional basic behavioral observations disclosing why these species do not always provide adequate control. The present review deals mainly with predators currently being used for augmentative biological control of *B. tabaci* such as *Macrolophus caliginosus* Wagner, *Nesidiocoris tenuis* (Reuter) and *Amblyseius swirskii* Athias-Henriot, and the commercially utilized parasitoids *Eretmocerus mundus* Mercet, *Er. eremicus* Rose and Zolnerowich and *Encarsia formosa* Gahan. Updates are also included on more elaborate studies with several other species that could potentially serve as commercial control agents in the greenhouse and the field. Finally, indigenous predators and parasitoids are present and active in all agroecosystems, even if in low numbers. The assessment of their contribution to biological control, and their ensuing conservation will hopefully help to suppress pest populations.

We hope this review will provide practical information as well as contribute to understanding of some basic questions relating to biological control, such as the value of parasitoids vs. predators and specific vs. more generalist parasitoids. However, no universal conclusions should be drawn, because each group of organisms could be suitable for utilization under different specific conditions.

## Predator Biology and Ecology

Recent efforts at identification, evaluation, and use of predators as biological control agents of *B. tabaci*, are summarized in this section as an update of the review published by Gerling et al. (2001). Table 15.1 compiles the new records of potential *B. tabaci* predators obtained since then, from two reviews covering China and the Neotropics as well as several other new publications (see references in Table 15.1). Of the more than 150 arthropod species currently described as *B. tabaci* predators, few have been studied in detail. Data on the biology and ecology of 7 newly recorded predator species – 2 Coccinelidae, 4 Heteroptera, and 1 Diptera – and 14 species already mentioned in Gerling et al. (2001) have been published from 2001 to the present, and are summarized in this section.

### *Coleoptera*

Sixteen new coccinellid species have been reported to feed on *B. tabaci*, mostly from China and the Neotropics. However, most of the recent work has focused on two well-known whitefly predators: *Serangium parcesetosum* Sicard and *Delphastus catalinae* (Horn). In choice experiments, *S. parcesetosum* consumed very few red spider mites, thrips, aphids or leafminers when late 4th instar nymphs (= "pupae") of either *B. tabaci, Trialeurodes vaporariorum* (Westwood) and/or *Trialeurodes ricini* (Misra) were available. Among these whitefly species, *S. parcesetosum* consumed more *B. tabaci* than *T. vaporariorum* but not more than *T. ricini* (Al-Zyoud and Sengonca 2004; Al-Zyoud 2007). Most predator species – e.g., *D. catalinae*,

**Table 15.1** Predators recorded for *B. tabaci* not included in Gerling et al. (2001). The predators are listed in the table with the name appearing in the original papers (except for misspellings). Vázquez (2002) lists known predators of *B. tabaci* in the Neotropical area from the primary literature. Ren et al. (2001) compiles new data of predators in China together with a review of previous literature (mostly in Chinese)

| Taxa | References |
|---|---|
| Acari | |
| Phytoseidae | |
| *Euseius ovalis* (Evans) | Borah and Rai (1989) in Nomikou et al. (2001) |
| Araneae | |
| Araneidae | |
| *Neoscona doenitzi* (Bösenberg and Strand) | Zhang et al. (2007a) |
| Linyphiidae | |
| *Erigonidium graminicolum* (Sundevall) | Zhang et al. (2007a) |
| Thomisidae | |
| *Misumenops celer* (Hentz) | Hagler and Naranjo (2005) |
| Coleoptera | |
| Coccinellidae | |
| *Axinoscymnus apioides* Kutnetsov and Ren | Wang et al. (2006) |
| *Axinoscymnus cardilobus* (Ren and Pang) | Ren et al. (2001) |
| *Clitostethus stenalis* (Pang and Gordon) | Ren et al. (2001) |
| *Coccidophilus* sp. | Vázquez (2002) |
| *Delphastus davidsoni* Gordon | Vázquez (2002) |
| *Harmonia axyridis* (Pallas) (=*Leis axyridis*) | Zhang et al. (2007a) |
| *Lemnia biplagiata* (Swartz) | Ren et al. (2001) |
| *Nephaspis hydra* Gordon | Vázquez (2002) |
| *Olla v-nigrum* Casey | Vázquez (2002) |
| *Phrynocaria congener* (Billberg) | Ren et al. (2001) |
| *Propylea japonica* (Thunberg) | Zhang et al. (2007a) |
| *Pullus ruficurdus* Erichson | Vázquez (2002) |
| *Scymnus hoffmanni* Weise | Zhang et al. (2007a) |
| *Serangium japonicum* Chapin | Ren et al. (2001) |
| *Serangium montazerii* Fürsch | Vatansever et al. (2003) |
| *Serangium* n.np | Asiimwe et al. (2007a) |
| *Stethorus minulatus* Gordon and Chapin | Silva and Bonani (2008) |
| Nitidulidae | |
| *Cybocephalus nipponicus* Endrödy-Younga | Ren et al. (2001) |
| Diptera | |
| Hybotidae | |
| *Drapetis* nr. *divergens* | Butler and Henneberry (1993) in Hagler (2002) |
| Syrphidae | |
| *Allograpta exotica* (Wiedemann) | Vázquez (2002) |
| *Ocyptamus mentor* (Curran) | Vázquez (2002) |
| *Toxomerus lacrymosus* Bigot | Vázquez (2002) |
| Heteroptera | |
| Anthocoridae | |
| *Orius laevigatus* (Fieber) | Arnó et al. (2008) |

**Table 15.1** (continued)

| Taxa | References |
|---|---|
| *Orius majusculus* (Reuter) | Arnó et al. (2008) |
| *Orius niger* Wolff | Bayhan et al. (2006) |
| *Orius sauteri* (Poppius) | Zhang et al. (2007a) |
| *Orius similis* Zheng | Ren et al. (2001) |
| Berytidae | |
| *Aknysus* sp. | Vázquez (2002) |
| *Jalysus spinosus* (Say) | Vázquez (2002) |
| Miridae | |
| *Camptotylus reuteri* Jacovlev | Jazzar and Hammad (2004) |
| *Campylomma chinensis* Schuh | Ren et al. (2001) |
| *Cyrtopeltis notatus* (Distant) | Vázquez (2002) |
| *Pseudatomoscelis seriatus* (Reuter) | Hagler and Naranjo (2005) |
| *Spanagonicus albofasciatus* (Reuter) | Hagler and Naranjo (2005) |
| Reduviidae | |
| *Zelus renardii* Kolenati | Hagler and Naranjo (1994) |
| Hymenoptera | |
| Vespidae | |
| *Polistes panamensis* Holmgren | Vázquez (2002) |
| Neuroptera | |
| Chrysopidae | |
| *Ancylopteryx octopunctata* Fabricius | Ren et al. (2001) |
| *Ceraeochrysa claveri* (Navás) | Vázquez (2002) |
| *Chrysocerca formosana* (Okamoto) | Ren et al. (2001) |
| *Chrysopa pallens* (Rambur) | Zhang et al. (2007a) |
| *Chrysoperla nipponensis* (=*Chrysopa sinica*) | Lin et al. (2006) |
| *Chrysoperla defreitasi* Brooks | Vázquez (2002) |
| *Chrysopodes collaris* (Schneider) | Vázquez (2002) |

*Nephaspis oculatus* (Blatchley) and *Axinoscymnus cardilobus* (Ren and Pang) – consumed immature stages, especially eggs (Liu and Stansly 1999; Ren et al. 2002; Huang et al. 2006; Legaspi et al. 2006). *Hippodamia convergens* Guérin-Méneville fed preferentially on whitefly eggs and adults over nymphs (Hagler et al. 2004) while *S. parcesetosum* fed more on late instar nymphs compared to eggs of *B. tabaci* (Al-Zyoud and Sengonca 2004). In contrast, *Propylea japonica* (Thunberg) successfully completed its development when feeding on *B. tabaci* nymphs, but not on whitefly eggs (Zhang et al. 2007b).

Coccinellids can perform well within greenhouse temperature ranges. *Serangium parcesetosum* completed nymphal development both at 18° and 30°C (Sengonca et al. 2004); *N. oculatus* completed development between 20–33°C (Ren et al. 2002); *D. catalinae* between 22–30°C (Legaspi et al. 2008); and *A. cardilobus* between 14–32°C (Huang et al. 2008). The estimated lower developmental threshold for the latter two species was 9–10°C. In contrast, longevity of *D. catalinae* when food was available was reduced from 174 days at 25°C to 16 days at either 5° or 35°C (Simmons and Legaspi 2004, 2007). No eggs hatched

when maintained at 5°C, while 48% hatched at 15°C, although none of the resulting larvae reached the pupal stage. In a follow-up study, Simmons and Legaspi (2004) found that adults and pupae of *D. catalinae* survived 24 h at 5° and 35°C, but not temperatures below zero; egg-hatch was inversely related to duration of exposure to 5°C between 24 and 72 h and, in a field study, a few individuals were still able to survive during winter when temperatures dropped to –8°C. Moreover, a significant linear relationship of prey consumption enhancement with temperatures between 14 and 35°C could be demonstrated, although predation was similar at most of the temperatures. Simmons et al. (2008) determined a negative effect of low relative humidity on oviposition, egg hatching, and immature survival.

## *Heteroptera*

Five new Anthocoridae, 2 Berytidae, 6 Miridae, and 1 Reduviidae species have been recorded as *B. tabaci* predators. Additionally, according to Perdikis et al. (2003) and Martínez-Cascales et al. (2006), most of the literature on *M. caliginosus* as a *B. tabaci* predator probably refers to *M. pygmaeus* (Rambur). However, we used the original name found in the references. Predatory Heteroptera colonize agroecosystems as diverse as greenhouses in the Mediterranean and cotton fields in the USA (Albajes and Alomar 1999; Naranjo 2001).

Polyphagy, which is well documented in Heteroptera, was confirmed in recent studies for *N. tenuis*. In laboratory experiments, it was able to complete nymphal development preying on a variety of arthropods including *B. tabaci*, *Ephestia kuehniella* Zeller, *Frankliniella occidentalis* (Pergande), or *Tetranychus urticae* Kock (Urbaneja et al. 2003).

Prey preference experiments have been conducted for the predators *M. caliginosus*, *Orius laevigatus* (Fieber), and *O. majusculus* (Reuter), which are often released in greenhouse crops (van Lenteren and Martin 1999; Castañé et al. 1999). *Macrolophus caliginosus* utilized *T. vaporariorum* rather than *B. tabaci* when similar stages of both whitefly species were offered (Bonato et al. 2006).

Even though the different species of *Orius* are considered to be mainly predators of thrips, field and laboratory studies show that they also prey readily and successfully on whiteflies. Both *O. laevigatus* and *O. majusculus* consumed all stages of *B. tabaci* and completed preimaginal development with high survival when fed exclusively on the whitefly in the laboratory. Both species preferred *F. occidentalis* over *B. tabaci*, but *O. majusculus* consumed more whiteflies than *O. laevigatus* in choice experiments (Arnó et al. 2008). Hagler and Naranjo (2005), who used immunological methods, found that more than 50% of the collected *Orius tristicolor* (White) individuals on cotton in Arizona had consumed eggs or adult females of *B. tabaci*. Likewise, Zhang et al. (2007a), who used molecular markers, determined that 67% of adults of *Orius sauteri* (Poppius) collected on cotton in China had consumed *B. tabaci*.

In laboratory assays, *M. caliginosus* fed preferably on older *B. tabaci* nymphs (Bonato et al. 2006), and *Lygus hesperus* Knight, an omnivore best known as a

key pest of cotton and other crops, was observed feeding on whitefly nymphs more frequently than on eggs and adults combined (Hagler et al. 2004). In contrast, those same authors determined that adults of *O. tristicolor* and *Geocoris punctipes* (Say) preyed on *B. tabaci* adults more frequently than on eggs and nymphs combined.

## *Neuroptera*

Seven new species of Neuroptera have been reported to prey on *B. tabaci*; some are considered important whitefly predators, especially in the neotropics (Vázquez 2002). Syed et al. (2005) determined that *B. tabaci* was better prey for *Chrysoperla carnea* (Stephens) than the cotton leafhopper *Amrasca devastans* (Distant), an important pest of cotton crops in Pakistan. Eggs and nymphs of *B. tabaci* were suitable prey for the development of *C. externa* (Hagen) and *Ceraeochrysa cincta* (Schneider) (Auad et al. 2001), although oviposition, fecundity and longevity of adults, egg and larval development, and egg viability were all influenced by the host plant (Silva et al. 2004a, b; Auad et al. 2005).

## *Diptera*

Four new species of Diptera have been mentioned as preying on *B. tabaci*. These species include *Drapetis* nr. *divergens* Loew (Empididae), which have been observed in Arizona cotton fields feeding on adult whiteflies, whereas alternative prey and the habitat of the egg, larval and pupal stages of this species have not yet been described (Hagler 2002; Hagler and Naranjo 2005). More is known about *Coenosia attenuata* Stein (Muscidae) that is present in European vegetable and ornamental greenhouses (Kühne 1998; Rodríguez-Rodríguez et al. 1994; Gilioli et al. 2005; Téllez and Tapia 2005; Arnó et al. 2006a). The adults catch whiteflies, fungus gnats, leafminers, and other insects on the wing while larvae feed on soil organisms (Kühne 2000). Hagler (2002) reported a consumption rate of 1.9 *B. tabaci* adults per hour for *D.* nr. *divergens.* For *C. attenuata* adults, consumption depends on the prey species, with a maximum of up to 7 adults of *Drosophila melanogaster* Meigen in 12 h, or 7 adults of *Bradysia paupera* Tuomikoski per day (Kühne 2000; Gilioli et al. 2005). Larval and pupal development lasted between 22 and 30 days at 25°C (Moreschi and Süss 1998; Moreschi and Colombo 1999; Kühne 2000). Gilioli et al. (2005) demonstrated that *C. attenuata* adults are active over a range of temperatures between 12 and 36°C.

## *Acarina*

Many phytoseiid mite species are polyphagous; with some known to effectively reduce *B. tabaci* populations on cotton and vegetable crops (Gerling et al. 2001). Developmental periods and oviposition rates of *Euseius scutalis* (Athias-Henriot) and *A. swirskii* were most favorable when they were feeding on *B. tabaci* compared

to other prey (Nomikou et al. 2001). They fed mainly on whitefly eggs and crawlers, but rarely on later immature stages. Feeding on pollen and honeydew enhanced survival, development, and reproduction (Nomikou et al. 2002, 2003a). Both mite species were able to suppress *B. tabaci* populations on cucumber (Nomikou et al. 2001, 2002). Polyphagy and the ability to feed on alternative foods were found to promote persistence in the crop even if *B. tabaci* was scarce, enabling the inoculative release of mites before pest colonization (Nomikou et al. 2002, 2004; Messelink et al. 2006).

Nomikou et al. (2005) showed that naïve adults with no whitefly experience discriminated between infested and clean cucumber plants, and that they were more aggregated on whitefly-infested plants in comparison to uninfested ones. However, adult female *B. tabaci,* previously exposed to *A. swirskii*, avoided cucumber leaves inhabited by mites while accepting uninhabited leaves (Nomikou et al. 2003b). In addition, Meng et al. (2006) showed that *B. tabaci* adults avoided plants with mites feeding on whitefly, but not plants with mites feeding on pollen, even if they had previously fed on *B. tabaci*. Avoidance was less if the mites feeding on whitefly had previously fed on pollen, compared to mites that had always fed only on whitefly.

## Parasitoid Biology and Ecology

This section summarizes recent efforts in identifying and studying parasitoids as biological control agents of *B. tabaci* since Gerling et al. (2001). Earlier data that had not been previously reviewed is also included.

Although individual parasitoid species might not act as key mortality factors, their effect can have an additive, decisive influence on whitefly-crop relationships. Described parasitoid species attacking *B. tabaci* include 46 *Encarsia*, 21 *Eretmocerus*, 3 *Amitus*, 1 *Neochrysocharis*, and 1 hyperparasitoid (*Signiphora*). An update to the former information concerning *Encarsia* and *Eretmocerus* is given in Tables 15.2 and 15.3. Recent work has added to the information on population dynamics, and the life history and utilization of some species, but most remain unstudied. Their use will require additional studies due to inherent differences in species bionomics, environmental conditions and cropping systems.

New records of parasitism incidence, rates of parasitism, new species, and new parasitoid locations appear continuously, primarily for the genera *Encarsia* (Evans 2007; Heraty et al. 2008) and *Eretmocerus* (Zolnerowich and Rose 2008) or both (Hernández-Suárez et al. 2003). Host ranges span from monophagy or narrow oligophagy as in *En. polaszeki* Evans to polyphagy as in *En. inaron* (Walker). Their use in biological control does not usually consider the degree of polyphagy, although Kirk et al. (2000) attributed host specificity to the decision to release the Spanish biotype of *Er. mundus* in Texas.

Recent geographical records include distribution of *En. inaron* in South America (Oliveira et al. 2003); *En. sophia* (Girault and Dodd) and *Er. mundus* in Uganda (Otim et al. 2005); two strains of *En. inaron* in Egypt (Abd-Rabou 2006); *En. desantisi* Viggiani, *En. nigricephala* Dozier, *En. pergandiella* Howard, and *Amitus* sp. in

**Table 15.2** *Encarsia* species reported as parasitizing whtieflies of the genus *Bemisia* not included in Gerling et al. (2001)

| Species | References | Remarks |
|---|---|---|
| *accenta* Schmidt and Naumann | Evans (2007) | *Bemisia* sp. |
| *adusta* Schmidt and Naumann | Evans (2007) | |
| *aferi* Schmidt and Polaszek | Heraty et al. (2007) | *Bemisia afer* |
| *aleurothrixi* Evans and Polaszek | Evans (2007) | |
| *asterobemisiae* Viggiani and Mazzone | Evans (2007) | |
| *davidi* Viggiani and Mazzone | Evans (2007) | |
| *estrellae* Manzari and Polaszek | Evans (2007) | |
| *galilea* Rivnay | Evans (2007) | *Bemisia afer* |
| *insignis* Schmidt and Polaszek | Heraty et al. (2007) | *Bemisia afer* group |
| *levadicola* Polaszek and Hernández | Evans (2007) | *Bemisia afer* |
| *macoensis* Abd-Rabou and Ghahari | Heraty et al. (2007) | |
| *silvestrii* Viggiani and Mazzone | Evans (2007) | *Bemisia* sp. |
| *smithi (Silvestri)* | Evans (2007) | |
| *synaptocera* Huang and Polaszek | Evans (2007) | |
| *Encarsia* sp. | Qiu et al. (2004a) | 4 apparently new species |

Nicaragua (Numes et al. 2006). Studies with *Encarsia* in Brazil revealed for the first time *En. aleurothrixi* Evans and Polaszek parasitizing *B. tabaci* (Oliveira et al. 2003).

Influences of plant species, location, seasons, and climate on parasitism rates and on the species complex of *B. tabaci* parasitoids in field crops were also demonstrated (e.g., López-Ávila et al. 2001; Ryckewaert and Alauzet 2002; Simmons et al. 2002; Vázquez 2002; Otoidobiga et al. 2004; Naranjo et al. 2004a; Sharma et al. 2004; Trujillo et al. 2004; Leite et al. 2005; Naranjo and Ellsworth 2005; Numes et al. 2006; Otim et al. 2006; Karut and Naranjo 2009).

Climatic differences influenced parasitism of *B. tabaci* on cassava in Uganda by *En. sophia* and *Er. mundus*, with the proportions of these two species varying at three different collecting sites (Otim et al. 2005). Possible influence on the existing parasitoid complex of *B. tabaci* by *En. sophia* was studied at three locations in the

**Table 15.3** *Eretmocerus* species reported as parasitizing species of the genus *Bemisia* not included in Gerling et al. (2001)

| Species | References | Remarks |
|---|---|---|
| *aegypticus* Evans and Abd-Rabou | Abd Rabou (2006) | |
| *californicus* Howard | Zolnerowich and Rose (2008) | |
| *corni* Haldeman (Maskell) | Zolnerowich and Rose (2008) | |
| *diversiciliatus* Silvestri | Zolnerowich and Rose (2008) | |
| *haldemani* Howard | Zolnerowich and Rose (2008) | |
| *nikolskajae* Myartseva | Abd Rabou (2006) | New association |
| *roseni* Gerling | Zolnerowich and Rose (2008) | "Non-*tabaci*" *Bemisia* |
| *rui* Zolnerowich and Rose | Zolnerowich and Rose (2008) | Monophagous |
| *serius* Silvestri | Zolnerowich and Rose (2008) | |
| *Eretmocerus* sp. | Qiu et al. (2004a) | Probably 2 new species |

Caribbean Basin where *En. sophia* was first recorded in Guadeloupe in 1997 (Pavis et al. 2003).

Influence of the plant on the success of the parasitoid measured both as parasitoid immature survivorship and adult fitness is a continuing theme (Inbar and Gerling 2008). Reese (1994) estimated hatching rates of 80% on cabbage, but only 42% on tobacco for *En. sophia* (as *transvena*). Demographic parameters estimated for *Er. mundus* on pepper and tomato were similar (171.1 eggs/female) (Urbaneja et al. 2007), but fecundity on cotton was estimated at only 81.7 eggs/female at 25°C (Ghahari et al. 2005). Different experimental conditions, and possibly host and parasitoid biotypes, might explain these divergent results. Gerling et al. (2006) showed that size of emerging *Er. mundus* was not correlated with size of the whitefly host. Since parasitoid size is generally a fitness parameter, this result suggested that other features of the host plant species might be influential in determining parasitoid fitness.

Many of the life-history studies on longevity, fecundity, sex ratio, immature mortality and development involving different species have been conducted under different conditions. Although this renders them invalid for comparison among species and strains, resulting numerical values provide a general idea of the parasitoids' physiological capabilities and potential of performance under the specified conditions. Moreover, the IPM practitioner is able to find varied and useful data to aid in making decisions on his specific project. For example, although the rates of increase ($r_m$) of *B. tabaci* in 19 different cases at 25–26°C were 0.130 $\pm$ 0.01 (average $\pm$ SEM), those of the parasitoids varied under the same temperatures, but on different host plants as follows: *Encarsia lutea* (Masi) ($n$=1) 0.178, *En. bimaculata* (Heraty and Polaszek 2000) 0.163 $\pm$ 0.0165 ($n$=5) and *Er. mundus* 0.20 $\pm$ 0.016 ($n$=5). In order to facilitate these types of comparisons, life history parameters from the literature for *A. bennetti*, 6 species of *Encarsia* and 6 of *Eretmocerus* are summarized in Tables 15.4 and 15.5 and in the following text.

## *Encarsia*

*Encarsia* females oviposit in any of the four whitefly nymphal instars but develop mainly in the fourth (e.g., *En. formosa*; Gelman et al. 2001) from which they emerge as adults. The first instar host nymph is the least suitable, resulting in highest parasitoid mortality and longest immature development. The third and early fourth instars are usually the most suitable for development (Gerling 1990). All *Encarsia* species – with the exception of *En. inaron* and the uniparental species – are autoparasitic, with males developing as parasitoids of other parasitoid immatures within the whitefly or occasionally other insects (Hunter and Woolley 2001). Four species attacking *B. tabaci* have been studied in recent years.

### *Encarsia formosa*

This uniparental species has been studied extensively around the world as the most frequently used parasitoid against *T. vaporariorum* under greenhouse conditions

**Table 15.4** Representative values of life history parameters measured for *Amitus bennetti* (*Ab*) and six most studied *Encarsia* species attacking *B. tabaci* at a temperature range of 25–30°C; *por.=porteri, per.=pergandiella*

| | *formosa* strain | | *bimaculata* | | | | | *sophia* | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Parameter | B | D | Mal | Fem | *lutea* | *per.* | *por.* | Mal | Fem | *Ab.* |
| Development (d) | 14[a,m] | 15[a] | 12.7[b,c] | 14[b,c] | 11.9[d] | 11.6[e] | 18.9[f] | 11[b,g] | 12[h,b,g] | 21.4[i] |
| Immature survival | 0.875[j] | | | | | | | 42–94%[h] | | |
| Longevity (days) | 50[a,m] | 23[a] | 5.4–8.4[c] | | 10.4[d] | | | 11.3**[,g] | | 6.3[i] |
| Gen. time (days) | 13[j] | | | | | | | | | |
| Day degrees (°C) | | | 181.4[c] | | | | | | | |
| Fecundity (eggs/fem.) | 141[j] | | 24.3[c] | | 32[d] | | | 48.6[h,k] | | 78.8[i] |
| Sex ratio (f/m) | | | | | | | | 4.1–9.2[h,k,l] | | |
| Day degrees | | | 181.4 ± 2.4[c] | | | | | | | |
| Developmental. threshold (°C) | | | 11.6 ± 0.31[c] | | | | | | | |
| Density dependence | | | | | | | | At low density[k] | | |
| Host killing/female | | | | | | | | 33.12*[,k] | | |
| $R_o$ 25°C | | | 18.21[c] | | | | | 15.5**[,k] | | |

*in addition to 48.6 eggs laid, during first 10 days of life
**outside temperature
[a]Qiu et al. (2004b)
[b]Antony et al. (2004)
[c]Qiu et al. (2006)
[d]Talebi et al. (2002)
[e]Liu and Stansly (1996)
[f]Viscarret and López (2004)
[g]Otim et al. (2008)
[h]Kapadia and Puri (1990)
[i]Joyce et al. (1999)
[j]Xu et al. (2003)
[k]Oster (1995)
[l]Reese (1994)
[m]Zhang et al. (2004)

(van Lenteren et al. 1996). Studies relating to *B. tabaci* became prevalent following outbreaks in European glasshouses (Drost et al. 1996) and include detailed examinations of its possible use in greenhouses. Although several strains of *En. formosa* have been found, their use for control of *B. tabaci* has yielded mixed results (Hoddle et al. 1997 and references therein).

### *Encarsia bimaculata*

This is a recently described (Heraty and Polaszek 2000) dominant parasitoid of *B. tabaci* in India and China. Life history was investigated by Antony et al. (2004) and temperature responses were examined in detail by Qiu et al. (2006).

**Table 15.5** Representative values of life history parameters measured for the six most studied *Eretmocerus* attacking *B. tabaci* at a temperature range of 25–30°C; *melanos=melanoscutum*, *queens=queenslandensis, nr. furu=nr. furuhashii*, *APF = Australian partenogenetic form*

| | *mundus* | | | | | |
|---|---|---|---|---|---|---|
| Parameter | Biparental | APF | *eremicus* [a] | *melanos* | *queens* | *nr. furu* [a] |
| Development (days) | 14.1[b–f] | | 23.1[g–j] | 17[k] | | 15.9–17.1[l] |
| Longevity (days) | 10.1[m,c–e] | | 22.7[g–i] | 13.2[k] | | 6.5–8.1[l] |
| Fecundity (eggs/fem.) | 171.1[m,d,e] | 109.3[n] | 28[g,j] | 138[k] | 106.4[n] | 35.4–46.4[l] |
| Imm. Surv. (%) | 75.5, 79.0[q] | | | | | 57.4–73.2[l] |
| Sex ratio (f/m) | 1.7[o,f] | | 1[p] | | | 1.07–1.41[l] |
| $R_o$ | 51.0, 63.8[q] | | 9.7–47.0[j] | | | 14.7–20.9[l] |
| $r_m$ | 0.219, 0.216[q] | | 0.115–0.212[j] | | | 0.133–0.157[l] |
| Density dependence | At low density[o] | | | | | |
| Host killing/female | 103.6[o] | | | | | |

[a]Ranges obtained with different plant species under equal conditions
[b]Urbaneja and Stansly (2004)
[c]Talebi et al. (2002)
[d]Ghahari et al. (2005)
[e]Headrick et al. (1996)
[f]Kapadia and Puri (1990)
[g]Headrick et al. (1999)
[h]Greenberg et al. (2000)
[i]Qiu et al. (2004b)
[j]Powell and Bellows (1992)
[k]Liu (2007)
[l]Qiu et al. (2005)
[m]Urbaneja et al. (2007)
[n]DeBarro et al. (2000)
[o]Fried (1997)
[p]Hunter and Kelly (1998)
[q]Urbaneja et al. (2007). First value tomato, second value sweet pepper

### *Encarsia porteri* (Mercet)

This South American species parasitizes *B. tabaci* on cotton, soybean and alfalfa in Argentina (Viscarret and López 2004), and has only been studied there.

### *Encarsia sophia* (= *En. transvena* Timberlake)

This widespread species consists of at least two distinct populations or cryptic species (Giorgini and Baldanza 2004). Gould et al. (2008) reported the introduction of *En. sophia* from several countries as well as their release in the USA. Different performance levels against *B. tabaci* were found to be dependent on the geographic origin. It was considered ineffective in south Texas (Goolsby et al. 2005), while a strain from a desert region of Pakistan established effectively in the Imperial Valley and Arizona (Naranjo 2008; Roltsch et al. 2008). Studies on bionomics include

published works by Oster (1995), Otim et al. (2008) and Reese (1994). These studies deal mainly with parasitoid behavior in relation to plant characteristics, density dependence, and sex ratios, and are discussed in the respective sections.

### Other *Encarsia* species

Other species in the genus have been studied to a lesser degree. Albergaria et al. (2003) used life tables to show that an undetermined species of *Encarsia* in South America was the main mortality factor of the 4th instar whitfely. Rodríguez-Rodríguez et al. (1994) included *En. lutea* in their studies of parasitoids in greenhouse vegetable crops, while Liu and Stansly (1996) studied the bionomics of *En. pergandiella*.

## *Eretmocerus*

All known *Eretmocerus* species oviposit under any of the four whitefly nymphal instars, but not under the pharate adults. The 1st instar larva penetrates into the host from underneath during the early 4th instar nymph (Gelman et al. 2005a). Second and 3rd instar nymphs are preferred for oviposition, and development is longer when the 1st instar nymph is attacked (Gerling 1966; Ghahari et al. 2005; Urbaneja and Stansly 2004). No differences were found in survivorship (85%) or offspring sex ratios (39.8%) for *Er. mundus* among progeny that developed from eggs laid under the different instars (Urbaneja and Stansly 2004). Unlike *Encarsia*, *Eretmocerus* are not autoparasitic. Most are biparental with a sex ratio that approximates 50%, while a few are uniparental, a condition that, at least sometimes is associated with infection by *Wolbachia*, e.g., *Er. mundus* (DeBarro et al. 2000).

Taxonomic identification of *Eretmocerus* species is difficult, resulting in probable misidentification and misrepresentations of host-parasitoid relationships (Zolnerowich and Rose 2008). Moreover, these authors indicate possible interbreeding of closely allied species introduced into the USA. The following species that attack *B. tabaci* have been studied and/or used in recent years.

### *Eretmocerus mundus*

This species, in its biparental form, occurs widely as an indigenous species in the Mediterranean basin (Urbaneja et al. 2007), Uganda (Otim et al. 2005), Ethiopia and Zimbabwe (Gerling, personal observations), and Thailand (Kirk et al. 2000). In addition, an endemic "Australian parthenogenetic form" (APF) was reported by DeBarro et al. (2000). This suggests existence of extensive genetic variation, and multiple forms or strains in diverse geographical locations. *Er. mundus* was introduced into the USA (Gould et al. 2008) for *B. tabaci* control where it has become a dominant component of the *B. tabaci* parasitoid fauna along with other introduced *Eretmocerus* species (see below, Gould et al. 2008). Although its reported host range

includes 12 different whitefly species (Zolnerowich and Rose 2008), attempts to rear it on *T. vaporariorum* have usually failed.

### *Eretmocerus eremicus* (= *Er.* nr. *californicus*)

This species originated and is prevalent in the southwestern USA. Both it and *Er. mundus* were grouped in laboratory studies as having high reproductive rates over a short period compared to *En. formosa* which reproduced at a lower rate but over a more prolonged period (Qiu et al. 2004b). It is widely used to control *B. tabaci* and *T. vaporariorum* in greenhouses exploiting the fact that it parasitizes both whitefly species (Greenberg et al. 2000; Gerling et al. 2001). It was introduced into Spanish and other European greenhouses for control of the two whitefly species, although *Er. mundus* with its natural prevalence outdoors in Spain gave better results when only *B. tabaci* was present (Stansly et al. 2004).

### *Eretmocerus queenslandensis* Naumann and Schmidt

This endemic Australian species was compared by DeBarro et al. (2000) with *Encarsia* spp. for efficiency on a number of host plants. Although the incidence of parasitism on *B. tabaci* by *Er. queenslandensis* was generally highest, it was outcompeted in the field by the uniparental (APF) *Er. mundus*.

### *Eretmocerus* sp. nr. *furuhashii*

This species was studied by Qiu et al. (2004a) who showed that it accounted for 82% of total parasitism in China. This study also explored its biology, including development, survivorship, and reproduction on glabrous and non-glabrous host plants.

### *Eretmocerus emiratus* Zolnerowich and Rose (Ethiopia)

*Eretmocerus emiratus* together with three additional species, *Er. melanoscutus* Zolnerowich and Rose, *Er. hayati* Zolnerowich and Rose, and *Er.* sp. nr. *emiratus* were introduced into the USA for biological control of *B. tabaci* (Zolnerowich and Rose 2008). All became established, with *Er. emiratus* from United Arab Emerits and *Er.* sp. nr. *emiratus* from Ethiopia becoming dominant in the desert regions of California and Arizona while *Er. hayati* from Pakistan dominated in south Texas (Gould et al. 2008) and surprisingly *Er. sp. nr. emiratus* from Sudan in Florida (P. Stansly, personal comm.).

### Other *Eretmocerus* sp.

McCutcheon and Simmons (2001) reported studies on an undescribed species in the USA with rates of parasitism on *B. tabaci* ranging from 0 to 29% and an optimum temperature range of 25–35°C.

## *Behavior*

### Dispersal

When *Er. mundus* and *En. sophia* were released in cages with two whitefly-infested leaves, both remained searching for 24 h on the first found leaf ignoring the alternative leaf (Fried 1997; Oster 1995, respectively). Female *Er. eremicus* responded more readily to plant cues in flight cages than males. Females also flew longer than males and unmated females longer than mated females (Blackmer and Cross 2001; Bellamy and Byrne 2001). Both sexes sustained flight in excess of 60 min. Males dispersed in a manner consistent with a simple diffusion model while females engaged in wind-directed flight soon after leaving the release sites. The differential flight responses between sexes could relate to the females' drive to locate hosts for oviposition while the males must locate mates (Blackmer and Cross 2001).

Movement of feral populations of *Er. eremicus* and *Encarsia* spp. from overwintering *B. tabaci*-infested refuges in the desert agricultural region of southeastern California was examined by Pickett et al. (2004) using a rubidium chloride marking technique. They found that 15–63% of *Er. eremicus* caught in adjacent cotton and cantaloupe originated from expressly planted refuges. Between 40 and 75% of the aphelinids in the refuges were *Encarsia* spp., but 98% of the marked and captured parasitoids in the adjacent cotton and cantaloupe were *Eretmocerus* spp., perhaps indicating superior dispersal capability for the latter.

### Functional Responses and Handling Times

*Eretmocerus mundus* showed positive functional responses – i.e., an increase in the percentage of hosts attacked per rising host density – on leaves with up to 35 hosts (Shimron 1991; Freid 1997). *Encarsia sophia* likewise showed a similar response within the range of 7–47 hosts/leaf, but not over the wider range of 7–365 hosts/leaf (Oster 1995). In both cases, parasitoid clutch size was used to explain these responses. Three species – *En. lutea*, *En. pergandiella*, and *Er. mundus* – fit the Holling Type II model when attacking nymphs of *B. tabaci* (Greenberg et al. 2001; Talebi et al. 2002). Temperature affected handling times, which were in the rank order: *En. pergandiella* > *Er. mundus* > *En. lutea*. Talebi et al. (2002) considered *En. lutea* more effective in controlling *B. tabaci* than *Er. mundus* because it had a relatively more favorable searching efficiency coefficient (1.825 at 25°C), as well as a favorable handling time coefficient (0.108 at 25°C).

### Influence of Host Volatiles and Chemical Cues on Behaviour

Arrestment in response to host-emitted chemicals was host-density dependent for *Er. eremicus*, but not for *En. luteola* Howard (Shimron et al. 1992). Mandour et al. (2003) showed that females *En. bimaculata* spent most time searching in patches treated with water extracts of *B. tabaci* adults, nymphs, or exuviae followed by searching in patches treated with other extractants and finally untreated patches

exhibiting both orthokinetic (random) and klinotaxic (directed) responses. In contrast, Siqueira and Farias (2003) found no response of naïve female *En. formosa* to volatiles from *B. tabaci* and tomato using a 4-port olfactometer.

### Foraging on the Leaf

Van Lenteren et al. (1987) showed the significance of parasitoid behavior studies in explaining performance. Ardeh et al. (2005) observed that oviposition of *Er. mundus* and *Er. eremicus* accounted for the longest duration of all host-handling behaviors, and was greater for 3rd instar hosts than younger hosts. They showed that females accepted the first three nymphal stages for either egg laying or host feeding, in agreement with Akiva (2008) among others. Gelman et al. (2005b) showed that *Er. mundus* also accepted 4th instar whiteflies, but only at their early stage. Mendelbaum (2004) compared egg load and foraging of recently emerged females after 4 and 8 h of exposure to hosts. Females tended to switch more from oviposition to other activities at 8 h than at 4 h, and were also most active during morning hours irrespective of their egg load. She concluded that egg load, rather than experience, influenced oviposition rate changes with time. Fried (1997), studied behaviors over longer durations and showed that although oviposition of *Er. mundus* decreased with age (1–4 days), behavioral sequences did not change. Oster (1995) found that cotton leaf pubescence hampered *En. sophia* efficiency, and that behavior changed significantly with age. Young (1-day-old) females spent the entire observation hour on the leaf searching and parasitizing hosts, while 4-day-old females stung but never parasitized hosts during the second half hour of observation. Otim et al. (2008) found that minor differences in leaf pubescence did not influence parasitization by either *Er. mundus* or *En. sophia* on cassava.

Fewer studies have been conducted on other *B. tabaci* parasitoids. Responses of *En. luteola* did not depend on honeydew concentration (Shimron 1991). Foraging activity by *En. pergandiella* varied during the day and by leaf surface. Foraging activity peaked around mid-day; moreover, most adults (80%) searched on the abaxial leaf surface during that time (Simmons and McCutcheon 2001). Even though parasitoid abundance varied among 7 diverse crops, daily foraging activity was similar on the crops (Simmons et al. 2002).

### Ovipositional Marking

Fried (1997) found that leg rubbing among naïve *Er. mundus* was performed on 43 (71%) of the 60 whitefly nymphs attacked. It was followed by whitefly emergence, death, or parasitoid emergence in 9, 23, and 67% of the 43 whitefly, respectively. Experienced females rubbed legs following all host encounters. The putative marking substance may be a C31 and/or C33 dimethylalkane which are major lipid components of hexane extracts from *Er. mundus* females (Buckner and Jones 2005). These compounds were detected in nymphs recently exposed to parasitoids, but not in control nymphs or in parasitized nymphs 10 d after exposure, indicating that the

dimethylalkanes were probably transferred onto nymphal cuticles by the ovipositing *Er. mundus* females.

### Host Feeding and Egg Production

Honeydew feeding can result in increased egg production even though the resulting eggs tend to be the lower quality hydropic (moisture absorbing) type (Burger 2002). Host feeding occurs in both *Encarsia* and *Eretmocerus* species and always causes the death of the host (Burger et al. 2005). *Encarsia* sting the host through the integument, while *Eretmocerus* penetrate the host through the vasiform oriface. This distinction correlates with the sharp-tipped ovipositor in *Encarsia* adapted to host piercing for oviposition vs. the blunt-ended *Eretmocerus* ovipositor used to slide the egg under the venter of the host (Gerling et al. 1998). Females of *En. formosa* and *Er. mundus* emerge with ready-to-lay eggs and are able to oviposit as soon as they emerge, but are clearly synovigenic. Given an average of $19.18 \pm 0.6$ eggs at emergence (Akiva 2008) and a lifetime egg production of $111.25 \pm 27.81$ eggs/female (Fried 1997), *Er. mundus* has an ovigenic index (OI) of 0.17 [OI= the proportion of the female's egg load at emergence to lifetime oviposition (Jervis et al. 2001)] as compared to 0.1 for *En. formosa* (Jervis et al. 2001). Akiva (2008) also showed that *Er. mundus* females continue to produce eggs and start to host-feed on their first post-emergence day, when their ovaries are still loaded with eggs, probably preparing nutrients for future egg development.

Estimated ratios of host feeding to oviposition are generally higher for *Encarsia* species: 20% for *En. formosa* (20%) vs. 7–9% in *Er. mundus* (van Lenteren et al. 1996; Urbaneja et al. 2007). Zang and Liu (2007) found that *En. sophia* exhibited superior host-feeding capacity ($\approx$ 3-fold) compared to *En. formosa* and *Er. melanoscutus.*

## Natural Enemy Interaction

As with all types of organisms, interactions among and within natural enemy groups can run the gamut from mutually beneficial to mutually injurious. Internecine interactions among intraguild predators or parasitoids could conceivably reduce biotic mortality of target pests and thus the effectiveness of biological control. One objective of the biological control practitioner is to manage the agroecosystem in a way that minimizes such negative interactions. Therefore, understanding interrelationships among natural enemies is important for optimizing the effectiveness of biological control.

### *Intraguild Predation*

The ladybeetle *S. parcesetosum* fed preferentially on unparasitized *B. tabaci* nymphs over nymphs parasitized by *En. formosa* 5 days earlier (Al-Zyoud and Sengonca 2004) or by *Er. mundus* 7 days after parasitoid oviposition (Al-Zyoud 2007).

Similarly, *S. japonicum* Chapin was capable of discriminating between whitefly nymphs containing advanced stages of parasitoids vs. unparasitized nymphs. However, no discrimination was observed against nymphs under which *Eretmocerus* sp. had oviposited 5 days previously. Consumption of parasitized nymphs subsequently decreased with parasitoid age (Sahar and Ren 2004). In choice experiments, adults and larvae of *D. catalinae* fed with equal frequency on nymphs parasitized by eggs or larvae of *En. sophia,* or unparasitized nymphs. However, parasitized hosts were rejected once they contained *En. sophia* pupae. Cage experiments showed a negative effect of this predator on the parasitoid populations and, consequently, the combined use of both natural enemies was not recommended (Zang and Liu 2007). Even worse, Naranjo (2007) showed that *H. convergens* was a discriminate predator of *Eretmocerus* immatures, preferring parasitized nymphs. Interactions among different predator species were observed by Al-Zyoud et al. (2005a). *Serengium parcesetosum* laid eggs in more protected portions of the leaf in the presence of *C. carnea*.

Naranjo (2007) examined predation of *G. punctipes* and *Orius insidiosus* (Say) on 4th instar *B. tabaci* nymphs, as well as nymphs parasitized by *Er.* sp. nr. *emiratus.* He found a significant preference for feeding on early 4th instar nymphs containing larval and pupal parasitoids, compared to unparasitized nymphs at that stage but not older 4th instars.

The predacious dipterans *C. attenuata* were compatible with *En. formosa* and the leaf miner parasitoid *Dacnusa sibirica* Telenga (Kühne 1998). However, Téllez and Tapia (2006) demonstrated that while *C. attenuata* adults did not feed on *O. laevigatus*, they did prey on the aphid parasitoid *Aphidius colemani* Viereck, the leafminer parasitoid *Diglyphus isaea* (Walker), the whitefly parasitoid *Er. mundus* and on the predator *N. tenuis.* The number of *A. colemani* and *D. isaea* attacked was higher when adults of *B. tabaci* were not present in the arena.

## *Parasitoid–Parasitoid Interactions*

Some parasitoid interactions – such as competition, host feeding, multiparasitism and autoparasitism – are counterproductive to effective biological control. Indeed, theory predicts that host density is always lower when a primary parasitoid acts alone than when an autoparasitoid is also present (Briggs and Collier 2001). Invasion of *En. sophia* into *Er. mundus* cultures results in drastic reductions of the latter (Gerling unpublished data). Collier and Hunter (2001) reported that both *Er. eremicus* and *En. sophia* engaged in multiparasitism with no advantage conferred to either. They also found that *En. sophia* reduced progeny of *Er. eremicus* by host feeding, although conspecific host feeding was also observed so that the net effect could not be predicted.

A similar problem regarding host preference occurs with predicting effects of autoparasitism. At least one autoparasitic species – *En. pergandiella* – preferred to lay male-producing eggs in heterospecific over conspecific secondary hosts. This might be the cause for disruption of *En. formosa* activity in whitefly control by this

species as reported from Spain (Gerling et al. 2001) and from Texas, although in the latter case, this was somewhat mitigated by the host species present (Bográn and Heinz 2002). Gerling and Rejouan (2004) found that younger pupae of *En. inaron, En. lutea*, and *En. sophia* were always more susceptible to host feeding or autoparasitism than older pupae, irrespective of melanization. The findings further defined the "window of opportunity" for male development described by Hunter and Kelly (1998) for *En. sophia*, and suggested an increasing probability with age of surviving competitive interactions among parasitoid pupae.

## *Entomopathogen-Parasitoid and Predator Interactions*

Most studies indicate that entomopathogens and parasitoids are compatible for use in biological control of insect pests. For instance, when the mycoinsecticide *Beauveria bassiana* Strain GHA was used to control *B. tabaci* in commercial melons, mortality was inflicted from both fungi and parasitoids with minimal impact on the populations of *Er. mundus* (Jaronski et al. 1998). Applications of *Aschersonia* spp., *B. bassiana, Paecilomyces* spp., *Verticillium lecanii, Acremonium* sp., *Conidiobolus* spp., *Entomophthora* sp., and *Zoophthora radicans* were generally considered compatible with *Encarsia* and *Eretmocerus* spp. (Wang and Huang 2006). Likewise, BotaniGard® (a formulation of *B. bassiana*) had no adverse effects on *En. formosa* (Murphy et al. 1998). Other studies have demonstrated the compatibility of entomopathogens and parasitoids, including *Paecilomyces fumosoroseus*, *V. lecanii* and *B. bassiana* with *Encarsia* spp. (Scholz-Dobelin and Stockmann 2003), *V. lecanii* with *En. formosa* (Jazzar and Hammad 2004), *B. bassiana* JW-1 strain and Naturalis-L® (ATCC 74040 strain of *B. bassiana*) with *Encarsia* spp. and *Eretmocerus* spp. (Wright and Knauf 1994; Wright and Kennedy 1996). However, Shipp et al. (2003) found that *B. bassiana* might reduce efficacy of *En. formosa* and *Er. eremicus* and warned that caution should be taken when this fungus in applied.

Regarding entomophatogen-predator interaction, Wang et al. (2005) demonstrated that crude toxins of the entomopathogenic fungus *V. lecanii* decreased feeding capacity, especially of the larvae, and subsequently reduced fecundity and longevity of female *D. atalinae*.

## *Natural Enemy-Plant Interactions*

*Bemisia tabaci* is very polyphaguous and its host plants can have a considerably marked influence on natural enemy activity (Inbar and Gerling 2008). Characteristics of host plants such as volatile emission, and especially leaf pubescence, may influence the behavior of coccinellid predators. Al-Zyoud et al. (2005b) found that the beetles preferred pubescent to glaborous host plants. They also found that larval development was shorter, mortality lower and longevity greater on cucumber compared to cotton. Liu (2005) found demographic parameters for *D. catalinae* on collards to be much more favorable than reported for other

hosts, such as hibiscus, poinsettia, and tomato (Hoelmer et al. 1993; Heinz et al. 1994; Heinz and Zalom 1996, respectively), and suggested that the more glaborous nature of collard best explained such differences. Guershon and Gerling (2006) studied the foraging behavior of this predator by comparing the effect of adding artificial hairs to a glabrous cotton leaf and observed that leaf hairs hampered searching. Furthermore, after encountering and consuming a smooth *B. tabaci* nymph, beetles were more apt to reject setose nymphs such as found most often on hirsute hosts. Thus, whitefly nymph setosity may be a strategy to avoid predation. Conversely, Legaspi et al. (2006) found that *D. catalinae* predation rates over 24 h were not significantly higher on glabrous leaves of cowpea and collard than on tomentose leaves of cotton, hibiscus, and tomato. They suggested that factors like volatile secondary compounds, rather than leaf structure, might also be involved in host plant suitability.

Many predatory Heteroptera also feed on plants and are referred to as *zoophytophagous*. In laboratory studies, *O. tristicolor* spent less time feeding on the plant and more on whitefly adults than *L. hesperus* (21 vs. 83% on plants and 79 vs. 17% on whiteflies, respectively). Other species spent an intermediate amount of their total feeding time feeding on plants (e.g., *G. punctipes* 66%) (Hagler et al. 2004). In some cases, phytophagy was found to injure the host plants, the level of damage being related to the abundance of arthropod prey. For example, *N. tenuis* caused significantly fewer necrotic rings on tomato when prey was available, both in laboratory and semi-field experiments (Arnó et al. 2006b; Sanchez 2008; Calvo et al. 2008).

Phytophagy enables longer survival and fecundity of predatory Heteroptera (Naranjo and Gibson 1996), but the more prey-dependent species require prey. A decrease in female survival and fertility of *M. caliginosus* was associated with decreasing whitefly availability on tomato and melon plants (Alomar et al. 2006). Poor predator establishment could be explained by low prey densities available on the crop. Jazzar and Hammad (2004) compared *B. tabaci* consumption rates by *Camptotylus reuteri* (Jakolev) and *M. caliginosus,* and concluded that *C. reuteri* was less dependent on *B. tabaci* densities than *M. caliginosus*. Urbaneja et al. (2005) demonstrated the inability of *N. tenuis* to survive on a strictly phytophagous diet and that it survived without prey longer on tomato than on pepper or eggplant.

Pavis et al. (2003) found that parasitoids were most diverse and efficient in controlling *B. tabaci* in Guadaloupe where crops were planted in small, diverse plots close to natural forest and only a few pesticides were used. Weeds favored the presence of parasitoids which reduced both the population of *B. tabaci* and the incidence of virus (Medina Balderas et al. 2002). Antony and Palaniswami (2002) reported that *Er. mundus* – which parasitized *B. tabaci* on sweetpotato – failed to develop on the population infesting cassava. Possibly, these effects were caused by genetic differences in the host population being expressed in distinct biotypes or cryptic species of *B. tabaci* known to colonize cassava (Burban et al. 1992; Legg 1996).

Low rates of parasitism have been associated with greater density and ridgidity of hairs on the leaves (Rajam et al. 1988). Oster (1995) reported a reduction in

parasitism by *En. sophia* on pubescent cotton varieties and Gruenhagen and Perring (2001) observed less parasitism of whiteflies on plants bearing trichomes, although the incidence of parasitism was similar on a glabrous melon and its pubescent (with non-glandular trichomes) isoline. Headrick et al. (1996) and McAuslane et al. (2000) found higher rates of parasitization by *Eretmocerus* species on hirsute varieties of melon and soybean, respectively, indicating that some parasitoids could be more effective on certain hirsute plant leaves.

Responses of parasitoids to leaf pubescence may be influenced by other plant characteristics. For example, Gruenhagen and Perring (2001) believed parasitism of *B. tabaci* to be lower on velvet leaf, *Aboutilon theofrasti,* than on four other plant species, due to the exudate from glandular trichomes which entrapped the parasitoids, providing an enemy-free space for the pest. Wax is another leaf factor affecting parasitoid activity. Parasitism (primarily by *Eretmocerus* spp.) was elevated in field plots of reduced-wax collard as compared with the same genotype with normal wax (Jackson et al. 2000). Similarly, parasitism by *En. pergandiella* was elevated (4.5-fold) in the laboratory on reduced-wax collard compared with normal collard, but no effect was observed with *Eretmocerus* sp. (since described as *Er. rui* Zolnerowich and Rose) (McAuslane et al. 2000).

### *Relevance of Interactions Between Natural Mortality Factors to Biological Control*

Naranjo (2007) using sensitivity analyses of field life table data, found only small negative effects of intraguild predation on mortality of *B. tabaci*. Hunter et al. (2002) observed that densities of *Er. eremicus* in cotton fields were higher in the absence of *En. sophia,* whereas *En. sophia* densities were unaffected by the presence of *Er. eremicus*, confirming laboratory results discussed above. However, releases of *Er. mundus* and *En. pergandiella* did not influence host suppression (Bográn and Heinz 2006). The two parasitoids were able to coexist for the duration of a field season when released simultaneously and at the same rate on *B. tabaci*-infested cotton plants in field cages. In contrast, releases of *En. formosa* together with *En. pergandiella* resulted in lower levels of host mortality than would be expected based on the observed mortality caused by individual parasitoid species (Bográn et al. 2002). Overall, the fact that one natural enemy could intervene with the controlling capacity of another – and that these effects could not be foreseen – must be considered and warrants more examination, especially under field conditions.

## Utilization, Monitoring, and Assessing the Impact of Natural Enemies

*Bemisia tabaci* often reaches pestiferous levels in the absence of control measures. When biological control is employed as a corrective measure, effectiveness must be monitored in order to gauge future practices.

## *Utilization*

### Predators

Mirid bugs are widely used in the Mediterranean as biological control agents of whiteflies. *Macrolophus caliginosus* and *N. tenuis* commonly colonize in large numbers in both field crops and greenhouses where little or no broad-spectrum insecticides are used, providing not only efficient control of whitefly populations but also contributing to the control of other secondary pests (Alomar et al. 2002; Vacante and Benuzzi 2002; Nannini 2003; Arnó et al. 2005; Calvo et al. 2008; Sanchez 2008). In vegetable greenhouses, success in using mirids for biological control of *B. tabaci* includes the inoculative and augmentative release of *M. caliginosus* (Carboni et al. 2002; Vacante and Benuzzi 2002; Gabarra et al. 2003; Trottin-Caudal and Capy 2003), and *N. tenuis* (Calvo and Urbaneja 2004). Calvo et al. (2008) compared two different release rates of *N. tenuis* – 0.1 and 4 individuals/plant – in large exclusion cages and found significant reduction of the *B. tabaci* populations (> 90%) with both release rates. However, bug feeding can weaken the apex and arrest plant growth. Although often observed only at the end of the season and resulting in natural pruning of the plant (Sanchez et al. 2006; Arnó et al. 2006b), plant feeding by these mirids can produce flower abortion and yield losses (Sánchez and Lacasa 2008; Arnó et al. 2010). (See section on natural enemy plant interactions).

*Bemisia tabaci* populations increase quickly under warm conditions and the combined releases of *Er. mundus* and *M. caliginosus* often improves whitefly control. For example, the combined use of the parasitoid and predator provided better results than the use of any single natural enemy, especially in spring when whitefly populations were very high in an experimental tomato greenhouse, (Gabarra et al. 2006). Trottin-Caudal et al. (2006) observed improved control of *B. tabaci* when *M. caliginosus* was included with *Er. mundus* in heated greenhouses during winter tomato production.

The newest commercially available predator for whitefly control is *A. swirskii* and is used alone or in combination with *Er. mundus.* Releases of this predaceous mite have been widely used with remarkable success for *B. tabaci* control in sweet pepper greenhouses in Spain (Calvo and Belda 2006; Calvo et al. 2006), and in open field eggplant in Florida (see Stansly and Natwick Chapter 17).

In ornamentals, two coccinellid species controlled *B. tabaci* populations in large cage experiments. On hibiscus, *N. oculatus* effectively controlled the whitefly when a 1:4 predator: prey ratio was observed (Liu and Stansly 2005). Inoculative releases of *S. parcesetosum* also maintained whitefly populations at low levels during 10 weeks. Whitefly control was primarily due to prolonged adult survival and continuous feeding of adult beetles on the whiteflies (Ellis et al. 2001).

### Parasitoids

Although there are numerous cases of exotic whitefly infestations being completely controlled through introduction of exotic parasitoids, such has not been the case for

*B. tabaci*. The most extensive effort was initiated in the early 1990s with collections of parasitoids as well as entomopathogens and some predaceous coccinellids coordinated by the USDA-ARS European Biological Control Laboratory in Montpellier, France (Gould et al. 2008). From the worldwide explorations in 28 countries, 55 parasitoid cultures were established at the USDA-APHIS Quarantine Facility in Mission in Texas, where they were characterized taxonomically and through RAPD-PCR and their biological attributes studied (Goolsby et al. 2008). Promising candidates were mass reared and released in Texas, Arizona and California and to a lesser extent in Florida. The best-performing species or geographic populations in the desert Southwest were those that originated from similar climatic regions: the Arabian Peninsula, arid northeastern Africa (*Er. emiratus* and *Er.* sp. nr. *emiratus*), and hot, dry regions bordering the Mediterranean (*En. sophia*) (Hoelmer and Roltsch 2008), whereas *Er. hayati* from Pakistan came to dominate in the Rio Grande Valley of Texas (Ciomperlik and Goolsby 2008). The apparent exception to this pattern of geoclimatic matching was Florida where an as yet undescribed species from Sudan, also released as *Er.* sp. nr. *emiratus* is now dominant (P. Stansly unpublished data). Although *B. tabaci* continued as a pest in this region, the indigenous parasitoid fauna attacking it was largely replaced with more specific and efficient Old World species that provided more effective biological control.

Augmentative release of parasitoids for control of *B. tabaci* in greenhouses has been also been studied (e.g. Hoddle et al. 1998; Stansly et al. 2004, 2005) and has been a common practice in protected agriculture (greenhouses and plastic-covered annuals, Stansly et al. 2004, 2005). Several commercial companies are engaged in the mass rearing and use of *Er. eremicus* and *Er. mundus* for both vegetable and ornamental greenhouses. More details are given in Stansly and Natwick (Chapter 17).

## *Monitoring and Impact Assessment*

This discussion is intended to complement the treatment of the methodology, application, and problem of monitoring and assessing natural enemy activity, provided by Naranjo (2001). Specific, organism-oriented work (predators and parasitoids) as well as general life table analyses are included.

### Predators

Additional predators of *B. tabaci* have been identified using ELISA methods in the field to test for a whitefly-specific egg protein in captured predators (Hagler and Naranjo 2005). The ELISA system has also been used to evaluate and compare the feeding activity of the predator complex in cotton fields under different insecticide treatment regimes (Hagler and Naranjo 2005). These tests relied on a very specific antibody and therefore, only predation on eggs or gravid females could be assessed (Hagler et al. 1993) and activity of the predators such as *L. hesperus* that feed mainly on *B. tabaci* nymphs was underestimated (Hagler et al. 2004). ELISA tests have also been used to monitor intercrop movements of predators previously marked with

specific IgG proteins (Hagler and Naranjo 2004). Overcoming the shortcomings of the stage-specific detection provided by ELISA, Zhang et al. (2007a, c) developed techniques to detect and quantify *B. tabaci* DNA within the predators' gut. They succeeded in identifying a number of new predator species of *B. tabaci* in China, and evaluating their importance in the cotton agroecosystem. Overall, specific difficulties arise when attempting to use serological or molecular marker techniques to quantify predation. Although the output of these techniques could be quantitative, actual estimation of prey consumed were complicated by uncontrolled factors such as prey size, metabolic differences among predator species, temperature, and digestion time since the last meal (Greenstone 1996; Naranjo and Hagler 1998; Agustí et al. 2000; Zhang et al. 2007c).

### Parasitoids

Recent field work has been characterized by attempting to monitor adult parasitoids in addition to the relatively straight forward method of collecting whiteflies and examining them for parasitism. Otoidobiga et al. (2003) observed captures of 0.14–13 *Eretmocerus* sp. individuals per yellow sticky card as levels of parasitism varied between 36 and 87% over different seasons. They also monitored year-round whitefly infestations in overlapping cotton crops in Burkina Faso (Otoidobiga et al. 2004). Although parasitoid populations increased with host density, no density-dependent trend was observed. Hoelmer and Simmons (2008) showed that the lower surface of horizontally placed sticky traps captured more *Er. emiratus* than the upper surface, and that females were captured in greater numbers than males, but found no significant correlation between trap capture of parasitoids and the number of parasitized *B. tabaci* on leaf samples. The effectiveness of yellow sticky traps at different heights and in different seasons was monitored on the population dynamics of *B. tabaci* and its parasitoids on tomato (Qiu and Ren 2006).

A cup trap equipped with a 530 nm lime green light-emitting diode (LED) caught more adult whiteflies, but fewer *Er. eremicus*, *Er. mundus*, *Eretmocerus* sp. and *En. formosa* when compared to sticky traps (Nombela et al. 2003; Chu et al. 2003, 2004a; Simmons et al. 2004). Hagler et al. (2002) used an ELISA assay to detect a protein marker in a mark-release-recapture study on dispersal of *Er. erimicus*. Most of the 40% marked and recaptured parasitoids were males, although an equal proportion of each gender was released, indicating greater dispersal of males. Additional methods include the rubidium chloride marking technique that Pickett et al. (2004) used to examine the movement of feral populations of *Er. eremicus* and *Encarsia* spp. from overwintering *B. tabaci*-infested refuges into adjacent crops of cantaloupe and cotton in the desert agricultural region of southeastern California.

## *Life Table Studies*

A more direct method for assessing and quantifying natural enemy effects is the use of life tables (see Naranjo et al. Chapter 6). In Arizona, cohort-based life table

studies in cotton demonstrated that predation occurring during the 4th nymphal instar was the key factor affecting *B. tabaci* populations (Naranjo and Ellsworth 2005). These authors also found a relatively large portion of mortality due to dislodgement that they associated with chewing predation and weather events. Life table studies conducted in a variety of crops and weed hosts also pointed to the important contribution of natural enemy mortality to *B. tabaci* population dynamics over the entire year (Naranjo et al. 2004a).

In contrast to the Arizona desert situation, life table studies on cassava in Uganda (Asiimwe et al. 2007b) and on cotton in Turkey (Karut and Naranjo 2009) showed parasitism to be the main source of natural enemy-induced mortality. Sources and rates of mortality were discernable based on visible symptoms and the use of marginal mortality rates to correct for contemporaneious mortality events. Although reliable data on natural enemies were obtained, the relative importance of different mortality factors was not always apparent; furthermore, predation on adult whiteflies was not ascertained.

## Conclusions

*Bemisia tabaci*-associated problems are here to stay and might even be increasing. However, the trend is toward greater use of non-insecticide based control methods including deployment of natural enemies (see also Antignus Chapter 13; Nombela and Muñiz Chapter 14). Here, we have updated the available information on the identity and use of natural enemies attacking *B. tabaci*. Numerous new species of predators and parasitoids have been found and studied. From these studies and the practical results of using natural enemies we can draw the following conclusions:

1. The identification of natural enemies and their quantitative role in the agroecosystem are important first steps. New horizons are being opened through the availability of new tools based on serological and molecular techniques, and time-tested methods such as life table analyses. These tools are continuously improving the ability to identify new natural enemies – especially predators – and to quantify their impact on the pest population. These techniques are providing a fuller picture of the role of natural enemies in suppression of *B. tabaci* in different agroecosystems.
2. Both predators and parasitoids are useful as control agents; however, each enemy has its' own specific conditions for optimal employment. Knowing these conditions is a prerequisite to their effective utilization as well as the inclusion of new organisms into our arsenal as was demonstrated in the case of *A. swirskii*. Moreover, the sensitivity to particular conditions such as plant species and pubescence can override other considerations: i.e. predators vs. parasitoids and host range of the natural enemy. The fact that most of the utilized predators are polyphagous, only *Er. mundus* among parasitoids can be considered truly monophagous, and that new natural enemies continue to be discovered and put to use, dictate that the search must continue.

3. Finally, testing and proving the quality and usefulness of natural enemies, especially parasitoids, has always been a weak point in their application. This is mostly due to the large number of organisms to be tested and complex tests required. Recently, it was demonstrated that a realitvely simple series of behavioral examinations and performance studies under field conditions can help in reducing the list of potential natural enemies. This may be done through utilizing a diagrammatic elimination scheme such as proposed by van Lenteren and Martin (1999) thus facilitating the introduction of new organisms for biological control.

**Acknowledgements** We wish to thank all those who helped both in discussion and review of earlier manuscript versions. Special thanks are due to Dr. J. Legaspi (USDA, ARS, Insect Behavior and Biocontrol Unit, Gainsville FL, USA) for reviewing the predator section, to an anonymous reviewer, and to Ms. Nomi Paz (Tel Aviv University, Department of Zoology) for linguistic assistance.

## Literature Cited

Abd-Rabou S. 2006. Hymenopterous parasitoids as a bioagent for controlling homopterous insects in Egypt. *J. Agric. Res.* 6:1–59.

Agustí N, de Vicente MC, Gabarra R. 2000. Developing SCAR markers to study predation on *Trialeurodes vaporariorum*. *Insect Mol. Biol.* 9:263–268.

Akiva I. 2008. Host feeding strategies in *Eretmocerus mundus* Mercet. M.Sc. Thesis, Tel Aviv University, 86pp.

Albajes R, Alomar O. 1999. Current and potential use of polyphagous predators. In *Integrated Pest and Disease Management in Greenhouse Crops*, ed. R Albajes, ML Gullino, JC van Lenteren, Y Elad, pp. 265–275. Dordrecht, The Netherlands: Kluwer.

Albajes R, Sarasúa MJ, Avilla J, Arnó J, Gabarra R. 2003. Integrated pest management in a Mediterranean region: the case of Catalonia. In *Integrated Pest Management in the Global Arena*, ed. KM Maredia, D Dakouo, D Mota-Sánchez, pp. 341–355. Oxon, UK: CABI Publishing.

de Albergaria NMMS, Cividanes FJ, Doria HOS. 2003. Ecological life table of *Bemisia tabaci* (Genn.) B-biotype (Hemiptera: Aleyrodidae). *Neotrop. Entomol.* 32:559–563.

Alomar O, Goula M, Albajes R. 2002. Colonisation of tomato fields by predatory mirid bugs (Hemiptera: Heteroptera) in northern Spain. *Agric. Ecosyst. Environ.* 89:105–115.

Alomar O, Riudavets J, Castañé C. 2006. *Macrolophus caliginosus* in the biological control of *Bemisia tabaci* on greenhouse melons. *Biol. Control* 36:154–162.

Al-Zyoud FA. 2007. Prey species preference of the predator *Serangium parcesetosum* Sicard (Col., Coccinellidae) and its interaction with another natural enemy. *Pakistan J. Biol. Sci.* 10: 2159–2165.

Al-Zyoud F, Sengonca C. 2004. Prey consumption preferences of *Serangium parcesetosum* Sicard (Col., Coccinelidae) for different prey stages, species and parasitized prey. *J. Pest Sci.* 77: 197–204.

Al-Zyoud F, Tort N, Sengonca C. 2005a. Influence of leaf portion and plant species on the egg-laying behaviour of the predatory ladybird *Serangium parcesetosum* Sicard (Col., Coccinellidae) in the presence of a natural enemy. *J. Pest Sci.* 78:167–174.

Al-Zyoud F, Tort N, Sengonca C. 2005b. Influence of host plant species of *Bemisia tabaci* (Genn.) (Hom., Aleyrodidae) on some of the biological and ecological characteristics of the entomophagous *Serangium parcesetosum* Sicard (Col., Coccinellidae). *J. Pest Sci.* 78: 25–30.

Antony B, Palaniswami MS. 2002. Influence of relative humidity and host biotypes on *Eretmocerus mundus* (Mercet) an aphelinid parasitoid of *Bemisia tabaci* (Genn.). *Insect Environ.* 8:4–5.

Antony B, Palaniswami MS, Kirk AA, Henneberry TJ. 2004. Development of *Encarsia bimaculata* (Heraty and Polaszek) (Hymenoptera: Aphelinidae) in *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) nymphs. *Biol. Control* 30:546–555.

Ardeh MJ, de Jong PW, van Lenteren JC. 2005. Selection of *Bemisia* nymphal stages for oviposition or feeding, and host-handling times of arrhenotokous and thelytokous *Eretmocerus mundus* and arrhenotokous *E. eremicus*. *BioControl* 50:449–463.

Arnó J, Gabarra R, Albajes R. 2005. Conservación de míridos depredadores para el control biológico en cultivos de tomate bajo invernadero: historia, éxitos y limitaciones. *Phytoma-España* 165:40–43.

Arnó J, Roig J, Gabarra, R 2006a. *Bemisia tabaci* biotype Q and its natural enemies in vegetable and ornamental crops. In *Proceedings of the 4th International Bemisia Workshop and International Whitefly Genomics Workshop,* ed. PA Stansly, CL McKenzie. *J. Insect Sci.* 8:04. December 3–8, Duck Key, Florida USA, http://www.insectscience.org/8.04/ref/abstract7.html

Arnó J, Castañé C, Riudavets J, Gabarra R. 2006b. Characterization of damage to tomato plants produced by the zoophytophagous predator *Nesidiocoris tenuis*. *Bull. OILB/SROP* 29: 249–254.

Arnó J, Roig J, Riudavets J. 2008. Evaluation of *Orius majusculus* and *O. laevigatus* as predators of *Bemisia tabaci* and estimation of their prey preference. *Biol. Control* 44:1–6.

Arnó J, Castañé C, Riudavets J, Gabarra R. 2010. Risk of damage to tomato crops by the generalist zoophytophagous predator *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae). *Bull. Entomol. Res.* 100:105–115.

Asiimwe P, Ecaat JS, Guershon M, Kyamanywa S, Gerling D, Legg JP. 2007a. Evaluation of *Serangium* n. sp. (Col., Coccinellidae), a predator of *Bemisia tabaci* (Hom., Aleyrodidae) on cassava. *J. Appl. Entomol.* 131:76–80.

Asiimwe P, Ecaat JS, Otim M, Gerling D, Kyamanywa S, Legg JP. 2007b. Life-table analysis of mortality factors affecting populations of *Bemisia tabaci* on cassava in Uganda. *Entomol. Exp. Appl.* 122:37–44.

Auad AM, Toscano LC, Boiça AL, Jr., de Freitas S. 2001. Aspectos biológicos dos estádios imaturos de *Chrysoperla externa* (Hagen) e *Ceraeochrysa cincta* (Schneider) (Neuroptera: Chrysopidae) alimentados con ovos e nimfas de *Bemisia tabaci* (Gennadius) Biótipo B (Hemiptera: Aleyrodiade). *Neotrop. Entomol.* 30:429–432.

Auad AM, Carvalho CF, Souza B, Trevizani R, Magalhaes CMFR. 2005. Desenvolvimento das fases imaturas, aspectos reprodutivos e potencial de predacao de *Chrysoperla externa* (Hagen) alimentada corn ninfas de *Bemisia tabaci* (Gennadius) biotipo B em tomateiro. *Acta Sci. Agron.* 27:327–334.

Bayhan E, Ulusoy MR, Brown JK. 2006. Host range, distribution, and natural enemies of *Bemisia tabaci* "B biotype" (Hemiptera: Aleyrodidae) in Turkey. *J. Pest Sci.* 79:233–240.

Bellamy DE, Byrne DN. 2001. Effects of gender and mating status on self-directed dispersal by the whitefly parasitoid *Eretmocerus eremicus*. *Ecol. Entomol.* 26:571–577.

Blackmer JL, Cross D. 2001. Response of *Eretmocerus eremicus* to skylight and plant cues in a vertical flight chamber. *Entomol. Exp. Appl.* 100:295–300.

Bográn CE, Heinz KM. 2002. Host selection by the heteronomous hyperparasitoid *Encarsia pergandiella*: multiple choice tests using *Bemisia argentifolii* as primary host. *Entomol. Exp. Appl.* 103:11–21.

Bográn CE, Heinz KM. 2006. Time delay and initial population density affect interactions between *Encarsia pergandiella* Howard and *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae). *Environ. Entomol.* 35:661–669.

Bográn CE, Heinz KM, Ciomperlik MA. 2002. Interspecific competition among insect parasitoids: field experiments with whiteflies as hosts in cotton. *Ecology* 83:653–668.

Bonato O, Couton L, Fargues J. 2006. Feeding preference of *Macrolophus caliginosus* (Heteroptera: Miridae) on *Bemisia tabaci* and *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). *J. Econ. Entomol.* 99:1143–1151.

Borah DC, Rai PS. 1989. Potentiality of *Amblyseius ovalis* (Acari: Phytoseiidae) as a biological control agent on *Bemisia tabaci* (Homoptera: Aleyrodidae). In *Progress in Acarology*, ed., CA Viraktamath, Vol. 2, pp. 375–379. Leiden, The Netherlands: GP Channabasavanna, E.J. Brill.

Briggs CJ, Collier TR. 2001. Autoparasitism, interference and pest-parasitoid population dynamics. *Theor. Popul. Biol.* 60:33–57.

Buckner JS, Jones WA. 2005. Transfer of methyl-branched hydrocarbons from the parasitoid, *Eretmocerus mundus*, to silverleaf whitefly nymphs during oviposition. *Comp. Biochem. Physiol. A, Mol. Integr. Physiol.* 140:59–65.

Burban C, Fishpool LD, Fauquet C, Fargette D, Thouvenel JC. 1992. Host-associated biotypes within West African populations of the whitefly *Bemisia tabaci* (genn), (Hom, Aleyrodidae). *J. Appl. Entomol.* 113:416–423.

Burger J. 2002. How to behave? Evolution of host-handling behavior in the whitefly parasitoid *Encarsia formosa*. Thesis, Wageningen University, Wageningen, Netherlands, 195pp.

Burger J, Kormany A, van Lenteren JC, Vet LEM. 2005. Importance of host feeding for parasitoids that attack honeydew-producing hosts. *Entomol. Exp. Appl.* 117:147–154.

Butler GD, Henneberry TJ. 1993. Sweetpotato whitefly natural enemies: parasite surveys in urban areas and cotton fields and identification of a new predator. *Arizona Agric. Exp. Stn. P-94*: 256–257.

Calvo J, Belda JE. 2006. Comparación de estrategias de control biológico de *Bemisia tabaci* Genn. (Homoptera: Aleyrodidae) en pimiento en condiciones de semicampo. *Bol. Sanidad Veg. Plagas* 32:297–311.

Calvo J, Urbaneja A. 2004. *Nesidiocoris tenuis*, un aliado para el control biológico de mosca Blanca. *Hort. Int.* 44:20–25.

Calvo J, Fernández P, Bolckmans K, Belda JE. 2006. *Amblyseius swirskii* (Acari: Phytoseidae) as a biological control agent of the tobacco whitefly *Bemisia tabaci* (Hom.: Aleyrodiade) in protected sweet pepper crops in southern Spain. *Bull. OILB/SROP* 29(4):77–82.

Calvo J, Bolckmans K, Stansly S, Urbaneja A. 2008. Predation by *Nesidiocoris tenuis* on *Bemisia tabaci* and injury to tomato. *BioControl* 54:237–246.

Carboni MA, Marchi S, Nannini M. 2002. Fattori che influenzano l'efficacia dei lanci inoculativi di *Macrolophus caliginosus* Wagner (Heteroptera: Miridae) nelle serre di pomodoro della Sardegna meridionale. *ATTI Giornate Fitopatol.* 1:475–480.

Castañé C, Riudavets J, Yano E. 1999. Biological control of thrips. In *Integrated Pest and Disease Management in Greenhouse Crops*, ed. R Albajes, ML Gullino, JC van Lenteren, Y Elad, pp. 244–253. Dordrecht, The Netherlands: Kluwer.

Chu CC, Chen TY, Simmons AM, Jackson CG, Alexander PA, Henneberry TJ. 2003. Development of light-emitting diode (led) traps for whiteflies and other insects. *Bull. OILB/SROP* 26:27–31.

Chu CC, Simmons AM, Chen T, Alexander PJ, Henneberry TJ. 2004. Lime green light-emitting diode equipped yellow sticky card traps for monitoring whiteflies, aphids, and fungus gnats in greenhouses. *Entomol. Sin.* 11:125–133.

Ciomperlik MA, Goolsby JA. 2008. Field evaluation of *Bemisia* parasitoids in Texas. In *Classical Biological Control of Bemisia tabaci in the United States – A Review of Interagency Research and Implementation*, ed. J Gould, KA Hoelmer, JA Goolsby, Vol. 4, pp. 147–159. Amsterdam, The Netherlands: Springer.

Collier TR, Hunter MS. 2001. Lethal interference competition in the whitefly parasitoids *Eretmocerus eremicus* and *Encarsia sophia*. *Oecologia* 129:147–154.

DeBarro PJ, Hart PJ, Morton R. 2000. The biology of two *Eretmocerus* spp. Haldeman and three *Encarsia* spp. Forster and their potential as biological control agents of *Bemisia tabaci* biotype B in Australia. *Entomol. Exp. Appl.* 94:91–102.

Drost YC, Elmula AF, Posthuma-Doodeman CJAM, van Lenteren JC. 1996. Development of criteria for evaluation of natural enemies in biological control: bionomics of different parasitoids of *Bemisia argentifolii*. *Bull. OILB/SROP* 19:31–34.

Ellis D, Mc Avoy R, Ayyash LA, Flanagan M, Ciomperlik M. 2001. Evaluation of *Serangium parcesetosum* (Coleoptera: coccidenilae) for biological control of silverleaf whitefly, *Bemisia argentifolii* (Homoptera: Aleyrodidae), on Poinsettia. *Florida Entomol.* 84:215–221.

Evans G. 2007. Parasitoids (Hymenoptera) associated with whiteflies (Aleyrodidae) of the world, 173pp. http://www.sel.barc.usda.gov:8080/1WF/parasitoidcatalog.pdf

Fried R. 1997. Reproductive biology of E*retmocerus mundus*. M.Sc. Thesis Tel Aviv University, 120pp.

Gabarra R, Arnó J, Albajes R. 2003. Integrated pest management in greenhouse tomatoes in Spain, pp. 198–202. *Tomate sous abri. Protection intégrée. Agriculture biologique.* Paris, France: Ctifl.

Gabarra R, Zapata R, Castañé C, Riudavets J, Arnó J. 2006. Releases of *Eretmocerus mundus* and *Macrolophus caliginosus* for controlling *Bemisia tabaci* on spring and autumn greenhouse tomato crops. *Bull. OILB/SROP* 29:71–76.

Gelman DB, Blackburn MB, Hu JS, Gerling D. 2001. Timing and regulation of molting/metamorphosis in the whitefly: cues for the development of its parasitoid, *Encarsia formosa*. In *Proceedings of the 2nd International Conference on Arthropods: Chemical, Physiological and Environmental Aspects,* December 21, 2002, ed. D Konopinska. University of Wroclaw.

Gelman DB, Gerling D, Blackburn MB, Hu JS. 2005a. Host-parasite interactions between whiteflies and their parasitoids. *Arch. Insect Biochem. Physiol.* 60:209–222.

Gelman DB, Gerling D, Blackburn MB. 2005b. Host-parasite interactions relating to penetration of *Bemisia tabaci* by its parasitoid *Eretmocerus mundus*. *J. Insect Sci.* 5:46.

Gerling D. 1966. Studies with whitefly parasites of southern California. II. *Eretmocerus californicus* Howard (Hymenoptera: Aphelinidae). *Can. Entomol.* 98:1316–1329.

Gerling D. 1990. Natural enemies of whiteflies: predators and parasitoids. In *Whiteflies their Bionomics, Pest Status and Management*, ed. D Gerling, pp. 147–186. Andover, UK: Intercept Ltd.

Gerling D, Quicke DLJ, Orion T. 1998. Oviposition mechanisms in the whitefly parasitoids *Encarsia transvena* and *Eretmocerus mundus*. *BioControl* 43:289–297.

Gerling D, Shoshan R, Guershon, M. 2006. Influence of host size upon the fitness of *Eretmocerus mundus*. In *Proceedings, 2006 Entomophagous Insects Workshop*, Newark DE, USA.

Gerling D, Alomar O, Arnó J. 2001. Biological control of *Bemisia tabaci* using predators and parasitoids. *Crop Prot.* 20:779–799.

Gerling D, Rejouan N. 2004. Age-related pupal defenses against congeneric internecine activity in *Encarsia* species. *Entomol. Exp. Appl.* 110:87–93.

Ghahari H, Huang J, Wang Z. 2005. Biology of a thelyotokous biotype of *Eretmocerus mundus* (Hymenoptera: Aphelinidae) on *Bemisia tabaci* (Homoptera: Aleyrodidae). *Insect Sci.* 12: 461–465.

Gilioli G, Baumgärtner J, Vacante V. 2005. Temperature influences on functional response of *Coenosia attenuata* (Diptera: Muscidae) individuals. *J. Econ. Entomol.* 98:1524–1530.

Giorgini M, Baldanza F. 2004. Species status of two populations of *Encarsia sophia* (Girault and Dodd) (Hymenoptera: Aphelinidae) native to different geographic areas. *Biol. Control* 30: 25–35.

Goolsby JA, De Barro PJ, Kirk AA, Sutherst RW, Canas L, Ciomperlik MA, Ellsworth PC, Gould JR, Hartley DM, Hoelmer KA, Naranjo SE, Rose M, Roltsch WJ, Ruiz RA, Pickett CH, Vacek DC. 2005. Post-release evaluation of biological control of *Bemisia tabaci* biotype "B" in the USA and the development of predictive tools to guide introductions for other countries. *Biol. Control* 32:70–77.

Goolsby JA, Legaspi BJ, Jr, Legaspi JC. 2008. Quarantine evaluation of parasitoids imported into the USA for biocontrol of *Bemisia tabaci* Biotype B. In *Classical Biological Control of Bemisia*

*tabaci in the United States – A Review of Interagency Research and Implementation*, ed. J Gould, KA Hoelmer, JA Goolsby, Vol. 4, pp. 121–128. Amsterdam, The Netherlands: Springer.

Gould J, Hoelmer KA, Goolsby JA (eds). 2008. *Classical Biological Control of Bemisia tabaci in the United States – A Review of Interagency Research and Implementation*, Vol. 4, 344pp. Amsterdam, The Netherlands: Springer.

Greenberg SM, Legaspi BC, Jones WA, Enkegaard A. 2000. Temperature-dependent life history of *Eretmocerus eremicus* (Hymenoptera: Aphelinidae) on two whitefly hosts (Hymenoptera: Aleyrodidae). *Environ. Entomol.* 29:851–860.

Greenberg SM, Legaspi BC, Jr., Jones WA. 2001. Comparison of functional response and mutual interference between two aphelinid parasitoids of *Bemisia argentifolii* (Homoptera: Aleyrodidae). *J. Entomol. Sci.* 36:1–8.

Greenstone MH. 1996. Serological analysis of arthropod predation: past, present and future. In *The Ecology of Agricultural Pests: Biochemical Approaches*, ed. WOC Symondson, JE Liddell, pp. 265–300. London, UK: Chapman and Hall.

Gruenhagen NM, Perring TM. 2001. Impact of leaf trichomes on parasitoid behavior and parasitism of silverleaf whiteflies (Homoptera: Aleyrodidae). *Southwestern Entomol.* 26:279–290.

Guershon M, Gerling D. 2006. Effects of plant and prey characteristics on the predatory behavior of *Delphastus catalinae*. *Entomol. Exp. Appl.* 121:15–21.

Hagler JR. 2002. Foraging behavior, host stage selection and gut content analysis of field collected *Drapetis* nr. *divergens*: a predatory fly of *Bemisia argentifolii*. *Southwestern Entomol.* 27: 241–249.

Hagler JR, Naranjo SE. 1994. Determining the frequency of heteropteran predation on sweetpotato whitefly and pink bollworm using multiple ELISAs. *Entomol. Exp. Appl.* 72:59–66.

Hagler JR, Naranjo SE. 2004. A multiple ELISA system for simultaneously monitoring intercrop movement and feeding activity of mass-released insect predators. *Int. J. Pest Manage.* 50: 199–207.

Hagler JR, Naranjo SE. 2005. Use of a gut content ELISA to detect whitefly predator feeding activity after field exposure to different insecticide treatments. *Biocontrol Sci. Technol.* 15: 321–339.

Hagler JR, Brower AG, Tu Z, Byrne DN, Bradley-Dunlop D, Enriquez FJ. 1993. Development of a monoclonal antibody to detect predation of the sweetpotato whitefly, *Bemisia tabaci*. *Entomol. Exp. Appl.* 68:231–236.

Hagler JR, Jackson CG, Henneberry TJ, Gould JR. 2002. Parasitoid mark-release-recapture techniques – II. Development and application of a protein marking technique for *Eretmocerus* spp., parasitoids of *Bemisia argentifolii*. *Biocontrol Sci. Technol.* 12:661–675.

Hagler JR, Jackson CG, Isaacs R, Machtley SA. 2004. Foraging behavior and prey interactions by a guild of predators on various lifestages of *Bemisia tabaci*. *J. Insect Sci.* 4:1–13. www/insectscience.org/14.11.

Headrick DH, Bellows TS, Perring TM. 1996. Host-plant effects on the behavior of *Eretmocerus* sp. nr. *californicus* females raised on melon. *Entomophaga* 41:15–26.

Headrick DH, Bellows TS, Perring TM. 1999. Development and reproduction of a population of *Eretmocerus eremicus* (Hymenoptera: Aphelinidae) on *Bemisia argentifolii* (Homoptera: Aleyrodidae). *Environ. Entomol.* 28:300–306.

Heinz KM, Zalom FG. 1996. Performance of the predator *Delphastus pusillus* on *Bemisia* resistant and susceptible tomato lines. *Entomol. Exp. Appl.* 81:345–352.

Heinz KM, Brazzle JR, Pickett CH, Natwick ET, Nelson JM, Parrella MP. 1994. *Delphastus pusillus* as a potential biological control agent for sweetpotato (silverleaf) whitefly. *California Agric.* 48:35–40.

Heraty JM, Polaszek A. 2000. Morphometric analysis and descriptions of selected species in the *Encarsia strenua* group (Hymenoptera: Aphelinidae). *J. Hymenoptera Res.* 9:142–169.

Heraty JM, Wolley A, Polaszek A. 2007. Catalog of the *Encarsia* of the world. http://cache.ucr.edu/∼heraty/Encarsia.cat.pdf

Heraty JM, Polaszek A, Schauff MA. 2008. Systematics and biology of *Encarsia*. In *Classical Biological Control of Bemisia tabaci in the United States – A Review of Interagency Research and Implementation*, ed. J Gould, KA Hoelmer, JA Goolsby, Vol. 4, pp. 71–87. Amsterdam, The Netherlands: Springer.

Hernández-Suárez E, Carnero A, Aguiar A, Prinsloo G, LaSalle J, Polaszek A. 2003. Parasitoids of whiteflies (Hymenoptera: Aphelinidae, Eulophidae, Platygastridae; Hemiptera: Aleyrodidae) from the Macaronesian archipelagos of the Canary Islands, Madeira and the Azores. *Syst. Biodiver.* 1:55–108.

Hoddle MS, van Driesche RG, Sanderson JP. 1997. Biological control of *Bemisia argentifolii* (Homoptera: Aleyrodidae) on poinsettia with inundative releases of *Encarsia formosa* Beltsville strain (Hymenoptera: Aphelinidae): can parasitoid reproduction augment inundative releases? *J. Econ. Entomol.* 90:910–924.

Hoddle MS, van Driesche RG, Sanderson JP. 1998. Biology and use of the whitefly parasitoid *Encarsia formosa*. *Annu. Rev. Entomol.* 43:645–649.

Hoelmer KA, Simmons AM. 2008. Yellow sticky trap catches of parasitoids of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in vegetable crops and their relationship to in-field populations. *Environ. Entomol.* 37:391–399.

Hoelmer KA, Osborne LS, Yokomi RK. 1993. Reproduction and feeding behavior of *Delphastus pusillus* (Coleoptera: Coccinellidae) a predator of *Bemisia tabaci* (Homoptera: Aleyrodidae). *J. Econ. Entomol.* 86:322–329.

Hoelmer KA, Roltsch WJ. 2008. Evaluation of exotic parasitoids and predators in field cages in California. In *Classical Biological Control of Bemisia tabaci in the United States – A Review of Interagency Research and Implementation*, ed. J Gould, KA Hoelmer, JA Goolsby, Vol. 4, pp. 129–145. Amsterdam, The Netherlands: Springer.

Huang Z, Ren SX, Yao SL. 2006. Life history of *Axinoscymnus cardilobus* (Col., Coccinellidae), a predator of *Bemisia tabaci* (Hom., Aleyrodidae). *J. Appl. Entomol.* 130: 437–441.

Huang Z, Ren S, Musa PD. 2008. Effects of temperature on development, survival, longevity, and fecundity of the *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) predator, *Axinoscymnus cardilobus* (Coleoptera: Coccinellidae). *Biol. Control* 46:209–215.

Hunter MS, Woolley JB. 2001. Evolution and behavioral ecology of hetronomous aphelinid parasitoids. *Annu. Rev. Entomol.* 46:251–290.

Hunter MS, Collier TR, Kelly SE. 2002. Does an autoparasitoid disrupt host suppression provided by a primary parasitoid? *Ecology* 83:1459–1469.

Hunter MS, Kelly SE. 1998. Hyperparasitism by an exotic autoparasitoid: secondary host selection and the window of vulnerability of conspecific and native heterospecific hosts. *Entomol. Exp. Appl.* 89:249–259.

Inbar M, Gerling D. 2008. Plant-mediated interactions between whiteflies, herbivores, and natural enemies. *Annu. Rev. Entomol.* 53:431–448.

Jackson DM, Farnham MW, Simmons AM, van Giessen WA, Elsey KD. 2000. Effects of planting pattern of collards on resistance to whiteflies (Homoptera: Aleyrodidae) and on parasitoid abundance. *J. Econ. Entomol.* 93:1227–1236.

Jaronski ST, Lord J, Rosinska J, Bradley C, Hoelmer KA, Simmons G, Osterlind R, Brown C, Staten R, Antilla L. 1998. Effect of a *Beauveria bassiana*-based mycoinsecticide on beneficial insects under field conditions. In *Brighton Crop Protection Conference: Pests and Diseases – 1998: Vol. 2: Proceedings of an International Conference*, Brighton UK, November 16–19, 1998. pp. 651–656 Farnham, UK: British Crop Protection Council.

Jazzar C, Hammad EAF. 2004. Efficacy of multiple biocontrol agents against the sweet potato whitefly *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) on tomato. *J. Appl. Entomol.* 128:188–194.

Jervis MA, Heimpel GE, Ferns PN, Harvey JA, Kidd NAC. 2001. Life-history strategies in parasitoid wasps: a comparative analysis of 'ovigeny.' *J. Anim. Ecol.* 70:442–458.

Joyce AL, Bellows TS, Headrick DH. 1999. Reproductive biology and search behavior of *Amitus bennetti* (Hymenoptera: Platygasteridae), a parasitoid of *Bemisia argentifolii* (Homoptera: Aleyrodidae). *Environ. Entomol.* 28:282–289.

Kapadia MN, Puri SN. 1990. Development, ralative proportions and emergence of *Encarsia transvena* (Timberlake) and *Eretmocerus mundus* Mercet, important paraitoids of *Bemisia tabaci* (Genadius). *Entomon* 15:235–239.

Karut K, Naranjo SE. 2009. Mortality factors affecting *Bemisia tabaci* populations on cotton the Çukurova plain, Turkey. *J. Appl. Entomol.* 133:367–374.

Kirk AA, Lacey LA, Brown JK, Ciomperlik MA, Goolsby JA, Vacek DC, Wendel LE, Napompeth B. 2000. Variation in the *Bemisia tabaci* s.l. species complex (Hemiptera: Aleyrodidae) and its natural enemies leading to successful biological control of Bemisia biotype B in the USA. *Bull. Entomol. Res.* 90:317–327.

Kühne S. 1998. Open rearing of generalist predators: a strategy for improvement of biological pest control in greenhouses. *Phytoparasitica* 26:277–281.

Kühne S. 2000. Räuberische Fliegen der Gattung *Coenosia* Meigen, 1826 (Diptera: Muscidae) und die Möglichkeit ihres Einsatzes bei der biologischen Schädlingsbekämpfung. *Studia Dipterologica Suppl.* 9:1–78.

Legaspi JC, Simmons AM, Legaspi BC, Jr. 2006. Prey preference by *Delphastus catalinae* (Coleoptera: Coccinellidae) on *Bemisia argentifolii* (Homoptera: Aleyrodidae): effects of plant species and prey stages. *Florida Entomol.* 89:218–222.

Legaspi JC, Legaspi BC, Jr, Simmons AM, Soumare M. 2008. Life table analysis for immatures and female adults of the predatory beetle, *Delphastus catalinae*, feeding on whiteflies under three constant temperatures. *J. Insect Sci.* 8:07. Available online: insectscience.org/8.07.

Legg JP. 1996. Host-associated strains within Ugandan populations of the whitefly *Bemisia tabaci* (Genn.) (Hom., Aleyrodidae). *J. Appl. Entomol.* 120:523–527.

Leite GLD, Picanco M, Jham GN, Moreira MD. 2005. Whitefly population dynamics in okra plantations. *Pesqui Agro. Brasileira* 40:19–25.

Lin KJ, Wu KM, Liu SB, Zhang YJ, Guo YY. 2006. Functional responses of *Chrysopa sinica*, *Propylea japonica* and *Leis axyridis* to *Bemisia tabaci*. *Chinese Bull. Entomol.* 43: 339–343.

Liu TX. 2005. Life history and life table analysis of the whitefly predator *Delphastus catalinae* (Coleoptera: Coccinellidae) on collards. *Insect Sci.* 12:129–135.

Liu TX. 2007. Life history of *Eretmocerus melanoscutus* (Hymenoptera: Aphelinidae), parasitizing nymphs of *Bemisia tabaci* Biotype B (Homoptera: Aleyrodidae). *Biol. Control* 42:77–85.

Liu TX, Stansly PA. 1996. Oviposition, development, and survivorship of *Encarsia pergandiella* (Hymenoptera: Aphelinidae) in four instars of *Bemisia argentifolii* (Homoptera: Aleyrodidae). *Ann. Entomol. Soc. Am.* 89:96–102.

Liu TX, Stansly PA. 1999. Searching and feeding behavior of *Nephaspis oculatus* and *Delphastus catalinae* (Coleoptera: Coccinellidae), predators of *Bemisia argentifolii* (Homoptera: Aleyrodidae). *Environ. Entomol.* 28:901–906.

Liu TX, Stansly P. 2005. Timing and release rates for control of *Bemisia tabaci* (Homoptera: Aleyrodidae) by *Nephaspis oculatus* (Coleoptera: Coccinellidae) under confined conditions. *J. Entomol. Sci.* 40:74–79.

López-Ávila A, Cardona Mejía C, García González J, Rendón F, Hernández P. 2001. Survey and identification of natural enemies of whiteflies in Colombia and Ecuador. *Rev. Colombiana Entomol.* 27:137–141.

Mandour NS, Ren SX, Qiu BL, Fazal S. 2003. Effects of extracts from nymphs, exuviae and adults of *Bemisia tabaci* B biotype on the behavior of *Encarsia bimaculata* Heraty et. Polaszek (Hymenoptera: Aphelinidae). *Acta Entomol. Sin.* 46:745–748.

Martinez-Cascales JI, Cenis JL, Cassis G, Sanchez JA. 2006. Species identity of *Macrolophus melanotoma* (Costa 1853) and *Macrolophus pygmaeus* (Rambur 1839) (Insecta: Heteroptera:

Miridae) based on morphological and molecular data and bionomic implications. *Insect Syst. Evol*. 37:385–404.

McAuslane HJ, Simmons AM, Jackson DM. 2000. Parasitism of *Bemisia argentifolii* on collard with reduced or normal leaf wax. *Florida Entomol.* 83:428–437.

McCutcheon GS, Simmons AM. 2001. Relationship between temperature and rate of parasitism by *Eretmocerus* sp. (Hymenoptera: Aphelinidae), a parasitoid of *Bemisia tabaci* (Homoptera: Aleyrodidae). *J. Agric. Urban Entomol.* 18:97–104.

Medina Balderas S, Ortega Arenas LD, González Hernández H, Villanueva Jiménez JA. 2002. Influence of weeds on the whitefly-virus-parasitoid complex in Veracruz, Mexico. *Manejo Integrado Plagas Agroecol.* 65:75–81 (in Spanish).

Mendelbaum S. 2004. Parameters influencing host-related behavior of the parasitoid wasp *Eretmocerus mundus*. Hymenoptera: Aphelinidae. M.Sc. Thesis, Tel Aviv University. 64pp.

Meng RX, Janssen A, Nomikou M, Zhang QW, Sabelis MW. 2006. Previous and present diets of mite predators affect antipredator behaviour of whitefly prey. *Exp. Appl. Acarol.* 38 (113–124):2006.

Messelink G, van Steenpaal SEF, Ramakers P. 2006. Evaluation of phytoseiid predators for control of western flower thrips on greenhouse cucumber. *BioControl* 51:753–768.

Moreschi I, Colombo M. 1999. Una metrodica per l'allevamento dei Ditteri predatori *Coenosia attenuata* e *C. strigipes*. *Inform. Fitopatol.* 7–8:61–64.

Moreschi I, Süss L. 1998. Osservazioni biologiche ed etologiche su *Coenosia attenuata* Stein e *Coenosia strigipes* Stein (Diptera Muscidae). *Boll. Zool. Agrar. Bachicoltura.* Serie II 30: 185–197.

Murphy BC, Morisawa T, Parrella MP. 1998. Insect-killing fungi: floriculture's IPM future? *Grower Talks* 61(10):60, 62, 64, 66, 68.

Nannini, M. 2003. The predatory capacity of *Macrolophus caliginosus* Wagner and *Nesidiocoris tenuis* Reuter (Heteroptera: Miridae) preying on whiteflies. In *Proceedings of the 3rd International* Bemisia *Workshop Barcelona*, March 17–20, 2003, p. 86.

Naranjo SE. 2001. Conservation and evaluation of natural enemies in IPM systems for *Bemisia tabaci*. *Crop Prot.* 20:835–852.

Naranjo SE. 2007. Intraguild predation on *Eretmocerus* sp nr. *emiratus*, a parasitoid of *Bemisia tabaci*, by three generalist predators with implications for estimating the level and impact of parasitism. *Biocontrol Sci. Technol.* 17:605–622.

Naranjo SE. 2008. Establishment and impact of exotic aphelinid parasitoids in Arizona: a life table approach. *J. Insect. Sci. (Tucson)* 8:36.

Naranjo SE, Ellsworth PC. 2005. Mortality dynamics and population regulation in *Bemisia tabaci*. *Entomol. Exp. Appl.* 116:93–108.

Naranjo SE, Gibson RL. 1996. Phytophagy in predaceous Heteroptera: effects on life history and population dynamics. In *Zoophytophagous Heteroptera: Implications for Life History and Integrated Pest Management*, ed. O Alomar, RN Wiedenmann, pp. 57–93. Lanham, MD, USA: Thomas Say Publications in Entomology, Entomological Society of America.

Naranjo SE, Hagler JR. 1998. Characterizing and estimating the impact of heteropteran predation. In *Predatory Heteroptera: Their Ecology and Use in Biological Control*, ed. M Coll, J Ruberson, pp. 170–197. Lanham, MD, USA: Thomas Say Publications in Entomology, Entomological Society of America.

Naranjo SE, Cañas L, Ellsworth PC. 2004a. Mortalidad de *Bemisia tabaci* en un sistema de cultivos múltiples. *Hort. Internacional* 43(Febrero):14–21.

Naranjo SE, Cañas L, Ellsworth, PC. 2009. Mortality and population dynamics of *Bemisia tabaci* within a multi-crop system. In *Proceedings of the Third International Symposium on Biological Control of Arthropods, Christchurch, New Zealand*, ed. PG Mason, DR Gillespie, CD Vincent, pp. 202–207. Publ. FHTET-2008-06: USDA Forest Service.

Naranjo SE, Ellsworth PC, Hagler JR. 2004b. Conservation of natural enemies in cotton: role of insect growth regulators for management of *Bemisia tabaci*. *Biol. Control* 30:52–72.

Nombela G, Chu CC, Henneberry TJ, Muñiz M. 2003. Comparison of three trap types for catching adult *Bemisia tabaci* whitefly and its parasitoid *Eretmocerus mundus* in tomato greenhouse. *Bull. OILB/SROP* 26:53–56.

Nomikou M, Janssen A, Schraag R, Sabelis MW. 2001. Phytoseiid predators as potential biological control agents for *Bemisia tabaci*. *Exp. Appl. Acarol.* 25:271–291.

Nomikou M, Janssen A, Schraag R, Sabelis MW. 2002. Phytoseiid predators suppress populations of *Bemisia tabaci* on cucumber plants with alternative food. *Exp. Appl. Acarol.* 27:57–68.

Nomikou M, Janssen A, Sabelis MW. 2003a. Phytoseiid predators of whiteflies feed and reproduce on non-prey food sources. *Exp. Appl. Acarol.* 31:15–26.

Nomikou M, Janssen A, Sabelis MW. 2003b. Herbivore host plant selection: whitefly learns to avoid host plants that harbour predators of her offspring. *Oecologia* 136:484–488.

Nomikou M, Janssen A, Schraag R, Sabelis MW. 2004. Vulnerability of *Bemisia tabaci* immatures to phytoseiid predators: consequences for oviposition and influence of alternative food. *Entomol. Exp. Appl.* 110:95–102.

Nomikou M, Meng RX, Schraag R, Sabelis MW, Janssen A. 2005. How predatory mites find plants with whitefly prey. *Exp. Appl. Acarol.* 36:263–275.

Numes C, Lucas E, Coderre D. 2006. Parasitism of *Bemisia tabaci* (Homoptera: Aleyrodidae) in vegetable crops in the dry Nicaraguan tropics. *Int. J. Trop. Insect Sci.* 26:57–63.

de Oliveira MRV, Amancio E, Laumann RA, Gomes LO. 2003. Natural enemies of *Bemisia tabaci* (Gennadius) B biotype and *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) in Brasilia, Brazil. *Neotrop. Entomol.* 32:151–154.

Oster, N. 1995. Factors influencing the parasitization efficiency of the parasitic wasp *Encarsia transvena*. M.Sc. Thesis, Tel Aviv University, 76pp.

Otim M, Legg J, Kyamanywa S, Polaszek A, Gerling D. 2005. Occurrence and activity of *Bemisia tabaci* parasitoids on cassava in different agro-ecologies in Uganda. *BioControl* 50: 87–95.

Otim M, Legg J, Kyamanywa S, Polaszek A, Gerling D. 2006. Population dynamics of *Bemisia tabaci* (Homoptera: Aleyrodidae) parasitoids on cassava mosaic disease-resistant and susceptible varieties. *Biocontrol Sci. Technol.* 16:205–214.

Otim M, Kyalo G, Kyamanywa S, Asiimwe P, Legg JP, Guershon M, Gerling D. 2008. Parasitism of *Bemisia tabaci* (Homoptera: Aleyrodidae) by *Eretmocerus mundus* (Hymenoptera: Aphelinidae) on cassava. *Int. J. Trop. Insect Sci.* 28:158–167.

Otoidobiga LC, Vincent C, Stewart RK. 2003. Susceptibility of field populations of adult *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) and *Eretmocerus* sp. (Hymenoptera: Aphelinidae) to cotton insecticides in Burkina Faso (West Africa). *Pest Manage. Sci.* 59:97–106.

Otoidobiga LC, Vincent C, Stewart RK. 2004. Relative abundance of *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) and its parasitoids, and the impact of augmentative release of *Eretmocerus* spp. (Hymenoptera: Aphelinidae) on the population dynamics of the pest in Burkina Faso (West Africa). *Int. J. Pest Manage.* 50:11–16.

Pavis C, Huc JA, Delvare G, Boissot N. 2003. Diversity of the parasitoids of *Bemisia tabaci* B-biotype (Hemiptera: Aleyrodidae) in Guadeloupe Island (West Indies). *Environ. Entomol.* 32:608–613.

Perdikis DC, Margaritopoulos JT, Stamatis C, Mamuris Z, Lykouressis DP, Tsitsipis JA, Pekas A. 2003. Discrimination of the closely related biocontrol agents *Macrolophus melanotoma* (Hemiptera: Miridae) and *M. pygmaeus* using mitochondrial DNA analysis. *Bull. Entomol. Res.* 93:507–514.

Pickett CH, Roltsch W, Corbett A. 2004. The role of a rubidium marked natural enemy refuge in the establishment and movement of *Bemisia* parasitoids. *Int. J. Pest Manage.* 50:183–191.

Powell DA, Bellows TS. 1992. Development and reproduction of two populations of *Eretmocerus* species (Hymenoptera: Aphelinidae) on *Bemisia tabaci* (Hymenoptera: Aphelinidae). *Environ. Entomol.* 21:651–658.

Qiu BL, Ren SX. 2006. Using yellow sticky traps to inspect population dynamics of *Bemisia tabaci* and its parasitoids. *Chinese Bull. Entomol.* 43:53–56.

Qiu BL, Ren SX, Lin L, Wang XM. 2004a. Species and dynamics of aphelinid parasitoids of *Bemisia tabaci* in Guangdong. *Entomol. Knowledge* 41:333–335.

Qiu YT, van Lenteren JC, Drost YC, Posthuma-Doodeman CJAM. 2004b. Life-history parameters of *Encarsia formosa*, *Eretmocerus eremicus* and *E. mundus,* aphelinid parasitoids of *Bemisia argentifolii* (Hemiptera: Aleyrodidae). *Eur. J. Entomol.* 101:83–94.

Qiu BL, DeBarro PJ, Ren SX. 2005. Development, survivorship and reproduction of *Eretmocerus* sp. nr. *furuhashii* (Hymenoptera: Aphelinidae) parasitizing *Bemisia tabaci* (Hemiptera: Aleyrodidae) on glabrous and non-glabrous host plants. *Bull. Entomol. Res.* 95:313–319.

Qiu BL, DeBarro PJ, Xu CX, Ren SX. 2006. Effect of temperature on the life history of *Encarsia bimaculata* (Hymenoptera: Aphelinidae), a parasitoid of *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Eur. J. Entomol.* 103:787–792.

Rajam B, Peter C, David BV. 1988. Influence of host plants on the parasitism of *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) by *Encarsia* sp. *Curr. Sci.* 57(22):1246–1247.

Reese S. 1994. Parasitierunsverhalten des heteronomen Hyperparasitoiden *Encarsia transvena* (Hymenoptera: Aphelinidae) des Weiszen Fliege *Bemisia tabaci* (Homoptera: Aleyrodidae). MSc Thesis, University of Kiel, 60pp.

Ren SX, Wang ZZ, Qiu BL, Xiao Y. 2001. The pest status of Bemisia tabci in China and non-chemical control strategies. *Entomol. Sin.* 8:279–288.

Ren SX, Stansly PA, Liu TX. 2002. Life history of the whitefly predator *Nephaspis oculatus* (Coleoptera: Coccinellidae) at six constant temperatures. *Biol. Control* 23: 262–268.

Rodríguez-Rodríguez MD, Moreno R, Tellez MM, Rodríguez-Rodríguez MP, Fernández-Fernández R. 1994. *Eretmocerus mundus* (Mercet), *Encarsia lutea* (Masi) y *Encarsia transvena* (Timberlake) (Hym.: Aphelinidae) parasitoides d*e Bemisia tabaci* (Homoptera: Aleyrodidae) en los cultivos horticolas protegidos almerienses. *Bol. Sanidad Veg. Plagas* 20:695–702.

Roltsch WA, Hoelmer KA, Simmons GS, Andress E. 2008. Release and recovery of exotic natural enemies of *Bemisia tabaci* (Biotype "B") (Hemiptera: Aleyrodidae) in Imperial Valley, California. In *Classical Biological Control of* Bemisia tabaci *in the United States – A Review of Interagency Research and Implementation*, ed. J Gould, KA Hoelmer, JA Goolsby, Vol. 4, pp. 204–224. Amsterdam, The Netherlands: Springer.

Ryckewaert P, Alauzet C. 2002. The natural enemies of *Bemisia argentifolii* in Martinique. *BioControl* 47:115–126.

Sahar F, Ren SX. 2004. Interaction of *Serangium japonicum* (Coleoptera: Coccinellidae), an obligate predator of whitefly with immature stages of *Eretmocerus* sp. (Hymenoptera: Aphelinidae) within whitefly host (Homoptera: Aleyrodidae). *Asian J. Plant Sci.* 3:243–246.

Sanchez JA. 2008. Zoophytophagy in the plant bug *Nesidiocoris tenuis*. *Agric. Forest Entomol.* 10:75–80.

Sanchez JA, Lacasa A. 2008. Impact of the zoophytophagous plant bug *Nesidiocoris tenuis* (Heteroptera: Miridae) on tomato yield. *J. Econ. Entomol.* 101:1864– 1870.

Sanchez JA, del Pino-Pérez M, Davó MM, Martinez-Cascales JI, Lacasa A. 2006. Zoophytophagy of the plantbug *Nesidiocoris tenuis* in tomato crops in the Southeast Spain. *Bull. OILB/SROP* 29:243–248.

Scholz-Dobelin H, Stockmann S. 2003. Mycoinsecticides against whitefly *Trialeurodes vaporariorum* in tomatoes. *Gesunde Pflanzen* 55:210–214.

Sengonca C, Al-Zyoud F, Blaeser P. 2004. Life table of the entomophagous ladybird *Serangium parcesetosum* Sicard (Coleoptera: Coccinellidae) by feeding on *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae) as prey at two different temperatures and plant species. *Z. Pflanzenkr. Pflanzenschutz* 111:598–609.

Sharma SS, Ram P, Saini RK. 2004. Population dynamics of *Bemisia tabaci* (Gennadius) and its parasitoid, *Encarsia lutea* (Masi), on cotton. *J. Cotton Res. Devel.* 18:102–103.

Shimron, O. 1991. Processes and cues in host parasitization of *Bemisia tabaci* (Homoptera) by its parasitoid *Eretmocerus* sp. and *Encarsia deserti* (Hymenoptera). PhD Thesis, Tel Aviv University, 147pp.

Shimron O, Hefetz A, Gerling D. 1992. Arrestment responses of *Eretmocerus* species and *Encarsia deserti* (hymenoptera: Aphelinidae) to *Bemisia tabaci* honeydew. *J. Insect Behav.* 5:517–526.

Shipp JL, Zhang Y, Hunt DWA, Ferguson G. 2003. Influence of humidity and greenhouse microclimate on the efficacy of *Beauveria bassiana* (Balsamo) for control of greenhouse arthropod pests. *Environ. Entomol.* 32:1154–1163.

Silva LD, Bonani JP. 2008. Occurrence of *Stethorus* (*Stethotrus*) *minutalus* Gordon and Chapin (Coleoptera: Coccinellidae) preying *Bemisia tabaci* biotype B (Hemiptera: Aleyrodidae) on cotton plant in Brazil. *Neotrop. Entomol.* 37:86–88.

Silva CG, Auad AM, Souza B, Carvalho CF, Bonani JP. 2004a. Aspectos biologicos de *Chrysoperla externa* (Hagen, 1861) (Neuroptera: Chrysopidae) alimentada com *Bemisia tabaci* (Gennadius, 1889) biotipo B (Hemiptera: Aleyrodidae) criada em tres hospedeiros. *Cienc. Agrotecnol.* 28:243–250.

Silva CG, Souza B, Auad AM, Bonani JP, Torres LC, Carvalho CF, Ecole CC. 2004b. Desenvolvimento das fases imaturas de *Chrysoperla externa* alimentadas com ninfas de *Bemisia tabaci* criadas em tres hospedeiros. *Pesqui. Agrop. Brasileira* 39:1065–1070.

Simmons AM, McCutcheon GS. 2001. Daily foraging incidence of *Encarsia pergandiella* (Hymenoptera: Aphelinidae) on cowpea. *J. Entomol. Sci.* 36:218–221.

Simmons AM, Legaspi JC. 2004. Survival and predation of *Delphastus catalinae* (Coleoptera: Coccinellidae), a predator of whiteflies (Homoptera: Aleyrodidae), after exposure to a range of constant temperatures. *Environ. Entomol.* 33:839–843.

Simmons AM, Legaspi JC. 2007. Ability of *Delphastus catalinae* (Coleoptera: Coccinellidae), a predator of whiteflies (Homoptera: Aleyrodiade), to survive mild winters. *J. Entomol. Sci.* 42:163–173.

Simmons AM, Abd-Rabou S, McCutcheon GS. 2002. Incidence of parasitoids and parasitism of *Bemisia tabaci* (Homoptera: Aleyrodidae) in numerous crops. *Environ. Entomol.* 31:1030–1036.

Simmons AM, Chu CC, Henneberry TJ. 2004. Yellow sticky cards equipped with light-emitting diodes: a natural enemies compatible management tool for whiteflies (Homoptera: Aleyrodidae) and other greenhouse vegetable pests. *J. Entomol. Sci.* 39:298–300.

Simmons AM, Legaspi JC, Legaspi BC. 2008. Response of *Delphastus catalinae* (Coleoptera: Coccinellidae), a predator of whiteflies (Homoptera: Aleyrodidae), to relative humidity: oviposition, hatch, and immature survival. *Ann. Entomol. Soc. Am.* 101:378–383.

de Siqueira KMM, de Farias AMI. 2003. Response of female *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) to host and plant-host odors. *Bragantia* 62:447–450.

Stansly PA, Calvo FJ, Urbaneja A. 2004. Biological control of *Bemisia tabaci* (Homoptera, Aleyrodidae) in protected tomato and pepper culture in southern Spain. In *Proceedings of the VII International Symposium on protected cultivation in mild winter climates: production, pest management and global competition*. Vol. I, *Acta Horticulturae* 659: 383–394.

Stansly PA, Calvo J, Urbaneja A. 2005. Release rates for control of *Bemisia tabaci* (Homoptera: Aleyrodidae) biotype "Q" with *Eretmocerus mundus* (Hymenoptera: Aphelinidae) in greenhouse tomato and pepper. *Biol. Control* 35:124–133.

Syed AN, Ashfaq M, Khan S. 2005. Comparison of development and predation of *Chrysoperla carnea* (Neuroptera: Chrysopidae) on different densities of two hosts (*Bemisia tabaci*, and *Amrasca devastans*). *Pakistan Entomol.* 27:41–44.

Talebi AA, Kamali K, Fathipour Y, Moharamipour S, Sahragard A, Khalghani J. 2002. Functional response of parasitoid wasps, *Encarsia lutea* and *Eretmocerus mundus* (Hym., Aphelinidae) to different densities of *B. tabaci* (Hom., Aleyrodidae) nymphs. *J. Agric. Sci. Islamic Azad Univ.* 8:83–94.

Téllez MM, Tapia G. 2005. Presencia y distribución de *Coenosia attenuata* (Diptera: Muscidae) en las principales zonas invernadas de la Provincia de Almería. *Bol. Sanidad Veg. Plagas* 31:335–341.

Téllez MM, Tapia G. 2006. Accion depredadora de *Coenosia attenuata* Stein (Diptera: Muscidae) sobre otros enemigos naturales en condiciones de laboratorio. *Bol. Sanidad Veg. Plagas* 32:491–498.

Trottin-Caudal Y, Capy A. 2003. Protection intégrée de la tomate sous serre en France. *Situation actuelle and perspectives. Tomate sous abri. Protection intégrée. Agriculture biologique*. pp. 208–212. Paris, France: Ctifl.

Trottin-Caudal Y, Chabrière C, Fournier C, Leyre JM. 2006. Current situation of *Bemisia tabaci* in protected vegetable crops in the South of France. *Bull. OILB/SROP* 29:53–58.

Trujillo HE, Arias B, Guerrero JM, Hernandez P, Bellotti A, Pena JE. 2004. Survey of parasitoids of whiteflies (Homoptera: Aleyrodidae) in cassava growing regions of Columbia and Ecuador. *Florida Entomol.* 87:268–273.

Urbaneja A, Sánchez E, Stansly PA. 2007. Life history of *Eretmocerus mundus* Mercet (Hym.: Aphelinidae), a parasitoid of *Bemisia tabaci* Gennadius (Hom: Aleyrodidae), on tomato and sweet pepper. *BioControl* 52:25–39.

Urbaneja A, Stansly PA. 2004. Host suitability of different instars of the whitefly *Bemisia tabaci* 'biotype Q' for *Eretmocerus mundus*. *BioControl* 49:153–161.

Urbaneja A, Tapia G, Fernández E, Sánchez E, Contreras J, Gallego A, Bielza P. 2003. Influence of the prey on the biology of *Nesidiocoris tenuis* (Hem.: Miridae). *Bull. OILB/SROP* 26:159.

Urbaneja A, Tapia G, Stansly PA. 2005. Influence of host plant and prey availability in the developmental time and survival of *Nesidiocoris tenuis* Reuter (Het.: Miridae). *Biocontrol Sci. Technol.* 15:513–518.

Vacante V, Benuzzi M. 2002. Pomodoro, la difesa biologica e integrate. *Colture Protette* 8:27–33.

van Lenteren JC, Hulspas-Jordaan PM, Li ZH, Ponti OMB. 1987. Leafhairs, *Encarsia formosa* and biological control of whitefly on cucumber. *Bull. OILB/SROP.* 2:92–96.

van Lenteren JC, Herman JW, van Roermund HJW, Sutterlin S. 1996. Biological control of greenhouse whitefly (*Trialeurodes vaporariorum*) with the parasitoid *Encarsia formosa*: how does it work?. *Biol. Control* 6:1–10.

van Lenteren JC, Martin NA. 1999. Biological control of whiteflies. In *Integrated Pest and Disease Management in Greenhouse Crops*, ed. R Albajes, ML Gullino, JC van Lenteren, Y Elad, pp. 202–216. Dordrecht, The Netherlands: Kluwer.

Vatansever G, Ulusoy MR, Erklc LB. 2003. Improving the mass rearing possibilities of *Serangium montazerii* Fursch (Coleoptera: Coccinellidae) on different host plants of *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae). *Turkish J. Agric.For.* 27:175–181.

Vázquez LL. 2002. Advances in the biological control of the whitefly *Bemisia tabaci* in the Neotropical region. *Manejo Integrado Plagas Agroecol.* 66:82–95.

Viscarret MM, López SN. 2004. Biological studies on *Encarsia porteri* (Mercet) (Hymenoptera: Aphelinidae) an heterotrophic parasitoid of the *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) complex. *Biol. Control* 30:236–240.

Wang LD, Huang J. 2006. Damage by and biological control of *Bemisia tabaci* (Homoptera: Aleyrodidae). *J. Fujian Agric. For. Univ. (Natural Science Edition).* 35:365–371.

Wang L, Huang J, You M, Guan X, Liu B. 2005. Effects of toxins from two strains of *Verticillium lecanii* (Hyphomycetes) on bioattributes of a predatory ladybeetle, *Delphastus catalinae* (Col., Coccinellidae). *J. Appl. Entomol.* 129:32–38.

Wang XM, Ren SX, Xu CX. 2006. Morphological characters and biology of *Axinoscymnus apioides* introduced from Brunei. *Chinese Bull. Entomol.* 43:810–813. (Abstract).

Wright JE, Kennedy FG. 1996. A new biological product for control of major greenhouse pests. *In Brighton Crop Protection Conference: Pests and Diseases – 1996*, Vol. 3. *Proceedings of an International Conference*, Brighton, UK, November, 18–21, 1996. pp. 45–52 British Farnham, UK: Crop Protection Council.

Wright JE, Knauf TA. 1994. Evaluation of Naturalis-L for control of cotton insects. In *Proceedings – Brighton Crop Protection Conference, Pests and Diseases*, 1994, Vol. 1. pp.885–892. UK: British Crop Protection Council, BCPC Publications, Brackwell.

Xu WH, Zhu GR, Li GL, Xu BY, Zhang YJ, Wu QJ. 2003. Influence of temperature on the biology of *Encarsia formosa* parasitizing the whitefly *Bemisia tabaci*. *Chinese J. Biol. Control* 19:103–106.

Zang LS, Liu TX. 2007. Intraguild interactions between an oligophagous predator, *Delphastus catalinae* (Coleoptera: Coccinellidae), and a parasitoid, *Encarsia sophia* (Hymenoptera: Aphelinidae), of *Bemisia tabaci* (Homoptera: Aleyrodidae). *Biol. Control* 41:142–150.

Zhang SZ, Guo JY, Wan FH, Zhang F. 2004. Effect of temperature on the development, survival and longevity of *Encarsia formosa*. *Chinese J. Biol. Control* 20:174–177.

Zhang GF, Lu ZC, Wan FH. 2007a. Detection of *Bemisia tabaci* remains in predator guts using a sequence-characterized amplified region marker. *Entomol. Exp. Appl.* 123:81–90.

Zhang SZ, Zhang F, Hua BZ. 2007b. Suitability of various prey types for the development of *Propylea japonica* (Coleoptera: Coccinellidae). *Eur. J. Entomol.* 104:149–152.

Zhang GF, Lu ZC, Wan FH, Lövei GL. 2007c. Real-time PCR quantification of *Bemisia tabaci* (Homoptera: Aleyrodidae) B-biotype remains in predator guts. *Mol. Ecol. Notes* 7:947–954.

Zolnerowich G, Rose M. 2008. The Genus *Eretmocerus*. In *Classical Biological Control of Bemisia tabaci in the United States – A Review of Interagency Research and Implementation*, ed. J Gould, KA Hoelmer, JA Goolsby, Vol. 4, pp. 89–109. Amsterdam, The Netherlands: Springer.

# Chapter 16
# Ecological Determinants of *Bemisia tabaci* Resistance to Insecticides

**Steven J. Castle, John C. Palumbo, Nilima Prabhaker, A. Rami Horowitz, and Ian Denholm**

## Introduction

The global importance of *Bemisia tabaci* (Gennadius) offers unique opportunities to examine patterns of infestation among diverse habitats and identify major factors that determine pest status. Its occurrence on field, vegetable and ornamental crops grown under open or protected conditions in temperate or tropical environments plays a critical role in the pest status of *B. tabaci*. Management practices also figure heavily into the arcane formula that ultimately determines the severity of infestation and degree of crop damage caused by *B. tabaci.* Decades of experience have taught valuable lessons regarding problems that arise when management practices are inadequate or inappropriate to meet the challenge of a *B. tabaci* onslaught. In some cases, inadequacy has taken the form of over-reactive management that responded to burgeoning *B. tabaci* infestations with brute-force application of insecticides. Situations in which outbreaks progressed despite full-scale chemical intervention earned *B. tabaci* a worldwide reputation as a foremost recalcitrant pest. Effective new modes of action, some characterized by pinpoint selectivity, have recently improved prospects for stable management of *B. tabaci* populations. However, insecticide resistance remains the biggest impediment to achieving détente with *B. tabaci*. Progress towards combating resistance requires an understanding of the conditions under which resistance arises and identifying tactical measures that most effectively counteract resistance. The present review will examine ecological characteristics of *B. tabaci* that influence patterns of resistance in various agricultural settings. These characteristics will be further examined in the context of past episodes of resistance so that ecological and operational factors that exacerbated or mitigated resistance can be explored, all the while keeping an eye towards the future of *B. tabaci* management.

The history of *B. tabaci* as an agricultural pest is replete with documented episodes of over-reaching insecticide use that resulted in resistance development

S.J. Castle (✉)
USDA-ARS, Arid-Land Agricultural Research Center, 21881 North Cardon Lane, Maricopa, AZ 85138, USA
e-mail: steven.castle@ars.usda.gov

P.A. Stansly, S.E. Naranjo (eds.), *Bemisia*: Bionomics and Management of a Global Pest,
DOI 10.1007/978-90-481-2460-2_16, 

and impairment of field control. Aggressive insecticide applications have often been held responsible for tipping the balance between manageable infestation and uncontrolled outbreak. The defining example of this occurred over 30 years ago in the Gezira region of Sudan. An institutional program of insecticide sprays on cotton contracted to the former Ciba-Geigy Corp led to massive outbreaks of *B. tabaci* during the 1970s over the entire Gezira scheme. While insecticide resistance was implicated (Dittrich et al. 1985) in the loss of field efficacy, catastrophic reduction in natural enemy populations was also identified (Eveleens 1983) as a factor responsible for unchecked *B. tabaci* populations.

Two decades later, cotton was again at the center of a resistance crisis in the state of Arizona (USA). High resistance levels to individual compounds had led to increasing dependence upon synergized pyrethroid treatments that most often involved fenpropathrin+acephate as the preferred combination. This treatment failed by the end of the 1995 cotton season and opened the door for a radical shift to much more selective chemistry that featured two insect growth regulators (IGRs), buprofezin and pyriproxyfen (Dennehy and Williams 1997). Both of these products had been used successfully in Israel in combination with biological control to produce effective and sustained management of *B. tabaci* (Horowitz and Ishaaya 1992). In Arizona, buprofezin and pyriproxyfen formed the centerpiece of a new integrated pest management (IPM) approach that included strict resistance management guidelines for conserving each IGR, action thresholds for timing applications of either product, and sampling plans for determining population densities in relation to treatment timing. The practical result was a phenomenal improvement in control of *B. tabaci* coupled with a dramatic decrease in insecticide use, no small feat considering that the number of insecticide sprays directed at *B. tabaci* during the 1995 season averaged 6.6 across central Arizona, but by 1997 had dropped to 2 and continued downward to 0.4 sprays by 1999.

The equally momentous philosophical result was the introduction of the novel concept of bioresidual activity following insecticide treatment. In the Arizona example, this terminology related to the phenomenon of significantly enhanced mortality of *B. tabaci* by natural enemies in cotton treated with the more selective IGRs compared to broad spectrum insecticide treatments (Ellsworth and Martinez-Carrillo 2001; Naranjo 2001). The significance of the bioresidual effect from a resistance management perspective is that following a well-timed application of a selective insecticide, suppression of the target population will be extended by the natural mortality exerted by greater numbers of biological control agents, thus avoiding or delaying subsequent treatments and thereby reducing exposure to the selective force of insecticides.

The new era of IPM for *B. tabaci* ushered in by the Israel and Arizona experiences has continued to be boosted by development and commercialization of effective insecticides, some of them highly selective against *B tabaci*. The challenge for IPM in general and insecticide resistance management (IRM) programs in particular will be to incorporate the newer chemistries into viable control programs that emphasize conservation of natural enemies and active ingredients. This task has been complicated to some degree by the expansion of certain classes of

insecticides such as the neonicotinoids that provide multiple choices of compounds and modes of application acting at the same target site, thereby elevating risks of cross-resistance (Nauen and Denholm 2005). The need for knowledgeable coordination of diverse modes of action is now greater than ever to minimize selection pressure against particular compounds or classes of insecticides. In addition to considering earlier resistance episodes as well as the characteristics of *B. tabaci* that led to resistance in the past, this review will emphasize the opportunities going forward to incorporate newer modes of action into sustainable management programs for *B. tabaci*.

## Ecological Characteristics of *B. tabaci* as a Resistance Recidivist

As the management crisis in the Sudan Gezira was playing out during the late 1970s, *B. tabaci* was being challenged by insecticides in many parts of the world. In a mini review of chemical control for *B. tabaci*, Sharaf (1986) included numerous references from India, the Middle East and North Africa where insecticides were being used for control of infestations and viral diseases vectored by *B. tabaci*. It is not surprising that other examples of resistance occurrences began to mount about the same time as the Sudan crisis was being reported. Previous reviews have discussed early cases of resistance in *B. tabaci* to conventional classes of insecticides that include organochlorines, organophosphates (OPs), carbamates and pyrethroids (Dittrich et al. 1990; Denholm et al. 1996). The pattern of resistance to newer conventional compounds as well as to new modes of action has continued to develop as novel materials have been implemented for *B. tabaci* control worldwide.

The question of why *B. tabaci*, among other select arthropod pests, continues to register new cases of resistance to new compounds in old places, or to old compounds in new places, must be addressed to advance a more complete understanding of the resistance phenomenon. Two fundamental possibilities come to mind regarding the repetitive occurrence of resistance in *B. tabaci*, i.e. (1) that the potential for resistance evolution in *B. tabaci* populations is intrinsically greater than for most other pest species, thereby supporting its reputation as a resistance recidivist, and/or (2) that the ecological potential of *B. tabaci* is much greater and is geographically more wide ranging than other arthropods so that resistance potential is increased probabilistically as a function of exposure. These two possibilities are obviously not exclusive of one another and it is likely that resistance in *B. tabaci* is a product of both. We will examine different ecological characteristics of *B. tabaci* that likely influence its propensity to develop resistance.

### *Polyphagy*

The typical understanding of *B. tabaci* as a broad feeder and colonizer of scores of plant species in many different families is derived from the great majority of

*B. tabaci* populations worldwide. However, *B. tabaci* is a conglomerate of genetically distinctive races, biotypes, or sibling species that vary in numerous traits including host range. One of the first clues that *B. tabaci* was more than a single homogeneous strain was the occurrence of isolated populations that appeared to have very narrow feeding ranges. The best-known example of this is a host race of *B. tabaci* found in Puerto Rico only on *Jatropha gossypifolia*, (Bird 1957). More recently, a biotype specializing on cassava was identified among other polyphagous biotypes in Africa (Burban et al. 1992; Legg 1996). Among the predominant polyphagous biotypes, there are also considerable host range differences that are poorly understood. Part of the difficulty in exploring host ranges is the sheer number of candidate plant species that may or may not serve as hosts. In addition, experimental host range is often a poor indicator of the realized host range in the agro-environment. A good example was seen with the indigenous biotype A of North America that was most likely competitively displaced during the invasion of biotype B in the late 1980s and early 1990s. The experimental host range of biotype A in the greenhouse included lettuce, squash and broccoli (Coudriet et al. 1985, 1986) but was restricted principally to cotton in the field except during fall when cotton crops were terminated and whiteflies emigrated onto lettuce and melons resulting in epidemics of lettuce infectious yellows virus (Duffus and Flock 1982). In contrast, the much more polyphagous biotype B readily colonizes broccoli, lettuce, cantaloupes and alfalfa in the field in addition to cotton (Perring et al. 1991). The proliferation of biotype B on all of these crops enabled much higher population densities year-round and was an important factor in the complete displacement of biotype A from the Southwestern USA and perhaps much of North America (Perring et al. 1993; Brown et al. 1995).

The consequences of polyphagy from a resistance perspective relate to the potential for greater exposure in a population that colonizes multiple crops within a few short generations. Intensive insecticide treatments that are sustained over consecutive generations due to perpetuation of populations across sequential, high value crops can result in continuous exposure and longer selection periods. However, diverse insecticide use across multiple crops may reduce selection pressure by one particular mode of action compared to a single main crop where overreliance on one mode of action may occur. Although experimental validation of these various scenarios represents a major challenge, at least one example from the literature supports the positive effect that polyphagy can have on resistance development. In Australian cotton, the absence of resistance to fenvalerate and endosulfan in *Helicoverpa punctigera* (Wallengren) compared to a progressive increase in *H. armigera* (Hübner) was attributed to the much greater polyphagy in *H. punctigera* that enabled it to more effectively exploit non-cropped areas and other untreated refuges (Forrester et al. 1993).

Another possibility dealing with host range breadth is that intrinsically greater polyphagy often correlates with ability to detoxify plant secondary compounds. Whatever the physiological mechanism that permits certain biotypes to utilize a wider host range may also preadapt those biotypes to resist other toxic chemistries. The biochemical preadaptation hypothesis (Gordon 1961; Rosenheim et al. 1996)

predicts that phloem and xylem feeders have less capacity towards resistance development to insecticides than leaf feeders due to reduced levels of defensive compounds that occur in the plant's vascular system. However, this hypothesis does not address the more specific example of monophagous vs. polyphagous phloem feeders where the polyphagous insect undoubtedly encounters more diverse secondary compounds and is therefore potentially better preadapted to defend against xenobiotics.

The distinction between narrow and broad-range feeders is an important one because of different strategies they employ with respect to the many noxious chemicals produced by their host plants. Just as most phytophagous insects have specialized on particular host plants (Strong et al. 1984), recent evidence points to some degree of specialization of their biochemical detoxification mechanisms. In the swallowtail butterfly family Papilionidae, substrate-specific CYP6B1 and CYP6B3 enzymes contribute to specialization of *Papilio polyxenes* on furanocoumarin-containing host plants, whereas CYP6B4 and CYPB17 enzymes in the polyphagous *P. glaucus* and *P. canadensis* have a much broader range of substrates (Mao et al. 2007). It has been conjectured that among polyphagous *B. tabaci* biotypes, Q may be more resistance prone than B. It is doubtful that any *B. tabaci* biotype exceeds the host range capacity of biotype B, but the difference may lie in specific plants that fall within the native range of biotype Q but not biotype B. If there is any credibility to the preadaptation hypothesis, then certain plant species potentially utilized by biotype Q having higher loads of secondary compounds may have selected for more potent detoxification capabilities in biotype Q that we now see expressed as higher resistance levels. At this point, it is still too early to determine if biotype Q is intrinsically predisposed to higher levels of resistance than biotype B, or if apparent differences between the two biotypes are due more to a history of heavy pesticide exposure in recent years that has selected for higher resistance levels in biotype Q.

## *r-Selection*

There are a host of ecological traits that identify an organism on the r-K selection continuum (Macarthur and Wilson 1967), a concept that continues to serve heuristically for identifying differences in life history patterns despite theoretical deficiencies (Stearns 1977). One of the problems with applying this concept to an insect such as *B. tabaci* is the difficulty of fully comprehending the degree to which it bears the characteristics of an r-selected life history. Most insect pests are recognized as r-selectionists by their ability to rapidly colonize unstable habitats such as agroecosytems and exploit crop resources through a combination of traits that include high reproductive rates, short generation times and effective dispersal. The fullest expression of these traits in an optimal environment is recognized in the biotically potent *B. tabaci* and evidenced by numerous historic outbreaks. Anecdotal evidence bears witness to its biotic potential such as photographs of *B. tabaci* "clouds" in California's Imperial Valley during the 1991 outbreak (see

Naranjo et al. Chapter 6) or by published observations such as "... flocks of the insect could be seen moving along the streets" in describing outbreak conditions in Brazil (Costa 1975).

The reproductive potential of *B. tabaci* is tremendous and drives populations to explosive levels under optimal conditions. Extreme densities of *B. tabaci* provide the ideal genetic testing ground whereby resistance (R) alleles already present in the population gene pool can be selected by insecticide treatments and rapidly increased each generation. The evolution of novel R alleles can also be favored by large populations in which normal background mutation rates can produce insecticide-resistant mutants. The probability of novel R alleles arising in the population is a density-independent process, but the chances for survival of rare alleles may improve in a larger gene pool and in a haplodiploid insect such as the *B. tabaci* male in which R alleles are exposed to selection without necessarily being masked in heterozygote genotypes (Denholm et al. 1998). The Allee effect represents an increased risk of extinction for a species, and by extension a genotype, because of inverse density dependence at low density (Courchamp et al. 1999; Liebhold and Tobin 2008). This effect is lost at high densities and the opportunity for novel R alleles to perpetuate is potentially enhanced.

In addition to high reproductive rates and large populations, generation times for *B. tabaci* can be very rapid in a high-fitness environment. During long, hot summer days in temperate latitudes, development from egg to adult can occur in as little as 13–14 days. Wagner (1995) measured a mean developmental time of $17.2 \pm 3.07$ days for *B. tabaci* at a constant temperature of 27.56°C. It has been estimated that there are 13 generations of *B. tabaci* per year in the desert agricultural regions of Southwestern United States (Palumbo et al. 2001), many of those occurring from April through November when melon, cotton and vegetable crops are being protected with insecticides. Generation time represents the interval during which selection takes place (Tabashnik 1990) and is often treated as a critical variable in simulation models.

## *Dispersal*

The nature of gene flow within and among populations is a critical aspect of resistance evolution, especially when a mosaic of selective forces within an environment is acting upon genotypes. Variation in insecticide treatment regimens among fields can result in a patchwork of different R alleles that mix according to dispersal rates between fields and subsequent mating interactions, potentially resulting in multifactorial resistance (Hemingway et al. 1987; Grafius 1995). There are also susceptible (S) alleles being carried by dispersing *B. tabaci* that are mixing in the gene pool and affecting the expression of resistance in the population. The degree to which SS genotypes are conserved in the metapopulation and encounter RR or RS individuals through dispersal and mating has figured prominently in theoretical studies of resistance evolution. Numerous simulation models have demonstrated how immigration of susceptible individuals can slow or suppress resistance (Comins 1977; Taylor

and Georghiou 1979; Tabashnik and Croft 1982). By the same token, migration of resistant individuals into untreated areas can also speed resistance development (Comins 1977) and reduce the advantage conferred by an untreated refuge.

Dispersal of *B. tabaci* in open fields has been evaluated in various studies by mass marking a population with fluorescent pigment dust and recovering individuals in traps placed at various distances and heights above the source canopy. Distances traveled have consistently been measured at the farthest trap distance of 2.7 km, prompting the suggestion that the effective migration distance may well be greater than this (Byrne et al. 1996). The majority of *B. tabaci* adults taking off from a crop are not migratory but rather foraging flyers (Isaacs et al. 1998). Although longer distance flights are associated with migratory forms, foraging flyers are also capable of traveling long distances in search of suitable host plants. Dispersing *B. tabaci* have been observed (by SJC) to move incrementally by temporarily adopting feeding and sheltering hosts that normally are not colonized, especially during mid day when temperatures may be too hot for sustained flight. As conditions become more suitable, foraging flights can continue so long as whiteflies derive sufficient nutrition and energy from their makeshift hosts. Thus, it is clear that *B. tabaci* is capable of traveling multi-kilometer distances when dispersing from one field to another. The number of dispersing whiteflies increases as infestations build in fields and crowding and host plant quality become factors. Higher densities of *B. tabaci* also elicit more frequent insecticide applications and increase selection pressure for higher resistance. Thus, a convergence of forces acts to spread R alleles, especially if insecticide treatments are relatively ineffective at reducing infestations.

## *Adaptability*

The wide distribution of *B. tabaci* in diverse habitats on six continents is alone evidence of its ecological adaptability. Perhaps our view of *B. tabaci* as a widely distributed pest species is distorted by the fact that all variants of *B. tabaci* are still being lumped under the one species. Even so, if focus is placed only on biotype B, its current worldwide distribution and range of habitats from temperate deserts to humid tropics is no less remarkable than *B. tabaci sensu lato*. One of the adaptations that greatly increase the geographic range of *B. tabaci* is its capacity to tolerate high temperatures and flourish in hot climates. The novel biochemical mechanism of synthesizing sorbitol from dietary carbohydrates offers effective protection against temperatures in excess of 40°C (Wolfe et al. 1998; Salvucci et al. 2000). In contrast to the cool temperature-adapted *Trialeurodes vaporariorum*, the thermoprotective mechanism of *B. tabaci* enables it to inhabit the warm climate zones where agricultural production is often large scale and year-round. Fertilizer-enriched crops expressing high rates of growth in warm environments provide an abundant resource for *B. tabaci* populations. Rapid growth of these populations in turn elicits a management response that sometimes involves excessive chemical treatments and

resistance development. Protected environments for growing vegetable or ornamental crops also create a rich resource for *B. tabaci* and are intensively treated with pesticides. The global expansion of protected agriculture and horticulture over the past two decades has provided new opportunities for biotypes B and Q in regions where it normally could not survive. The increased global distribution of protected agricultural products that first brought biotype B to much of the world is now also distributing biotype Q. What was formerly known as an outdoor crops pest has now become a major pest of glasshouses and plastic culture environments of the type made famous by the Almeria region of Spain. Some of the most prominent cases of resistance have occurred in Almeria and other protected agriculture regions of the world in recent years (Cahill et al. 1996; Elbert and Nauen 2000; Nauen et al. 2002; Rauch and Nauen 2003). The tremendous range expansion of *B. tabaci* over the past two decades, in particular biotypes B and Q, has subjected populations to heavy insecticide exposures and fueled the way for resistance development.

## *Integrated Control*

The analysis of the Sudan crisis by Eveleens (1983) argued that intensive pesticide use severely disrupted natural enemy populations that had previously held *B. tabaci* populations in check. This sentiment was echoed additional times (Sundaramurthy 1992; Johnson et al. 1982; Byrne and Devonshire 1993) and was considered by many to be the largest factor responsible for crisis situations (Byrne et al. 1990). This raises the question of whether it is feasible to control field populations of *B. tabaci* with whitefly parasitoids and other biological control agents without use of insecticides. Hoelmer (1996) suggested a number of factors to explain why *B. tabaci* has continued to present difficult problems for biological control, including dispersal behavior, diversity of cropping patterns and the many crop plants colonized by *B. tabaci.* These are critical points towards understanding both the success of *B. tabaci* in a particular agro-ecosystem and the challenges posed in terms of regulating *B. tabaci* populations by natural enemies. Permissive agro-ecosystems for biotype B are characterized by a favorable mix of temporally overlapping vegetable and field crops grown year-round and a hot, arid climate with mild winters, e.g. Imperial Valley, California. A less favorable system may not contain the optimal blend of crops or might be characterized by a cooler and wetter climate inhibitory to rapid population growth of *B. tabaci*, e.g. the Rhône Valley in France. The challenges for biological control in the permissive environment may be insurmountable, whereas in the less favorable environment may be minimal and therefore more easily overcome. It is the permissive environments of the world, forged by a combination of climate and cropping patterns, in which *B. tabaci* will always present the biggest challenge to management and probably always require chemical intervention to avoid economic damage.

The synoptic population model (Southwood and Comins 1976) was developed to help in the understanding of essential dynamical features of insect populations (Southwood 1977). Among numerous parameters of the model are habitat

stability and life-history strategy characterized by the r-K continuum. A key feature of the model is the "natural enemy ravine" in which K-strategist pests are generally held in check by natural enemies, whereas r-strategist pests will frequently achieve pest level if they invade the ephemeral crop early enough. Southwood (1977) suggested that control of inherently booming populations of r-pests will almost always rely upon insecticides because of instability in the system that r-strategists so effectively exploit. Growth rates of r-selected pests such as *B. tabaci* in a permissive environment will preclude regulation by natural enemies, and dispersal to new crops will introduce the instability that thwarts natural enemies in their searches for prey. Well-timed application of insecticides is necessary to prevent *B. tabaci* from climbing the epidemic ridge as depicted in the three-dimensional synoptic model. The killing power of insecticides reduces the population growth rates of the r-selected pests, not only by lowering their population densities, but in some cases by slowing development of surviving immatures and/or reducing reproductive rates. The ideal insecticide may be one that can accomplish these actions against the r-selected pest without eliminating natural enemy populations in the process. The slowing of growth and reproductive rates without diminishing biological control agents could take away some of the "r-selectiveness" inherent in the pest and provide an opportunity for natural control to effectively regulate the r-pest populations.

## Agro-Ecology of Resistance

The nature of the agro-environment in which ecological traits of *B. tabaci* find their expression is critical to the maintenance of susceptibility or development of resistance. Management practices, in particular chemical control, are another major determinant of the resistance path that might vary considerably in length and directness. That resistance is something other than monolithic was astutely recognized by Sawicki (1987) who suggested that both a shift in susceptibility to an insecticide and control failure could be considered manifestations of resistance, but that the former need not result in loss of control. With *B. tabaci*, many of the ecological traits and environmental conditions discussed earlier have combined with loss of susceptibility to produce unambiguous control failures at a frequency rarely seen in the vast majority of agricultural pests.

### *Agro-Environment*

#### Closed Versus Open Systems

Expansion of the pestiferous habitat of *B. tabaci* from open to protected agriculture began with the introduction of biotype B into Florida sometime in the mid-1980s. Biotype B spread rapidly to other states in the USA on transported plant material grown in glasshouses and nurseries before eventually breaking out into open fields. However, it has remained a significant pest of ornamentals and other plants in protected agriculture in North America even as it rose to super pest levels in open

agriculture. The same mechanism of spread by live plant traffic has continued with the invasion of additional continents by biotype B (Oliveira et al. 2001). Perhaps a more significant example of the adoption of protected agriculture by *B. tabaci* is the story of biotype Q that rose to major pest status in the protected agriculture environment of Almeria, Spain. Prior to the development of intensive, protected agriculture in southern Spain beginning in the early 1980s, biotype Q was likely an innocuous insect that had little impact on open agriculture. As the protected agricultural industry grew in southern Spain and other Mediterranean locations, so did the pest status of biotype Q. As with biotype B, considerable spread of biotype Q to multiple continents has occurred with subsequent reports of resistance problems associated primarily with protected agriculture. However, there are areas in open agriculture where biotype Q has become well established and where resistance may have played a factor in sustaining biotype Q at higher densities than biotype B (Horowitz et al. 2005; Khasdan et al. 2005; Chu et al. 2007). It is likely that resistance to pyriproxyfen in biotype Q in Israel first evolved in glasshouses before appearing in field populations where resistance has been maintained in some areas by regular use of pyriproxyfen (Horowitz et al. 2002).

Crops grown in glasshouses or under similar protected environments are often high value with extremely low damage or infestation thresholds, sometimes referred to as the "aesthetic threshold" (Oetting and Buntin 1996). Aggressive chemical management is commonly exerted to prevent establishment and buildup of *B. tabaci* and other arthropod populations. Avoidance of pest populations by propagation within a clean greenhouse and the use of insect proof screens and filters on air intake systems can prevent or substantially delay the growth of pest populations and reduce the dependency on insecticides (see also Stansly and Natwick Chapter 17). However, intensive production schedules often preclude establishment of pest-free environments at the time of crop start-up, and sub-standard greenhouses in many areas provide access to external pests. In Almeria and other intensive production areas of southern Spain, greenhouse structures enclosed mostly by plastic have been used for the intensive production of vegetables for domestic and export markets (Moreno et al. 1994). Open vents and other points of vulnerability have enabled encroachment by various pests, foremost among them being *B. tabaci* biotype Q. Elevated greenhouse temperatures permit rapid population growth rates that too often require insecticide treatments for suppression. Climate conditions in southern Spain and throughout the Mediterranean basin generally support year-round populations of *B. tabaci* so that movement into porous greenhouses can occur at any time that new crops are being established. However, once established within a greenhouse, population increase is mostly due to internal growth dynamics as higher temperatures and favorable hosts promote faster growth inside greenhouses. The semi-closed populations may be subjected to intensive insecticide pressure and reduced gene flow due to lower densities outside the greenhouses and impeded movement into and out of greenhouses. Consequently, high resistance levels have been detected in biotype Q populations originating from southern Spain (Elbert and Nauen 2000; Nauen et al. 2002; Rauch and Nauen 2003). In the Israeli example, high resistance to pyriproxyfen (>500-fold) developed in *B. tabaci* following three successive applications in

a rose greenhouse (Horowitz et al. 1999). Pyriproxyfen resistance did not develop nearly so fast in Israeli cotton fields, having benefited by the resistance management restriction that permitted only a single use per year in cotton. However, after 4 years of use in the Ayalon Valley of Israel, resistance to pyriproxyfen was comparable to that observed in the rose greenhouse (Horowitz et al. 2002). Resistance was less pronounced in other cotton growing regions such as the western Negev, a result possibly explained by differences in agroecology and topography among growing regions. It was suggested that Ayalon Valley is isolated with few alternative hosts other than sprayed crops, thus forming a closed system tantamount to a "greenhouse effect" (Horowitz et al. 2002).

### Intermittent Monoculture Versus Continuous Polyculture

Differences in agricultural environments such as those briefly described for Israel affect the phenology and growth of *B. tabaci* populations and the intensity of insecticide treatments used for control. Agricultural regions vary from one site to another in terms of the scale, intensity, duration, and types of crops grown. The more diverse the cropping within a region, the greater likelihood that different types of crops will overlap to provide a continuous food resource for a polyphagous pest like *B. tabaci*. Spatial scale can be an important determinant of cropping diversity within a region, especially in warm temperate or tropical regions where climate permits year-round agriculture. Greater areas of individual crops can be grown, but a large agricultural region also provides the luxury of land that can lie fallow or in preparation for a subsequent crop having a different growing season, thus enabling sequential crop production and ensuring a continuous food supply for highly mobile populations of *B. tabaci*. Areas with year-round production of diverse crops are continuous polycultures and represent optimal environments for *B. tabaci* populations. This description fits the Imperial Valley that has an arable land area over 200,000 ha with rotational plantings of vegetables, cucurbits and cotton and a continuous crop of alfalfa that alone occupies ca. 40% of the entire land area. This kind of resource base coupled with the hot and arid climate of the Imperial Valley drives *B. tabaci* populations to densities as high as any place on earth and forces intensive use of insecticides for crop protection. Chemical treatments are applied over crops during each growing season, causing sustained exposure across many generations. Although heavy and continuous insecticide pressure should be among the most difficult situations in terms of conserving susceptibility to insecticides, *B. tabaci* populations in the Imperial Valley and the adjacent growing region of Yuma, Arizona have actually remained variably susceptible to most treatments used against them (Castle et al. 1996; Palumbo et al. 2001; Prabhaker et al. 2005). Differences in susceptibility among populations have been frequently observed according to location or season (Prabhaker et al. 1992), but there has not been the type of resistance crisis in Yuma or the Imperial Valley that occurred in central Arizona in 1995 (Dennehy and Williams 1997).

In contrast to the continuous polyculture, intermittent monoculture is characterized by a dominant crop within an agricultural region. Although perennial crops are

often densely planted to form a monoculture, the concern with *B. tabaci* is more with annual crops planted new each year, especially where one crop occurs in excess of all others. In many regions, cotton is planted as a single dominant and irrigated crop during summer growing seasons that may alternate with winter cereal crops that are non-hosts for *B. tabaci*. Essentially then, there is only one major crop during the annual cycle that *B. tabaci* populations infest to potentially damaging levels. This is the nature of the Ayalon Valley in Israel where it is relatively isolated and surrounded by inhospitable and non-cropped habitat (Horowitz et al. 2002). In common with the monoculture habitat of the Ayalon Valley is the intensive cotton-growing region of central Arizona. At the time of the resistance crisis in 1995 (Dennehy and Williams 1997), 85% of the cropped area of Pinal County was planted to cotton with only 13% planted to alfalfa (Fig. 16.1). This agricultural area of central Arizona is surrounded by desert vegetation and also is sparsely populated, so there are not many alternative host plants for *B. tabaci* other than the major cotton crop and the minor alfalfa crop. Consequently, a large proportion of the population was undoubtedly exposed to the intensive insecticide treatments that were applied through the early 1990s, culminating in treatment failures in 1995. A similar scenario was experienced in the cotton-intensive regions of Pakistan (Ahmad et al. 2001). In contrast, only 4% of

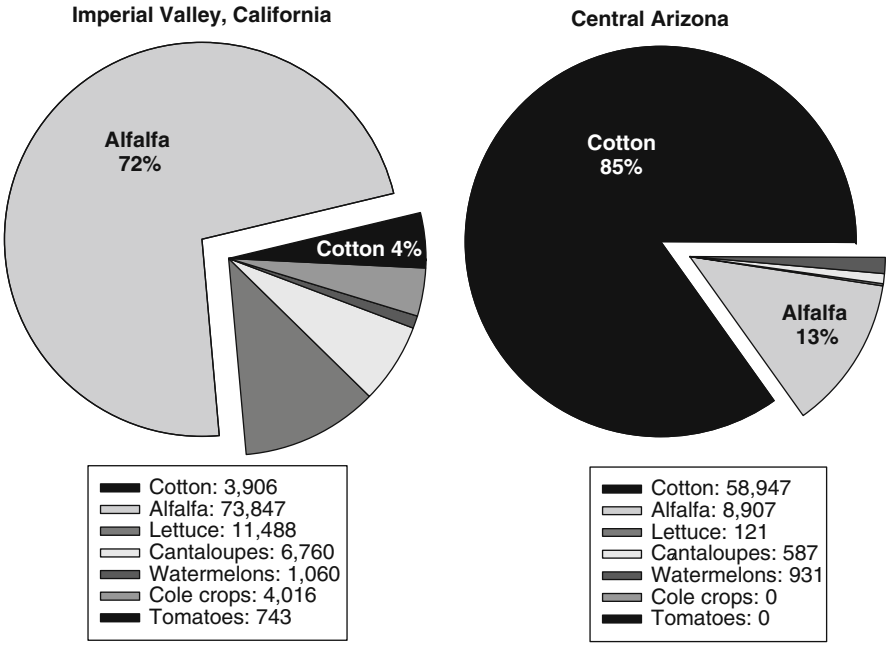



**Fig. 16.1** Relative areas (ha) of *Bemisia tabaci* host crops grown in the Imperial Valley, California and in Pinal County, central Arizona in 1995. The cotton crops in both locations were intensively sprayed with insecticides whereas the alfalfa crops were effectively untreated for the control *B. tabaci.* Percentages are calculated on the basis of total *B. tabaci* host crop areas for each location

*B. tabaci* host crops in 1995 were represented by cotton in the Imperial Valley, and this does not include a large number of ornamental hosts that make up the landscape of small to medium sized cities throughout the Imperial Valley. Although large numbers of whiteflies are treated by insecticides in cotton and other crops that *B. tabaci* infests, overall they are a minor proportion of the meta-population in the Imperial Valley, much of it unexposed to insecticides on alfalfa and ornamentals.

The well-developed capacity for dispersal of *B. tabaci* when populations are increasing and treatments become more intense, especially during summer cotton season, helps to promote the pairing of resistant and susceptible genotypes and mitigate the within-cotton field selection pressure favoring homozygous or hemizygous resistant genotypes. It is something of a paradox that the diversity of the continuous polyculture in the Imperial Valley and other similar habitats promotes rapid and pestiferous population growth of *B. tabaci* that requires aggressive chemical treatments to avoid economic damage in sensitive crops, but simultaneously provides a vast untreated refuge area in alfalfa and ornamental hosts that conserves susceptible genotypes and helps moderate resistance occurrences. In the cotton monoculture of central Arizona, *B. tabaci* populations develop high population densities much later in the summer due to the paucity of spring-planted vegetable or cucurbit crops that generate earlier population pressure. Thus, efforts to control *B. tabaci* populations are concentrated in cotton as opposed to being spread over several crop seasons from spring through fall in the continuous polyculture. It may be a bit counterintuitive that *B. tabaci* populations targeted in a single crop are more prone to resistance development than the longer duration infestations and more continuous generations targeted in multiple crops. But this has been the pattern in the Imperial Valley that has not experienced the severe resistance that occurred in the cotton monoculture of central Arizona (Dennehy and Williams 1997). The agricultural contrast between the Imperial Valley and central Arizona is similar to the situation in Israel where the cotton monoculture of the Ayalon Valley in the 1990s contrasted to the more diverse cropping of the western Negev region. Arguably greater selection pressure in the Ayalon Valley resulting from a higher proportion of the regional *B. tabaci* population being exposed to pyriproxyfen treatments resulted in more rapid and greater occurrence of pyriproxyfen resistance than the Negev region (the "ecological bottleneck" effect sensu Denholm et al. 1998). Greater dispersal of *B. tabaci* among a wider range of host crops and ornamental plants in the Negev meant that a lower proportion of the overall population was being exposed to pyriproxyfen, thereby conserving a greater proportion of susceptible genotypes.

## Patterns of Resistance

### *Stable Versus Unstable Resistance*

Insecticide resistance is a dynamic process influenced by the frequency of resistance genes and the biotic and environmental forces that determine their inclination

and trajectory within a population, i.e. its quantitative expression. Resistance is also highly variable depending on the nature of the mechanism that confers resistance, i.e. its qualitative expression. The relative constancy of resistance from one generation to the next depends in part on the balance between positive and negative selection forces that either reinforce or reduce gene frequencies. A gene that arises within a population by mutation or immigration may be advantageous in a particular environment, e.g. insecticide-treated crops, but in the absence of insecticide applications causes a relative loss of fitness compared to susceptible genotypes and a subsequent decline in the frequency of that gene. Susceptibility can be regained in a population as occurred with *B. tabaci* populations in central Arizona relative to the synergized pyrethroid mixture of fenpropathrin+acephate. Following the resistance crisis in 1995 and the adoption of the IGR-based strategy beginning in 1996, resistance monitoring surveys conducted in 1996 indicated that whiteflies were already substantially more susceptible to fenpropathrin+acephate (Dennehy and Williams 1997). Similarly, a decline in pyriproxyfen resistance was observed in the Ayalon Valley of Israel following cessation of its use after the 1997 season. In the western Negev region, susceptibility to pyriproxyfen was regained following greater use of neonicotinoid insecticides on predominantly B-biotype populations (Horowitz et al. 2002).

Resistance often increases in conjunction with the application of a selecting mode of action but recedes if the population shifts to a different crop where insecticides are not used or a different mode of action is substituted. The reversion of resistance may be due to the disadvantage of individuals with major genes for insecticide resistance (Via 1986), i.e. the fitness cost. Another possibility is that original selective regimes prior to the onset of pesticide treatments tend to be restored when those treatments are suspended (Uyenoma 1986). However, a return to pesticide susceptibility upon suspension of treatments should not always be assumed due to the potential for limited or no selective differential to occur between susceptible and resistant genotypes. Moreover, the interval for restoration of susceptibility may be considerably extended relative to the interval to resistance due to a lower intensity of selection for susceptibility (National Research Council 1986).

Recession of resistance may commonly occur when a resistant population collected in the field is reared in the laboratory free from exposure to insecticides. In the case of one *B. tabaci* population collected in Maricopa, Arizona, the initial imidacloprid bioassay conducted on the day of collection (01 September 2004) indicated a susceptible population (Fig. 16.2). Field populations in central Arizona during late summer are often under tremendous environmental stress brought on by deteriorating host quality in late season cotton fields and extremely high temperatures. After culturing on cotton without exposure to insecticides, resistance to imidacloprid was subsequently revealed following 1–2 generations in stress-free conditions in the greenhouse on high quality host plants. The highest resistance in this colony was observed nearly 5 months later, after which time the colony became increasingly susceptible to imidacloprid (Fig. 16.2). In the absence of imidacloprid or other neonicotinoid exposure, the gene(s) conferring resistance in this laboratory strain probably declined to a low frequency or disappeared altogether.

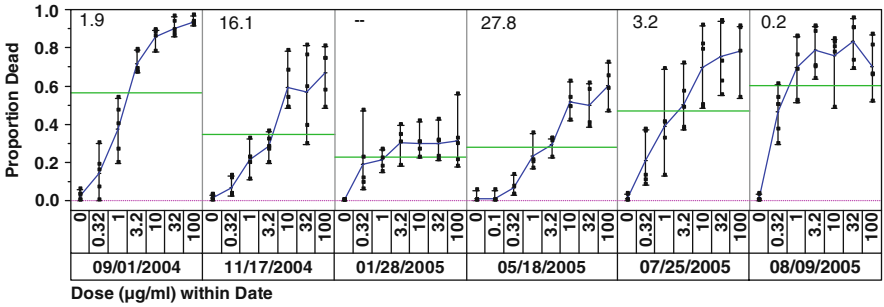



**Fig. 16.2** A series of imidacloprid bioassays conducted on a field-collected (01 September 2004) strain of *B. tabaci* demonstrates unstable resistance when maintained on cotton as a laboratory colony free from insecticide exposure. After establishment as a robust laboratory colony, resistance expression increased through 28 January 2005 before gradually receding 10 months later. In each *panel*, points represent individual replicates at particular doses. The *vertical lines* connecting these points indicate the range of mortality within a dose. The *line* connecting doses joins the mean mortality at each dose at the point of its intersection with the *vertical range lines*. *Horizontal lines* in each *panel* indicate the mean mortality among all doses; $LC_{50}$s are given in *upper left corners* of each *panel* (SJ Castle unpublished data)

The often-observed pattern of declining resistance in an unselected laboratory colony is not universal. Resistance levels in neonicotinoid-resistant Q biotypes have been reported to remain stable when maintained in the laboratory without selection pressure (Nauen et al. 2002; Rauch and Nauen 2003). The principal resistance mechanism in these strains appeared to be metabolic as indicated by elevated activity of P450-dependent monooxygenases (Rauch and Nauen 2003). A similar pattern of stable resistance was observed with a Q-type sample collected in an Almeria greenhouse in October 2002, shipped to the University of California, Riverside quarantine facility, and maintained in isolation without insecticide exposure for 18 months before conducting toxicological bioassays. The first tests conducted in April 2004 and thereafter revealed extraordinary levels of resistance to neonicotinoid insecticides (Fig. 16.3A, Prabhaker et al. 2005). A follow-up test was performed using acetamiprid and imidacloprid with or without simultaneous exposure to the synergist piperonyl butoxide (PBO). The unchanging response of the biotype Q strain from Spain with respect to PBO exposure (Fig. 16.3B) may indicate a resistance mechanism other than oxidative metabolism or the expression of a monooxygenase unaffected by PBO inhibition. Evidence of target site resistance to neonicotinoids has been exceedingly scarce (ffrench-Constant et al. 2004), but recently was demonstrated unequivocally in the brown planthopper *Nilaparvata lugens* (Liu et al. 2005). Further characterization of the biotype Q strain at UC Riverside was terminated due to contamination and eventual displacement by biotype B.

## *Rates of Resistance Evolution*

Theoretical models of resistance evolution have most often dealt with the simplest genetics by assuming a single locus with two alleles in a diploid organism

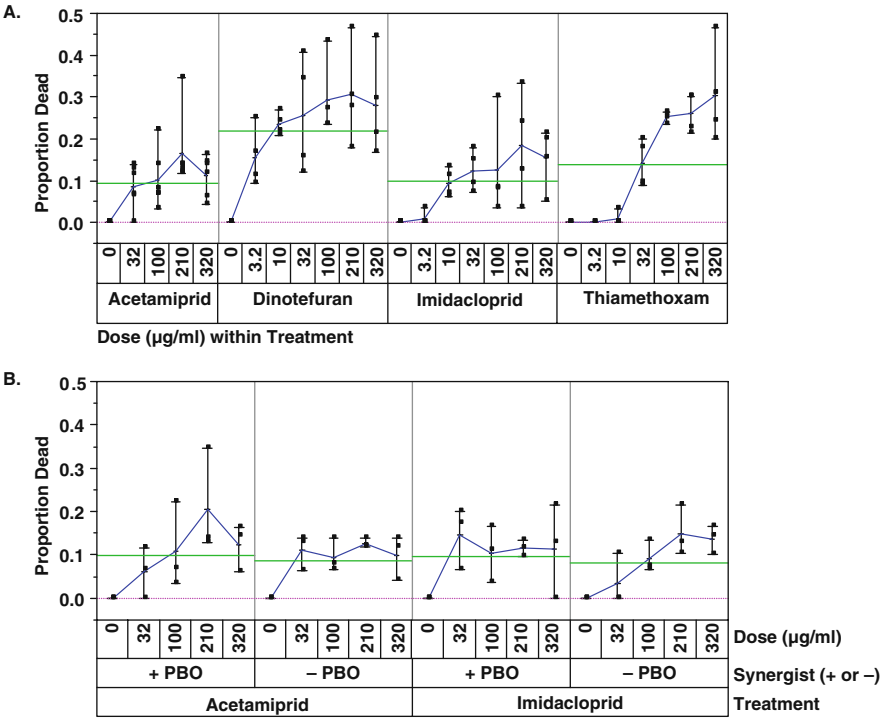



**Fig. 16.3** Bioassay test results from a *B. tabaci* biotype Q colony collected from Almeria, Spain and (**A**) challenged by four neonicotinoid insecticides 18 months after establishment as a laboratory colony without insecticide exposure. (**B**) Simultaneous exposure to the synergist piperonyl butoxide during bioassay had no effect on mortality (from Prabhaker et al. 2005). In each *panel*, points represent individual replicates at particular doses. The *vertical lines* connecting these points indicate the range of mortality within a dose. The *line* connecting doses joins the mean mortality at each dose at the point of its intersection with the *vertical range lines*. *Horizontal lines* in each *panel* indicate the mean mortality among all doses

(Georghiou and Taylor 1977; Taylor 1983; Tabashnik and Croft 1985; May and Dobson 1986). The time to resistance predicted by these models of course varies depending upon parameters used for model construction and how they are adjusted for hypotheses testing. Tabashnik (1990) reviewed the factors emphasized in numerous resistance models under biological, operational, and economic categories and focused on three specific factors that influence time to resistance. He summarized from different models that resistance will evolve faster as (a) reproductive potential increases, (b) immigration of susceptible individuals decreases, and (c) insecticide use increases. High reproductive rates and rapid turnover of generations in *B. tabaci* enables selection for resistance to proceed over a shorter time period than what might be expected for a species with a longer generation time. Examples of 24 species of apple orchard arthropods (Tabashnik and Croft 1985) and seven other arthropod species exposed to soil applications of aldrin and dieldrin (Georghiou and

Taylor 1986) were compiled by Tabashnik (1990) to reveal a significant relationship between number of generations per year and the rate of resistance development. Another survey of resistance cases revealed that the absolute time required for a significant degree of resistance to appear varied between 5 and 100 generations but with most cases between 5 and 50 generations (May and Dobson 1986). Despite general empirical support of the negative correlation between generation time and resistance, there are important exceptions, most notably the Colorado potato beetle that has only 2–4 generations per year. A subsequent analysis of empirical data by Rosenheim et al. (1990, 1991) departed from the emphasis placed on generation time and concluded instead that resistance evolution depends on the absolute time elapsed rather than on the number of generations elapsed. It was acknowledged that generation time can influence the rate of resistance evolution but in a more interactive manner with genetic, ecological and operational factors rather than in a simple, uniform way as treated in previous investigations. However, some caution may be advised regarding conclusions drawn from literature on reported cases of resistance. For example, the Rosenheim and Tabashnik (1990, 1991) studies relied upon a compiled list of pesticide-resistant arthropods (Georghiou 1981) that is composed largely of anecdotal reports of resistance rather than laboratory-substantiated cases that used accepted bioassay techniques. The practice of identifying the resistance status of particular species, and in some cases characterizing major vs. minor resistance, is already problematic given the relativity involved with using one strain as a reference for another. Further dependence upon largely unsubstantiated reports of resistance could prove constraining to theoretical conclusions drawn from such data.

Some examples of resistance in *B. tabaci* fully support the pattern of major resistance occurring in a 5–50 generation timeframe (May and Dobson 1986). Returning to the resistance episode in central Arizona in 1995, major resistance to the fenpropathrin+acephate mixture developed over a relatively brief time interval based on the time when this mixture was first available for commercial use in 1993 to when resistance was documented in 1994 (Dennehy et al. 1995). Following outbreaks of *B. tabaci* in central Arizona cotton in 1992, emergency registration of fenpropathrin was granted to Arizona for the 1993 season. Research demonstrating the superior efficacy of fenpropathrin+acephate (Ellsworth et al. 1994; Watson et al. 1995) provided the underpinning for the rapid adoption of this mixture as the standard treatment for control of *B. tabaci* in cotton. Whitefly control in cotton was much improved during the 1993–94 seasons, but warning signs in the form of reduced mortality at diagnostic doses in bioassays began to appear by the end of the 1994 season (Dennehy et al. 1995). Resistance to this particular mixture was already developing within *B. tabaci* populations but without the high frequencies that would occur the following year resulting in field failures and the well-documented resistance crisis of 1995 in Arizona cotton (Dennehy and Williams 1997). If one assumes that *B. tabaci* has 12 generations per year in central Arizona, perhaps six of them occurring in cotton, then an estimate of 12–15 generations before decreased mortalities began to show up in bioassays at the end of the 1994 season does not seem unreasonable. However, other pyrethroids such as bifenthrin and esfenvalerate

were being used against *B. tabaci* in Arizona cotton prior to registration of fenpropathrin and perhaps should be factored into the estimate of the number of generations to resistance. In this case, the estimated time to resistance would double to 24–30 generations by including 1991–92 exposures. Unfortunately, resistance to fenpropathrin+acephate was not well characterized with respect to cross resistance potential to other pyrethoid+organophosphate mixtures or to individual compounds in each insecticide class, and therefore it is not known how specific the resistance mechanism was to the fenpropathrin+acephate mixture.

In contrast to the rapid progression to control-failure resistance observed in central Arizona in 1995, whitefly populations in the more diverse cropping systems of western Arizona remained relatively susceptible to the fenpropathrin+acephate mixture despite having identical histories in terms of the period of time that fenpropathrin+acephate was used on cotton (Dennehy and Williams 1997). However, as described earlier in the continuous polyculture vs. intermittent monoculture discussion, diverse agricultural conditions in Yuma and the Imperial Valley, California were more favorable for sustaining susceptible whiteflies. Although the whitefly pressure in the diverse systems was even greater than in the cotton-intensive systems of central Arizona, susceptibility was nevertheless retained to a greater degree in the continuous polyculture system despite equivalent insecticide exposures in cotton of both systems. A comparison of bioassay data between *B. tabaci* populations collected in central Arizona at the University of Arizona's Maricopa Agricultural Center and at various locations in the Imperial Valley during October 1995 revealed the disparity in susceptibility to fenpropathrin+acephate (Fig. 16.4A). Emergency use registration for fenpropathrin was granted to California 1 year later than Arizona and therefore the comparison in susceptibility between the two locations is biased towards the shorter use history in California. Nevertheless, monitoring of whitefly populations in the Imperial Valley from 1994 to 98 for susceptibility to fenpropathrin+acephate and other insecticide treatments revealed only modest progress towards resistance compared to the case in central Arizona (Fig. 16.4b). In this example and others, e.g. resistance to pyriproxyfen in Arizona (Dennehy et al. 2002, 2007; Li et al. 2003), the gradual loss of susceptibility to particular insecticide treatments is manifested through reduced mortality, often at the lower doses in bioassays. Unlike the major resistance to fenpropathrin+acephate in central Arizona in 1995 in which drastic increases in survivors in bioassays already occurred by the end of the second season (1994), only incremental reduction of susceptibility was observed during five consecutive cotton seasons in the Imperial Valley as evidenced by the migration of log dose-probit (ldp) lines towards higher concentrations of fenpropathrin (Fig. 16.4B). The composite *ldp* line for 1995 shifted towards lower fenpropathrin concentrations relative to 1994 following the first full year of imidacloprid use on fall vegetable (1994) and spring cantaloupe (1995) crops before moving towards higher concentrations in subsequent years.

With the pyriproxyfen example, a resistance monitoring program established in 1996 during the first year of commercial use tracked susceptibility at multiple sites in Arizona using two diagnostic concentrations, 0.01 and 0.1 μg/ml. Pyriproxyfen, a juvenile hormone analog, inhibits egg hatching and shows activity against other

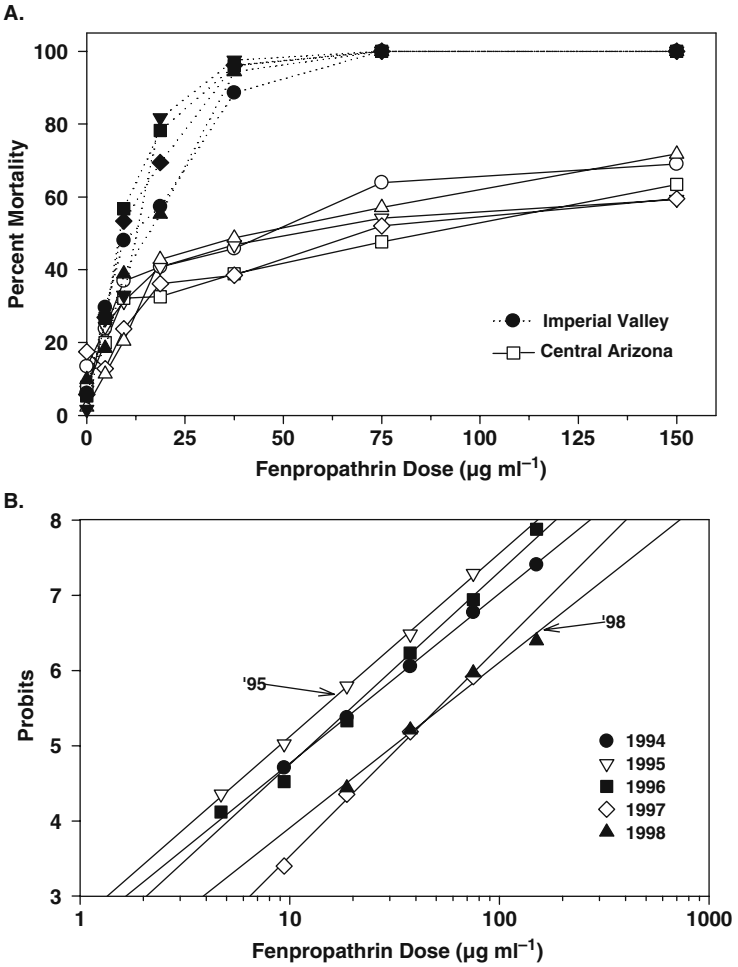



**Fig. 16.4** Responses of *B. tabaci* populations in October 1995 from (**A**) a continuous polyculture (Imperial Valley, California – *closed symbols, dotted lines*) compared to an intermittent monoculture (Central Arizona – *open symbols, solid lines*) to the fenpropathrin+acephate mixture (fenpropathrin dose only presented) used predominantly in both systems. In contrast to the rapid development of major resistance in Central Arizona, resistance in the Imperial Valley over a 5 year period (**B**) developed more gradually and without major control failures. Composite log dose-probit lines for each year show the gradual migration towards reduced susceptibility (Data from Castle et al. 2001)

stages of *B. tabaci* (Ishaaya and Horowitz 1992, 1995). As a condition of proactive resistance management guidelines for both pyriproxyfen and buprofezin, the use of either compound was limited to a single application in cotton per season. The bioassay for pyriproxyfen measured percentage egg hatch at each of the two doses and showed no significant reduction on a statewide basis through 1998. In

the 4th year of pyriproxyfen use in Arizona, a significant decrease in mortality was observed statewide at the 0.01 μg/ml dose and at a single location (Buckeye) for the 0.1 μg/ml dose (Li et al. 2003). From 2002 to 2004, statewide survival at the higher dose jumped from 5.5 to 20% with no additional progression observed in 2005 (Dennehy et al. 2007). Although these are clear signs of resistance developing slowly to pyriproxyfen, there has been no noticeable decline in performance against whiteflies in cotton. Such gradual changes in susceptibility to pyriproxyfen in Arizona and fenpropathrin+acephate in the Imperial Valley may represent examples of polygenic resistance where relatively minor genes for resistance have been selected over time. Further study of the genetic mechanisms involved, e.g. Via's (1986) tolerance curves, would be required to confirm whether multiple genes are involved with the type of mundane resistance that is often overlooked in theoretical treatments of single, major gene resistance. However, continuous progression of susceptibility loss will eventually incur a pest management cost.

## *Cross Resistance*

The development and commercial proliferation of compounds within an insecticide mode of action (MoA) class broadens the spectrum of activity and expands the number of pest species that can be controlled. Although all compounds within a class utilize the same target site, differences in physico-chemical properties of individual compounds confer intrinsically greater or lesser activity across species based on variation in their biological properties. Many active ingredients are further tailored using different product formulations for applications in various crops or against different pests. The inevitable result of this expansion of compounds and formulated products within a class is more frequent exposure to insecticides having the same MoA, leading to an increased risk of resistance. There is the added danger that cross-resistance problems arise with the onset of resistance, especially among members of the same MoA class that share a common target site as well as a similar molecular structure prone to attack by metabolic enzymes.

The neonicotinoids (MoA group 4a; www.irac-online.org) are a good example of a class that has expanded in recent years, resulting in more frequent exposure and increased selection pressure. Despite concerns expressed over the proliferation of neonicotinoids and the resistance management complications raised by having multiple neonicotinoid products available in the same crop (Cahill and Denholm 1999; Dennehy and Denholm 1998), new registrations have increasingly enabled repeated neonicotinoid use without having to use any single compound more than once per crop season. Unfortunately, high levels of resistance to imidacloprid have become common in the horticultural crop production regions of the Mediterranean including Spain (Elbert and Nauen 2000; Nauen et al. 2002; Rauch and Nauen 2003) and Cyprus (Roditakis et al. 2005). An outcome of imidacloprid resistance has been a propensity for cross resistance against other neonicotinoid compounds to occur in

some populations. For example, a Q-biotype population collected from Almeria in 1999 and maintained in the laboratory for 2 years demonstrated cross resistance to thiamethoxam and acetamiprid after having developed resistance to imidacloprid (Nauen et al. 2002). Additional testing of this strain and other Q-type strains showed extreme resistance to imidacloprid but a diminishing degree of cross resistance to other compounds in the order imidacloprid > thiamethoxam > acetamiprid (Rauch and Nauen 2003). This study also reported a biotype B strain from Israel that showed highest resistance to thiamethoxam with diminishing cross resistance to imidacloprid and acetamiprid.

The expression of cross-resistance among neonicotinoids has been less definitive in Israel or the Southwestern USA. Populations of *B. tabaci* in Israeli cotton, vegetables, and ornamentals grown have been treated intensively with acetamiprid since 1996, yet no appreciable resistance to this compound had developed as late as 2003 (Horowitz et al. 2004). In contrast, thiamethoxam had not been used in Israel during this same period, yet cross-resistance factors based on comparison of $LC_{50}$s between field strains and a laboratory susceptible strain ranged as high as 237-fold for thiamethoxam. Further investigation in the laboratory using an acetamiprid-selected strain again demonstrated extremely high cross-resistance to thiamethoxam, whereas the thiamethoxam-selected strain did not show cross-resistance to acetamiprid (Horowitz et al. 2004; Ishaaya et al. 2005). In *B. tabaci* biotype B populations exposed for many years to imidacloprid-treated vegetable and melon crops in California and Arizona, only limited or no cross-resistance to dinotefuran or acetamiprid has been observed, although somewhat higher levels have been seen with thiamethoxam (Prabhaker et al. 2005). This generally follows the pattern seen in *B. tabaci* populations from southern Spain that have been exposed for many more years to imidacloprid and express resistance levels much higher to this compound than to newer neonicotinoids.

As pointed out by Horowitz et al. (2004), resistance conferred by the same mechanism can vary substantially even among closely related compounds. In the respective examples from southern Spain and the Southwestern USA discussed here, the mechanism conferring resistance to imidacloprid has been assumed to be the same mechanism responsible for cross-resistance to the other tested neonicotinoid compounds. However, it is conceivable that unknown resistance mechanisms could be contributing or principally responsible for the cross-resistance observed, even though elevated P450 monooxygenase levels correlated positively with levels of imidacloprid resistance in different strains (Rauch and Nauen 2003; Karunker et al. 2008). The example from Israel of no resistance to the heavily used acetamiprid but strong resistance to the lightly used thiamethoxam suggests the possibility of an alternate resistance mechanism to which only thiamethoxam was vulnerable through cross-resistance. Unpredictable occurrences like this one seem possible in light of the report of pyriproxyfen resistance in Q type strains in the laboratory being sustained by treatments of neonicotinoids in contrast to untreated subsets of the same colony that declined in resistance to pyriproxyfen (Horowitz et al. 2005).

## Biotype

Perspectives on *B. tabaci* as a taxonomic entity have remained fluid ever since the invasion of biotype B into North America forced the realization that dramatic biological differences were present in an otherwise morphologically invariant insect (see Gill and Brown Chapter 1; Brown Chapter 2). The recognition of variation in esterase banding patterns along with inducement of squash silverleaf symptoms between indigenous and invasive biotypes resulted in the identification of A and B biotypes, respectively, and set off a flurry of activity to "biotype" geographical populations worldwide (Costa and Brown 1991; Brown et al. 1995; Perring 2001). Molecular methods that rely primarily on nucleotide sequence differences in the mitochondrial cytochrome oxidase I (mtCO1) gene have been used more recently to elucidate genetic variation among global populations of *B. tabaci.* Phylogenetic analyses performed on sequence data deposited in GenBank and other genomic databases have revealed distinctive macro-geographical groupings. These provisional groupings are themselves dynamic at present as more sequences are becoming available and phylogenetic reconstruction methods are improving. For example, De Barro et al. (2005) estimated a global phylogeny that resolved into six distinctive "races" having broad geographical identities. A subsequent reanalysis (Boykin et al. 2007) of the same data set using Bayesian techniques revealed seven groupings, or clades, rather than the six groupings in the original determination. By incorporating newer sequences into a still larger data set, a total of 12 clades were ultimately resolved in the Boykin et al. (2007) evaluation. Interestingly, the Mediterranean/Asia Minor/Africa group described by De Barro et al. (2005) was split into the Mediterranean/Asia Minor/Africa-B Biotype and Mediterranean-Q Biotype groups in the Boykin et al. (2007) analysis. Besides casting doubt on speculation by De Barro et al. (2005) that *B. argentifolii* is a junior synonym of *B. tabaci* that should be discontinued, separation of Mediterranean region whiteflies into two phylogenetic groups reinforces the probability that unique traits are present among biotypes identified in each group, foremost among them the B and Q biotypes.

Among traits that most often have been considered in biotype comparisons is the propensity to develop resistance to insecticides. The rapid spread of biotype B populations in North America during the early 1990s and inability to adequately manage infestations fueled speculation over potential differences between biotypes to resist insecticides. However, performance-related comparisons between biotypes, whether based on life history traits (Bethke et al. 1991; Perring et al. 1993) or tolerance to insecticides (Costa et al. 1993), were compromised by culturing differences and pesticide exposure histories (Denholm et al. 1996) between biotypes A and B. Only longstanding colonies of biotype A remained available for testing following its complete displacement by biotype B, whereas more recently collected field strains of biotype B were used in these comparisons. Not only were A-type colonies maintained free of insecticide exposure, other problems associated with long term colonies such as inbreeding depression (Roush 1986) may have affected performance in insecticide bioassays or life history studies.

Determination of intrinsic differences among biotypes to resist insecticides is especially challenging because of variation in past exposures to toxins, synthetic or natural. The global distribution of biotypes has resulted in differential insecticide exposures according to the type and intensity of agriculture practiced in a given region. Intensive agricultural production and pesticide-intensive management practiced in the Sudan from the 1950s through the 1970s produced the first example of *B. tabaci* resistance associated with a management crisis. During this same period of time, no resistance problems were being reported with the whitefly currently referred to as biotype Q in southern Spain because the plastic-culture industry and intensive insecticide-based management now common there had not yet established. The roles have now reversed as biotype Q has become notorious for its high resistance while the situation in the Sudan is unknown due to a decline in pest management information that developed following the resistance crisis of the late 1970s (Dittrich et al. 1985).

In contrast to the intensification of agriculture that occurred within the endemic regions of particular *B. tabaci* biotypes such as the Q type, the spread from indigenous to new territories has ensured the widest possible exposure to insecticides. Biotypes B and Q are both well-known for their multiple resistance traits and invasiveness that have brought each biotype into sympatry with the other on multiple continents. Their overlap in various territories has generated a kind of natural laboratory to determine if one or the other biotype is more fit under particular conditions of climate, cropping and chemical management. Initial reports of high resistance to neonicotinoids in biotype Q strains from Europe (Nauen et al. 2002) and to pyriproxyfen in biotype Q strains from Israel (Horowitz et al. 2002) generated considerable speculation that biotype Q has a greater intrinsic potential for resistance. Subsequent detection of high neonicotinoid resistance in a B-type population from Israel (Rauch and Nauen 2003) Guatemala (Byrne et al. 2003) and Florida (Schuster et al. 2008) reflected the potential for high resistance to new chemistry that had been raised by an earlier report of resistance to imidacloprid selected in the laboratory from a biotype B strain collected in California (Prabhaker et al. 1997). However, further characterization of both B-type and Q-type populations in Israel suggested that Q biotype was favored by more intensive use of pyriproxyfen or neonicotinoids possibly because selection to insecticides in B biotype is slower than in the Q type (Horowitz et al. 2005). Following the discovery of biotype Q in the USA (Dennehy et al. 2005), prognostication of serious resistance problems (Dalton 2006) developing in horticultural environments and eventually in agriculture stemmed not only from the reputation of Q biotype in Europe and Israel, but because toxicological evaluations of Q infestations in the USA indicated unusually high resistance to insecticides representing multiple modes of action (Dennehy et al. 2005). Significant management problems have not yet materialized despite its recovery from greenhouses in 24 states (http://mrec.ifas.ufl.edu/lso/BEMISIA/positive_states.htm), and in fact new findings of Q infestations in the USA are becoming more rare.

The tendency to view Q-type as more resistance prone than B-type has persisted with recent reports of resistance to newer modes of action (Roditakis et al.

2005; Prabhaker et al. 2005; Horowitz et al. 2005; Rauch and Nauen 2003; Nauen et al. 2002). Virtually all of the highly resistant Q strains that have been reported originated from protected agriculture conditions in southern Europe with the exception of pyriproxyfen-resistant Q strains from the Ayalon Valley and Carmel Coast regions of Israel. With respect to the protected agriculture situation, it seems plausible that Q type whiteflies represented the original whitefly pest problem in southern Spain as the industry developed in an endemic region for Q type. Agricultural intensification required an increase in pest management inputs that resulted in heavy selection pressure over a period of many years. The intensity of selection was no doubt exacerbated by semi-closed conditions where very little refuge from insecticide treatments was available inside or out of the plastic greenhouses. At some point, possibly in the early 1990s, B biotype was introduced to southern Spain, but apparently never developed high populations in the presence of its more insecticide-resistant relative and native resident of Spain, biotype Q (Guirao et al. 1997; Moya et al. 2001; Rua et al. 2006). Unlike other situations where invading B type populations rapidly displaced indigenous biotypes (De Barro et al. 2006; Liu et al. 2007), intensive selection for resistance under conditions of protected agriculture in southern Spain provided biotype Q with a more completely selected trait, or set of resistance traits, relative to the invading population of B types.

The situation in Israel regarding differential expression of resistance in B and Q biotypes is perhaps more uncertain than the Spanish situation. The most fundamental question may concern the original distributions of B and Q biotypes and whether one or both have been introduced into Israel, perhaps bringing along insecticide resistance genes. In addition to the aforementioned phylogenetic analyses that postulate all or part of the native ranges of Q and B biotypes in the Mediterranean region, respectively (De Barro et al. 2005; Boykin et al. 2007), further evidence for biotype B originating from a geographic region that includes Israel is contained in reports of squash silverleafing that predate the earliest reports of this phenomenon from Florida during the late 1980s (Simons et al. 1988; Yokomi et al. 1990). Leaf silvering of various species of squash (*Cucurbita* spp.) was first recognized and reported as a serious disorder in Israel (Be'eri and Kapuler 1963; Ayyalon 1969; Burger et al. 1983). It is interesting to note that both in Israel (Paris et al. 1987) and Florida (Simons et al. 1988) the silvering symptoms of squash were considered to be a physiological disorder exacerbated by drought (Burger et al. 1983) and not related to infestations by B biotype. It was subsequently determined that an association between squash leaf silvering and feeding by whitefly nymphs existed (Yokomi et al. 1990), but not until Costa and Brown (1991) made the unequivocal distinction between biotypes A and B was it known that feeding on squash by biotype B nymphs was responsible for squash silverleaf disorder. Retrospectively, this determination establishes biotype B in Israel where squash silverleaf had been long recognized. There is no such supporting evidence to place biotype Q in Israel prior to the time of its collection from a rose greenhouse in 1991 and subsequently from the field in 1999 and 2000 (Horowitz et al. 2003). Recent surveys (Erdogan et al. 2008; Bayhan

et al. 2006) from another eastern Mediterranean country, Turkey, detected the presence of biotype B but not biotype Q or the indigenous biotype TC first identified by Bedford et al. (1994). It was estimated that biotype B first invaded Turkey in 1999 and has since displaced the indigenous biotype TC (Bayhan et al. 2006).

The recorded incidence of squash silverleaf symptoms in Israel for more than 40 years provides strong evidence for Asia Minor as indigenous territory for biotype B. Perhaps biotypes B and Q have coexisted in Israel over this time span and longer, but a more plausible scenario may be that intensive agricultural commerce over the past two decades between southern Spain and other European and Mediterranean countries has resulted in the recent dissemination of biotype Q from its native range that includes the Iberian peninsula. An important distinction between biotypes B and Q is that the initial dissemination of biotype B into North America occurred prior to the advent of newer insecticide modes of action and even before full commercial development of the pyrethroids had been completed. In contrast, deployment of the neonicotinoids began in Spain in the early 1990s and has been followed by IGRs and other newer modes of action. The intensity of insecticide use has created a pressure cooker environment that has favored selection for multiple resistance to more diverse insecticides compared to the mid 1980s when biotype B first invaded North America. Monitoring for resistance to imidacloprid in Almeria, Spain first showed reduced susceptibility in strains collected in 1994 and 1995 (Cahill et al. 1996), but by 2000 had increased to >100-fold resistance in various populations from Spain (Nauen et al. 2002; Rauch and Nauen 2003). Moreover, biotype Q collections from greenhouses in Germany and Italy also showed a high degree of cross resistance to neonicotinoids that engendered speculation that both populations had arrived on imported plants possibly originating from Spain (Nauen et al. 2002). Similarly, the initial recovery and testing of biotype Q in Arizona (USA) in 2004 revealed high resistance to multiple modes of action including neonicotinoids and pyriproxyfen (Dennehy et al. 2005) indicating that resistance genes were already present in the new immigrants. With these probable examples of resistant individuals being exported to new localities, other cases of biotype Q resistance remote from Spain should be considered for the possibility that resistance genes were already present in the founding population. This appears to be the case with recent outbreaks of biotype Q in China, possibly resulting from a single importation in 1999 (Ma et al. 2007), and which are also characterized by high levels of resistance to neonicotinoids (L Chen unpublished data). If biotype Q in Israel turns out to be an immigrant from Spain rather than a native, then it may be that recently observed differences between biotypes B and Q in resistance expression result more from exposure history rather than biotype identity. On the other hand, improved understanding of phylogenetic differences now renders more plausible than ever the possibility that basic genetic differences between biotypes explain the different patterns of resistance observed in B and Q biotypes. However, studies on a limited number of B and Q strains have suggested that imidacloprid resistance is due to over-expression of the same monoxygenase gene in both biotypes (Karunker et al. 2008).

## Resistance Management

Various pesticide use strategies (National Research Council 1986; Roush 1989; Tabashnik 1990; Denholm and Rowland 1992) have been proposed for preventing or mitigating resistance in arthropods. These remain largely untested in the real world due to the difficulty of experimentally manipulating whole systems to compare strategies. Computer simulation has most often been relied upon to evaluate the efficacies of competing strategies for delaying resistance. Avoidance of resistance altogether seems out of the realm of possibility even when minimal pesticide use is included in models. In terms of practical examples of resistance management, the Australian program for *H. armigera* was remarkable for its disciplined implementation and thorough documentation of program outcomes (Forrester et al. 1993). Essentially a rotational strategy partitioned into three temporal stages, the Australian program successfully avoided crippling resistance problems in cotton to pyrethroids and endosulfan, the principle concerns of their resistance management strategy. Resistance progression was nonetheless charted annually and attributed principally to an oxidative metabolic resistance mechanism to pyrethroids rather than the more intractable nerve insensitivity mechanism that predominated prior to implementation of the resistance management program. The principle cause given for the gradual rise in resistance through seven seasons was contamination of refugia by resistance genes (Forrester et al. 1993). Emigration of surviving *H. armigera* moths from treated cotton into surrounding untreated crops and wild hosts to overwinter as eggs, then back into the new cotton crop the following spring each year throughout the 7 year study period resulted in the progressive increase in resistance.

The pattern of gradualism in *H. armigera* resistance in Australian cotton is akin to that observed in *B. tabaci* to pyriproxyfen in Arizona (Dennehy et al. 2007), fenpropathrin + acephate in the Imperial Valley (Castle et al. 2001) and imidacloprid in Yuma, Arizona (Palumbo unpublished data). As described by Forrester et al. (1993), the resistance management strategy did not overcome the problem entirely, but did allow more time for development and implementation of alternative control measures. Similarly, new insecticides available for *B. tabaci* along with greater awareness of resistance avoidance strategies in their deployment have permitted significant advances in whitefly management. A much wider selection of novel compounds has become available that provides outstanding opportunities for sustainable control of *B. tabaci*. However, the requirements for a knowledgeable and well-trained pest management community are also greater than ever to obtain maximum efficacy from each treatment while minimizing selective forces that encourage resistance. The basic principles of managing resistance have been presented in numerous review articles and book chapters and widely disseminated among pest management practitioners. The challenge is to integrate these principles to the fullest extent within pest management programs that limit reliance upon chemical control. Although each cropping system will have unique challenges, the following provides basic objectives that should, in principal, have universal application.

## *Minimize Insecticide Use*

If the only certain way to avoid insecticide resistance is to suspend insecticide use altogether, it follows that greater restraint with insecticide applications will limit resistance progression better than less restrained use. The problem is that even modest insecticide use can lead to resistance occurrence as was evident in the *H. armigera* example or the *B. tabaci* example in Israel and Arizona with respect to pyriproxyfen treatments. In the latter case, label restrictions permitted only a single application of pyriproxyfen per cotton season, amounting to roughly one treated generation out of 12 per year, yet decreasing susceptibility was recorded by the 4th year that has continued to progress (Dennehy et al. 2007). Additional registrations of pyriproxyfen in leafy vegetables and cantaloupes in Arizona have raised the potential for exposure across an increasing number of *B. tabaci* generations, highlighting the difficulty of limiting exposure to particular modes of action in a multi-crop pest. This matter becomes even more serious for a class of insecticides such as the neonicotinoids that features multiple compounds registered for use on multiple crops utilized by *B. tabaci*. The potential for prolonged exposure to compounds that have the same mode of action becomes much greater as does the cross-resistance potential. It is therefore important that non-chemical tactics be promoted as essential components of a holistic pest management program directed against infestations in both cropped and non-cropped areas.

There are numerous ways to characterize control tactics that don't involve chemical treatments or biological control but instead rely upon manipulation of the environment to render it unfavorable to the pest, a process that has been termed cultural control by Dent (1991). An elaboration of cultural control involves integrating a thorough understanding of pest ecology with traditional cultural methods to produce ecological management (Pedigo 1999). Although it may be difficult to appreciate the incremental effects that various cultural practices have on population growth, a concerted effort on the part of growers in a community can almost certainly influence population growth rates and the number of insecticide sprays necessary to control pests. Adjustments to all phases of pest management made by growers following outbreaks of *B. tabaci* are critical to the process of regaining control. Changes in cultural practices made in the Imperial Valley of California following the 1991 outbreak included earlier planting and plow-down dates, spatial and temporal isolation of sequential crops, reduction in acreages of vulnerable crops, elimination of non-crop hosts of *B. tabaci* including weeds and ornamentals, and additional adjustments that included more vigilant chemical control. Many of the preventative measures designed to avoid infestations were incorporated into a conceptual framework of whitefly IPM using a pyramid structure (Ellsworth and Martinez-Carrillo 2001, Fig. 17.1). The avoidance layer is the most complex layer of the IPM pyramid and is further subdivided into three inter-related areas that include areawide impact, exploitation of pest biology and ecology, and crop management. Altogether the avoidance approaches included in the IPM pyramid of Ellsworth and Martinez-Carrillo (2001) represent a comprehensive set of tactics for minimizing insecticide use by restricting population growth well in advance of economic thresholds.

Improving biological control of *B. tabaci* is another pathway towards minimizing insecticide use and lowering the risk of resistance. The challenges for establishment and maintenance of robust populations of biocontrol agents are immense in intensive agricultural regions with diverse cropping (Hoelmer 1996). The inherent instability due to rapid turnover of crops confers a distinct advantage to an adept colonizer like *B. tabaci*. Dispersal to new crops and development of infestations ahead of lagging natural enemy populations keep *B. tabaci* on the epidemic ridge as characterized in Southwood's synoptic population model. Historical dependence upon insecticides to counter rapid colonization of crops has been at odds with conserving natural enemies and promoting biological control. However, improving compatibilities of newer insecticides with biological control agents is providing opportunities for fuller integration of pest management approaches. The first example of a comprehensive management program for *B. tabaci* that involved selective insecticides with reduced impact on natural enemies of *B. tabaci* was the Israeli IPM-IRM program for cotton. Beginning with buprofezin in 1989 and followed by pyriproxyfen in 1991, the Israeli program has achieved a reduction in insecticide use against the entire range of cotton pests (Horowitz et al. 1994; Horowitz and Ishaaya 1996). By relying upon the selective activity of both IGRs, a higher level of natural enemy activity has resulted in improved control of *B. tabaci* despite some resistance problems with pyriproxyfen in the Ayalon Valley (Horowitz et al. 1999, 2002).

The successful implementation of IGRs in Israel provided the foundation for launching the IGR-based IPM-IRM program for *B. tabaci* in Arizona after the resistance crisis in 1995 (Dennehy and Williams 1997). As previously mentioned, insecticide use in Arizona declined steadily from 1996 to 2000 following the launching of buprofezin and pyriproxyfen, thus building onto the example set in Israel. Intensive investigations conducted in Arizona cotton fields from 1997 to 2001 provided a more complete understanding of how the selective activity of both IGRs helped to conserve natural enemies and result in proportional increases in natural mortality relative to insecticide-induced mortality. A life table approach conducted in large scale experimental field plots that compared conventional vs. IGR insecticide treatments revealed the additional mortality within cohorts of *B. tabaci* contributed by natural enemies (Naranjo and Ellsworth 2009b). In particular, recovery of biological control in field plots after an application of either buprofezin or pyriproxyfen was more robust than in conventional insecticide-treated plots. The continuation of natural enemy-induced mortality in IGR treated plots led to the concept of bioresidual to describe the extended period of suppression through proper use of IGRs (Ellsworth and Martinez-Carrillo 2001; Naranjo 2001). The thorough experimental field work to document the relatively higher levels of natural mortality following IGR treatments provides a compelling example for rethinking past approaches to *B. tabaci* control. Conceptualization of the bioresidual effect represents an important philosophical advance in pest management theory. It effectively conveys one of the central goals of integrated pest management, i.e. to incorporate multi-tactic approaches in a cohesive and compatible manner that effectively suppresses pest populations. The largest constraint to achieving this ideal has often been the severe disruption to biological control that occurs following broad spectrum

insecticide use. The selective activities of buprofezin and pyriproxyfen in addition to more recent chemistry, such as spiromesifen and spirotetramat, are making it possible to integrate chemical and biological control of *B. tabaci* more completely than ever as revealed by the Arizona example. The term bioresidual brilliantly reflects the desired effect of prolonged biological control following treatment with selective insecticides and should help to focus goals of IPM programs as they are developed and refined (see Naranjo and Ellsworth 2009a).

## *Diversify Insecticide Use*

In pest management situations that require multiple applications of pesticides to suppress target populations, a longstanding rule to diversify treatments has been aimed at reducing selection pressure by any one active ingredient or class of chemistry acting at a specific target site. Although accomplished easily on paper, the challenge of rotating treatments in practice can be formidable if growers and pest managers find one treatment clearly superior to others. The tendency to over rely upon a single best treatment increases with escalating pest pressure, leaving behind resistance management guidelines in favor of a solution at any cost to avoid damaging infestations. This was likely the case in 1995 when too many Arizona cotton growers resorted to repeated sprays of synergized pyrethroids to combat rapidly building infestations of *B. tabaci* (Dennehy and Williams 1997), counter to resistance management guidelines that had been implemented prior to the 1995 season advising rotation of insecticides (Dennehy et al. 1995). Examples like this one underscore the maxim that the best resistance management is good pest management. Steady suppression of target populations according to plan helps to avoid a sense of urgency that triggers deviation from recommended treatment practices. With an explosive pest such as *B. tabaci*, resistance and pest management programs can rapidly deteriorate into chaos once control on a large scale has been lost. Resistance management will be the first casualty of a *B. tabaci* outbreak and must therefore be implemented within the context of the most effective and sustainable IPM program. A well designed pest management program is especially crucial in high risk regions where chronic outbreak conditions exist.

The capacity to diversify insecticide treatments against *B. tabaci* has increased dramatically over the past 10–15 years. At the time of the early 1990s, outbreaks of B-biotype in the USA, treatment options were limited to a selection of conventional chemistry representing organophosphate, carbamate, pyrethroid and organochlorine insecticide groups. Only three modes of action (MoA) were represented among these four groups as OPs and carbamates both target acetylcholinesterase. This situation has changed dramatically as the number of MoAs available for *B. tabaci* control has more than tripled to ten according to the most recent update by the Insecticide Resistance Action Committee (http://www.irac-online.org). Borrowing from the IRAC chart that provides insecticide groupings according to MoA, Table 16.1 presents a modified subset of this scheme showing only those MoAs having activity against whiteflies. This listing is specific to active ingredients that are

**Table 16.1** Insecticides registered for use in USA against *Bemisia tabaci* on three different crops by IRAC group number and mode of action. It should be noted that many additional compounds are registered for use in each crop, particularly OPs and carbamates, but do not specify *B. tabaci* on their labels

| Group | Sub-group | Mode of action | Chemical sub-group or active ingredient | Insecticide name | | |
|---|---|---|---|---|---|---|
| | | | | Broccoli | Cantaloupe | Cotton |
| 1 | A | Acetylcholine esterase inhibitors | Carbamates | – | – | Oxamyl<br>Aldicarb |
| | B | | Organophosphates | – | – | Acephate<br>Chlorpyrifos |
| 2 | A | GABA-gated chloride channel antagonists | Cyclodiene organochlorines | Endosulfan | Endosulfan | Endosulfan |
| 3 | | Sodium channel modulators | Pyrethroids, Pyrethrins | Cyfluthrin<br>Bifenthrin<br>Cypermethrin<br>λ cyhalothrin<br>Fenpropathrin | Cyfluthrin<br>Bifenthrin<br>λ cyhalothrin<br>Fenpropathrin | Esfenvalerate<br>Bifenthrin<br>Cypermethrin<br>λ cyhalothrin<br>Fenpropathrin |
| 4 | A | Nicotinic Acetylcholine receptor agonists/antagonists | Neonicotinoids | Imidacloprid<br>Thiamethoxam<br>Acetamiprid<br>Dinotefuran | Imidacloprid<br>Thiamethoxam<br>Acetamiprid<br>Dinotefuran | Imidacloprid<br>Acetamiprid<br>Dinotefuran |
| 7 | | Juvenile hormone mimics | Pyriproxyfen | Pyriproxyfen | Pyriproxyfen | Pyriproxyfen |
| 9 | B | Compounds of unknown or nonspecific mode of action (selective feeding blockers) | Pymetrozine | Pymetrozine | Pymetrozine | Pymetrozine |
| 15 | | Chitin synthesis inhibitor | Novaluron | Novaluron | – | – |
| 16 | | Chitin synthesis inhibitor | Buprofezin | – | Buprofezin | Buprofezin |
| 18 | B | IGR, ecdysone agonists/molting disruptors | Azadiractin | Azadiractin | Azadiractin | Azadiractin |
| 23 | | Inhibitors of lipid synthesis | Tetronic acid derivatives | Spiromesifen<br>Spirotetramat | Spiromesifen | Spiromesifen |
| 28 | | Ryanodine receptor modulators | Diamides | Rynaxypyr | Rynaxypyr | |

registered for use on three different crops grown in Arizona (USA), but the diversity of MoAs available are generally representative worldwide. While the availability of insecticides representing ten different MoAs bodes well for diversifying insecticides within a crop season, it potentially complicates the issue of maintaining diversity across commodities (Palumbo et al. 2003) due to the repetition of product registrations on multiple crops as seen for lettuce, melons and cotton in Arizona (Table 16.1).

In addition to the now wide selection of MoAs available for control of *B. tabaci*, the different physico-chemical properties of compounds belonging to each MoA group offer much versatility in terms of application procedures and timings with respect to crop and pest population phenologies. As a sample scenario of choices available to pest managers, a selection of products and their respective MoAs have been arranged in a sequence of crop-stage intervals beginning with pre-plant through pre-harvest for a broccoli crop grown in Arizona (Table 16.2). Broccoli and other leafy vegetable crops planted in the early fall growing season are often subject to peak densities of *B. tabaci* as they emerge from the soil. Soil applications of neonicotinoid insecticides at planting have been critical to success gained in management of *B. tabaci* in the irrigated desert valleys of the Southwestern USA. Solubility differences among imidacloprid, thiamethoxam and dinotefuran (0.51, 4.1 and 39.8 g/l, respectively) influence the uptake rate and persistence in the crop. Application of these compounds through drip irrigation enables pest managers an even greater degree of control in terms of the timing of an application and whether it is split into multiple applications or applied at full rate in a single application. A second MoA represented by the diamide group is now also available as a soil-applied systemic insecticide alternative to the neonicotinoids. Following at-plant soil applications or early season drip irrigation applications of neonicotinoids or diamides, many whitefly treatment options featuring different MoAs remain for safeguarding the crop through harvest. The IGRs buprofezin and pyriproxyfen represent separate MoAs that could be used following diminishment of early season systemic insecticide treatments as *B. tabaci* populations begin to rebuild in the crop (Table 16.2). The tetronic acid derivative treatments spiromesifen and spirotetramat provide a still different MoA for a maturing crop, with spirotetramat giving the unique benefit of being fully phloem mobile and therefore systemic within the plant following foliar application. Depending on which MoA was used early season, 4A (neonicotinoid) or 28 (diamides), still other treatment options exist mid to late season (Table 16.2). A foliar treatment using an MoA 4A insecticide could be used on a maturing crop if an MoA 28 insecticide had been used early season, or vice versa if MoA 4A was used as an early season soil systemic treatment. The guiding resistance management objective for each crop season is to avoid using any MoA more than one time and to limit chemical control to the fewest number of MoAs possible per season. Incorporating compounds with good bioresidual activity into the chemical management strategy will encourage higher levels of biological control and help reduce the number of chemical treatments.

**Table 16.2** A selection of insecticides or classes of insecticides representing eight modes of action that are registered for control of *Bemisia tabaci* during different stages of a broccoli crop in Arizona (USA). The intent is to show the diversity of compounds available for managing *B. tabaci* and the time during the season they may be most effectively used. This is not to suggest that each compound should be used during a single season, rather that options exist for this crop and many other vegetable, melon, and field crops

| | | Pre-plant | Stand Establishment | | Thinning to Heading | | | | Heading to Harvest | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Insecticide | IRAC MOA | Soil | Emer-gent | 2-4 leaf | 5-10 leaf | 10-15 leaf | 15-20 leaf | 20-25 leaf | Early button | 2-4" head | 4-6" head |
| Imidacloprid | 4A | | | | | | | | | | |
| Thiamethoxam | | | | | | | | | | | |
| Dinotefuran | | | | | | | | | | | |
| Acetamiprid | | | | | | | | | | | |
| Pyrethroids | 3 | | | | | | | | | | |
| Ops/Carbamates | 1 | | | | | | | | | | |
| Endosulfan | 2A | | | | | | | | | | |
| Pymetrozine | 9B | | | | | | | | | | |
| Pyriproxyfen | 7 | | | | | | | | | | |
| Spiromesifen | 23 | | | | | | | | | | |
| Rynaxypyr | 28 | | | | | | | | | | |

## *Refine Insecticide Use*

Development and commercialization of effective new MoAs have greatly expanded the arsenal available to growers and pest managers for combating recalcitrant *B. tabaci* infestations. Not only are the new products more effective control agents at reduced rates compared to conventional insecticides, their generally narrower activity spectra make possible a more finessed approach to pest management. As the Israeli and Arizona examples have shown with the IGRs buprofezin and pyriproxyfen, treatments have proven less destructive to biological control agents while simultaneously providing better direct control of *B. tabaci* (Naranjo et al. 2004). The thorough research that identified the bio-residual phenomenon for the IGRs has yet to be performed for newer MoAs, but preliminary findings suggest that some also show a high degree of compatibility with beneficial insects. For example, spiromesifen (MoA group 16) has been classified by Koppert Biological Systems as harmless to adult *Encarsia formosa* and *Orius laevigatus* (http://www.koppert.nl/Side_effects.html). Other compounds such as acetamiprid vary in their selectivity depending on the predator or parasitoid species under consideration. In Arizona cotton, acetamiprid was found to depress populations of fewer predator taxa than conventional treatments, but for eight taxa the level of reduction was approximately equivalent (Naranjo and Akey 2005). Determining toxicity profiles of specific compounds to key beneficial insects in crop systems enables more knowledgeable decisions to be made regarding the timing of insecticide applications. Moreover, understanding of the modes of action of all chemistry in the repertoire of pest managers is essential to attaining optimal performance of each treatment against *B. tabaci* with minimal impact on beneficial insects. Refinement of insecticide use is a process of identifying patterns of target pest infestations in crops requiring protection, developing sampling plans and economic thresholds for when treatments should be applied, and incorporating ecotoxicological information regarding impact on beneficial insects to minimize collateral damage from an insecticide treatment. Whilst these are also elements of good IPM, they more specifically relate to responsible use of insecticides.

Advancements made in management of *B. tabaci* have largely occurred through development of more effective insecticides and the more knowledgeable implementation of those insecticides. Commercialization of imidacloprid, the first neonicotinoid insecticide, made possible the transformation of *B. tabaci* from a perpetual outbreak pest in the intensive vegetable and melon production regions of the Southwestern USA to a pest that can be well managed (Palumbo et al. 2003), albeit one that continually challenges. The effectiveness of soil applications of imidacloprid was greatly enhanced by research that demonstrated the importance of placement in the seed furrow at time of planting. The relative insolubility of imidacloprid compared to thiamethoxam or dinotefuran required that placement in the root zone of emerging seedlings be optimal for protection against whitefly hordes attacking tender vegetation. At the same time, this characteristic of imidacloprid enabled persistent uptake by growing plants and provided extended protection

against unyielding whitefly pressure that previously would have required multiple spray applications of conventional compounds.

A different solution was required in the cotton-intensive regions of central Arizona where a delayed phenology of *B. tabaci* populations relative to vegetable growing regions of western Arizona and the Imperial Valley in California precluded at-planting applications of imidacloprid to cotton. Other factors including a much higher plant density per hectare limited imidacloprid's effectiveness in cotton. During the lead-up to the resistance crisis in 1995, much research was being pursued on the spatial and temporal characteristics of *B. tabaci* infestations and identifying economic thresholds in cotton. Binomial sampling plans were developed from the large body of data on infestation patterns and presented in the scientific peer-reviewed literature as well as in extension publications for growers and pest managers. Pocket guides were developed to aid in-field use of the sampling program and provided reminders of action threshold densities. In the meantime, insecticide field trials conducted over multiple years were exploring the activities of buprofezin and pyriproxyfen against *B. tabaci* and determining the most effective timing during development of an infestation. Fortunately, when untenable resistance to synergized pyrethroids resulted in a significant failure to the 1995 cotton crop, the research infrastructure was mature and the knowledge base replete with information necessary for crafting an entirely new system of whitefly management. The same information was instrumental in the granting of a Section 18 emergency exemption by the USA. Environmental Protection Agency for both IGRs (Ellsworth et al. 1996; Dennehy et al. 1996; Ellsworth and Martinez-Carrillo 2001). A remarkable feature of the emergency registration mandated that each IGR could only be used once per season and that pest managers had to complete a training session as a condition of product use. This latter element represented extraordinary foresight to recognize the potential for ineffectual use of the IGRs that might occur without first educating the pest management community about their unique modes of action and how they should be deployed to gain maximum impact against *B. tabaci*. Workshops were set up around the state of Arizona during spring 1996 to train potential users by demonstrating on live plants the developmental stages that had to be targeted for each compound to be most effective. A new set of sampling guidelines were developed that focused on the stages to be targeted and were discussed at length before the grower and pest manager audiences (see review by Naranjo and Ellsworth 2009a).

The conditions for presenting such information before a receptive audience were perhaps unique given the widespread cotton failures that occurred the previous year. The desperation that many growers felt after the 1995 season no doubt contributed to a willing adoption of the new management system for whiteflies and the implementation of the use guidelines for each IGR as instructed during training sessions. It was a situation in which growers and pest managers looked to the research community for a solution to the problems of 1995, and therefore were supportive of the restrictive conditions of IGR use as mandated in the Section 18 permit. The program delivered to them was nothing short of a complete IPM package that contained rigorous sampling and threshold information, proactive resistance management components, and enhanced biological control relative to conventional insecticides, the magnitude of which was only fully revealed in follow-up life table

studies conducted in the field (Naranjo and Ellsworth 2009b). The bold mark of success of this program has been the steep decline in insecticide use observed during the first year of implementation and that continued to decrease thereafter (Ellsworth and Martinez-Carrillo 2001; Naranjo and Ellsworth 2009a). Furthermore, there has been a complete absence of resistance episodes of the magnitude observed in 1995. Cotton yields and quality improved tremendously in 1996 and have not experienced destructive whitefly attack ever since. By incorporating knowledge-based elements of pest management into a comprehensive control program, insecticide use was dramatically refined with resulting improvement and sustainability of *B. tabaci* management.

Other novel MoAs new to the market or in the developmental pipeline will also require the kind of knowledgeable implementation carried out for the IGRs if they are to be used to their fullest potential. The increasing number of highly effective choices available for pest managers makes possible continuing improvement in management of whiteflies even in the most outbreak-prone locations. However, a dilemma arises in terms of how best to make use of the diversity of the MoAs available, especially in year-round production systems in which multiple compounds from a single MoA are registered for use on consecutive crops. This situation is most apparent with the heavily populated neonicotinoid class that now has seven commercialized products, as many as four to five with registration on the same spectrum of crops infested by *B. tabaci*. But it is also representative of MoA groups that contain only a single compound that are registered for use against *B. tabaci* in multiple crops. Recognizing the increased potential for resistance through multiple uses of neonicotinoid compounds within a single crop and then again in subsequent crops, a set of cross-commodity guidelines were developed for neonicotinoid insecticides in Arizona (Palumbo et al. 2003). Various crop communities were identified across the state and use guidelines were developed for each with the goal of limiting dependence upon neonicotinoids for whitefly control. Calendar windows were relied upon to designate critical crop periods where a soil-applied neonicotinoid was essential for protection, but only with the provision that no foliar neonicotinoids be applied to the same crop. Even in the most intensive production systems, protracted neonicotinoid-free periods were identified while advising rotation to other MoAs. This type of expansive overview of annual total insecticide use and patterns of use must be taken into consideration by the pest management community to avoid overuse of individual MoAs. Guidelines that are well-grounded in experimental findings are essential for determining the best possible whitefly management strategy while also avoiding overdependence on particular MoAs.

## Conclusions

Recognition of *B. tabaci* as a resistance-prone pest of both protected and open agriculture has become widespread over the past 30 years and continues to be a dominant issue in the formulation of effective management strategies. The discovery and implementation of newer insecticides with novel modes of action

have been indispensable in restoring control of *B. tabaci* in areas where population outbreaks were exacerbated by resistance. An important challenge going forward, however, will be to deploy these myriad chemistries in an effective and restrained manner that conserves active ingredients by reducing resistance risks. Proliferation of compounds within insecticide classes has complicated resistance management strategies due to market pressures that make available multiple products representing a single mode of action within a crop season. For areas where consecutive crops are grown throughout the year and the same assemblages of insecticides are registered for each crop, the potential for continuous exposure to a single mode of action is magnified with each additional product registration. There is no other class of insecticides where this concern has greater relevance than for the neonicotinoids. While the registration of additional neonicotinoid insecticides in multiple crops provides growers with yet more effective options for whitefly control, the pressure exerted on a single target site potentially reaches a breaking point if stringent guidelines for conservation of this crucial chemistry are not formulated and adhered to. The same warning applies to all insecticides that are registered in multiple crops grown sequentially throughout the year.

Given the reality of the pesticide marketplace and the inevitable saturation by competing products with the same MoA, it is incumbent upon the end user to be familiar with the insecticides available and the potential pitfalls through their misuse. Knowledgeable deployment of newer and more selective insecticides will be fundamental to the successful management of *B. tabaci*. The complexity involved in selecting an insecticide to use at a particular time during a crop season has increased dramatically with the development of novel modes of action. The lethal activity of newer compounds that disrupts growth and developmental processes in *B. tabaci* is rarely apparent immediately following an application. It is therefore essential from a pest management perspective that pest managers understand the basic similarities and differences among insecticides, have a general idea of how each mode of action induces mortality and what to expect following treatment, and be aware of the probable impact on natural enemies of *B. tabaci* so that bioresidual effects can be maximized following treatments. From a resistance management perspective, each grower and/or pest manager should consider developing an individual plan of action for whitefly-vulnerable crops that will be grown throughout the year. Central to this plan would be a provisional insecticide use strategy that firstly anticipates the number of treatments that may be required in all crops throughout the year, and secondly devises a deployment schedule that minimizes overlap in modes of action while maximizing effectiveness of each insecticide application. Actual treatments should of course rely solely upon scouting information that justifies treatment action, but the preplanned schedule would provide a structural framework for avoiding repetitious use of a single mode of action.

On a more fundamental level, improved understanding of resistance mechanisms from the molecular to ecological scales will provide the basis for anticipating and contravening resistance occurrences. The critical importance of the ecological milieu to the scale of resistance has been aptly illustrated for *B. tabaci* (Denholm et al. 1998) as well as other resistant pests (Daly 1993; Forrester and Fitt 1992).

From examples provided herein, the simple observation that resistance does not take on the same form or behavior in every situation where insecticides are used to control *B. tabaci* provides incentive to better understand the ecological determinants of resistance. Retrospective appraisal of conditions under which resistance became unmanageable vs. inconsequential appears to support basic theory concerning the importance of pesticide-free refuges for maintaining susceptible genotypes. This is not to suggest that whole agricultural systems need to be redesigned so as to be more conducive ecologically to the avoidance of resistance. But it does point to a certain predictive power by studying the agroecology for clues as to what proportion of a regional population is subjected to constant vs. more intermittent insecticide pressure. Scrupulous observance of counter resistance methods should be incorporated anywhere that insecticide applications are made against *B. tabaci*, but the stakes for rapid development of major resistance are arguably higher in those areas where crop diversity is reduced and alternative unsprayed hosts are less available. However, the insidious nature of slower, but progressively developing minor resistance should not be underestimated. The elapsed time to loss of insecticide efficacy may be longer, but the ultimate impact is no less costly once an active ingredient has been undermined by resistance. The end result may have greater finality in the case of polygenic resistance that will likely be less reversible than the often unstable resistance caused by a single major resistance gene.

## Literature Cited

Ahmad M, Arif MI, Ahmad Z, Denholm I. 2001. Cotton whitefly (*Bemisia tabaci*) resistance to organophosphate and pyrethroid insecticides in Pakistan. *Pest Manag. Sci.* 58:203–208.

Ayyalon A. 1969. Growing green summer squash for export. *Ext. Serv. Bull*. 68/100/19. Israel Ministry of Agriculture, Tel Aviv, Israel, 22pp.

Bayhan E, Ulusoy MR, Brown JK. 2006. Host range, distribution, and natural enemies of *Bemisia tabaci* 'B biotype' (Hemiptera: Aleyrodidae) in Turkey. *J. Pest. Sci.* 79:233–240.

Bedford ID, Briddon RW, Brown JK, Rosell RC, Markham PG. 1994. Geminivirus transmission and biological characterisation of *Bemisia tabaci* (Gennadius) biotypes from different geographic regions. *Ann. Appl. Biol.* 125:311–325.

Be'eri Y, Kapuler B. 1963. Summer squash. Growing vegetables. Part B. *Sifriyyat Hassadeh Publ*., Tel Aviv, pp. 235–240 [in Hebrew].

Bethke JA, Paine TD, Nuessly GS. 1991. Comparative biology, morphometrics, and development of two populations of *Bemisia tabaci* (Homoptera: Aleyrodidae) on cotton and poinsettia. *Ann. Entomol. Soc. Am.* 84:407–411.

Bird J. 1957. A whitefly-transmitted mosaic of *Jatropha gossypifolia*. *Tech. Papers, Agric. Exp. Stn. (Puerto Rico)* 22:5–35.

Boykin LM, Shatters RG, Rosell RC, McKenzie CL, Bagnall RA, De Barro P, Frohlich DR. 2007. Global relationships of *Bemisia tabaci* (Hemiptera: Aleyrodidae) revealed using Bayesian analysis of mitochondrial COI DNA sequences. *Mol. Phylogen. Evol.* 44:1306–1319.

Brown JK, Frohlich DR, Rosell RC. 1995. The sweetpotato or silverleaf whiteflies: biotypes of *Bemisia tabaci* or a species complex? *Annu. Rev. Entomol.* 40:511–534.

Burban C, Fishpool LDC, Fauquet C, Fargette D, Thouvenel JC. 1992. Host-associated biotypes within West African populations of the whitefly *Bemisia tabaci* (Genn.) (Hom., Aleyrodidae). *J. Appl. Entomol.* 113:416–423.

Burger Y, Paris HS, Nerson H, Karchi Z, Edelstein M. 1983. Overcoming the silvering disorder of *Cucurbita*. *Cucurbit Gen. Coop. Rep.* 6:70–71.

Byrne DN, Bellows TS, Parrella MP. 1990. Whiteflies in agricultural systems. In *Whiteflies: Their Bionomics, Pest Status and Management*, ed. D Gerling, pp. 227–261. Andover, UK: Intercept.

Byrne DN, Rathman RJ, Orum TV, Palumbo JC. 1996. Localized migration and dispersal by the sweet potato whitefly, *Bemisia tabaci*. *Oecologia* 105:320–328.

Byrne FJ, Castle SJ, Prabhaker N, Toscano NC. 2003. Biochemical study of resistance to imidacloprid in B-biotype *Bemisia tabaci* from Guatemala. *Pest Manag. Sci.* 59:347–352.

Byrne FJ, Devonshire AL. 1993. Insensitive acetylcholinesterase and esterase polymorphism in susceptible and resistant populations of the tobacco whitefly, *Bemisia tabaci*. *Pestic. Biochem. Physiol.* 45:34–42.

Cahill M, Denholm I. 1999. Managing resistance in the chloronicotinyl insecticides – rhetoric or reality. In *Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor*, ed. I Yamamoto, JE Casida, pp. 253–270. Tokyo: Springer.

Cahill M, Gorman K, Day S, Denholm I, Elbert A, Nauen R. 1996. Baseline determination and detection of resistance to imidacloprid in *Bemisia tabaci* (Homoptera: Aleyrodidae). *Bull. Entomol. Res.* 86(4):343–349.

Castle S, Henneberry T, Toscano PN, Birdsall S, Weddle D. 1996. Silverleaf whiteflies show no increase in insecticide resistance. *California Agric.* 50:18–23.

Castle SJ, Ellsworth PC, Prabhaker N, Toscano NC, Henneberry TJ. 2001. Relative susceptibility of whiteflies to Danitol+Orthene over a 5-year period. In *Cotton – A College of Agriculture Report Series P-125*, ed. J Silvertooth, pp. 269–277. Tucson: University of Arizona.

Chu D, Jiang T, Liu GX, Jiang DF, Tao YL, Fan ZX, Zhou HX, Bi YP. 2007. Biotype status and distribution of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Shandong Province of China based on mitochondrial DNA markers. *Environ. Entomol.* 36:1290–1295.

Comins HN. 1977. The development of insecticide resistance in the presence of immigration. *J. Theor. Biol.* 64:177–197.

Costa AS. 1975. Increase in the populational density of *Bemisia tabaci*, a threat of widespread virus infection of legume crops in Brazil. In *Tropical Diseases of Legumes*, ed. J Bird, K Maramorosch, pp. 27–29. New York, USA: Academic Press.

Costa HS, Brown JK. 1991. Variation in biological characteristics and in esterase patterns among populations of *Bemisia tabaci* Genn. and the association of one population with silverleaf symptom development. *Entomol. Exp. Appl.* 61:211–219.

Costa HS, Brown JK, Sivasupramaniam S, Bird J. 1993. Regional distribution, insecticide resistance, and reciprocal crosses between the 'A' and 'B' biotypes of *Bemisia tabaci*. *Insect Sci. Appl.* 14:255–266.

Coudriet DL, Prabhaker N, Kishaba AN, Meyerdirk DE. 1985. Variation in developmental rate on different hosts and overwintering of the sweetpotato whitefly, *Bemisia tabaci* (Homoptera: Alleyrodidae). *Environ. Entomol.* 14:516–519.

Coudriet DL, Meyerdirk DE, Prabhaker N, Kishaba AN. 1986. Bionomics of sweetpotato whitefly (Homoptera: Aleyrodidae) on weed hosts in the Imperial Valley, California. *Environ. Entomol.* 15:1179–1183.

Courchamp F, Clutton-Brock T, Grenfell B. 1999. Inverse density dependence and the Allee effect. *Trends Ecol. Evol.* 14:405–410.

Dalton R. 2006. The Christmas invasion. *Nature* 443:898–900.

Daly JC. 1993. Ecology and genetics of insecticide resistance in *Helicoverpa armigera*: interactions between selection and gene flow. *Genetica* 90:217–226.

De Barro PJ, Trueman JW, Frohlich DR. 2005. *Bemisia argentifolii* is a race of *B. tabaci* (Hemiptera: Aleyrodidae): the molecular genetic differentiation of *B. tabaci* populations around the world. *Bull. Entomol. Res.* 95:193–203.

De Barro PJ, Bourne A, Khan SA, Brancatini VAL. 2006. Host plant and biotype density interactions – their role in the establishment of the invasive B biotype of *Bemisia tabaci*. *Biol. Inv.* 8:287–294.

Denholm I, Rowland MW. 1992. Tactics for managing pesticide resistance in arthropods: theory and practice. *Annu. Rev. Entomol.* 37:91–112.

Denholm I, Cahill M, Byrne FJ, Devonshire AL. 1996. Progress with documenting and combating insecticide resistance in *Bemisia*. In *Bemisia*: *1995 Taxonomy, Biology, Damage, Control and Management*, ed. D Gerling, RT Mayer, pp. 577–603. Andover, Hants, UK: Intercept Ltd.

Denholm I, Cahill M, Dennehy TJ, Horowitz AR. 1998. Challenges with managing insecticide resistance in agricultural pests, exemplified by the whitefly *Bemisia tabaci*. *Phil. Trans. R. Soc. Lond. B* 353:1757–1767.

Dennehy TJ, Williams L. 1997. Management of resistance in *Bemisia* in Arizona cotton. *Pestic. Sci.* 51:398–406.

Dennehy TJ, Denholm I. 1998. Goals achievements and future challenges of the Arizona whitefly resistance management program. In *Proceedings of the 1998 Beltwide Cotton Production Research Conference*, pp. 68–72. Memphis, TN: National Cotton Conference.

Dennehy TJ, Simmons A, Russell J, Akey D. 1995. Establishment of a whitefly resistance documentation and management program in Arizona. *Cotton – A College of Agriculture Report, Series P-99*, pp. 287–297. The University of Arizona, Tucson, AZ.

Dennehy TJ, Ellsworth PC, Nichols RL. 1996. The 1996 whitefly resistance management program for Arizona cotton. *IPM Publication Series No. 8. The University of Arizona, Cooperative Extension. Publication #196008*. The University of Arizona, Tucson, AZ.

Dennehy TJ, Zaborac M, DeGain B, Holley D, Nichols RN, Li AY, Ellsworth P, Palumbo J. 2002. Six years of successful management of whitefly resistance in Arizona cotton. In *Proceedings Beltwide Cotton Conferences*. National Cotton Council, Memphis, TN.

Dennehy TJ, DeGain BA, Harpold VS, Brown JK, Fabrick JA, Byrne FJ, Nichols RL. 2005. New challenges to management of whitefly resistance to insecticides in Arizona. In *2005 Vegetable Report*, ed. DN Byrne, P Baciewicz. The University of Arizona, Tucson. http://ag.arizona.edu/pubs/crops/az1382/az1382_2.pdf

Dennehy TH, DeGain BA, Harpold VS. 2007. Biotype designations and insecticide susceptibility of southwestern *Bemisia tabaci*. *Cotton Report (Series P-151)*, pp. 127–154. The University of Arizona, Tucson, AZ.

Dent D. 1991. *Insect Pest Management*. UK: CABI.

Dittrich V, Hassan SO, Ernst GH. 1985. Sudanese cotton and the whitefly: a case study of the emergence of a new primary pest. *Crop Prot.* 4:161–176.

Dittrich V, Ernst GH, Ruesch O, Uk S. 1990. Resistance mechanisms in sweetpotato whitefly (Homoptera: Aleyrodidae) populations from Sudan, Turkey, Guatemala, and Nicaragua. *J. Econ. Entomol.* 83(5):1665–1670.

Duffus JE, Flock RA. 1982. Whitefly-transmitted disease complex of the desert southwest. *California Agric.* 36:4–6.

Elbert A, Nauen R. 2000. Resistance in *Bemisia tabaci* (Homoptera: Aleyrodidae) to insecticide in southern Spain with special reference to neonicotinoids. *Pest Manag. Sci.* 56:60–64.

Ellsworth P, Moore L, Watson TF, Dennehy T. 1994. Insect pest management for cotton. *The University of Arizona, Cooperative Extension. Publication #194022*. The University of Arizona, Tucson, AZ.

Ellsworth PC, Dennehy TJ, Nichols RL. 1996. Whitefly management in Arizona cotton 1996. *IPM Series No. 3, Univ. Arizona Coop. Ext. Publ. 196004*. The University of Arizona, Tucson, AZ.

Ellsworth PC, Martinez-Carrillo JL. 2001. IPM of *Bemisia tabaci*: a case study from North America. In *Special Issue: Challenges and Opportunities for Pest Management of Bemisia Tabaci in the new century*, ed. SE Naranjo, PC Ellsworth. *Crop Prot.* 20:853–869.

Erdogan C, Moores GD, Gurkan MO, Gorman KJ, Denholm I. 2008. Insecticide resistance and biotype status of populations of the tobacco whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) from Turkey. *Crop Prot.* 27:600–605.

Eveleens KG. 1983. Cotton-insect control in the Sudan Gezira: analysis of a crisis. *Crop Prot.* 2:273–287.

Forrester NW, Fitt GP. 1992. Management of insecticide resistance in *Heliothis armigera* in Australia – ecological and chemical countermeasures. In *Resistance '91: Achievements and Developments in Combating Pesticide Resistance*, ed. I Denholm, AL Devonshire, DW Hollomon, pp. 88–99. London and New York: Elsevier Applied Science.

Forrester NW, Cahill M, Bird LJ, Layland JK. 1993. Management of pyrethroid and endosulfan resistance in *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Australia. *Bull. Entomol. Res* Supplement No. 1:1–132.

ffrench-Constant RH, Daborn PJ, Le Goff G. 2004. The genetics and genomics of insecticide resistance. *Trends Genet.* 20:163–170.

Georghiou GP, Taylor CE. 1977. Operational influences in the evolution if insecticide resistance. *J. Econ. Entomol.* 70:653–658.

Georghiou GP. 1981. *The Occurrence of Resistance to Pesticides in Arthropods*. Rome: Food and Agriculture Organization of the United Nations.

Georghiou GP, Taylor CE. 1986. Factors influencing the evolution of resistance. In *Pesticide Resistance: Strategies and Tactics for Management*, ed. National Research Council, pp. 157–169. Washington, DC: National Academy Press.

Gordon HT. 1961. Nutritional factors in insect resistance to chemicals. *Annu. Rev. Entomol.* 6:27–54.

Grafius EJ. 1995. Is local selection followed by dispersal a mechanism for rapid development of multiple insecticide resistance in the Colorado potato beetle? *Am. Entomol.* 41:104–109.

Guirao P, Beitia F, Cenis JL. 1997. Biotype determination of Spanish populations of *Bemisia tabaci* (Homoptera: Aleyrodidae). *Bull. Entomol. Res.* 87:587–593.

Hemingway J, Jayawardena KGI, Weerasinghe I, Herath PRJ. 1987. The use of biochemical tests to identify multiple insecticide resistance mechanisms in field-selected populations of *Anopheles subpictus* Grassi (Diptera: Culicidae). *Bull. Entomol. Res.* 77:57–66.

Hoelmer KA. 1996. Whitefly parasitoids: can they control field populations of *Bemisia*? In *Bemisia: 1995 Taxonomy, Biology, Damage, Control and Management*, ed. D Gerling, RT Mayer, pp. 451–476. Andover, Hants, UK: Intercept Ltd.

Horowitz AR, Ishaaya I. 1992. Susceptibility of the sweetpotato whitefly (Homoptera: Aleyrodidae) to buprofezin during the cotton season. *J. Econ. Entomol.* 85:318–324.

Horowitz AR, Forer G, Ishaaya I. 1994. Managing resistance in *Bemisia tabaci* in Israel with emphasis on cotton. *Pestic. Sci.* 42:113–122.

Horowitz AR, Ishaaya I. 1996. Chemical control of *Bemisia tabaci*-management and application. In *Bemisia: 1995 Taxonomy, Biology, Damage, Control and Management*, ed. D Gerling, RT Mayer, pp. 537–556. Andover, Hants, UK: Intercept Ltd.

Horowitz AR, Denholm I, Gorman K, Cenis JL, Kontsedalov S, Ishaaya I. 2003. Biotype Q of *Bemisia tabaci* identified in Israel. *Phytoparasitica* 31:94–98.

Horowitz AR, Kontsedalov S, Ishaaya I. 2004. Dynamics of resistance to the neonicotinoids acetamiprid and thiamethoxam in *Bemisia tabaci* (Homoptera: Aleyrodidae). *J. Econ. Entomol.* 97:2051–2056.

Horowitz AR, Kontsedalov S, Khasdan V, Ishaaya I. 2005. Biotypes B and Q of *Bemisia tabaci* and their relevance to neonicotinoid and pyriproxyfen resistance. *Arch. Insect Biochem. Physiol.* 58:216–225.

Horowitz AR, Kontsedalov S, Denholm I, Ishaaya I. 2002. Dynamics of insecticide resistance in *Bemisia tabaci*: a case study with the insect growth regulator pyriproxyfen. *Pest Manag. Sci.* 58:1096–1100.

Horowitz AR, Mendelson Z, Cahill M, Denholm I, Ishaaya I. 1999. Managing resistance to the insect growth regulator, pyriproxyfen, in *Bemisia tabaci*. *Pestic. Sci.* 55:272–276.

Isaacs R, Byrne DN. 1998. Aerial distribution, flight behaviour and eggload: their inter-relationship during dispersal by the sweetpotato whitefly. *J. Anim. Ecol.* 67:741–750.

Ishaaya I, Horowitz AR. 1992. Novel phenoxy juvenile hormone analog (pyriproxyfen) suppresses embryogenesis and adult emergence of sweetpotato whitefly (Homoptera, Aleyrodidae). *J. Econ. Entomol.* 85:2113–2117.

Ishaaya I, Horowitz AR. 1995. Pyriproxyfen, a novel insect growth regulator for controlling whiteflies: mechanisms and resistance management. *Pestic. Sci.* 43:227–232.

Ishaaya I, Kontsedalov S, Horowitz AR. 2005. Biorational insecticides: mechanism and cross-resistance. *Arch. Ins. Biochem. Phys.* 58:192–199.

Johnson MW, Toscano NC, Reynolds HT, Sylvester ES, Kido KR, Natwick, ET. 1982. Whiteflies cause problems for southern California growers. *California Agric*. 9–10: 24–26.

Karunker I, Benting J, Lueke B, Ponge T, Nauen R, Roditakis E, Vontas J, Gorman K, Denholm I, Morin S. 2008. Over-expression of cytochrome P450 *CYP6CM1* is associated with high resistance to imidacloprid in the B and Q biotypes of *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Ins. Biochem. Mol. Biol.* 38:634–644.

Khasdan V, Levin I, Rosner A, Morin S, Kontsedalov S, Maslenin L, Horowitz AR. 2005. DNA markers for identifying biotypes B and Q of *Bemisia tabaci* (Hemiptera: Aleyrodidae) and studying population dynamics. *Bull. Entomol. Res.* 95:605–613.

Legg JP. 1996. Host-associated strains within Ugandan populations of the whitefly, *Bemisia tabaci* (Genn) (Hom, Aleyrodidae). *J. Appl. Entomol.* 120:523–527.

Li AY, Dennehy TJ, Nichols RL. 2003. Baseline susceptibility and development of resistance to pyriproxyfen in *Bemisia argentifolii* (Homoptera: Aleyrodidae) in Arizona. *J. Econ. Entomol.* 96:1307–1314.

Liebhold AM, Tobin PC. 2008. Population ecology of insect invasions and their management. *Annu. Rev. Entomol.* 53:387–408.

Liu Z, Williamson MS, Lansdell SJ, Denholm I, Han Z, Millar NS. 2005. A nicotinic acetylcholine receptor mutation conferring target-site resistance to imidacloprid in *Nilaparvata lugens* (brown planthopper). *Proc. Natl. Acad. Sci.* 102:8420–8425.

Liu SS, De Barro P, Xu J, Luan JB, Zang LS, Ruan YM, Wan FH. 2007. Asymmetric mating interactions drive widespread invasion and displacement in a whitefly. *Science* 318:1769–1772.

Ma D, Gorman K, Devine G, Luo WC, Denholm I. 2007. The biotype and insecticide-resistance status of whiteflies, *Bemisia tabaci* (Hemiptera: Aleyrodidae), invading cropping systems in Xinjiang Uygur Autonomous region, northwestern China. *Crop Prot.* 26:612–617.

MacArthur RH, Wilson EO. 1967. *The Theory of Island Biogeography*. Princeton, NJ: Princeton University Press.

Mao W, Schuler MA, Berenbaum MR. 2007. Cytochrome P450s in *Papilio multicaudatus* and the transition from oligophagy to polyphagy in the Papilionidae. *Ins. Mol. Biol.* 16:481–490.

May RM, Dobson AP. 1986. Population dynamics and the rate of evolution of pesticide resistance. In *Pesticide Resistance: Strategies and Tactics for Management*, ed. National Research Council, pp. 170–193. Washington, DC: National Academy Press.

Moreno R, Garijo C, Rodriguez MD, Garcia S, Téllez MM, Garcia E, Rodriguez MP. 1994. IPM development on tomato under plastic in the south of Spain. *Bull. OILB/SROP (IOBC/WPRS)* 17:22–26.

Moya A, Guirao P, Cifuentes D, Beitia F, Cenis JL. 2001. Genetic diversity of Iberian populations of Bemisia tabaci (Hemiptera: Aleyrodidae) based on random amplified polymorphic DNA polymerase chain reaction. *Mol. Ecol.* 10:891–897.

Naranjo SE. 2001. Conservation and evaluation of natural enemies in IPM systems for *Bemisia tabaci*. In *Special Issue: Challenges and Opportunities for Pest Management of Bemisia Tabaci in the New Century*, ed. SE Naranjo, PC Ellsworth. *Crop Prot.* 20:835–852.

Naranjo SE, Akey DH. 2005. Conservation of natural enemies in cotton: comparative selectivity of acetamiprid in the management of *Bemisia tabaci*. *Pest Manag. Sci.* 61:555–566.

Naranjo SE, Ellsworth PC. 2009a. Fifty years of the integrated control concept: moving the model and implementation forward in Arizona. *Pest Manag. Sci.* 65:1267–1286.

Naranjo SE, Ellsworth PC. 2009b. The contribution of conservation biological control to integrated control of *Bemisia tabaci* in cotton. *Biol. Control.* 51:458–470.

Naranjo SE, Ellsworth PC, Hagler JR. 2004. Conservation of natural enemies in cotton: role of insect growth regulators for management of *Bemisia tabaci*. *Biol. Control* 30:52–72.

National Research Council. 1986. *Pesticide Resistance: Strategies and Tactics for Management*. Washington, DC: National Academy Press.

Nauen R, Stumpf N, Elbert A. 2002. Toxicological and mechanistic studies on neonicotinoid cross resistance in Q-type *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Pest Manag. Sci.* 58:868–875.

Nauen R, Denholm I. 2005. Resistance of insect pests to neonicotinoid insecticides: current status and future prospects. *Arch. Insect Biochem. Physiol.* 58:200–215.

Oetting RD, Buntin GD. 1996. *Bemisia* damage expression in commercial greenhouse production. In *Bemisia: 1995 Taxonomy, Biology, Damage, Control and Management*, ed. D Gerling, RT Mayer, pp. 201–208. Andover, Hants, UK: Intercept Ltd.

Oliveira MRV, Henneberry TJ, Anderson P. 2001. History, current status, and collaborative research projects for *Bemisia tabaci*. *Crop Prot.* 20:709–723.

Palumbo JC, Horowitz AR, Prabhaker N. 2001. Insecticidal control and resistance management for *Bemisia tabaci*. In *Special Issue: Challenges and Opportunities for Pest Management of Bemisia tabaci in the New Century*, ed. SE Naranjo, PC Ellsworth. *Crop Prot*. 20(9):739–765.

Palumbo JC, Ellsworth PC, Dennehy TJ, Nichols RL. 2003. Cross-commodity guidelines for neonicotinoid insecticides in Arizona. *The University of Arizona, Cooperative Extension. IPM Series No. 17, AZ1319*. The University of Arizona, Tucson AZ.

Paris HS, Nerson H, Burger Y. 1987. Leaf silvering of *Cucurbita*. *Can. J. Plant Sci.* 67:593–598.

Pedigo LP. 1999. *Entomology and Pest Management*. USA: Prentice Hall.

Perring TM. 2001. The *Bemisia tabaci* species complex. *Crop Prot.* 20:725–737.

Perring TM, Cooper A, Kazmer DJ, Shields C, Shields J. 1991. New strain of sweetpotato whitefly invades California vegetables. *California Agric.* 45:10–12.

Perring TM, Cooper AD, Rodriguez RJ, Farrar CA, Bellows TS. 1993. Identification of a whitefly species by genomic and behavioral studies. *Science* 259:74–77.

Prabhaker N, Toscano NC, Castle SJ, Henneberry TJ. 1997. Selection for imidacloprid resistance in silverleaf whiteflies from the Imperial Valley and development of a hydroponic bioassay for resistance monitoring. *Pestic. Sci.* 51:419–428.

Prabhaker N, Toscano NC, Perring TM, Nuessly G, Kido K, Youngman RR. 1992. Resistance monitoring of the sweetpotato whitefly (Homoptera: Aleyrodidae) in the Imperial Valley of California. *J. Econ. Entomol.* 85:1063–1068.

Prabhaker N, Castle SJ, Toscano N, Henneberry TJ. 2005. Assessment of cross-resistance potential among neonicotinoid insecticides in *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Bull. Entomol. Res.* 95:535–543.

Rauch N, Nauen R. 2003. Identification of biochemical markers linked to neonicotinoid cross resistance in *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Arch. Insect Biochem. Physiol.* 54:165–176.

Roditakis E, Roditakis NE, Tsagkarakou A. 2005. Insecticide resistance in *Bemisia tabaci* (Homoptera: Aleyrodidae) populations from Crete. *Pest Manag. Sci.* 61:577–582.

Rosenheim JA, Tabashnik BE. 1990. Evolution of pesticide resistance: interactions between generation time and genetic, ecological, and operational factors. *J. Econ. Entomol.* 83:1184–1193.

Rosenheim JA, Tabashnik BE. 1991. Influence of generation time on the rate of response to selection. *Am. Nat.* 137:527–541.

Rosenheim JA, Johnson MW, Mau RFL, Welter SC, Tabashnik BE. 1996. Biochemical preadaptations, founder events, and the evolution of resistance in arthropods. *J. Econ. Entomol.* 89:263–273.

Roush RT. 1986. Inbreeding depression and laboratory adaption in *Heliothis virescens* (Lepidoptera: Noctuidae). *Annu. Entomol. Soc. Am.* 79:583–587.

Roush RT. 1989. Designing resistance management programs: how can you choose? *Pestic. Sci.* 26:423–441.

Rúa P, Simón B, Cifuentes D, Martinez-Mora C, Cenis JL. 2006. New insights into the mitochondrial phylogeny of the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) in the Mediterranean Basin. *J. Zoo Systemat. Evol. Res*. 44:25–33.

Salvucci ME, Stecher DS, Henneberry TJ. 2000. Heat shock proteins in whiteflies, an insect that accumulates sorbitol in response to heat stress. *J. Thermal Biol.* 25:363–371.

Sawicki RM. 1987. Definition, detection and documentation of insecticide resistance. In *Combating Resistance to Xenobiotics*, ed. MG Ford, DW Holloman, BPS Khambay, RM Sawicki, pp. 105–117. Chichester, UK: Ellis Horwood.

Schuster DJ, Mann RS, Toapanta M, Cordero R, Thompson S, Morris RF. 2008. Monitoring of imidacloprid resistance in biotype B of *Bemisia tabaci* in Florida. In *4th International Bemisia Workshop International Whitefly Genomics Workshop*, ed. PA Stansly, CL McKenzie, 53 pp. *J. Insect Sci*. 8:4, available online: http://www.insectscience.org/8.04/ref/abstract83.html

Sharaf N. 1986. Chemical control of *Bemisia tabaci*. *Agric. Ecosyst. Environ.* 17:111–127.

Simons JN, Stoffella PJ, Shuler KD, Raid RN. 1988. Silver-leaf of squash in South Florida. *Proc. Fla. State Hort. Soc.* 101:397–399.

Southwood TRE, Comins HN. 1976. A synoptic population model. *J. Anim. Ecol.* 45:949–965.

Southwood TRE. 1977. Entomology and mankind. *Am. Scient.* 64:30–39.

Stearns SC. 1977. The evolution of life-history traits: a critique of the theory and a review of the data. *Annu. Rev. Ecol. Syst.* 8:145–171.

Strong DR, Lawton JH, Southwood R. 1984. *Insects on Plants*. Cambridge: Harvard Press.

Sundaramurthy VT. 1992. Upsurgence of whitefly *Bemisia tabaci* Gen. in the cotton ecosystem in India. *Outlook Agric.* 21:109–115.

Tabashnik BE, Croft BA. 1982. Managing pesticide resistance in crop-arthropod complexes: interactions between biological and operational factors. *Environ. Entomol.* 11:1137–1144.

Tabashnik BE, Croft BA. 1985. Evolution of pesticide resistance in apple pests and their natural enemies. *Entomophaga* 30:37–49.

Tabashnik BE. 1990. Modeling and evaluation of resistance management tactics. In *Pesticide Resistance in Arthropods*, ed. RT Roush, BE Tabashnik, pp. 153–182. New York: Chapman & Hall.

Taylor CE. 1983. Evolution of resistance to insecticides: the role of mathematical models and computer simulations. In *Pest Resistance to Pesticide*, ed. GP Georghiou, T Saito, pp. 163–173. New York: Plenum.

Taylor CE, Georghiou GP. 1979. Suppression of insecticide resistance by alteration of gene dominance and migration. *J. Econ. Entomol.* 72:105–109.

Uyenoyama MK. 1986. Pleiotropy and the evolution of genetic systems conferring resistance to pesticides. In *Pesticide Resistance: Strategies and Tactics for Management,* ed. National Research Council, pp. 207–221. Washington, DC: National Academy Press.

Via S. 1986. Quantitative genetic models and the evolution of pesticide resistance. In *Pesticide Resistance: Strategies and Tactics for Management*, ed. National Research Council, pp. 222–235. Washington, DC: National Academy Press.

Wagner TL. 1995. Temperature-dependent development, mortality, and adult size of sweetpotato whitefly biotype B on cotton. *Environ. Entomol.* 24:1179–1188.

Watson TF, Tellez MA, Peña M. 1995. Chemical control of the sweetpotato whitefly in cotton. In *Cotton: A College of Agriculture Report*, ed. J Silvertooth, pp. 259–270. Tucson, AZ: The University of Arizona.

Wolfe GR, Hendrix DL, Salvucci ME. 1998. A thermoprotective role for sorbitol in the silverleaf whitefly, *Bemisia argentifolii*. *J. Insect Physiol.* 44:597–603.

Yokomi RK, Hoelmer KA, Osborne LS. 1990. Relationship between the sweetpotato whitefly and the squash silverleaf disorder. *Phytopathology* 80:895–900.

# Chapter 17
# Integrated Systems for Managing *Bemisia tabaci* in Protected and Open Field Agriculture

**Philip A. Stansly and Eric T. Natwick**

## Introduction

The combined efforts of disparate entities have produced notable advances in management of *Bemisia tabaci* (Gennadius) over the decades. On the one hand, entomologists and academicians have focused on specific problems, approaches and solutions, and designed experiments with replicated treatments to provide statistically valid results that hold up to scientific scrutiny. On the other hand, growers must integrate information from all disciplines into a profitable cropping and marketing system in order to survive. The gap between these extremes is often filled by crop consultants or agrochemical sales representatives who may have limited interests, focus or experience. There is clearly a need for more and better information on how management practices can be integrated to provide the desired level of pest suppression while still maintaining a balanced and profitable cropping system. The key challenge to implementing new practices will be to sustain or improve current levels of productivity while minimizing impact on the environment and biodiversity.

Integrated pest management (IPM) as conceived by Stern et al. (1959) stressed the rational combination of chemical, biological, and other control methods. These authors also introduced the key concept of the economic injury level (EIL), essentially the equivalence point between the cost and benefit of pesticide use. This approach has been widely adopted for low cost agronomic crops, but less so in high value vegetable and ornamental crops, many of which are susceptible to attack by *B. tabaci*. There are a number of reasons why this is so. On the one hand, pesticides may represent a small fraction of the total cost of production, often less than 5%. Therefore, controlling their cost has little effect on profit. On the other, it is difficult to base decisions on projected earnings because commodity prices often fluctuate unpredictably in a given season. Furthermore, these fluctuations in price do not always have the predicted effect on EIL because of the counteracting

P.A. Stansly (✉)
Department of Entomology and Nematology, Southwest Florida Research and Education Center, University of Florida/IFAS, 2685 SR 29N., Immokalee, FL 34142, USA
e-mail: pstansly@ufl.edu

P.A. Stansly, S.E. Naranjo (eds.), *Bemisia*: Bionomics and Management of a Global Pest,
DOI 10.1007/978-90-481-2460-2_17, 

effect of price on demand. When prices go down the EIL should go up proportionately, but buyers can afford to be more exigent and therefore less tolerant of insect injury, effectively lowering the threshold. As a result, the need for insect control can increase when it can be least afforded. Product scarcity raises prices but also increases buyer tolerance for damage which augments supply. This tends to counteract the expected decrease in EIL, even though the grower will attempt to maintain production to take advantage of the good market. Therefore, the grower's strategy may be to maximize yield to take advantage of ephemeral high prices, rather than to control costs.

An "action threshold" requiring less rigorous criteria has often been employed to help rationalize management decisions when definition of an economic threshold based on EIL seems impractical. Action threshold has been defined as the level of pest populations at which control should be implemented to avoid significant damage to the crop (Dik and Albejas 1999). All that is required is demonstration of significant crop loss with no economic consideration. Even so, we are unaware of any action threshold that has been set for an insect that acts as a disease vector, including *B. tabaci*. Such a threshold would have to be based on the number of immigrating whiteflies that are viruliferous, a difficult estimate to obtain. This uncertainty leads to an attitude that any number of whiteflies is too many, especially in high value commodities and where viral transmission is a threat. Thus risk management has come to replace cost management as the goal of whitefly control in high value crops.

While the inability to define thresholds in high value crops tends toward intensified use of pesticides, the counteracting tendency is provided by legal restrictions and increased liability. Public concern for health risks associated with pesticides is evidenced by the increasing market for organic produce, valued in the USA at $10.3 billion and in the EU at $13 billion (Dimitri and Oberholtzer 2005). Clearly, reduced dependence on insecticides will require strengthening the remaining two legs of the IPM triangle, biological and cultural control. The opportunities and constraints for accomplishing this are often distinctly different for protected and open field crops. In particular, protected (greenhouse) horticulture offers the possibility of both excluding pest populations and confining natural enemies. Control over environmental conditions, to the extent that it can be achieved in protected horticulture, can also be used to favor beneficial organisms.

The many advantages of protected horticulture in terms of yield, quality, and duration of production have contributed to its increasing prominence in many high value crops on which *B. tabaci* is a significant pest. The division of this chapter into two sections recognizes the distinctive management challenges represented by protected versus open field cropping systems. The agronomic crops (cotton and alfalfa) will be considered as part of open field systems with special attention given to area-wide management. An emphasis on cropping systems rather than particular crops is in concert with the more general approach of this chapter, as it is mostly in the finer details that commodity distinctions come into play, especially with regard to augmentative biological control as it is practiced in protected vegetable production. Nevertheless, open field horticulture can benefit from experience in the greenhouse

environment and efforts at adapting some of these augmentative control tactics to field grown vegetable production will also be discussed.

## Biologically Based Management of *B. tabaci* in Protected Vegetable Crops

### *Key Pests of Greenhouse Vegetables*

The objective of protected agriculture to provide an ideal environment for plant growth and development year round also provides favorable conditions for arthropod pests. These conditions favor non-diapausing pests with high reproductive rates. The diversity of crop plants found in greenhouses favors polyphagous species with efficient detoxification systems. Constant selection pressure from insecticides on confined populations, coupled with high rates of increase can result in rapid development of resistant populations common among greenhouse pests. Rapid population growth is typical of small pest species whose often cryptic habits make them difficult to detect. Many have attained global distributions in recent years thanks to the world wide trade in greenhouse grown commodities, especially ornamentals. Consequently, many greenhouse pests tend to be small, polyphagous, capable of rapid population growth, resistant to insecticides, and globally distributed (Table 17.1).

**Table 17.1** Principal pests of greenhouse vegetable production in approximate order of importance

| Tomato | Pepper | Beans | Cucurbits |
|---|---|---|---|
| **Whiteflies** | Thrips | **Whiteflies** | **Whiteflies** |
| Spider mites | **Whiteflies** | Spider mites | Spider mites |
| Russet mite | Noctuidae | Thrips | Thrips |
| Leafminers | Broadmite | Aphids | Leafminers |
| Gelechiidae | Aphids | Noctuidae | Aphids |

Whiteflies: *B. tabaci* predominating in the tropical and subtropics, *T. vaporiariorum* in temperate regions
Thrips: Primarily *Frankliniella occidentalis* with *Thrips tabaci* and *T. palmi* being more localized problems
Spidermites: *Tetranichus* spp., principally *T. urticae*
Leafminers: *Liriomyza* spp. especially *L. trifolii*, followed by *L. huidobrensis* an *L. sativae*, all with world wide distributions and *L. bryoniae* still with an Old World distribution
Russet mite: *Aculops lycropersici*
Broadmite: *Polyphagotarsonemus latus*
Aphids: most often *Myzus persicae*, or *Aphis gossypii*, as well as *Macrosiphus euphorbiae* (especially Solanaceae) and *Aulacorthum solani* (especially pepper)
Noctuidae such as *Spodoptera literalis*, *S. exigua*, *Chrysodeixis* spp. and *Tricoplusia ni*, and Gelechiidae (*Tuta absoluta, Keiferia lycopersicella*)

Growers and pest control consultants cannot afford to focus on a single pest, leading to a tendency for calendar sprays with broad spectrum insecticides. Yet, it is also clear that one or the other of the whitefly species *Trialeurodes vaporariorum* (Westwood) and *B. tabaci* can be considered a key pest in many greenhouse crops. This is especially true when whiteflies are acting as virus vectors, which is often the case when tomatoes and cucurbits are grown, but also in some regions with beans, peppers and other crops (Polston and Anderson 1997). Consequently, effective whitefly control may often be the top pest management priority. This situation in most major greenhouse growing regions justifies our focus on *B. tabaci.*

## *Damage to Vegetable Crops Caused by* Bemisia tabaci

In ascending order by damage potential, plants may experience direct injury from sap removal, buildup of honeydew and sooty mold, physiological disorders, and transmission of plant viruses (see Section III of this volume). Honeydew can be washed off during packing, but sooty mold blackens the leaves, interfering with transmission of light to the chloroplasts, and also causes cosmetic damage that may downgrade product acceptability and value (Howard et al. 1994). Physiological disorders caused by nymphal feeding include tomato irregular ripening and squash silverleaf that may affect fruit color as well as foliage (Schuster et al. 1996). Like sooty mold, both disorders can significantly downgrade fruit quality and value in ways that cannot be corrected in the packing house. The worst consequence of whitefly infestation is often the early and devastating appearance plant viruses such as *Tomato yellow leaf curl virus* (TYLCV), *Tomato chlorosis virus* (ToCV), *Cucurbit yellow stunting disorder virus* (CYSDV), *Cucumber vein yellowing virus* (CVYV), *Squash vein yellowing virus* (SqVYV), and *Bean golden mosaic virus* (BGMV) (see Moriones and Navas-Castillo Chapter 8; Morales Chapter 9; Wintermantel Chapter 10). In fact, more than 150 plant viruses are known to be transmitted by whiteflies and the number continues to grow (Polston and Anderson 1997; Jones 2003). Therefore, protection from whitefly attack early in the crop cycle may be the most important pest management task facing the grower or consultant.

## *Greenhouse Exclusion Technology*

Greenhouse construction runs the gamut from simple polyethylene tunnels to elaborate structures of plastic or glass fitted with computerized controls for climate control, irrigation and fertilization. Even more germane to pest management in greenhouses is the capacity to exclude insect pests with fine netting and/or UV absorbing films (Antignus Chapter 13). However, there is a tradeoff between exclusion and ventilation as finer netting means less air exchange with the outside and consequent rise of temperature during the heat of the day (Harmanto and Tantau 2006; Teitel 2006). High rates of evaporation from the substrate and evapotranspiration from the crop maintain high humidity that can reach 100%. Poor ventilation

may be especially acute in greenhouses with screened ventilation retrofitted rather than included in the original design. The result is often replacement of a pest problem with a fungal disease problem.

It may be necessary to entirely screen sides and ends of the greenhouse and ventilate large portions of the roof to create an insect barrier in tropical or subtropical areas and still maintain reasonable growing conditions. However, even with screened surface areas as large as the floor space, mesh sizes small enough to exclude whiteflies may not allow for sufficient ventilation rate (Alvarez et al. 2006; Harmanto and Tantau 2006). In such cases, forced ventilation with fans may be necessary to improve air exchange. However, the insect excluding ability of a particular netting is inversely proportional to air approach velocity. Therefore, air must be drawn into the greenhouse as uniformly as possible over the entire screened surface to minimize pest penetration. Netting can also become plugged with dust and debris, requiring periodic cleaning and/or replacement. Additional cooling can be provided in dry climates by evaporation using fogging or fan and pad evaporation systems. However, the latter must also be housed in screen enclosures sufficiently large to allow for the required air flow. Increasing gutter height is another way of improving air circulation and homogeneity of physical conditions in the greenhouse (Raya et al. 2006).

Thoracic width is generally the criterion used to determine mesh size for pest exclusion. The commonly accepted value for *B. tabaci* is 239 μm (Bethke and Paine 1991). Although increasing exclusion capability generally results in increased air resistance (static pressure), these two characteristics of screens are not always well correlated (Bell and Baker 2000). Rectangular interstices are increasingly used to minimize air resistance while maintaining exclusion ability (Cabrera et al. 2006). Commonly used netting to exclude *B. tabaci* is constructed of woven, UV stabilized (and often UV absorbing) polyethylene with 10 threads per cm in the vertical plane and 22 in the horizontal plane, providing openings of approximately 200 by 700 μm (Table 17.2). This mesh size has been shown to provide reliable exclusion of *B. tabaci* while allowing free entry of the parasitoid *E. mundus* (Hanafi et al. 2007).

**Table 17.2** Thorax width of greenhouse pests (Bethke and Paine 1991) and corresponding mesh sizes of insect nettings in microns that could be used to exclude them

| Insect | Thorax (μ) | Screen | Hole size (μm) | Static pressure |
|---|---|---|---|---|
| Flower thrips | 192 | Bugbed® | 135×135 | High |
| *B. tabaci* | 240 | Projar 22×10 | 230×900 | Moderate |
| Melon aphid | 340 | Green-tek antivirus | 266×818 | Moderate |
| Leafminer | 640 | Lumite 32×32 | 530×530 | Low |

## *Host Plant Resistance*

Effective mechanisms of plant resistance to TYLCV in tomato were first demonstrated in Israel over 40 years ago. However, the process of incorporating resistant

characteristics from tomato relatives such as *Solanum habrochaites* and *S. pennellii* into the many varieties preferred by growers using classical breeding techniques has been understandably slow (Ji et al. 2007; see Nombela and Muñiz Chapter 14 for a review of plant resistance to the vector). The technology exists to speed this process markedly (Beachy 1997), but unfortunately, widespread prejudice against genetically engineered crops has impeded development (Baker and Burnham 2001). Thus, the grower must often content himself with what he considers to be horticulturally inferior cultivars if he is to incorporate TYLCV resistance into his program. Nevertheless, the risk of loss from TYLCV is so great that considerable adoption of these cultivars has occurred (Stansly et al. 2004a, b; Polston and Lapidot 2007; Ozores-Hampton et al. 2008). Rejection of genetically modified crops in the marketplace may also explain why no horticulturally acceptable cultivars have been developed that resist or tolerate other whitefly-borne viruses of tomato such as ToCV or *Tomato infectious chlorosis virus* (TICV).

Effective mechanisms for plant resistance to CYSDV have not been incorporated into commercial melon varieties, although resistant cucurtbit germ plasm is known. CYSDV incidence has been partially managed in the desert southwest USA. through reduction of the *B. tabaci* vector with a summer host free period between the spring and fall melon cropping seasons (Gilbertson 2007) and more recently in Arizona, 2008, with a grower-imposed host free period (D. Byrne, personal communication).

## *Biological Control of* Bemisia tabaci

### Entomopathogenic Fungi

Three species of entomopathogenic fungi active against *B. tabaci* are available commercially, *Paecilomyces fumosoroseus* = *Isaria fumorosea*, *Verticillium lecanii* and *Beauveria bassiana* (Table 17.3). The first two are naturally found infecting whiteflies whereas *B. bassiana* is only seen infecting whiteflies when applied as part of a formulation.

Entomopathogenic fungi are easy to apply although good coverage is required on the abaxial foliar surfaces where whiteflies reside. These fungi present essentially no risk to human health and most studies show that they are relatively innocuous to other natural enemies (Goettel et al. 2001; Vestergaard et al. 2003; Zimmerman 2008). Registration is often expedited in the USA, although not in Europe, where, unlike the USA, efficacy is a required criterion. Use of fungal products is compatible with many insecticides and resistance to mycopesticides has not yet been reported. However, fungi are slow acting compared to chemical insecticides, exhibit poor adulticidal activity, and are incompatible with many commonly used fungicides. In addition, they are relatively expensive, have limited shelf life, and are dependent on favorable environmental conditions (Inglis et al. 2001; Faria and Wraight 2001; Vidal et al. 2003).

**Table 17.3** Commercial formulations of entomopathogenic fungi for whitefly control. Modified from Faria and Wraight (2001)

| Fungus | Product | Company | Country |
|---|---|---|---|
| *Beauveria bassiana* | BotaniGard | Laverlam International/Bioworks | USA |
| | Ago Biocontrol Beauveria | Ago Biocontrol | Columbia |
| | Bea-Sin | Agrobiologicos de Noroeste S.A. de C. V. | Mexico |
| *Isaria fumorosea* = *(Paecilomyces fumosoroseus)* | PFR-97 | Certis | USA |
| | PreFerRal | Biobest N.V. | Belgium |
| | Pae-Sin | Agrobiologicos de Noroeste S.A. de C. V. | Mexico |
| *Verticillium lecanii* | Ago Biocontrol Verticillium | Ago Biocontrol | Columbia |
| | Mycotal | Koppert Biological Systems | Holland |

### Hymenoptera: Aphelinidae

The pioneering work with *Encarsia formosa* Gahan on the greenhouse whitefly, *T. vaporariorum* (Van Lenteren and Woets 1988; Hu et al. 2002) ushered in the augmentative biological control strategy for whiteflies. While *En. formosa* will also attack *B. tabaci* (Enkegaard 1993; Hu et al. 2003), it is not as effective against this host as are many *Eretmocerus* spp. (Bosclair et al. 1990; Szabo et al. 1993; Gerling et al. 2001; Hoddle 2004). This may in part be due to the high temperature sensitivity of *En. formosa* which is at a disadvantage above 20°C (Qui et al. 2004). Furthermore, the more proovigenic *Eretmocerus* spp. have higher reproductive rates than the synovogenic *En. formosa* (Jervis et al. 2001; Qui et al. 2004; Urbaneja et al. 2007, Arnó et al. Chapter 15) and also are able to locate patches of *B. tabaci* more quickly (Hoddle et al. 1998). Therefore, interest has turned to *Eretmocerus*, in particular *Er. eremicus* and *Er. mundus* for control of *B. tabaci* (Stansly 2004a, b, 2005a, b).

*Eretmocerus eremicus* is a New World species that attacks both *B. tabaci* and *T. vaporariorum* with apparently equal facility (Greenberg et al. 2002; Soler-Gamborena and van Lenteren 2004). Therefore, it is especially useful for controlling mixed infestations of the two whiteflies. It has also been used to control pure infestations of *B. tabaci*, albeit with limited success (van Driesche et al. 2001, 2002). *Er. eremicus* was displaced by *Er. mundus* in greenhouses in Spain where both species were released (Stansly et al. 2004a, b, 2005a, b). Although immigration from outside the greenhouse explained this displacement in part, behavioral traits such as willingness to multi-parasitize hosts parasitized by the other species (Ardeh et al. 2005) may have assisted *Er. mundus* in competition with *Er. eremicus*. Coincidentally, native *Eretmocerus* spp. have been largely displaced by introduced old world species in open agriculture in the American Southwest, Florida and elsewhere in the USA and in Australia (Stansly unpublished data; Naranjo 2008; De Barro and Coombs 2008).

Successful management of *B. tabaci* using *Er. mundus* was demonstrated in large-scale commercial trials in protected pepper production facilities near Cartagena in southern Spain (Stansly et al. 2004b, 2005a). The primary pest, western flower thrips, *Frankliniella occidentalis*, was being controlled biologically in many of these greenhouses which facilitated acceptance of whitefly biological control. Control in tomato was also shown to be possible, although higher release rates were required to obtain the same level of control as in pepper (Stansly et al. 2005b). Large scale field trials in commercial greenhouses supported this conclusion, although results were somewhat compromised in tomato by pesticide use (see Table 17.4), presumably in response to the greater threat posed by the pest in its role as a virus vector in that crop (Stansly et al. 2004a, b).

### Heteroptera: Miridae

The availability and use of predators alone and in combination with other control agents is discussed in detail by Arnó et al. (Chapter 15) and is summarized here. Three species of Miridae are widely used for augmentative biological control of *B. tabaci*: two European species *Macrolophus caliginosus* and *Nesidiocoris tenuis* and the American species, *Dicyphus hesperus*. All are naturally found on hirsute hosts and adapt best to tomato and to a lesser extent eggplant, but not at all to pepper. Effective control of *B. tabaci* by *N. tenuis* was demonstrated in large cage studies (Calvo et al. 2008a, b). However, trials in experimental and commercial greenhouses were less successful (Nannini 2001) and high release rates were required for satisfactory control. Best results were obtained when releases were made early during the warm season and/or reinforced with releases of *En. formosa*.

Establishment of mirid predators is generally slow and all feed on plants when prey is scarce (Alomar and Albajes 1996; Urbaneja et al. 2005; Sanchez 2008; Calvo et al. 2008a, b). Nevertheless, *D. hesperus* has been shown to prefer tomato leaves to fruit, so potential for damage is relatively low (McGregor et al. 2000). Shipp and Wang (2006) observed that damage to tomato by *D. hesperus* increased exponentially when a ratio of 1:10 (predator: prey) was exceeded. Calvo et al. (2008a, b) showed that the ratio of *B. tabaci* nymphs and *N. tenuis* individuals was the best predictor of incidence of damage in the form of necrotic rings on the peduncle. Alomar and Albajes (1996) provided a decision chart indicating that insecticidal control against *Dicyphus tamanini* was required when it exceeded 4 per plant and adult whitefly were less than 20 per plant.

### Acari: Phytoseiidae

The most recent breakthrough in whitefly biological control has been development and commercialization of the predatory mite, *Amblyseius swirskii* (Nomikou et al. 2001a; Calvo et al. 2008a, b). In contrast to the mirids, *A. swirskii* seems to be well-adapted to every vegetable crop host except tomato, including pepper, cucumber, and eggplant (Nomikou et al. 2001b; Calvo et al. 2008a, b: Stansly and Castillo

2009). The ability to feed on alternate hosts is a distinct advantage and significant suppression of broadmite and western flower thrips has also been observed (Messelink et al. 2005; Tal et al. 2007). The mites also feed on pollen and therefore could be released preventively before pests are present Nomikou et al. 2003; Hoogerbrugge et al. 2007).

Nomikou, et al. (2001b) showed that populations of *B. tabaci* were reduced 16- to 21-fold on plants receiving the mites compared to those that did not, 9 weeks after *A. swirskii* had been released on cucumber plants provided with *Typhus* sp. pollen. Similar results were reported by Belda and Calvo (2006) and Calvo et al. (2008a, b). Whiteflies were virtually eliminated from pepper plants having received eight whitefly adults per week over a 3-week period followed by a single release of either 25 or 50 mites per plant. Messelink et al. (2008) found better suppression of *T. vaporariorum* was achieved following release of *A. swirskii* on cucumber when western flower thrips was also present, presumbably because the additional food source allowed higher populations of the mite to be maintained. Belda and Calvo (2006) and Calvo et al. (2008a, b) reported that the best biological control strategy for *B. tabaci* in eggplant was the combination of *A. swirskii* and *E. mundus*. Effectiveness, host range and compatibility of *A. swirskii* with other natural enemies has lead to widespread adoption of biological control in greenhouse pepper and other protected vegetable crops in Spain and elsewhere, and greatly furthered acceptance of biological control as a viable strategy for management of greenhouse pests (van der Blom 2007).

## *Compatibility of Various Pest Control Practices*

While the threat of whitefly-transmitted viruses motivates reliance on insecticidal control, consumer demand for produce grown with little or no pesticides provides incentive for alternative management. Additional impetus comes from the example of successful biological control in vegetable greenhouse industries of northern Europe, especially the Netherlands (Bolckmans 1999). However, biological control may not seem like a viable alternative in the face of insect-borne virus were it not for compatible technologies that can provide protection early in the crop cycle. These include insect excluding structures alluded to above and in a previous chapter, spunbonded or embossed floating row covers (Natwick and Laemmlen 1993; Orozco-Santos et al. 1995), crop free periods, and the availability of disease resistant or tolerant cultivars.

Excluding structures and crop free periods were classified under cultural control practices designed to provide refuge from the pest in space or time, respectively, by Hilje et al. (2001). However, there is an important difference between the two practices in that excluding structures can be implemented at the level of the individual farm whereas crop free periods must be implemented area-wide. A single farm out of phase with the rest can provide sufficient inoculum and vectors to infect nearby growing areas. Nevertheless, well implemented crop free periods have succeeded in

reducing levels of whitefly and TYLCV and CYSDV to tolerable levels in open field systems such as southwest Florida, the Arava Valley in Israel and the Dominican Republic (Hilje et al. 2001), but not to our knowledge in an area dominated by protected horticulture.

## *Compatibility of Pesticides with Biological Control*

Development of a totally pesticide-free cropping system for vegetable production is a daunting task, given market demands for blemish-free produce. Even organic crops are frequently sprayed, often more than conventional crops, due to the reduced efficacy of permitted products such as soaps, oils, plant extracts, mineral and fermentation products. It can also be said that no pesticide, regardless of how apparently benign, is totally without some negative impact on biological control agents (http://www.koppert.nl/Side_effects.html). Nevertheless, there is a wide spectrum of selectivity among active ingredients, with considerable variation among natural enemies and their life stages in susceptibility to any particular pesticide. Given the large number of products, natural enemies of interest, possible effects of life stage, environmental conditions, host plants, and time of exposure, the possible combinations are essentially unlimited and preclude certainty that any single pest management decision is the best one. Defining these effects for all life stages of natural enemies of interest and the myriad of products on the market has occupied the energies of many investigators. Much of this information has been summarized by the biological control industry in indices available on-line such as the one cited above. For example, the Koppert database gives two numbers, the first representing relative impact on a scale of 1–4 and the second the interval of residual effect in weeks.

Stansly et al. (2004a) used this guide to evaluate the impact of pesticide applications on *Eretmocerus* spp. in Spanish greenhouses characterized as either IPM or conventional based on whether or not they employed biological control or relied totally on insecticides for pest management. *Eretmocerus mundus* and *E. eremicus* were released in separate sections of 10 IPM greenhouses to control *B. tabaci*, Each application was valued as the sum of ratings (1–4) for pupae and for adults of *E. eremicus* or closest related species given – usually *En. formosa* – and the mean number of weeks of residual effect. The sum of these three numbers was termed an impact rating and varied between 2 (most selective) and 18 (least selective). Impact ratings for all applications made while the crop was being monitored were summed for each greenhouse and then divided by the number of weeks to give an index of incompatibility during the period of study. The index ranged 1.1–8.7 in IPM greenhouses and 1.1–35.7 in conventional greenhouses (Table 17.4). A low incompatibility index usually corresponded to successful biological control in the IPM greenhouses as judged by parasitism rates and pest populations. The index also proved useful for rating the pest management program on a broad spectrum vs. selective continuum.

**Table 17.4** Mean (±SE) number of pesticide applications, the number broad spectrum insecticides, selective insecticides/acaracides or fungicides used in those applications, the sum of side effect ratings for all pesticides used, and index of incompatibility of pesticide regime by management system on tomato crops in 19 Spanish greenhouses, 2001–2002. The management system designation of conventional or IPM was based on whether or not biological control was employed (Stansly et al. 2004a)

| | Management designation | | | |
|---|---|---|---|---|
| | Conventional ($N = 9$) | | IPM ($N = 10$) | |
| Applications (No.) | 40.3 | ±6.9 | 16.2 | ±2.1 |
| Broad spectrum (No.) | 9.2 | ±4.1 | 0.4 | ±0.4 |
| Selective (No.) | 15.0 | ±4.1 | 7.0 | ±1.6 |
| Fungicides (No.) | 16.1 | ±2.6 | 8.8 | ±1.4 |
| Side effects (Sum) | 276.4 | ±59.1 | 60.0 | ±9.7 |
| Incompatibility index | 11.4 | ±3.2 | 3.0 | ±0.5 |

## Area-Wide Management of Whitefly in Open Field Crops

The advances described above leading to the acceptance of biologically-based management of *B. tabaci* and other pests in protected vegetable production provide a stark contrast to the realities of most open field cropping systems. Nevertheless, some commonalities exist. Both production systems may occur in a mosaic of different crop types and phenologies within which polyphagous pests like *B. tabaci* move freely (see Naranjo et al. Chapter 6). Therefore, prevention or avoidance of severe whitefly infestations through cultural manipulation of crops or area-wide management is often required. These techniques have been used successfully in conjunction with an integrated control system featuring both biological and chemical control in the desert southwest of the USA and adjoining Mexico (Ellsworth and Martinez-Carrillo 2001; Naranjo and Ellsworth 2009a). These authors proposed a model of whitefly management that organizes all *B. tabaci* control tactics into a multi-level, multi-component pyramid and defines three major keys as "sampling", "effective chemical use", and "avoidance" (Fig. 17.1). Insect growth regulators ([IGRs] buprofezin and pyriproxyfen) in cotton and imidacloprid in vegetables and melons were key chemical tactics, integrated with sampling plans, action thresholds, and resistance management guidelines.

An area-wide or community-based management approach for *B. tabaci* can be successful in reducing the risk of damage to cotton and other crops (cucurbits, *Brassica*, lettuce and alfalfa). However, this approach relies on cooperation of growers within a defined geographic area or community to reduce intercrop movement and buildup of *B. tabaci* populations and to manage insecticide-resistance. Therefore, organized and sustained grower education was the key to the area-wide adoption and deployment of this IPM plan (Ellsworth and Martinez-Carrillo 2001).

Adjusting planting and harvest dates to avoid the heaviest migration periods and crop overlap has been a successful strategy. In the Rio Grande Valley of Texas, short

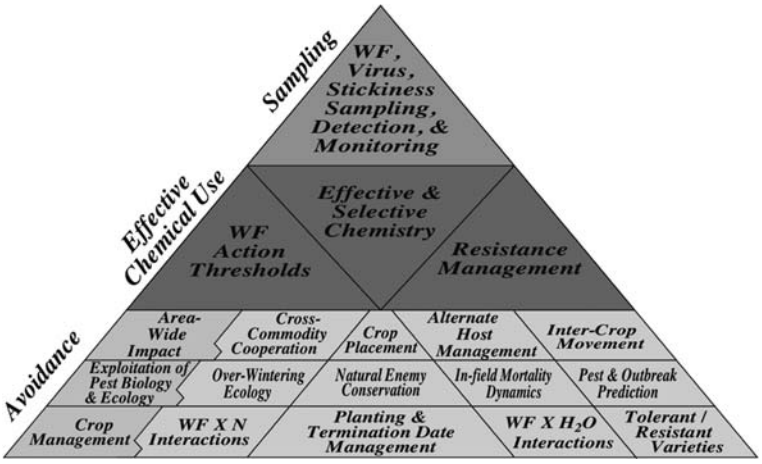



**Fig. 17.1** Interaction of key aspects of whitefly management employed in the North American desert agroecosystem based on sampling, appropriate thresholds, effective chemistry and resistance management, and avoidance strategies that include exploitation of pest biology and ecology, biological control, crop management and area-wide impact (Ellsworth and Martinez-Carrillo 2001; with permission from Elsevier)

season, highly determinate cotton varieties were used to shorten the production season and successfully avoid late season *B. tabaci* infestations that lead to sticky cotton (Cook and Scott 1995). Early or delayed plantings may also be used, depending on the crop and migration patterns. Geographic manipulation of crops has been used to avoid heavy periods of *B. tabaci* migrations. Susceptible crops such as lettuce and *Brassica* spp. should not be sown near infestation sources such as cotton or melon, which themselves should not be sown in close proximity to each other nor near urban landscape plants that are heavily infested with *B. tabaci*. The urban landscape can also be a source of whitefly-transmitted viruses, such as TYLCV, as documented in California (Rojas et al. 2007).

Selection of crop varieties for each area is a key component in making this approach successful. Crops resistant to whitefly infestation or nymphal development help to limit insect population growth, reduce damage to the resistant crop and reduce mass migrations to other crops. An example is the development of the whitefly-resistant alfalfa variety UC-Impalo-WF (Teuber et al. 1997), which provides high levels of resistance to whiteflies (Jaing et al. 2003; Jaing and Walker 2007). Adjusted planting and harvest dates are also key components in establishment of a host-free period for management of *B. tabaci* and its vectored virus pathogens. These practices are being used to manage whitefly-transmitted virus diseases such as CYSDV (Natwick et al. 2008). Good sanitation practices are also key components critical for establishment of host-free periods and for reducing whitefly adult intercrop migration. Crop residues from winter vegetable crops (primarily *Brassica* sp. and *Lactuca* sp.) and melons crops should be shredded and turned into the soil immediately following harvest. Control of weed species that

harbor *B. tabaci* in non-crop areas including head rows and fallow fields may also be helpful. However, weeds may also serve as sources of whitefly predators and parasitoids that play an important role in whitefly suppression and should be conserved (Godfrey et al. 2008). The Arizona area-wide management plan promotes the shortest possible growing season for cotton, winter vegetable crops (cole crops and lettuce), melon crops (spring and fall), the shortest acceptable alfalfa cutting cycles, encourages geographic separation between susceptible crops, and the maximum time between whitefly host crops and cotton planting (Palumbo et al. 1999).

## *Management System for Cotton in the Desert Southwest USA*

Worldwide, whiteflies cause serious economic damage to cotton via direct feeding that removes phytosynthates and nutrients, by the deposition of sugary excrement "honeydew" on lint (sticky and stained cotton), and by transmission of viral pathogens. Several biotypes of *B. tabaci* are major pest problems in cotton worldwide, mostly in warm desert regions (Munro 1987). Cotton grown in the desert Southwest of the United States is infested by the B biotype of *B. tabaci*, but cotton grown in Mediterranean countries and in China may also be infested with the Q biotype and other indigenous biotypes (Liu et al. 2007). More temperate cotton growing regions and those with higher rainfall do not receive as much direct feeding damage and lint contamination from *B. tabaci*, but may be susceptible to virus pathogens it transmits such as *Cotton leaf curl virus* (CLCuV) (Mansor et al. 1993). Several other whitefly species may infest cotton, especially greenhouse whitefly, *T. vaporariorum* and bandedwinged whitefly, *T. abutiloneus* (Haldeman). These usually do not cause economic damage in cotton, so correct identification is important. *B. tabaci* adults are approximately 0.8 to 1.5 mm long, yellowish, with white wings that are held somewhat vertically tilted, or roof-like, over the body, generally not meeting over the back. *T. vaporariorum* adults are similar in size and color, but hold their wings flatter over the back with no space separating the two pairs of wings when at rest. *T. abutiloneus* adults are easily distinguished from the aforementioned species, having brownish bands across the wings. Greenhouse and bandedwinged whitefly nymphs and pupae have a marginal fringe of wax filaments or long waxy rods on the dorsum of their scale-like body that is lacking in *B. tabaci*. Also the oval body of the *B. tabaci* pupa tapers down to the leaf surface rather than being ridged like the other two species. *B. tabaci* biotypes can only be reliably distinguished by molecular techniques (Gill 2007).

Integrated management of whiteflies in cotton needs to begin before planting, relying as much as possible on cultural and biological controls and later use of insecticides only when needed. Overuse of insecticides for whitefly control in cotton, and using a single class of pesticide or another class with the same mode of action, has lead to development of insecticide-resistance in *B. tabaci*, in the desert southwest of the United States and in other countries (Dennehy and Williams 1997; Castle et al. Chapter 16).

## Cultural Control

Crop rotation patterns, special considerations for type and spatial arrangement of crops planted, and other cultural treatments can be used to increase host-free periods or reduce inter-crop migrations as a means to control whitefly populations (Hilje et al. 2001; Ellsworth and Martinez-Carrillo 2001). Choosing where to plant cotton is important for whitefly management. Cotton should be planted at least one-half mile upwind from other key host crops (e.g., melons, cole crops, and tomatoes), from key ornamental plants, and from key weed species that harbor populations of *B. tabaci* (Godfrey et al. 2008). Cotton should not be grown as a perennial crop which would provide overwintering sites for *B. tabaci* and for whitefly-transmitted virus pathogens such as the new world *Cotton leaf crumple virus* (CLCrV) (Dickson et al. 1954; Seo et al. 2006) and the old world CLCuV (Mansor et al. 1993). Because cotton worldwide is generally grown as an annual, *B. tabaci* must migrate to other crops, ornamental plants and weeds to overwinter. In the desert southwest of the USA, populations of *B. tabaci* that overwinter in vegetable crops, ornamental plants and weeds migrate to spring melon crops where populations begin to increase rapidly with warmer weather (Watson et al. 1992). Problems in cotton develop as populations of migratory *B. tabaci* move into the crop in late spring and early summer (Chu et al. 2005a, 2007; Ellsworth and Martinez-Carrillo 2001). Once temperatures warm up in summer, populations can build rapidly with the highest populations occurring in mid- to late-summer (Chu et al. 2001, 2007; Naranjo and Ellsworth 2009a). Naranjo et al. (Chapter 6) provide further discussion of the seasonal cycle of *B. tabaci.*

Water and fertility management play important roles as cultural tactics in whitefly management. Over use of both water and nitrogen fertilizer can greatly exacerbate damage from *B. tabaci* infestations by increasing whitefly numbers and honeydew production (Bi et al. 2001, 2005). Although *B. tabaci* developed higher populations on water-stressed cotton compared with well-watered cotton (Flint et al. 1996), individuals feeding on well-watered plants produced more honeydew and sugars per gram of honeydew (Henneberry et al. 2002).

Crop termination through cessation of irrigation and chemical defoliation are cultural tools for whitefly management. Nuessly et al. (1994) found that *B. tabaci* can continue to increase up to 6 weeks after the final cotton irrigation even following defoliation because red eye nymphs were able to continue development to the adult stage on cotton leaves that abscised and fell from the plants. In the low desert production areas of California, a final cotton irrigation on July 21 followed by defoliation on August 20, followed by sugarbeets and vegetable crops planted after September 15, provided only a one to 2 week host crop-free period. However, early cotton crop termination and defoliation, including an herbicide to prevent regrowth, helped limit additional whitefly buildup and reduced whitefly migration from cotton to other crops.

Whitefly population levels as monitored by year-round trapping decreased following the implementation of a mandatory short season cotton production program in the Imperial Valley for pink bollworm management that included cotton defoliation by September 1 (Chu et al. 2001, 2007). It was not possible to attribute

yearly declines entirely to the short season cotton program, but the program was almost certainly a contributing factor. Defoliation in mid-September when approximately 95% of the crop matured and early harvest is important to avoid sticky lint in upland cotton, *Gossypium hirsutum* (Henneberry et al. 1998). Early defoliation and harvest to avoid sticky lint at harvest may not be as practical for Pima cotton, *G. barbadense*, due to its later fruiting and lack of a distinct termination of the first cotton fruiting cycle.

### Host Plant Resistance

Hirsute cotton varieties are generally more susceptible than glabrous varieties (Pollard and Saunders 1956; Mound 1965; Butler and Henneberry 1984; Flint and Parks 1990; Norman and Sparks 1997; Chu et al. 1999). It has also been well documented that cotton genotypes with okra-leaf shape are generally less susceptible to *B. tabaci* colonization than genotypes with normal palmate leaf shape (Berlinger 1986; Chu et al. 2005b). The wild cotton, *G. thurberi* Todaro, has resistance to *B. tabaci* (Walker and Natwick 2006) and can be bred and manipulated to cross with *G. hirsutum* to produce more resistant cotton (Beasley 1940). Slow maturing Pima cotton is generally more susceptible than faster maturing upland cotton, although Natwick et al. (1995) also found whitefly susceptibility differences among Pima cotton varieties. Therefore, a glabrous or okra-leaf upland cotton that is determinate in its fruiting cycle provides a better fit for an area management scheme. Whitefly populations will build up more slowly, and early termination helps to avoid potentially higher *B. tabaci* infestation levels in the fall. These factors allow for a host-free period between cotton and winter vegetable crops in the Southwestern USA.

### Biological Control

*B. tabaci* is indigenous to many cotton systems where a full suite of natural enemies would be expected to occur. Where *B. tabaci* is an introduced pest, indigenous natural enemies have adopted it as prey, and numerous exotic parasitoids and a few coccinellid predator species have often been introduced. Although biological control alone has yet to solve the whitefly problem in cotton, natural enemies can still play an important role in cotton IPM systems (Naranjo and Ellsworth 2005, 2009a). The initially depauperate parasitoid complex attacking *B. tabaci i*n the USA desert Southwest was later enriched through release of exotic species of *Eretmocerous* and *Encarsia* (Gould et al. 2008; Roltsch et al. 2008) a few of which became established. Using multiple ELISAs, Hagler and Naranjo (1994) determined that several predator species in Arizona cotton also prey on *B. tabaci* eggs and adult females, but the most common in the southwestern United States are *Geocoris* spp. and *Orius tristicolor* (Say). Other whitefly predators found in cotton include several species of lady beetles such as the convergent lady beetle, *Hippodamia convergens*, the seven-spotted lady beetle, *Coccinella septempunctata*, *Collops* spp. (Coleoptera: Melyridae), several lacewing species, and an empidid fly, *Drapetis* nr. *divergens*, which is a voracious predator of adult whiteflies (Hagler 2002; Hagler and Naranjo 2005). Use

of insecticides is a limiting factor in establishment of effective biological control in cotton (Gerling and Naranjo 1998); however, use of selective insecticides such as the IGRs early in the cotton season can minimize the risk of destroying whitefly natural enemies (Naranjo et al. 2004) and allowing them to contribute significantly to pest control (Naranjo and Ellsworth 2009b). IGRs allow increased benefit to cotton growers and the environment while also reducing the risk of insecticide-resistance by decreasing the number of insecticide treatments needed for whitefly control.

## Monitoring and Treatment Decisions

Whitefly control with foliar insecticides in cotton and other crops is complicated by two factors: (1) adults and nymphs are found mostly on the abaxial leaf surface, often escaping contact with spray droplets, and (2) *B. tabaci* has developed resistance to many insecticides. Whiteflies need to be monitored on abaxial surfaces of leaves from early squaring to harvest. Prior to registration of IGRs for whitefly control, only adults were monitored for treatment decisions in cotton. Later, nymphal action thresholds were also established (Naranjo and Flint 1994; Ellsworth and Martinez-Carrillo 2001; Naranjo and Ellsworth 2009a). When whiteflies are first found in sweep samples, sampling via leaf-turn method (Naranjo and Flint 1995; Ellsworth et al. 1995) should begin and continue through crop termination. All parts of the cotton field should be checked; however, field margins should be checked for whitefly adults as often as twice weekly during critical periods, especially early in the season when populations can build up as nearby host crops are being harvested or are senescing. Action thresholds as high as ten and as low as three adults per cotton leaf, sampling the fifth main stem leaf from the top, have been published (Naranjo et al. 1996; Naranjo et al. 1998). Chu and Henneberry (1999) found that initiating chemical control at four adults per leaf-turn produced higher lint yields and less sticky lint compared to initiating chemical control at 15 adults per leaf-turn. The relationship between pest density and yield for cotton is fairly straight forward. However, the relationship between stickiness and treatment thresholds is not consistent due to the onset and duration of the whitefly infestation, relative humidity, and possible occurrence of rainfall during the period cotton bolls are open; all factors that contribute to the level of stickiness (Naranjo et al. 1998).

Nymphs must be present to justify treatment with IGRs. Whitefly nymphs can be monitored on the abaxial leaf surface by placing a ca. 2.5 cm ring between the central and left-side main veins and checking for presence or absence of large nymphs. A leaf is scored as infested if any third and fourth instar nymphs are present within the ring, and the action threshold is 40% infested leaves (Ellsworth and Martinez-Carrillo 2001). Although five adults per cotton leaf is the generally accepted action threshold for conventional insecticidal control of *B. tabaci* in Arizona and Southern California, Naranjo et al. (2002) suggested that predator conservation may be enhanced by raising the initial threshold to delay the first application or initially using more selective materials such as IGRs (Naranjo et al. 2004).

Early season treatment for *B. tabaci* nymphs with selective insecticides such as IGRs (buprofezin and pyriproxyfen), and lipid synthesis inhibitors such as spiromesifen, minimizes impact of insecticidal control on whitefly parasitoids and predators (Naranjo et al. 2004; Ellsworth et al. 2006). Long-term pest suppression afforded by use of selective insecticides such as the IGRs is a combination of several weeks of chemical residual control and many additional weeks of control from conserved natural enemies. This effect has been coined the bioresidual and it is the mechanism allowing long-term pest control (Naranjo and Ellsworth 2009a, b). Limiting IGRs with the same mode of action to no more than one application per season is the strategy being employed to reduce the rate of selection for resistance to these materials. Classification of insecticides by mode of action is available from the Insect Resistance Action Committee (IRAC) http://www.irac-online.org/. This strategy was adopted from the Israeli system originally suggested by Horowitz et al. (1994). Foliar applied neonicotinoid insecticides such as acetamiprid are also effective against *B. tabaci*, but are more disruptive to predators and parasitoids of whiteflies and other cotton pests (Naranjo and Akey 2005). Furthermore, neonicotinoids in general might better be saved for use as drenches in vegetable crops where they are most effective. A cross-commodity insecticide resistance plan has been developed for the low desert production areas of the southwestern USA which takes into account differing crop mixtures and classes of chemistry to try and preserve insecticidal efficacy in cotton and many other crops grown in the area (Palumbo et al. 2001, 2003). Deferring broad spectrum insecticides until later in the season is a way to preserve and build populations of natural enemies of whitefly and secondary pests that might otherwise be released from their natural enemies.

A quick knockdown of adults obtained with tank mixes of pyrethroids such as bifenthrin or fenpropathrin in combination with endosulfan or an organophosphate is sometimes required to protect open bolls from contamination with honeydew caused by a massive influx of whitefly adults from other cotton fields (Natwick 1993; Chu et al. 1998). Henneberry et al. (1998) found that timing of defoliation in relation to the last insecticide application or detectable increase in population of *B. tabaci* can be an important tool to manage the cotton crop to avoid sticky lint. Extending the cotton season may increase yield, but lower profit to the grower due to sticky lint.

## *Management in Alfalfa in the Desert Southwest USA*

The B biotype of *B. tabaci* first became an economically important pest of alfalfa in California and Arizona during the summer of 1991 (Natwick and Robinson 1993). *B. tabaci* can cause economic damage to alfalfa in the low desert regions of Southern California and Arizona from July through September. This perennial crop serves as a transitional host between crop harvests of melons, cole crops, lettuce and cotton (Yee et al. 1997). Palumbo et al. (2000) demonstrated the nature of the damage to alfalfa: reduced growth rate, diminished forage yield, and contamination of hay

with honeydew causing harvest and bailing problems. *B. tabaci* also reduces hay quality by removal of plant assimilates and contamination of hay with sooty molds that grow on honeydew. Most of the damage, however, is restricted to two forage harvest periods during the summer coinciding with peak adult populations and dispersal from alternate hosts (Yee et al. 1997). Definitive monitoring and treatment guidelines have not been developed for whitefly control in alfalfa, and no insecticides are registered for whitefly control in this crop, nor would they be cost-effective due to relatively low profit margins for alfalfa hay. Large acreages of alfalfa grown for forage in Arizona and California preclude an area-wide management approach that incorporates cultural practices such as strip-cutting, summer fallowing or shortened harvest cycles. The economic infeasibility of treating with insecticides coupled with the impracticality of cultural practices over such large areas has focused attention on host plant resistance to *B. tabaci* (Palumbo et al. 2000). Breeding efforts by Teuber et al. (1997) lead to the release of a whitefly-resistant alfalfa cultivar (UC-Impalo-WF), and continuing research by Jaing et al. (2003) may lead to the release of improved whitefly-resistant alfalfa cultivars.

## *Vegetables in the USA Desert Southwest and Elsewhere*

Fall vegetable and melon plantings as well as sugar beets were decimated during the 1980s in the Southwest USA by *Lettuce infectious yellows virus* (LIYV) transmitted by *B. tabaci*, later designated "biotype A" (Duffus et al. 1986). Biotype B, first detected in Florida in 1986, eventually displaced biotype A, bringing even greater infestation levels of *B. tabaci*, but an abatement of LIYV due to lower vector efficiency in transmitting the virus (Cohen et al. 1992). When cotton crops were terminated, huge cloud of whiteflies were observed moving directly into newly planted vegetable and melon crops (Blua et al. 1994; Nuessly et al. 1994). To break this cycle, a combination of early termination of cotton and delayed planting of vegetable and melon crops was recommended to reduce the overall impact of whitefly populations and virus incidence on fall plantings of vegetables.

Whiteflies first appeared as a problem in Florida fruiting vegetables in 1987 with the advent of *B. tabaci* biotype B. Appearing first in poinsettia and termed the poinsettia strain, biotype B attacked plants never before seen as whitefly hosts in Florida, including tomato, eggplant, potato, and various cucurbits, causing the silverleaf symptom in squash, a disorder not previously attributed to whiteflies (Maynard and Cantliffe 1990; Yokomi et al. 1990; Schuster et al. 1996). Irregular ripening soon followed and millions of dollars worth of tomatoes were dumped, often at their market destination. The begomovirus *Tomato mottle virus* (ToMoV) appeared in 1989 and TYLCV in 1994. However, use of imidacloprid drenches began the same year and whitefly related problems abated for several years (Stansly 1996).

Soil applied imidacloprid and other neonicotinoids are still key insecticides for protecting open field vegetables and other crops in the USA and elsewhere. Soil

drenches are often followed by regular foliar applications of various insecticides, including IGRs. Use of neonicotinoids is increasing due to several factors: (1) patent expiration on imidacloprid and the ensuing decrease in cost, (2) availability of additional neonicotinoids that further depress prices or are more effective as foliar sprays, and (3) appearance of new virus diseases such as SqVYV in watermelon and *Cucurbit leaf crumple virus* (CuLCV) that motivate use on these crops.

Increased use of neonicotinoids has placed the burden on users to adopt science-based plans for sustaining their efficacy and sharing their use among different agricultural interests. Through identification of crop communities (i.e., "multi-crop", "cotton-intensive", and "cotton/melon") common to agriculture to the southwest desert region, plans for use of neonicotinoids and other chemistries have been developed that should allow more effective use, while helping to avoid resistance (Palumbo et al. 2001, 2003). Therefore, whitefly management on cole crops and lettuce in the desert agricultural valleys of Arizona and southeastern California depends on avoidance of *B. tabaci* sources such as cotton and melon crops in addition to use of neonicotinoid insecticides at planting.

To this same end, neonicotinoid insecticides are recommended for use only during the first 6 weeks of the crop cycle in the Florida fruiting vegetable system, regardless of whether the application is foliar or soil drench at planting (Schuster et al. 2007). The objective is to relax selection for resistance against neonicotinoids during the latter part of the crop cycle. Selective versus broad-spectrum insecticides are recommended for the next third of the crop cycle to conserve natural enemies. Cultural recommendations that apply anywhere whitefly-borne viruses are an issue include rapid crop destruction and establishment of a minimum 2 month crop-free period during the summer, practices to assure production of virus and whitefly free transplants, and use of TYLCV resistant tomato and pepper cultivars. Pepper is included because some cultivars have been shown to be non-symptomatic hosts of TYLCV (Polston et al. 2006). Also recommended are ultraviolet light reflective ("aluminized") mulches and living mulches that have been shown to protect crops from whiteflies and other visually orienting pests early in the crop cycle (Csizinszky et al. 1999; Hilje et al. 2001; Stapleton and Summers 2002; Hilje and Stansly 2008; Nyoike et al. 2008; see Antignus Chapter 13). All these practices are aimed at reducing the whitefly population and therefore the need for insecticidal control in the crop.

### Action Thresholds for Whiteflies in Open-Field Vegetables

Economic thresholds are difficult to establish in high value crops for the reasons mentioned above. Economic injury levels of four nymphs per leaf and one adult per 3 × 45 cm beat tray were obtained in a study of open field tomato in Brazil (Gusmao et al. 2006). However, the study was conducted in processing tomatoes valued at only $US181.78 per ton. This economic injury level would scale down in proportion to the increased value of tomato crops destined for the fresh market.

An economic injury level of 18 adult *B. tabaci* per cucumber plant in the four-leaf stage was determined for China (Chen et al. 2005). This threshold would relate to

market and environmental conditions in China when study was done, and also would have to be revised if CYSDV were to appear as it has in American desert southwest cucurbit production areas. Such a threshold would have to take into account the damage potential of the virus disease which typically is greatest when the crop is young and decreases subsequently (Schuster et al. 1996). Moreover, the threshold would have to be based, not only on number whiteflies per sample unit, but also the proportion carrying virus and capable of transmitting the disease. No such thresholds have been proposed to our knowledge.

By resorting to an "action" rather than economic threshold, consideration of unstable or difficult to estimate parameters inherent in the economic injury level, such as crop value and cost of control can be avoided. It may be sufficient to show a minimum level infestation associated with significant loss of crop yield or quality. Action thresholds also depend on efficacy of the control tactic. Therefore, the action threshold of one whitefly nymph per two leaflets to prevent irregular ripening of tomato with IGRs was established by comparison with an imidacloprid-treated standard (Schuster 2002).

Action thresholds can vary greatly, presumably due to different experimental conditions. Action thresholds for melon of one large nymph per 15 $cm^2$ of leaf area or one adult per leaf in Texas and three adults per leaf in Arizona have also been established (Riley and Palumbo 1995). Later, Nava-Camberos et al. (2001) revised these based on number of insecticide treatments to range from 0.02 to 3.92 adults/leaf, or from 0.2 to 54.4 nymphs/6.5 $cm^2$ leaf surface! We were unable to find any other action or economic thresholds for *B. tabaci* on vegetables in the literature. Clearly this basic IPM concept is still underutilized in vegetable IPM for the many reasons mentioned.

## Role of Biological Control and Adaptation of Augmentative Control Practices

The lack of usable thresholds and the reliance on broad-spectrum insecticidal control of *B. tabaci* in most vegetable crops seem to leave little room for biological control. Still, there clearly exists an important role for natural enemies in reducing populations outside the crop or in crops not treated with broad-spectrum insecticides. For example, Stansly et al. (1997) and Brewster et al. (1997) observed apparent parasitism levels of 80% or more on organically grown eggplant and tomato in southwest Florida. Combined levels of predation and parasitism on weeds around tomato fields in west-central Florida averaged from 40 to 90% (Schuster et al. 1998). As a result of this mortality, as well as the fact that crop resources are not present to support population growth, whitefly populations fall precipitously during fallow periods in southwest Florida (Stansly 1996). This is the basis for recommending the crop free period as a critical component of whitefly management (Hilje et al. 2001).

Just as in protected horticulture, the presence of whitefly-transmitted viruses in field grown crops reduces the threshold for whitefly infestation to an undetermined level, presumably below what can be reasonably achieved by conservation or augmentative biological control (Dik and Albajes 1999). Other impediments to

establishing biologically based management systems in fruiting vegetable production are dependence on insecticides and negative expectations regarding effectiveness of biological control (Stansly et al. 2004a, b). Presently, vector-borne disease in south Florida is primarily a problem in tomato (TYLCV) and watermelon (SqVYV). Additionally, biological control of whiteflies in tomato is a special challenge requiring a suite of natural enemies, some of which, e.g. the mirid predators, are poorly adapted to other crops (Urbaneja et al. 2005). Nevertheless, recent success with augmentative biological control in crops not affected by whitefly-borne virus disease has demonstrated good potential for this approach (Van der Blom 2007).

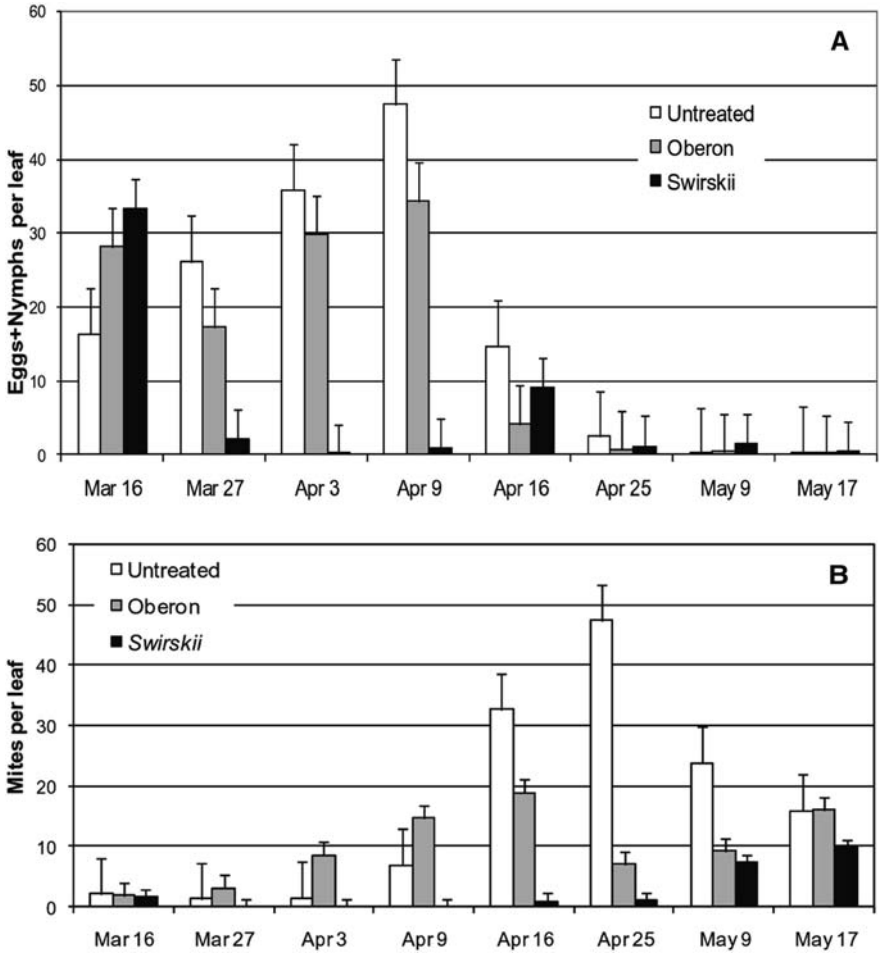



**Fig. 17.2** Numbers of adult and immature whitefly per leaf (**A**) and of broad mites (**B**, all stages) on small plots of eggplant in southwest Florida in spring 2007. *A. swirskii* released 16 March and spiromesifen (Oberon 2SC) applied twice as a foliar spray at 1.3 L/ha one and 2 weeks later (Stansly and Castillo 2009)

Preliminary results in Florida have shown that, although eggplant growers spend an estimated $1,520/ha on insecticides, the principal pests of eggplant in Florida (mites, whiteflies and thrips) can all be controlled biologically with predaceous mites at comparable or even reduced cost (Stansly and Castillo 2009). We evaluated *A. swirski* on eggplant in experimental plots and on a commercial farm in southwest Florida. In one experiment, *A. swirskii* provided better control of both *B. tabaci* and *Polyphagotarsonemus latus* than the widely used insecticide/acaracide spiromesifen (Oberon®) (Fig. 17.2). Eggplant receiving *A. swirskii* yielded significantly more fruit at first harvest than untreated plants or even eggplants receiving two acaricide sprays (data not shown). However, as plants grew older, they became heavily infested with spider mites (*Tetranichus urticae*). Subsequent experiments demonstrated that all three pests could be controlled with mixtures of *A. swirskii* and *Neoseulus* = *Amblyseius californicus* (Stansly and Castillo, 2009).

Although a relatively minor crop, working systems in eggplant may set the stage for acceptance of biological control in other crops including cucumber and pepper. Pepper and eggplant share a number of pests such as broad mite and western flower thrips. Pepper is also attacked by beet armyworm, *Spodoptera exigua* (Hübner), although this pest can be controlled by selective insecticides that are relatively compatible with whitefly natural enemies. Unfortunately, broad spectrum insecticides used to control for pepper weevil, *Anthonomus eugenii* Cano, are incompatible with predaceous mites and minute pirate bugs that otherwise frequently colonize pepper. However, pepper weevil infestation can be minimized by cultural practices such as field sanitation, summer fallows, and control of back nightshade, its main alternate host. Pests of cucurbits such as whiteflies and spider mites can also be managed by predaceous mites and lepidopteron pests by compatible insecticides. Thus, the door is open to implement biological control in a number of key fruiting vegetable crops in areas such as south Florida.

## Conclusions

Integrated, biologically-based management of *B. tabaci* has become a reality in some greenhouse grown vegetables not affected by whitefly vectored virus. This is particularly true in Spain and elsewhere where *A. swirskii* has played a major role in controlling *B. tabaci* in pepper. In contrast, biological control of *B. tabaci* remains a challenge in some other crops, especially tomato, to which mite predators are poorly adapted and in which whitefly-borne virus is a major threat. However, more acceptable virus resistant varieties coupled with improvements in insect exclusion technology should open the door to greater use of available natural enemies such as *Eretmocerus* spp. and the mirid predators that have demonstrated good potential to control *B. tabaci* in greenhouse grown tomato.

In contrast, augmentative biological control has made few inroads in open field crops where insecticidal control is still the norm and augmentation is seen as too costly, unreliable or difficult to implement. Fallow periods free of crop hosts of

*B. tabaci* and it's vectored viruses have been the basis for successful management in Florida, the Dominican Republic and Israel in recent times, and in cotton and tobacco production in Africa as far back as the 1930s. With the advent of effective control with neonicotinoids and insect growth regulators, area wide management of insecticide use and modification of cropping patterns has been key to maintaining the viability of open field vegetable and cotton production in the face of the continual threat of insecticide resistance. Few efforts at augmentative biological control have been reported from these agroecosystems, although indigenous natural enemies often play an important role in the crop as well as by in reducing pest populations in unsprayed weeds and during fallow periods. Recent advances in implementation of augmentative biological control using predaceous mites in field grown vegetable crops such as eggplant and pepper, and successful integrated control program for cotton in Arizona where native predators contribute significant control, may point the way to more biologically based management strategies in agronomic crops such as alfalfa, soybean and cotton, especially where whitefly-borne virus disease is not a major issue. The challenge in any crop is to integrate management strategies for all potentially damaging pests and diseases into an economically and environmentally viable system. Whitefly control may only be one of many components in such systems, although it is often a key component. Future success will depend on continued advances in application of basic biology and ecology to practical pest management solutions.

## Literature Cited

Alomar O, Albajes R. 1996. Greenhouse whitefly (Homoptera: Aleyrodidae) predation and tomato fruit injury by the zoophytophagous predator Dicyphus tamaninii (Heteroptera: Miridae). In *Zoophytophagous Heteroptera: Implications for Life History and Integrated Pest Management*, ed. O Alomar, R Wiedanmann, pp. 155–177. *Proceedings*, Lanham: Thomas Say Publications in Entomology. E.S.A.

Alvarez AJ, Valera DL, Molina-Aiz FD. 2006. A method for the analysis of the geometric characteristics of protection screens. *Acta Hort. (ISHS)* 719:557–564. http://www.actahort.org/books/719/719_65.htm

Ardeh MJ, de Jong PW, van Lenteren JC. 2005. Intra- and interspecific host discrimination in arrhenotokous and thelytokous *Eretmocerus* spp. *Biol. Control* 33:74–80.

Baker GA, Burnham TA. 2001. Consumer response to genetically modified foods: market segment analysis and implications for producers and policy makers. *J. Agric. Resour. Econ.* 26:387–403.

Beachy RN. 1997. Mechanisms and applications of pathogen-derived resistance in transgenic plants. *Curr. Opin. Biotechnol.* 8:215–220.

Belda J, Calvo J. 2006. Eficacia de *Amblyseius swirskii* (Acarii: Phytoseiidae) en el control biológico de *Bemisia tabaci* (Genn.) (Hom. Alerodidae) y *Frankliniella occidentalis* (Pergande) (Thys: Thripidae) en pimiento en condiciones de semi-campo. *Bol. San. Veg. Plagas* 32:283–296.

Bell ML, Baker JR. 2000. Comparison of greenhouse screening materials for excluding whitefly (Homoptera: Aleyrodidae) and thrips (Thysanoptera: Thripidae). *J. Econ. Entomol.* 93:800–804.

Beasley JO. 1940. The origin of American tetraploid *Gossypium* species. *Am. Nat.* 74:285–286.

Berlinger MJ. 1986. Host plant resistance to *Bemisia tabaci*. *Agric. Ecosyst. Environ.* 17:69–82.

Bethke JA, Paine TD. 1991. Screen hole size and barriers for exclusion of insect pests of glasshouse crops. *J. Entomol. Sci.* 26:169–177.

Bi JL, Ballmer GR, Hendrix DL, Hennneberry TJ, Toscano NC. 2001. Effect of cotton nitrogen fertilization on *Bemisia argentifolii* populations and honeydew production. *Entomol. Exp. Appl.* 99:25–36.

Bi JL, Lin DM, Lii KS, Toscano NC. 2005. Impact of cotton planting date and nitrogen fertilization on *Bemisia argentifolii* populations. *Insect Sci.* 12:31–36.

Blua MJ, Perring TM, Nuessly GS, Duffus JE, Toscano NC. 1994. Seasonal cropping pattern effects on abundance of *Bemisia tabaci* (Homoptera: Aleyrodidae) and incidence of lettuce infectious yellows virus. *Environ. Entomol.* 23:1422–1427.

Bolckmans JKF. 1999. Commercial aspects of biological pest control in greenhouses. In *Integrated Pest and Disease Management in Greenhouse Crops*, ed. R Albajes, M Lodovica Gullino, JC van Lenteren, Y Elad, pp. 310–318. Dordrecht, Netherlands: Kluwer Academic Publishers.

Bosclair J, Brueren GJ, van Lenteren JC. 1990. Can *Bemisia tabaci* be controlled with *Encarsia formosa*? *SROP/WPRS Bull.* 5:32–35.

Brewster CC, Allen JC, Schuster DJ, Stansly PA. 1997. Simulating the dynamics of *Bemisia argentifolii* (Homoptera: Aleyrodidae) in an organic cropping system with a spaciotemporal model. *Environ. Entomol.* 26:203–216.

Butler GD, Jr, Henneberry TJ. 1984. *Bemisia tabaci*: effect of cotton leaf pubescence on abundance. *Southwest. Entomol.* 9:91–94.

Cabrera FJ, Lopez JC, Baeza EJ, Pérez-Parra JJ. 2006. Efficiency of anti-insect screens placed in the vents of Almería greenhouses. *Acta Hort. (ISHS)* 719:605–614. http://www.actahort.org/books/719/719_70.htm

Calvo J, Bolckmans K, Belda JE. 2008a. Controlling the tobacco whitefly *Bemisia tabaci* (Genn.) (Hom.: Aleyrodidae) in horticultural crops with the predatory mite *Amblyseius swirskii* (Athias-Henriot). In *4th International Bemisia Workshop International Whitefly Genomics Workshop*, ed. PA Stansly, CL McKenzie, p. 53. *J. Insect Sci*. 8:4. www.insectscience.org/8.04

Calvo J, Bolckmans K, Stansly PA, Urbaneja A. 2008b. Predation by *Nesidiocoris tenuis* on *Bemisia tabaci* and injury to tomato. *Biocontrol* 54:237–246.

Chen BB, Ren SX, Musa PH, Chen C. 2005. A study on economic threshold of *Bemisia tabaci*. *Acta Agric. Univ. Jiangxiensis* 27:234–237. (in Chinese)

Chu CC, Natwick ET, Henneberry TJ, Lee R. 1998. Effects of pyrethroid insecticides alone and in mixtures on *Bemisia argentifolii* (Homoptera: Aleyrodidae) and cotton, cauliflower, and broccoli yields. *J. Agric. Assn. China* 184:57–66.

Chu CC, Henneberry TJ. 1999. *Bemisia argentifolii*: action thresholds, upland cotton yields and cotton lint stickiness in the Imperial Valley, California. *Southwest. Entomol.* 24:79–86.

Chu CC, Cohen AC, Natwick ET, Simmons GS, Henneberry TJ. 1999. *Bemisia tabaci* (Hemiptera: Aleyrodidae) biotype B colonization and leaf morphology relationships in upland cotton. *Aust. J. Entomol.* 38:127–131.

Chu CC, Henneberry TJ, Natwick ET, Ritter D, Birdsall SL. 2001. Efficacy of CC traps and seasonal activity of adult *Bemisia argentifolii* (Homoptera: Aleyrodidae) in Imperial and Palo Verde Valleys, California. *J. Econ. Entomol.* 94:47–54.

Chu CC, Barnes E, Natwick ET, Chen TY, Henneberry TJ. 2005a. *Bemisia tabaci* population dynamics in the Imperial Valley, California, 1996 to 2002. *Egypt. J. Agric. Res.* 83:89–95.

Chu CC, Natwick ET, Henneberry TJ. 2005b. Okra- and normal-leaf upland cotton *Bemisia tabaci* (Homoptera: Aleyrodidae) colonization, 1996–2002. *Egypt. J. Agric. Res.* 83:109–117.

Chu CC, Barnes E, Natwick ET, Chen TY, Ritter D, Henneberry TJ. 2007. Trap catches of sweetpotato whitefly (Homoptera: Aleyrodidae) in the Imperial Valley, California, from 1996 to 2002. *Insect Sci.* 14:165–170.

Cohen S, Duffus JE, Liu HY. 1992. A new *Bemisia tabaci* biotype in the southwestern United States and its role in silverleaf of squash and transmission of lettuce infectious yellows virus. *Phytopathology* 82:86–90.

Cook CG, Scott AW, Jr. 1995. Registration of C21S781-2 cotton germplasm. *Crop Sci.* 35:598.

Csizinszky AA, Schuster DJ, Polston JE. 1999. Effects of ultraviolet-reflective mulches on tomato yields and on the silverleaf whitefly. *HortScience* 34:911–914.

De Barro PJ, Coombs MT. 2008. Post-release evaluation of *Eretmocerus hayati* Zolnerowich Rose in Australia. *Bull. Entomol. Res.* 24:1–14.

Dennehy TJ, Williams L. 1997. Management of resistance in *Bemisia* in Arizona cotton. *Pestic. Sci.* 51:398–406.

Dimitri C, Oberholtzer L. 2005. Market-led versus government-facilitated growth development of the U.S. and EU organic agricultural sectors. USDA-ARS WRS 0505. http://www.ers.usda.gov/publications/WRS0505/wrs0505.pdf

Dik AJ, Albajes R. 1999. Principals of epidemiology, population biology, damage relationships, and integrated control of diseases and pests. In *Integrated Pest and Disease Management in Greenhouse Crops*, ed. R Albajes, M Lodovica-Gullino, JC van Lenteren, Y Elad, pp. 69–81. Amsterdam, The Netherlands: Springer.

Dickson RC, Johnson MM, Laird EF. 1954. Leaf crumple, a virus disease of cotton. *Phytopathology* 44:479–480.

Duffus JE, Larsen RC, Liu HY. 1986. Lettuce infectious yellows virus – a new type of whitefly-transmitted virus. *Phytopathology* 76:97–100.

Ellsworth PC, Diehl JW, Dennehy TJ, Naranjo SE. 1995 (Rev. 11/2000). Sampling sweetpotato whiteflies in cotton. *IPM Series No. 2. Publ. No. 194023*. University of Arizona, College of Agriculture and Life Sciences, Cooperative Extension, Tucson, Arizona. http://cals.arizona.edu/crops/cotton/insects/wf/wfsampl.html

Ellsworth PC, Martinez-Carrillo JL. 2001. IPM for *Bemisia tabaci*: a case study from North America. *Crop Prot.* 20:853–869.

Ellsworth PC, Palumbo JC, Naranjo SE, Dennehy TJ, Nichols RL. 2006. Whitefly management in Arizona cotton 2006. Cooperative Extension, IPM Series No. 18. The University of Arizona, Tucson, http://Cals.Arizona.Edu/Pubs/Insects/Az1404.Pdf

Enkegaard A. 1993. *Encarsia formosa* parasitizing the Poinsettia strain of the cotton whitefly, *Bemisia tabaci*, on Poinsettia: bionomics in relation to temperature. *Entomol. Exp. Appl.* 69:251–261.

Faria M, Wraight SP. 2001. Biological control of *Bemisia tabaci* with fungi. *Crop Prot.* 20:767–778.

Flint HM, Naranjo SE, Leggett JE, Henneberry TJ. 1996. Cotton water stress, arthropod dynamics, and management of *Bemisia tabaci* (Homoptera: Aleyrodidae). *J. Econ. Entomol.* 89:1288–1300.

Flint HM, Parks NJ. 1990. Infestation of germplasm lines and cultivars of cotton in Arizona USA by whitefly nymphs (Homoptera: Aleyrodidae). *J. Entomol. Sci.* 25:223–229.

Gerling D, Naranjo SE. 1998. The effect of insecticide treatments in cotton fields on the levels of parasitism of *Bemisia tabaci*. *Biol. Control* 12:33–41.

Gerling D, Alomar O, Arnó J. 2001. Biological control of *Bemisia tabaci* using predators and parasitoids. *Crop Prot.* 20:779–799.

Gilbertson RL. 2007. Introduction of *Cucurbit yellow stunting disorder virus* (CYSDV) into the Imperial Valley (and Yuma, Arizona): assessment of the threat to melon production and disease management. In *California Melon Research Board 2007 Annual Report*, pp. 23–30.

Gill RJ. 2007. Can molecules solve the *Bemisia* conundrum when morphology cannot? A taxonomist's perspective. In *4th International Bemisia Workshop International Whitefly Genomics Workshop*, 2008, ed. PA Stansly, CL McKenzie. *J. Insect Sci*. 8:4.

Godfrey LD, Goodell PB, Natwick ET, Haviland DR. 2008. Insects and mites. In *UC IPM Pest Management Guidelines: Cotton*. UC ANR Publication 3444. http://www.ipm.ucdavis.edu/PMG/r114300311.html

Goettel MS, Hajek AE, Siegel JP, Evans HC. 2001. Safety of fungal biocontrol agents. In *Fungi as Biocontrol Agents: Progress, Problems and Potential*, ed. TM Butt, CW Jackson, N Magan, pp. 347–376. Wallingford, UK: CABI.

Gould J, Waldner D, Colletto N, Merten P. 2008. Release and recovery of four species of *Eretmocerus* against *Bemisia tabaci* biotype B in Arizona. In *Classical Biological Control of*

*Bemisia tabaci in the United States – A Review of Interagency Research and Implementation*, ed. J Gould, KA Hoelmer, JA Goolsby, Vol. 4, pp. 191–204. Amsterdam, the Netherlands: Springer.

Greenberg SM, Jones WA, Liu TX. 2002. Interactions among two species of *Eretmocerus* (Hymenoptera: Aphelinidae), two species of whiteflies (Homoptera: Aleyrodidae), and tomato. *Environ. Entomol.* 31:397–402.

Gusmao MR, Picanco MC, Guedes RNC, Galvan TL, Pereira EJG. 2006. Economic injury level and sequential sampling plan for *Bemisia tabaci* in outdoor tomato. *J. Appl. Entomol.* 130:160–166.

Hagler JR, Naranjo SE. 1994. Determining the frequency of heteropteran predation on sweetpotato whitefly and pink bollworm using multiple ELISAs. *Entomol. Exp. Appl.* 72:59–66.

Hagler JR. 2002. Foraging behavior, host stage selection and gut content analysis of field collected *Drapetis* nr. *divergens*: a predatory fly of *Bemisia argentifolii*. *Southwest. Entomol.* 27: 241–249.

Hagler JR, Naranjo SE. 2005. Use of a gut content ELISA to detect whitefly predator feeding activity after field exposure to different insecticide treatments. *Biocontrol Sci. Technol.* 15: 321–339.

Hanafi A, Bouharroud R, Amouat S, Miftah S. 2007. Efficiency of insect nets in excluding whiteflies and their impact on some natural biological control agents. *Acta Hort. (ISHS)* 747:383–388. http://www.actahort.org/books/747/747_47.htm

Harmanto HJ, Tantau SVM. 2006. Influence of insect screens with different mesh sizes on ventilation rate and microclimate of greenhouses in the humid tropics. *Agricultural Engineering International: The CIGR Ejournal*. Manuscript BC 05 017. Vol. VIII, http://www.cigrjournal.org/index.php/Ejounral

Henneberry TJ, Jech LF, Hendrix DL. 1998. Seasonal distribution of *Bemisia argentifolii* (Homoptera: Aleyrodidae) honeydew sugars and Pima and Upland cotton lint and lint stickiness at harvest. *Southwest. Entomol.* 23:105–121.

Henneberry TJ, Jech LJ, De La Torre TM. 2002. Effects of cotton plant water stress on *Bemisia tabaci* strain B (Homoptera: Aleyrodidae) honeydew production. *Southwest. Entomol.* 27: 117–133.

Hilje L, Costa HS, Stansly PA. 2001. Cultural practices for managing *Bemisa tabaci* and associated viral diseases. In *Challenges and Opportunities for Pest Management of Bemisia tabaci in the New Century*, ed. S Naranjo, P Ellsworth. *Crop Prot*. 20(9):801–812.

Hilje L, Stansly PA. 2008. Living mulch ground covers for management of *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) and *Tomato yellow mottle virus* (ToYMoV) in Costa Rica. *Crop Prot.* 27:10–16.

Hoddle MS, van Driesche RG, Elkinton JS, Sanderson JP. 1998. Discovery and utilization of *Bemisia argentifolii* patches by *Eretmocerus eremicus* and *Encarsia formosa* (Beltsville strain) in greenhouses. *Entomol. Exp. Appl.* 87:15–28.

Hoddle MS. 2004. Biological control of whiteflies on ornamental crops. In *Biocontrol in Protected Culture*, ed. K Heinz, RG Van Driesche, MP Parrella, pp. 149–170. Batavia, IL, USA: Ball Publishing.

Hoogerbrugge H, Calvo J, van Houten Y, Bolckmans K. 2007. Biological control of the tobacco whitefly *Bemisia tabaci* with the predatory mite *Amblyseius swirskii* in sweet pepper crops. *IOBC/WPRS Bull.* 28:119–122.

Horowitz AR, Forer G, Ishaaya I. 1994. Managing resistance in *Bemisia tabaci* in Israel with emphasis on cotton. *Pestic. Sci.* 42:113–122.

Howard RJ, Garland JA, Seaman WL. 1994. *Diseases and Pests of Vegetable Crops in Canada, An Illustrated Compendium*, 554pp. Ottawa: The Canadian Phytopathological Society and The Entomological Society of Canada.

Hu JS, Gelman DB, Blackburn MB. 2002. Growth and development of *Encarsia formosa* (Hymenoptera: Aphelinidae) in the greenhouse whitefly, *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae): effect of host age. *Arch. Insect Biochem. Physiol.* 49:125–136.

Hu JS, Gelman DB, Blackburn MB. 2003. Age-specific interaction between the parasitoid, *Encarsia formosa* and its host, the silverleaf whitefly, *Bemisia tabaci* (Strain B). *J. Insect Sci.* 3:28.

Inglis GD, Goettel MS, Butt TM, Strasser H. 2001. Use of hyphomycetous fungi for managing insect pests. In *Fungi as Biocontrol Agents. Progress, Problems and Potential*, ed. TM Butt, CW Jackson, N Magan, pp. 1–8. Wallingford: CABI.

Jaing YX, Zareh N, Walker GP, Teuber LR. 2003. Characterization of alfalfa germplasm expressing resistance to silverleaf whitefly, *Bemisia argentifolii*. *J. Appl. Entomol.* 127:447–457.

Jaing YX, Walker GP. 2007. Identification of phloem sieve elements as the site of resistance to silverleaf whitefly in resistant alfalfa genotypes. *Entomol. Exp. Appl.* 125:307–320.

Jervis MA, Heimpel GE, Ferns PN, Harvey JA, Kidd NAC. 2001. Life-history strategies in parasitoid wasps: a comparative analysis of 'ovigeny'. *J. Anim. Ecol.* 70:442–458.

Ji YF, Scott JW, Hanson P, Graham E, Maxwell DP. 2007. Sources of resistance, inheritance, and location of genetic loci conferring resistance to members of the tomato-infecting begomoviruses. In *Tomato Yellow Leaf Curl Virus Disease Management, Molecular Biology, Breeding for Resistance*, ed. H Czosnek, pp. 343–362. Amsterdam, The Netherlands: Springer.

Jones DR. 2003. Plant viruses transmitted by whiteflies. *Eur. J. Plant Path.* 109:195–219.

Liu SS, De Barro PJ, Xu J, Luan JB, Zang LS, Ruan YM, Wan FH. 2007. Asymmetric mating interactions drive widespread invasion and displacement in a whitefly. *Science* 318:1769–1772.

Mansor S, Bedford I, Pinner MS, Stanley J, Markham PG. 1993. A whitefly transmitted geminivirus associated with cotton leaf curl diseases in Pakistan. *Pakistan J. Bot.* 25:105–107.

Maynard DN, Cantliffe DJ. 1990. Squash silverleaf and tomato ripening: new vegetable disorders in Florida. *Vegetable Crops Fact Sheet* VC-37, IFAS, University of Florida.

McGregor RR, Gillespie DR, Park CG, Quiring DMJ, Foisy MRJ. 2000. Leaves or fruit? The potential for damage to tomato fruits by the omnivorous predator, *Dicyphus hesperus Entomol*. *Exp. Appl.* 95:325–328.

Messelink G, van Steenpaal S, van Wensveen W. 2005. *Typhlodromips swirskii* (Athias-Henriot) (Acari: Phytoseiidae): a new predator for thrips control in greenhouse cucumber. *IOBC/WPRS Bull.* 28:183–186.

Messelink GJ, Maanen RV, van Steenpaal SEF, Janssen A. 2008. Biological control of thrips and whiteflies by a shared predator: two pests are better than one. *Biol. Control* 44:372–379.

Mound LA. 1965. Effect of leaf hair on cotton whitefly populations in the Sudan Gezira. *Empire Cotton Growing Rev.* 42:33–40.

Munro JM. 1987. *Cotton*, 2nd Edition, 436pp. UK: Longman Scientific & Technical.

Nannini M. 2001. Experiments on greenhouse whitefly control by the mirid bugs *Macrolophus caliginosus* wagner and *Cyrtopeltis tenuis* (Reuter) in protected winter tomato crops. *Acta Horticulturae* 559:769–774.

Naranjo SE. 2008. Establishment and impact of exotic Aphelinid parasitoids in Arizona: a life table approach. *J. Insect Sci.* 8:36.

Naranjo SE, Akey D. 2005. Conservation of natural enemies in cotton: comparative selectivity of acetamiprid in the management of *Bemisia tabaci*. *Pest Manag. Sci.* 61:555–566.

Naranjo SE, Ellsworth PC. 2005. Mortality dynamics and population regulation in *Bemisia tabaci*. *Entomol. Exp. Appl.* 116:93–108.

Naranjo SE, Ellsworth PC. 2009a. Fifty years of the integrated control concept: moving the model and implementation forward in Arizona. *Pest Manag. Sci.* 65:1267–1286.

Naranjo SE, Ellsworth PC. 2009b. The contribution of conservation biological control to integrated control of *Bemisia tabaci* in cotton. *Biol. Control.* 51:458–470.

Naranjo SE, Flint HM. 1994. Spatial distribution of premaginal *Bemisia tabaci* (Homoptera: Aleyrodidae) in cotton and development of fixed-precision, sequential sampling plans. *Environ. Entomol.* 24:254–266.

Naranjo SE, Flint HM. 1995. Spatial distribution of adult *Bemisia tabaci* (Homoptera: Aleyrodidae) in cotton, and development and validation of fixed-precision sampling plans for estimating population density. *Environ. Entomol.* 24:261–270.

Naranjo SE, Chu CC, Henneberry TJ. 1996. Economic injury levels for *Bemisia tabaci* (Homoptera: Aleyrodidae) in cotton: impact of crop price, control costs, and efficacy of control. *Crop Prot.* 15:779–788.

Naranjo SE, Ellsworth PC, Chu CC, Henneberry TJ, Riley DG, Watson TF, Nichols RL. 1998. Action thresholds for the management of *Bemisia tabaci* (Homoptera: Aleyrodidae) in cotton. *J. Econ. Entomol.* 91:1415–1426.

Naranjo SE, Ellsworth PC, Chu CC, Henneberry TJ. 2002. Conservation of predatory arthropods in cotton: role of action thresholds for *Bemisia tabaci* (Homoptera: Aleyrodidae). *J. Econ. Entomol.* 95:682–691.

Naranjo SE, Ellsworth PC, Hagler JR. 2004. Conservation of natural enemies in cotton: role of insect growth regulators for management of *Bemisia tabaci*. *Biol. Control* 30:52–72.

Natwick ET. 1993. Silverleaf whitefly control in cotton using various insecticides in the Imperial Valley of CA. In *Proceeding of the Beltwide Cotton Conference*, ed. DJ Herber, DA Richter, pp. 722–727. Memphis, TN: National Cotton Council.

Natwick ET, Laemmlen FF. 1993. Protection from phytophagous insects and virus vectors in honeydew melons using row covers. *Fla. Entomol.* 76:120–126.

Natwick ET, Robinson F. 1993. Alfalfa host plant resistance to silverleaf whitefly (formerly sweetpotato whitefly strain B). In *Proceedings of the Alfalfa Seed Production Symposium*, ed. S Mueller, pp. 60–63.

Natwick ET, Chu CC, Perkins HH, Henneberry TJ, Cohen AC. 1995. Pima and upland cotton susceptibility to *Bemisia argentifolii* under desert conditions. *Southwest. Entomol.* 20:429–438.

Natwick ET, Turini T, Gilbertson R. 2008. Whitefly transmitted virus diseases in southeastern California. *CAPCA Advisor* 11(1):24–27.

Nava-Camberos U, Riley DG, Harris MK. 2001. Density-yield relationships and economic injury levels for *Bemisia argentifolii* (Homoptera: Aleyrodidae) in cantaloupe in Texas. *J. Econ. Entomol.* 94:180–189.

Nomikou M, Janssen A, Schraag R, Sabelis MW. 2001a. Phytoseiid predators as potential biological control agents for *Bemisia tabaci*. *Exp. Appl. Acarol.* 25:270–290.

Nomikou M, Janssen A, Schraag R, Sabelis MW. 2001b. Phytoseiid predators suppress populations of *Bemisia tabaci* on cucumber plants with alternative food. *Exp. Appl. Acarol.* 27:57–68.

Nomikou M, Janssen A, Sabelis MW. 2003. Phytoseiid predators of whiteflies feed and reproduce on non-prey food sources. *Exp. Appl. Acarol.* 31:15–26.

Norman JW, Jr, Sparks AN, Jr. 1997. Cotton leaf hairs and silverleaf whiteflies in the lower Rio Grande Valley of Texas. In *Proceedings Beltwide Cotton Conferences*, ed. P Dugger, DA Richter, pp. 1063–1064. Memphis, TN: National Cotton Council.

Nuessly GS, Meyerdirk DE, Coudriet DL, Henneberry TJ. 1994. The effect of short season cotton production schedules on *Bemisia tabaci* (Gennadius). *Southwest. Entomol.* 19:209–217.

Nyoike TW, Liburd OE, Webb SE. 2008. Suppression of whiteflies, *Bemisia tabaci* (Hemiptera: Aleyrodidae) and incidence of *cucurbit leaf crumple virus*, a whitefly-transmitted virus of zucchini squash new to Florida, with mulches and imidacloprid. *Fla. Entomol.* 91:460–465.

Orozco-Santos M, Perez-Zamora O, Lopez-Arriaga O. 1995. Floating row cover and transparent mulch to reduce insect populations, virus diseases and increase yield in cantaloupe. *Fla. Entomol.* 78:493–501.

Ozores-Hampton M, McAvoy E, Simonne E, Stansly P. 2008. Evaluation of TYLCV virus-resistant varieties under commercial conditions in Southwest Florida. In *Proceedings of the Florida Tomato Institute*, pp. 12–16. http://gcrec.ifas.ufl.edu/tomatoproceedings08.pdf

Palumbo JC, Ellsworth PC, Dennehy TJ, Umeda K. 1999. Cross commodity management of whiteflies and chemical efficacy in Arizona. In *Vegetable Report.* Series P-117, AZ 1143, ed. DN Byrne, pp. 108–120. The University of Arizona, College of Agriculture, Tucson, AZ.

Palumbo JC, Toscano NC, Blua MJ, Yoshida HA. 2000. Impact of *Bemisia* whiteflies (Homoptera: Aleyrodidae) on alfalfa growth, forage yield, and quality. *J. Econ. Entomol.* 93:1688–1694.

Palumbo JC, Ellsworth PC, Dennehy TJ, Nichols RL. 2003. Cross-commodity guidelines for neonicotinoid insecticides in Arizona, *IPM Series No. 17*, Cooperative Extension, The University of Arizona, Tucson, http://cals.arizona.edu/pubs/insects/az1319.pdf

Palumbo JC, Horowitz AR, Prabhaker N. 2001. Insecticidal control and resistance management for *Bemisia tabaci*. *Crop Prot.* 20:739–765.

Pollard DG, Saunders JH. 1956. Relations of some cotton pests to jassid resistant Sakel. *Empire Cotton Growing Rev.* 33:197–202.

Polston JE, Anderson PK. 1997. The emergence of whitefly transmitted geminiviruses in tomato in the western hemisphere. *Plant Dis.* 81:1358–1369.

Polston JE, Cohen L, Sherwood TA, Ben-Joseph R, Lapidot M. 2006. *Capsicum* species: symptomless hosts and reservoirs of tomato yellow leaf curl virus (TYLCV). *Phytopathology* 96:447–452.

Polston JE, Lapidot M. 2007. Management of tomato yellow leaf curl virus: US and Israel perspectives. In *Tomato Yellow Leaf Curl Virus Disease Management, Molecular Biology, Breeding for Resistance*, ed. H Czosnek, pp. 252–262. Amsterdam, The Netherlands: Springer.

Qui YT, van Lenteren JC, Drost YC, Posthuma-Doodeman CJAM. 2004. Life history parameters of *Encarsia formosa*, *Eretmocerus eremicus* and *E. mundus*, aphelinid parasitoids of *Bemisia argentifolii* (Homoptera: Aleyrodidae). *Eur. J. Entomol.* 101:83–94.

Raya V, Parra M, Cid MC. 2006. Influence of changes in cover and height on the climate of Canary-screenhouses for tomato growth: preliminary results. *Acta Hort. (ISHS)* 719:535-542. http://www.actahort.org/books/719/719_62.htm

Riley DG, Palumbo JC. 1995. Action thresholds for *Bemisia argentifolii* (Homoptera: Aleyrodidae) in cantaloupe. *J. Econ. Entomol.* 88:1733–1738.

Rojas MR, Kon T, Natwick ET, Polston JE, Akad F, Gilbertson RL. 2007. First report of *Tomato yellow leaf curl virus* associated with tomato yellow leaf curl disease in California. *Plant Dis.* 91:1056.

Roltsch WJ, Hoelmer KA, Simmons GS, Andress E. 2008. Release and recovery of exotic natural enemies of *Bemisia tabaci* (biotype "B") (Hemiptera: Aleyrodidae) in Imperial Valley. In *California Classical Biological Control of Bemisia tabaci in the United States – A Review of Interagency Research and Implementation*, ed. J Gould, KA Hoelmer, JA Goolsby, Vol. 4, pp. 205–224. Amsterdam, The Netherlands: Springer.

Sanchez JA. 2008. Zoophytophagy in the plantbug *Nesidiocoris tenuis*. *Agric. For. Entomol.* 10:75–80.

Schuster DJ, Stansly PA, Polston JE. 1996. Expressions of plant damage from *Bemisia*. In *Bemisia 1995: Taxonomy, Biology, Damage Control and Management*, ed. D Gerling, RT Mayer, pp. 153–165. Andover, Hants, UK: Intercept Ltd.

Schuster DJ, Evans GA, Bennett FD, Stansly PA, Jansson RK, Leibee GL, Webb SE. 1998. A survey of parasitoids of *Bemisia* spp. whiteflies in Florida, the Caribbean, and Central and South America. *Int. J. Pest Manag.* 44:255–260.

Schuster DJ. 2002. Action threshold for applying insect growth regulators to tomato for management of irregular ripening caused by *Bemisia argentifolii* (Homoptera: Aleyrodidae). *J. Econ. Entomol.* 95(2):372–376.

Schuster DJ, Stansly PA, Polston JE, Gilreath PR, McAvoy E. 2007. Management of whiteflies, whitefly-vectored plant virus, and insecticide resistance for vegetable production in southern Florida. *ENY-735 (IN695)*, IFAS Extension, University of Florida, Gainesville.

Seo YS, Zhou YC, Turini TA, Cook CG, Gilbertson RL, Natwick ET. 2006. Evaluation of cotton germplasm for resistance to whitefly and cotton leaf crumple (CLCr) disease and etiology of CLCr in California's Imperial Valley. *Plant Dis.* 90:877–884.

Shipp JL, Wang K. 2006. Evaluation of *Dicyphus hesperus* (Heteroptera: Miridae) for biological control of *Frankliniella occidentalis* (Thysanoptera: Thripidae) on greenhouse tomato. *J. Econ. Entomol.* 99:414–420.

Soler-Gamborena R, van Lenteren JC. 2004. Reproduction and development of *Eretmocerus eremicus* (Hymenoptera: Aphelinidae) on *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). In *Proceedings of the Section Experimental and Applied Entomology of the Netherlands Entomological Society (NEV)* 15, ed. R Soler-Gamborena, JC van Lenteren, pp. 111–117.

Stansly PA. 1996. Seasonal abundance of silverleaf whitefly in Southwest Florida vegetable fields. *Proc. Fla. State Hort. Soc.* 108:234–242.

Stansly PA, Schuster DJ, Liu TX. 1997. Apparent parasitism of *Bemisia argentifolii* (Homoptera: Aleyrodidae) by Aphelinidae (Hymenoptera) on vegetable crops and associated weeds in South Florida. *Biol. Control.* 9:49–57.

Stansly PA, Sanchez PA, Rodriguez JM, Canizares F, Nieto A, Lopez-Leyva MJ, Fajardo M, Suarez V, Urbaneja A. 2004a. Prospects for biological control of *Bemisia tabaci* (Homoptera, Aleyrodidae) in greenhouse tomatoes of southern Spain. *Crop Prot.* 23:701–712.

Stansly PA, Calvo FJ, Urbaneja A. 2004b. Biological control of *Bemisia tabaci* (Homoptera, Aleyrodidae) in protected tomato and pepper culture in southern Spain. In *Proceedings of the VII Internatational Symposium on Protected Cultivation in Mild Winter Climates: Production, Pest Management and Global Competition*. Vol. I, *Acta Hort*. 659:383–394. http://www.actahort.org/books/659/659_51.htm

Stansly PA, Calvo J, Urbaneja A. 2005a. Augumentative biological control of *Bemisia tabaci* biotype "Q" in greenhouse pepper using *Eretmocerus* spp. (Hym. Aphelinidae). *Crop Prot.* 24:829–835.

Stansly PA, Calvo J, Urbaneja A. 2005b. Release rates for control of *Bemisia tabaci* (Homoptera: Aleyrodidae) Biotype "Q" with *Eretmocerus mundus* (Hymenoptera: Aphelinidae) in greenhouse tomato and pepper. *Biol. Control* 35:124–133.

Stansly PA, Castillo J. 2009. Control of broad mite *Polyphagotarsomeus latus* and the whitefly *Bemisia tabaci* in open field pepper and eggplant with predaceous mites. In *Proceedings of the Working Group "Integrated Control in Protected Crops, Mediterranean Climate"*, ed. C Castañe, D Perdikis. *IOBC WPRS Bull*. 49:145–152.

Stapleton JJ, Summers CG. 2002. Reflective mulches for management of aphids and aphid-borne virus diseases in late season cantaloupe (*Cucumis melo* L. var. *cantalupensis*). *Crop Prot.* 21:891–898.

Stern VM, Smith RF, van den Bosch R, Hagen KS. 1959. The integrated control concept. *Hilgardia* 29:81–101.

Szabo P, van Lenteren JC, Huisman PWT. 1993. Development time, survival and fecundity of *Encarsia formosa* on *Bemisa tabaci* and *Trialeurodes vaporariorum*. *IOBC/WPRS* 16:173–176.

Tal C, Coll M, Weintraub PG. 2007. Biological control of *Polyphagotarsonemus latus* (Acari: Tarsonemidae) by the predaceous mite *Amblyseius swirskii* (Acari: Phytoseiidae). *IOBC/WPRS Bull.* 30:111–115.

Teitel M. 2006. The effect of screens on the microclimate of greenhouses and screenhouses – a review. *Acta Hort*. (ISHS) 719:575–586. http://www.actahort.org/books/719/719_67.htm

Teuber LR, Rupert ME, Gibbs LK, Taggard KL. 1997. Breeding resistant alfalfa holds promise for silverleaf whitefly management. *Calif. Agric.* 51(3):25–29.

Urbaneja A, Tapia G, Stansly PA. 2005. Influence of host plant and prey availability in the developmental time and survival of *Nesidiocoris tenuis* Reuter (Het: Miridae). *Biocontrol Sci. Technol*. 15:513–518.

Urbaneja A, Sanchez E, Stansly PA. 2007. Life history of *Eretmocerus mundus* Mercet (Hym.: Aphelinidae), a parasitoid of *Bemisia tabaci* Gennadius (Hom: Aleyrodidae), on tomato and sweet pepper. *Biocontrol* 52:25–39.

Van der Blom J. 2007. Control de plagas en hortícolas protegidas: el año de la transición. *Horticultura* 200:36-43. http://www.horticom

van Driesche RG, Hoddle MS, Lyon S, Sanderson JP. 2001. Compatibility of insect growth regulators with *Eretmocerus eremicus* (Hymenoptera: Aphelinidae) for whitefly (Homoptera: Aleyrodidae) control on poinsettias. II. Trials in commercial poinsettia crops. *Biol. Control* 20:132–146.

van Driesche RG, Lyon S, Jacques K, Smith T, Lopes P. 2002. Comparative cost of chemical and biological whitefly control in poinsettia: is there a gap? *Fla. Entomol.* 85:488–493.

Van Lenteren JC, Woets J. 1988. Biological and integrated control in greenhouses. *Annu. Rev. Entomol.* 33:239–269.

Vestergaard S, Cherry A, Keller S, Goettel M. 2003. Safety of hyphomycete fungi as microbial control agents. In *Environmental Impacts of Microbial Insecticides: Needs and Methods for*

*Risk Assessment*, ed. HMT Hokkanen, AE Hajek, pp. 35–62. The Netherlands, Dordrecht: Kluwer Academic publisher.

Vidal C, Fargues J, Rougier M, Smits N. 2003. Effect of air humidity on the infection potential of hyphomycetous fungi as mycoinsecticides for *Trialeurodes vaporariorum*. *Biocontrol Sci. Technol.* 13:183–198.

Walker GP, Natwick ET. 2006. Resistance to silverleaf whitefly, *Bemisia argentifolii* (Hem., Aleyrodidae), in *Gossypium thurberi*, a wild cotton species. *J. Appl. Entomol.* 130:429–436.

Watson TF, Silvertooth JC, Tellez A, Lastra L. 1992. Seasonal dynamics of sweetpotato whitefly in Arizona. *Southwest. Entomol.* 17:149–167.

Yee WL, Toscano NC, Palumbo JC, Blua MJ, Yoshida HA. 1997. Seasonal population trends of *Bemisia argentifolii* (Homoptera: Aleyordidae) on alfalfa in southern California and Arizona. *Environ. Entomol.* 26:241–249.

Yokomi RK, Hoelmer KA, Osborne LS. 1990. Relationship between the sweetpotato whitefly and the squash silverleaf disorder. *Phytopathology* 80:895–900.

Zimmerman G. 2008. The entomopathogenic fungi *Isaria farinosa* (formerly *Paecilomyces farinosus*) and the *Isaria fumosorosea* species complex (formerly *Paecilomyces fumosoroseus*): biology, ecology and use in biological control. *Biocontrol Sci. Technol.* 18:865–901.

# Section V
# Prospects for the Application of Genomics

**Judith K. Brown**

## Introduction

This final section contains a single chapter, *The Whitefly Genome White Paper*, whose foundations were put into place by a group of scientists attending the First International Whitefly Genomics Workshop, which followed the 4th International *Bemisia* Workshop, held in Duck Key, Florida in 2006. The Genome White Paper is the last chapter in this book, both to signify the present limitations in technology use that exist in whitefly science, and to set the stage for a new era in whitefly research that will utilize multiple approaches to elucidate new levels of knowledge with which biological concepts will be merged.

The *Bemisia tabaci* (Gennadius) sibling species group comprises phenotypically diverse "biological types," some that readily adapt to new environments and may become invasive, and others that are more benign and readily displaced by invasive types. Phenotypic variants, or "biotypes" exhibit variable behaviors, that include but aren't limited to host preference, fecundity, dispersal, vector competency, and endosymbiont composition. In constrast *B. tabaci* "biotypes" have no set of morphological characters that distinguishes them from one another, despite evidence of potentially high genetic variability, as revealed by molecular markers. Although this has given rise in the short term to a taxonomic conundrum, it has nonetheless underscored the value of initiating genome level studies of the *B. tabaci* sibling species group, as a unique system for elucidating the functional genomics underlying cryptic species development, and processes underlying speciation. The relative importance of genome sequence, versus gene expression, in determining this phenotypic variation will only be fully elucidated when the whitefly genome sequence is available for study. Clearly such an approach has relevance to other cryptic insect species, a number of which also are are homopterans (Hemiptera), which also may spawn invasive genotypes. However, *B. tabaci* is unusual among homopterans in employing haplo-diploid reproduction instead of true parthenogenetic and/or true sexual reproduction, opening doors for comparisons of mechanisms operating in

---

Judith K. Brown (Section Editor)

this type of reproduction in other insect orders, most notably the Hymenoptera and Thysanoptera.

Monoculture cropping systems and high inputs of insecticides have favored the selection of invasive biotypes of *B. tabaci* in zones of endemism, and in exotic locales owing to the human transport of infested hosts within and between continents. The most notorious invasive insect of these recognized to date is the extremely polyphagous B biotype, which is thought to orginate in northern Africa/Middle East. Other well-known examples having an extensive host range are the A biotype, native to the USA/Mexico southwestern deserts, and the Q biotype from southern Spain. These three biotypes exhibit clear behavioral and genetic differences that can be exploited in a comparative genomics approach to elucidate the connections between genetic makeup and gene expression. Another example, the Ug2 cassava colonizing biotype that invaded East-Africa (ca. 1990), and is spreading the cassava mosaic disease (CMD) caused by a complex of begomoviruses (*Geminiviridae*) throughout east and central Africa, with eight countries affected to date. Because cassava is the staple food of many subsaharan Africans, CMD has caused a crisis owing to a shortage of cassava. Thus, multiple invasive *B. tabaci* biotypes, polyphagous and monophagous, are recognized, and are excellent starting points for *The Whitefly Genome Project*, whose initial outputs could illuminate genes key to host range, invasiveness, cryptic speciation, and virus-vector specificity, among others.

Of fundamental evolutionary interest is the ancient association and co-evolution of *B. tabaci*-begomovirus complexes that has resulted in a high degree of virus-vector specificity. As a result, the highest priority is to discover the suite of whitefly-encoded proteins that are crucial for virus acquisition, circulation in the vector, and virus transmission, as well as competency. These protein-protein interactions involve receptor-mediated processes acting at the gut-hemolymph and hemolymph-salivary gland interfaces that dictate the specificity of this circulative transmission pathway. The identification of the (putative) gut and salivary gland "receptors", as well as key proteins that modulate whitefly-mediated transmission, is expected to lend valuable insights in the development of novel disease abatement strategies based on interference with key steps in the transmission pathway. In addition, it is plausible that the whitefly responds to invading begomoviruses as hostile entities and reacts to their presence by activating general or specific stress-response cascades. Thus, whitefly functional genomics would provide a model for studying innate immunity and affiliated cellular pathways conserved in arthropod-virus complexes (Mesaroviv et al. 2004). Such pathways and derived characters are represented across most or all kingdoms, making them invaluable to the vector biology community for uncovering evolutionarily conserved, responsive molecules and pathways relevant to animal and human health. Presently, such functional genomics studies are hindered because the complete *B. tabaci* genome sequence is not available.

The *B. tabaci* sibling species group is a biologically unique organism, and a tropical relative to other agriculturally important phloem feeding homopterans, including aphids, leafhoppers, and mealybugs. It is, however, distinct from many of its closest relatives by inhabiting entirely different niches and by developing unique

behavioral mechanisms. *B. tabaci* harbors obligate and facultative endosymbionts known to contribute to nutrient uptake owing to amino acid-poor phloem sap, and perhaps also to whitefly host adaptation to stress, and thereby to "biotype formation". This plasticity is thought to have allowed *B. tabaci* to readily adapt to modern agricultural settings, making it one of the highest-priorty pests and plant virus vectors in temperate and subtropical cropping systems. Even so, the fundamental biology and genetics of this important complex is poorly understood owing to the lack of a genomics toolbox. The proposed *Whitefly Genome Project* will alleviate this knowledge gap by facilitating key studies to elucidate for the first time the genetic basis underlying biotype formation, phenotypic plasticity, invasivenes, host preference (e.g., monophagy versus polyphagy), virus-vector specificity, and insecticide resistance, among others.

We propose to initiate *The Whitefly Genome Project* by first providing at least 20X coverage – and greater, with expected advancements in sequencing technologies – of the genome for the now cosmopolitan – albeit, exotic – B biotype, a well-studied polyphagous haplotype whose relatives are native to Sub-Saharan Africa and the Middle East. In addition, the project objectives include sequencing the transcriptome of the B biotype to serve as a reference sequence for future comparisons of as many as 30 known biotypes, representing all world regions and habitats where *B. tabaci* is extant, in order to carry out an insightful comparative genomics analysis of the genome and transcriptome of phenotypically distinct *B. tabaci*. More than thirty biotypes are recognized and more are being discovered. Examples of others are the invasive cassava-associated Ug2 driving the cassava mosaic disease pandemic in eastern Africa and the endemic Ug1, the polyphagous Spanish Q and its relatives from the Mediterranean that recently have invaded non-native territories, the Sida biotype from Puerto Rico, monophagous biotypes from Benin (Aystasia), Puerto Rico (Jatropha), Spain and West Africa (S), Italy and nearby islands (T), a B biotype relative, the Ms biotype from Reunion Island (relative of the "non-B" from Uganda), the Australian natives, WAN and EAN, and ZHI and ZH2 from China.

The next steps are to procure financial and intellectual support, and enthusiasm throughout the community to initiate *The Whitefly Genome Project* along with the timely interactive development of the multiple -omics toolbox that will meet the broad needs of the scientific community, related industry partners, and public and private sector stakeholders.

## Consortium Partner Countries and Participants (2009)

*Australia* (O. Edwards, P. De Barro), *Brazil* (A.K. Inoue-Nagata, R.O. Resende), *China* (F. Yang, X. Zhou, S.S. Liu), *Chile* (M. Rosales Villavicencio, N. Mejia), *Cyprus* (M. Hadjistylli, L. Papayiannis), *France* (H. Delatte), *Germany* (R. Nauen; S. Winter), Israel (H. Czosnek, M. Ghanim, V. Khasdan, M. Lapidot, S. Morin), *Italy* (G.P. Acotto, P.C. Caciagli), *Morocco* (H. Abdelhaq, B. Rachid), *South Africa*

(M.E.C. Rey), *Spain* (E. Moriones, J. Navas Castillo, D. Janssen), *The Netherlands* (J. Wijbrandi, P. Bleeker), *United Kingdom* (I. Bedford, I. Denholm, A. McCaffery), and *United States* (J.K. Brown, D. Frohlich, D. Gang, C. McKenzie, N. Merchant-ARL, G. Roderick, J. Polston, R. Rosell, R. Shatters, C. Soderlund, L. Walling) [*Additional partners are welcome*].

## History of Synergistic Activities

- In September 2004 the *International Whitefly Genome Consortium* was founded during the *Second European Whitefly Symposium* hosted in Dubrovnik, Croatia. The *co-Founders* were: Judith K. Brown (The University of Arizona, Tucson, AZ) and Henryk Czosnek (The Hebrew University of Jerusalem, Israel). *The Consortium Founding Members* were Jelle Wijbrandi (Keygene), Ralf Nauen (Bayer CropScience-AG), Shai Morin (The Hebrew University of Jerusalem), Stephan Winter (BBA Brunschweig, Germany), Jesus Navas-Castillo and Enrique Moriones (Consejo Superior de Investigaciones Cientificas, Spain), Paul de Barro (CSIRO Entomology, Australia), Ian Bedford (John Innes Centre, UK), John Stanley (John Innes Centre, UK), M.N. Maruthi (NRI-UK), Murad Ghanim (The Volcani Center, Israel), Vadim Khasdan, (Ben-Gurion University, Israel), Ian Denhom (Rothamsted, UK), Alan McCaffery (Syngenta), Abdelhaq Hanafi and Bouharroud Rachid (Complexe Horticole, Morocco).
- In January 2005 the *International USDA Insect Genetics Workshop* was convened, and *B. tabaci* was among the insects featured (www.intl-pag.org/13/13-insect.html).
- In December 2006 the *Fourth International* Bemisia *Workshop* was held in Duck Key, FL, followed by a USDA-supported workshop, "The Whitefly Genome Project" that hosted the third meeting of the Whitefly Genome Consortium.
- During October 2008 the *Third European Whitefly Symposium* was hosted in Almeria, Spain, and offer a special Symposium entitled "Whitefly functional genomics and proteomics".
- In November 2009 the *Fifth International* Bemisia *Workshop* was held in Guangzhou, China, where new genomics technologies were highlighted.
- The Sixth International *Bemisia* Workshop is tentatively expected to take place in Crete.

## Literature Cited

Mesarovic MD, Sreenath SN, Keene JD. 2004. Search for organizing principles: understanding in systems biology. *Systems Biol*. 1:19–27.

# Chapter 18
# The Whitefly Genome – White Paper: A Proposal to Sequence Multiple Genomes of *Bemisia tabaci*

**Henryk Czosnek and Judith K. Brown**

## Introduction

The whitefly, *Bemisia tabaci* (Gennadius 1889), complex (Brown et al. 1995) can cause extensive damage to crop and ornamental plants. Damage can be biotype specific, including induction of physiological disorders due to feeding alone. More importantly, whitefly transmitted plant viruses cause diseases, which affect fiber, vegetable, and ornamental crops. Most whiteflies in general colonize woody species, while very few utilize herbaceous plants. In this respect, *B. tabaci* is unique in colonizing herbaceous plant species.

Crop losses attributed to B biotype *B. tabaci* infestation, together with pesticide costs, have surpassed $1 billion annually in the USA (Gerling and Mayers 1996; USDA 2004 Statistics). The B biotype, an exotic haplotype that now occurs worldwide owing to its widespread introduction, has a propensity to outbreak and can achieve enormous densities. The B and other biotypes of importance in cropping systems exhibit resistance to modern insecticides (Cahill et al. 1995; Brown 2001; Morin et al. 2002; Horowitz et al. 2005; Ma et al. 2007). Although whiteflies have only recently become economically important, they have lived on Earth for more than 130 million years (MY) (Campbell et al. 1996), an estimate based on evidence of an ancestor trapped in amber and discovered in Lebanon (Fig. 18.1). Anatomical features do not appear to have changed much since then (Schlee 1970).

More than 500 crop plants are colonized by *B. tabaci* (Cock 1993), underscoring the potentially damaging effect of this cryptic species in agricultural systems. A female whitefly may lay 300–400 eggs in her 4-weeks lifespan (Byrne et al. 1990). With a propensity to exploit current agricultural practices, this once little-known insect has become a near-celebrity as a "superbug" both throughout the subtropics and in temperate zones with mild climates that support multiple cropping cycles per year.

H. Czosnek (✉)
Faculty of Agriculture, The Robert H. Smith Institute for Plant Sciences and Genetics in Agriculture, The Hebrew University of Jerusalem, Rehovot, Israel
e-mail: czosnek@agri.huji.ac.il

P.A. Stansly, S.E. Naranjo (eds.), *Bemisia*: Bionomics and Management of a Global Pest,
DOI 10.1007/978-90-481-2460-2_18, 

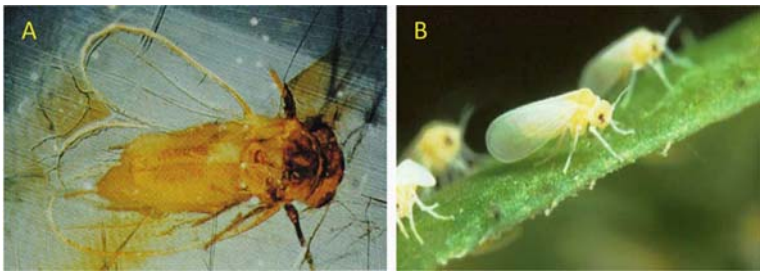



**Fig. 18.1** (**A**) Primitive whitefly *Bernaea neocomica* (Schlee) in 130 MY old Lebanese amber (Stuttgart Natural History Museum); (**B**) *Bemisia tabaci* (Gennadius) from Arizona

*B. tabaci* and other polyphagous whiteflies of tropical origin, also in the family Aleyrodidae, differ from their temperate aphid counterparts (Aphididae), which also have garnered much attention as pests and plant virus vectors. Aphids proliferate mainly in temperate climates during the spring and summer and, following migratory flights, often over-winter as eggs, mostly on woody plants. *B. tabaci* on the other hand employs adaptive dispersal behaviors ranging from short-distance "flitting" between understory plants, to long-distance flight (e.g. arid/seasonal rainfall and scarcity of "food" versus dry subtropics and plentiful food) (Cohen et al. 1988; Brown 2001).

As genomics, proteomics, and metabolomics research is expanded beyond model organisms, the methodologies developed through studies of model arthropods are becoming available for application to non-model systems, many of which are important agriculture pests. Directing these technologies to solving applied problems offers new opportunities for developing approaches to reduce the damage caused by agricultural pests. Elucidating the functional genomics of the *B. tabaci* complex has become essential for devising novel, sustainable pest control strategies, and directed interference of whitefly-mediated virus transmission. Hence, availability of the complete genome sequence is now crucial for facilitating valuable genomics, proteomics, and functional genomics applications (Eisen et al. 1998).

Whitefly genomics research is expected to open important avenues into the discovery of novel strategies for whitefly management based in an improved understanding of molecular, cellular, and biological processes. The genome sequence will synergize projects underway to develop and sequence *B. tabaci* expressed sequence tags (EST) or cDNA libraries for functional genomics and proteomics analysis. The benefits are far reaching, including better resolution of the *B. tabaci* species complex systematics that contribute to their hallmark plasticity, identifying genes that combat abiotic and biotic stresses that often lead to invasiveness and insecticide resistance, and understanding the basis for whitefly-virus specificity (De Risi et al. 1997; Werling and Jungi 2003).

An annotated genome sequence, together with microarray capability and other functional genomics tools, will facilitate the analysis of whitefly genetics and of metabolic pathways. It will help elucidate the functional characterization of genes,

their differential expression, localization of transcripts in situ, and determination of the proteome. Altogether, availability of the whole genome sequence will be instrumental in elucidating the biochemical processes that contribute to pest status. Below we summarize in greater detail the rationale for undertaking *The Whitefly Genome Project*. The case was made throughout this book for the importance of *B. tabaci* as a serious insect pest that undermines food and fiber production. Here we describe a number of unique biological and genetic attributes that will be elucidated at a deeper level than is presently possible.

## Homopteran Model

### *Cryptic Species*

The taxonomy of many agriculturally important insects is confounded by the absence of morphological characters that enable their immediate recognition or identification; these are referred to as cryptic species. Cryptic species by definition pose a problem for identification, complicate systematic and phylogenetic classification, and confound population genetics studies. This has led to the misidentification of certain "cryptic species", and hence their rapid emergence as invasive species, some with serious economic consequences (Pimentel et al. 2000). This paradigm holds true for the *B. tabaci* complex (Frohlich et al. 1999), including several morphologically indistinguishable relatives presently not classified in the genus *Bemisia* (Costa and Brown 1991; Bedford et al. 1994; Brown et al. 1995; De Barro et al. 2000; De Barro 2005; Gill 1990; Martin 2003; Rosell et al. 1997).

The genus *Bemisia* has been synonymized by Russell (1957) who combined several genera and ~18 species into the *B. tabaci* specific epithet based on fourth instar morphology (Gill 1990; Mound and Halsey 1978). Another complicating factor is that the external morphology of the fourth instar (pupae) can be altered owing to its unusual ability to mimic leaf surface topology (Bedford et al. 1994; Mound 1963).

One contemporary systematist (see Gill and Brown Chapter 1) has suggested that *B. tabaci* is simply so perfectly adapted to its varied habitats that it neither requires nor benefits from external changes, sparing its adaptive energies for subtle genetic modification (probably at the level of gene expression), to accommodate ever-changing habitats. It is also believed that a portion of this plasticity and fitness is attributable to diverse bacterial endosymbionts (Buchner 1965; Costa et al. 1995; Dobson et al. 1999; Douglas 1998).

Clearly, the taxonomic debate involving *B. tabaci* challenges the scientific community. The availability of whitefly genome sequences, together with functional genomics and gene expression studies, promises to shed important light on the processes underlying cryptic species morphology and phenotypic plasticity. That other "cryptic" animals exist, many of which are insects, makes the *B. tabaci* genome ideal for elucidating the genomics basis for genetic diversification that occurs without the "expected," corresponding morphological changes.

## *Systematics Model – Sibling Species Group*

*B. tabaci* is troublesome as well as fascinating to systematists, biologists, and geneticists (Bedford et al. 1994; Burban et al. 1992; Brown 2000; Costa and Brown 1991; Costa et al. 1993; Frohlich et al. 1996; 1999; Gawel and Bartlett 1993; Gill 1990; Perring et al. 1993; Rosell et al. 1997; Wool et al. 1993). Phenotypic variability in the *B. tabaci* complex is manifest in a variety of ways. Variability can be observed as differences in plant host-preference, host range, fecundity, dispersal behavior, vector competency, phytotoxic feeding effects, endosymbiont composition, invasiveness, and insecticide resistance, among others (Bedford et al. 1994; Brown et al. 1995; Burban et al. 1992; Costa and Brown 1991; Costa et al. 1995; Costa and Russell 1975; Sseruwagi et al. 2005).

Host specialization was described first by Bird (1957) who studied the host range and virus-vector transmission of two haplotypes in Puerto Rico, which he termed based on their differences, "host races." Other host range studies have further contributed to the recognition that certain populations in Africa for example, are restricted to cassava, while others do not feed on it at all (Costa and Russell 1975; Sseruwagi et al. 2005). In contrast, several biotypes recently troublesome in agricultural crops have host ranges of several hundred species (Cock 1993). The taxonomic debate is becoming more subdued as to whether *B. tabaci* is a sibling species complex comprising host races or biotypes, or a complex of different species (species complex). Based on new information about molecular phylogeny and evidence for discontinuous or incomplete gene flow, it is herein referred to as a sibling species complex (at least for now).

At a genetic level, comparisons based on the mitochondria cytochrome oxidase I gene (mtCO1) between *B. tabaci* and other well-defined insect species (Simon et al. 1994) have revealed an unexpected degree of variation within the former by as much 26% (Fig. 18.2). This knowledge has aided in the delineation of at least four major phylogeographic clades, within which are sister clades that generally group with a basis in phylogeography. In contrary cases it is either known or suspected that human movement has been responsible for haplotype relocation (Berry et al. 2004; Brown 2000; Brown et al. 1995; Frohlich et al. 1999; Viscarret et al. 2003).

## *Subtropical Homopteran Model – Comparative Biology*

To date, no complete genome sequence has been determined for a homopteran of truly tropical origin, even though thousands of such species are known. Whiteflies therefore offer an opportune model study system. In comparison to analogous temperate insect species economic impact, and biological intrigue, they are poorly studied in every way. Whiteflies are distributed predominatly in habitats between the 30th degree parallel, north and south. The geographical range of the *B. tabaci* complex spans the dry tropics, subtropics, and mild climates regions, including deserts, which experience seasonal rainfall. To inhabit this diverse range of

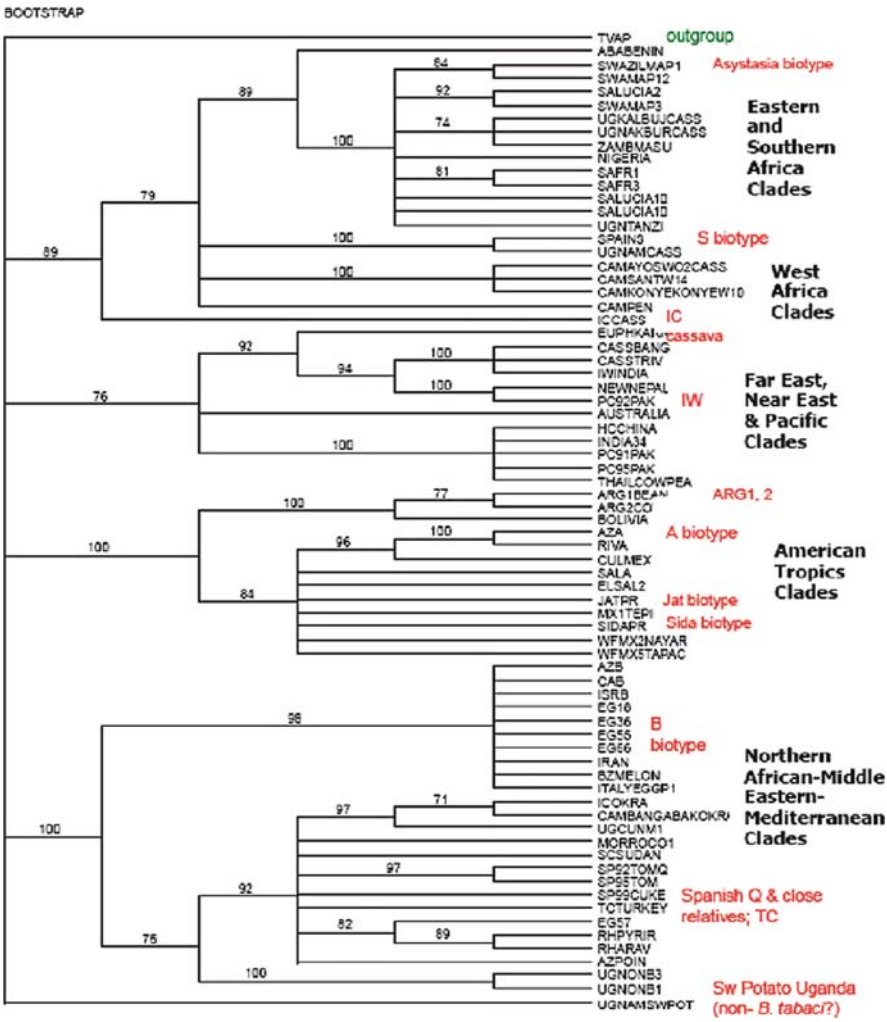



**Fig. 18.2** Phylogenetic tree showing the four major clades (**bold black**) comprising representative haplotypes of *B. tabaci* sibling species group based on the mitochondria cytochrome oxidase I (mtCO1) sequence (780 bases). The major geographic groups diverge at ~2–26% (Brown 2007). A number of sister clades are represented within each major clade depending on the choice of sequence and algorithm used for the analysis. The phylogenetic placements of selected recognized biological types are shown in *red letters* (not inclusive)

conditions, whitefly development slows greatly during cold periods and accelerates with increasing temperature.

*B. tabaci* is an ideal tropical model homopteran for the many reasons described herein, and much can be learned through comparative studies with its temperate/

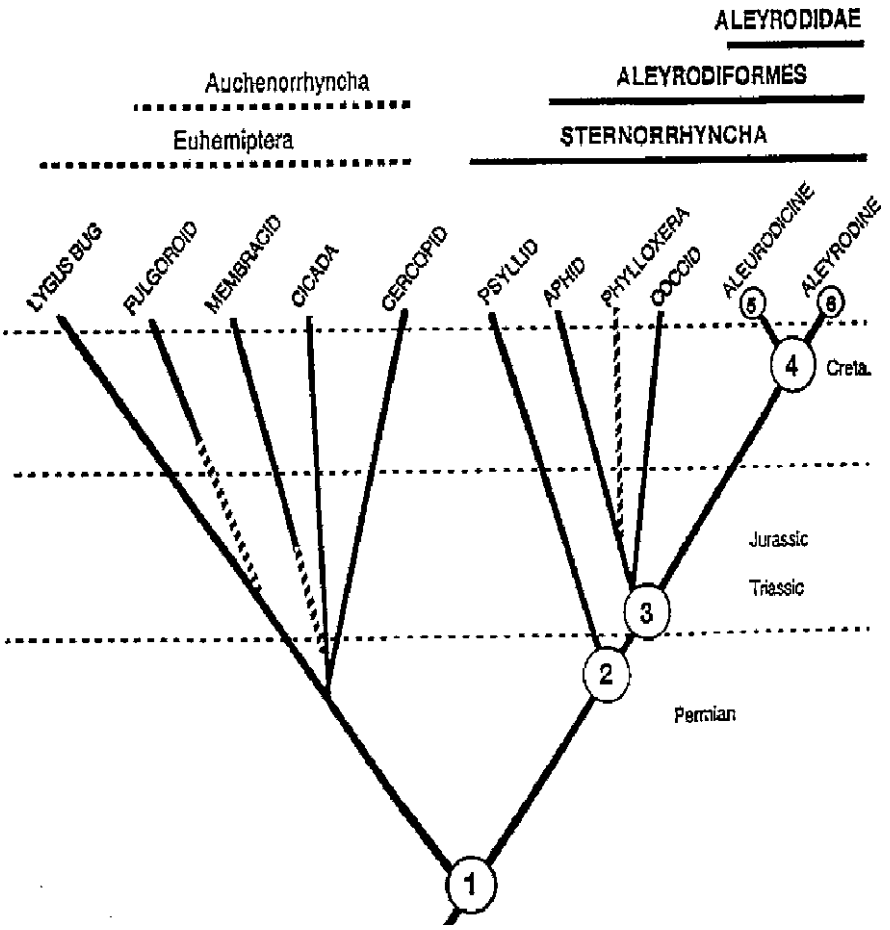



**Fig. 18.3** Phylogenetic lineages reconstructed using the 18S rRNA gene (Campbell et al. 1996) for the most important homopteran insect vectors, all specializing in feeding in the plant vasculature (*left to right*): glassy winged sharpshooter (Pierce's disease-bacterium), citrus psyllid (greening-bacterium), aphids (Luteoviruses, Potyviruses, Cucumoviruses), the whitefly *B. tabaci* (Closterovirus-Crinivirus, Carlaviruses, Geminivirus-Begomoviruses; Potyviruses). Note the distinct evolutionary lineages and ecological niches of these vascular feeders

subtropical counterparts; aphids, leafhoppers, and psyllids (Campbell et al. 1996; Fig. 18.3). Comparative genomic and functional genomics between multiple homopteran families will yield new and important discoveries about insects that share a common co-evolutionary history, and specialize feeding on the vascular tissue of higher plants (phloem or xylem). Indeed the majority of plant virus vectors are homopterans are, owing to vascular specialization of these insects. They also share the requirement for bacterial symbionts that produce amino acids that are in low supply in plant sap (Douglas 1998). Further, a "c-type" primary endosymbiont

synthesizes a conserved GroEL homologue 60S heat shock protein that interacts with plant virus particles while *en route* in the blood to the salivary glands (van der Heuvel et al. 1994; Morin et al. 1999, 2000, see also Rosell et al. Chapter 5). In contrast, a striking difference resides in their reproductive system, which for whiteflies and some scale insects is haplodiploid and through eggs, while many aphids bear live young parthenogenetically, and may have a brief sexual stage (Campbell 1993; Clark et al. 1992; Costa et al. 1995; Czosnek et al. 2002; Munson 1991; Moran and Telang 1998).

## *Reproduction – Haplodiploidy, Reproductive Isolation, and Speciation*

*B. tabaci* is haplodiploid and so fertilized eggs give rise to diploid females, whereas, males are produced from unfertilized eggs. Haplodiploidy is not uncommon among insects, and in fact is found in all Hymenoptera including ants, wasps and bees. Homopterans employ diverse types of reproductive systems. Many utilize parthenogenesis e.g. aphid mothers produce live, clonal offspring without mating. In contrast, whiteflies and most other Homoptera produce eggs that are attached to the leaf surface, giving rise to first instars referred to as crawlers. Whitefly crawlers seek a suitable vascular bundle as their feeding site and become sessile. After several molts they eclose as adults. In this system diploid (2N) females are produced from fertilized eggs, while haploid (1N) males are produced from unfertilized eggs (Blackman and Cahill 1998). When the number of males in a population decline, fertilization is reduced, causing an increased number of male offspring to be produced in the next generation. Hence, sex ratio bias is frequently reported for alternate generations of this whitefly (Byrne et al. 1990; Gill 1990; Martin 2003). Changes of sex ratios (from predominantly males to predominantly females) have been reported to occur during the tomato-growing season in relation to *Tomato yellow leaf curl virus* infection in the Jordan Valley (Cohen et al. 1988).

Haplodiploidy, together with the effects of "parasitic bacteria," which induce a condition referred to as cytoplasmic incompatibility (CI) can further confound sex ratio and population dynamics in interesting ways. Among the best-known CI-inducing bacteria are *Wolbachia* spp. (Werren 1997, 1998; Southamer et al. 1999), which has been associated with a number of *B. tabaci* populations (Zchori-Fein and Brown 2002). An example of the recently discovered divergent CI-inducer *Cardinium* spp. (Weeks and Breeuwer 2003; Weeks et al. 2003; Zchori-Fein and Perlman 2004; Provencher et al. 2005) has been identified in the A biotype (Caballero and Brown 2008). When males that harbor the bacterium mate with uninfected females, female offspring are eliminated (Tram and Sullivan 2002). CI-induced reproductive isolation clearly can obfuscate reproductively compatible haplotypes, obstruct gene flow, and introduce induce sex bias in populations. How such factors influence gene flow and invasiveness in *B. tabaci* has not been studied at the genomic level. Thus, an immediate goal is to understand the genetic mechanisms

involved in haplodiploidy and CI-induction, an important stepping-stone by which novel means of modulating whitefly reproduction and sex ratios may be revealed (Blair et al. 2000; Brown and Czosnek 2002).

### *B. tabaci* Biotypes as a Model for Ongoing Speciation

The origin of *B. tabaci* biotypes is intriguing. The question of whether they constitute the same or different species is a heated debate. Mating and production of viable F1 generations that produce fertile offspring is the common denominator for several definitions of species. Sexual selection followed by mating incompatibility is one of the mechanisms of sympatric speciation; that is, the formation of two or more descendant species from a single ancestral species all occupying the same geographic location (Kirkpatrick and Ravigné 2002).

Mating between whitefly biotypes has been intensively studied but results are not always definitive. Several inter-biotype crossings that resulted in unsuccessful F1 hybrids have been documented. For example A and B biotypes from the United States were crossed without producing an F1, suggesting a lack of gametic transfer in this haplodiploid insect (Costa et al. 1993; Perring et al. 1993; Perring and Symmes 2006). Further studies with the B biotype provided evidence of reproductive incompatibility among members of the *B. tabaci* complex. Reciprocal crosses performed between biotype B from the United States, biotype K from Pakistan, biotype M from Turkey, biotype D from Nicaragua and biotype ZHJ1 from China resulted in no F1 hybrids (Bedford et al. 1994; Liu et al. 2007). Compatible crosses among *B. tabaci* biotypes have been documented as well. Hybrids have been identified in crosses between biotype B from the United States and biotype L (SUD) from Sudan (Byrne et al. 1995). Similar results were recorded between a native Australian population and biotype B (Gunning et al. 1997) and between biotypes B and Q in Spain (Ronda et al. 2000). In this latter example, the authors did not indicate whether the F1 hybrids were fertile. Contrary to these findings, fertile F1 hybrids between biotype A, biotype B, and the Jatropha biotype have been reported (Caballero et al. 2001). In Israel, although B and Q biotypes coexist in the same regions, B/Q F1 has not been reported (Horowitz et al. 2003; 2005). Recently, begomovirus transmission has been described to occur during mating between females and males of the same biotype, whether B or Q. However, no transmission was found when male Q and female B were caged together, and vice versa, indicating mating incapability (Ghanim and Czosnek 2000; Ghanim et al. 2007b). It is clear from these results that various levels of reproductive incompatibility operate in the *B. tabaci* sibling species group. Several mechanisms could result in this isolation. It has been suggested that the incompatibility found between the Q and B biotypes may be caused by a different bacterial symbiont load (Cheil et al. 2007). Prezygotic mechanisms also can drive reproductive incompatibility, and one such mechanism that may be operational in the *B. tabaci* species complex is the mate recognition system (Butlin 1995). Interestingly, several researchers observed that although males and females of distinct *B. tabaci* populations did not mate, they engaged in extensive courtship behavior (De Barro and Hart 2000; Maruthi et al. 2004; Perring and Symmes 2006).

Thus some haplotypes may share essential behaviors in common, while others share only some in common but lack the necessary signals that result in mating between the variants. These findings do not provide any definite answer to whether B and Q biotypes have a pre- or post-zygotic barrier.

A sequence comparison of the DNA sequences of the nuclear and organelle genomes, and of the endosymbiotic bacteria of the major *B. tabaci* biotypes, associated with comparative functional genomics studies, for example, by heterologous hybridizations on a B biotype microarray, may shed some light on the question of *B. tabaci* speciation and of the mechanism of speciation in general.

## Whitefly-Begomovirus Interactions: A Cross Kingdom Model

Some of the most difficult to control and poorly understood pathogens of humans and animals are transmitted by an arthropod vector. The disease cycle of most parasites and pathogens involves general mechanisms such as stimulation of humoral and innate immune systems of the host, in addition to specific responses to the pathogen. Elucidating the pathogenesis host response in human- and other animal-viral interactions (e.g., Avian influenza, AIDS, and Ebola) has been challenging.

Recent advances in elucidating conserved innate immune mechanisms across higher and lower-level eukaryotes have begun to illuminate basic tenants by which organisms sense and respond to foreign invasion. Even so, the study of innate immune systems of mammalian and lower order animals is confounded by humoral responses that mask functions of the innate pathway. Therefore, discerning the two pathways from one another is difficult and makes it necessary to identify and characterize key cellular receptors and the associated pathogen ligands that confer specificity to multiple types of host-pathogen interactions. Disruption of ligand-receptor binding and perturbation of the pathogen life cycle at the weakest points in the process offers lucrative means to abate diseases that undermine humoral immunity (Gillespie et al. 1997; Girardin et al. 2002). Owing to the potential for cross-kingdom conservation, implementing whitefly-begomovirus complexes as simple study systems lacking humoral immunity may reveal conserved biochemical and cellular hallmarks of host-pathogen-vector interfaces.

Independent but converging information suggests that the whiteflies and begomoviruses have interacted over geological times (Czosnek 2007). First, an ancestor of the modern whitefly has been found in ~130 MY old amber in Lebanon (Schlee 1970, Fig. 18.1). Second, geminiviral DNA sequences are known to be present in the genome of tobacco plants, probably owing to illegitimate recombination during *Nicotiana* speciation, about 25 MY ago (Bejarano et al. 1996). Third, the endosymbiotic bacteria that produce the GroEL homologue heat shock protein necessary for the survival of begomoviruses in their insect vector (Morin et al. 1999), have probably been associated with whiteflies for over a million years (Baumann et al. 1993). And fourth, with the drift of continents, whitefly-begomovirus complexes have evolved into geographically separated, co-adapted virus-vector complexes (Bradeen et al. 1997).

It is inevitable that during this long-lasting virus-vector relationship, the virus on the one hand has evolved a conformation that ensures both its survival and efficient transmission by its whitefly vector. On the other hand, the vector has evolved strategies to avoid possible deleterious effects of the virus. Functional genomics will be instrumental in (1) studying the interactions underlying the circulative transmission of begomoviruses within vector and non-vector whitefly species, (2) identifying the cellular determinants involved in transmission, and (3) deciphering the evolutionary history of begomovirus-whitefly complexes.

Begomoviruses are transmitted by *B. tabaci* in a circulative manner. Virus particles ingested through the stylets enter the esophagus and the filter chamber, are transported through the gut into the hemocoel, reach the salivary glands and are finally egested during feeding, about 8–12 h after the beginning of an acquisition access period (Rosell et al. 1995, 1999; Ghanim et al. 2001a). Path and velocity of translocation constitute intrinsic properties of the vector, not of the virus. Once ingested by the whitefly, vector particles cross the gut barrier to the hemolymph and engage a GroEL homologue 60S heat shock protein encoded by a primary endosymbiont (Morin et al. 1999, 2000). "Chaperonin" proteins such as the Hsp60 have been described in aphids, and may serve to stabilize virions during membrane transit and/or to mask antigenic sites on the capsid to thwart host recognition while en route in the hemolymph to the salivary glands (van den Heuvel et al. 1994, Gupta 1995).

Despite the co-adaptation of *B. tabaci* with begomoviruses for which it serves as the exclusive vector (Brown and Idris 2005), circulation of virions in the insect body does not appear to have a neutral outcome. Instead, the circulation of virions in the whitefly vector body has been shown to influence the expression of specific whitefly genes. However, only hints of these interactions have been uncovered to date, suggesting that many more will be discovered using a functional genomics approach. These genes may represent targets against which novel strategies can be devised for blocking the virus-host interaction. Several lines of evidence inspire this rationale: (1) Begomoviruses such as *Tomato yellow leaf curl virus* (TYLCV) and *Tomato mottle virus* (ToMoV) influence whitefly fecundity (McKenzie 2002; Rubinstein and Czosnek 1997). (2) Virions translocate in a circulative manner requiring receptor-mediated translocation (Czosnek et al. 2002). (3) TYLCV, but not ToMoV, produces viral transcripts within the whitefly (Sinisterra et al. 2005). (4) TYLCV induces the synthesis of whitefly antiviral factors (Cohen 1967; Cohen and Marco 1970) and virus-specific transcripts (Sinisterra et al. 2005). (5) The TYLCV coat protein has a nuclear targeting signal allowing it to penetrate insect cell nuclei (Kunik et al. 1997).

Begomoviruses studied thus far, including TYLCV, may be retained in their whitefly vector for several weeks (e.g. *Tomato yellow leaf curl Sardinia virus* – TYLCSV, Caciagli et al. 1995; Jiang et al. 2000) or for the entire life of the vector (e.g. TYLCV and *Tomato yellow leaf curl Chinese virus* – TYLCCNV, Rubinstein and Czosnek 1997; Jiu et al. 2007). The long-term association of begomoviruses with *B. tabaci* has been shown to have consequences on host longevity and fertility. The life span of female whiteflies (A biotype) fed for 24 h on the bipartite *Squash leaf curl virus* (SLCV)-infected cucurbits was on average 25% shorter than

that of whiteflies fed on the same virus source for 4 h only (Cohen et al. 1989). The deleterious effects of the direct association between the whitefly vector and TYLCV was established by comparing longevity and fertility of viruliferous and non-viruliferous insects reared on cotton (a virus non-host plant), following a short exposure to TYLCV-infected tomato plants (Rubinstein and Czosnek 1997). The life span of the viruliferous insects was shorter by 5–7 days compared to that of non-viruliferous whiteflies (out of 28–32 days). A decrease of 25–50% in the number of eggs laid (depending on the age of the adult) by viruliferous whiteflies also was found. Similar observations – the invasive B and local ZHJ1 – showing a decrease in longevity and fertility were made in China with two biotypes fed on tomato plants infected with TYLCCNV (Jiu et al. 2007). Both biotypes were similarly affected by TYLCCNV – 40% reduction in longevity and 35% in the number of eggs. In contrast to TYLCV and TYLCCNV, the bipartite begomovirus ToMoV actually increased egg production of the B biotype vector (McKenzie 2002). Whiteflies that acquired ToMoV produced more eggs than their non-viruliferous counterparts. These observations indicate that some begomoviruses have deleterious effects on the insect host, while others may actually increase fitness.

At least one whitefly species that colonizes some of the same hosts as *B. tabaci*, the greenhouse whitefly *Trialeurodes vaporariorum*, is capable of ingesting, but not transmitting begomoviruses. For this whitefly-virus combination at least, one barrier to transmission has been shown to occur at the gut-hemocoel interface (Czosnek et al. 2002). Transmission would occur if the virus made it to the salivary glands, so it is thought that receptors mediate begomovirus translocation into the salivary glands of *B. tabaci*. However, the specific receptor involved in this translocation across the double membrane of the primary salivary glands has not been identified. Genomics and proteomics approaches are needed to elucidate these important receptors.

The characteristic high degree of specificity in this study system parallels relationships also seen for a number of arthropod vector-pathogens of human, animal, and other plant hosts. The whitefly genome sequence, when determined, will offer a simplified, parallel animal model for studying innate immunity and cellular pathways that are globally significant to arthropod-virus interactions. Virus-whitefly complexes and therefore interactions they reveal are expected to be selectively involved in transmission specificity. The availability of the whitefly genome sequence will make possible surveys of global cross-kingdom interactions that utilize ligand-receptor mediated mechanisms to invoke generalized and specific defenses against invasion. Indeed, begomoviruses are multi-trophic opportunists, employing not only plant-encoded proteins for exploitation of the host for replication, movement, and gene silencing suppression, but also whitefly proteins during their transmission phase.

Exploiting knowledge of cellular mechanisms and molecular interactions in common with the whitefly-begomovirus transmission pathway could also aid in elucidating the basis for virus-vector specificity and defense responses in human-pathogen complexes (Blair et al. 2000). The proposed Whitefly Genome effort will benefit and synergize a global, trans-disciplinary user community.

## The Whitefly Transcriptome

### *Genome Size of the Whitefly,* B. tabaci

Availability of the sequence of the *B. tabaci* nuclear genome, combined with bioinformatics comparisons with sequences from other whitefly biotypes and species, and other insects will provide estimates of (1) the degree of synteny between these insects, (2) gene density in the whitefly genome, (3) the mean number of introns/exons in whitefly genes, as well as characterize the various repetitive DNA families and their mode of dispersion in the genome (Sakharkar and Kangueane 2004).

The whitefly *B. tabaci* has a relatively large genome compared to that of other insects, including the few pests of agricultural crops for which a genome is available (Brown et al. 2005, Table 18.1). It is likely that *B. tabaci* has a number of genes close to those of *Drosophila* and of other sequenced insect genomes, ca. 15,000 (Holt et al. 2002). Therefore much of the whitefly genome may be found to comprise non-coding, sometimes repetitive, DNA. Sequencing the whitefly genome will allow the user community to determine the order of genes in whitefly euchromatin regions. This will allow direct comparisons with that of fully sequenced genomes of other insects, facilitate the first estimations of degrees of synteny between the whitefly and other model insect genomes, and allow assessment of the likelihood that these models can serve as backbones for assembling the whitefly genome. It also will facilitate calculations of gene density in the whitefly genome, differentiate the various repetitive DNA families and note their dispersion in the genome. For example, in human and mouse (3 Mbp, 30,000 genes) a gene is found every 100,000 bp, while a gene is found every 9,000 bp in *Drosophila* (0.14 Mbp, 14,100 genes) and 4,000 bp in *Arabidopsis* (0.11 Mbp, 25,500 genes). Hence it can be estimated that in *B. tabaci*

**Table 18.1** Estimates of the nuclear DNA content (haploid) of selected insects, C-value in pg (1 pg DNA = 980 Mbp)

| Insect species | Nuclear DNA content |
|---|---|
| Honey bee *Apis mellifera* | 0.17 |
| Fruit fly *Drosophila melanogaster* | 0.18 |
| Flour beetle *Tribolium castaneum* | 0.21 |
| Monarch butterfly *Danaus plexippus* | 0.29 |
| Pea aphid *Acyrthosiphon pisum* | 0.31 |
| Green peach aphid *Myzus persicae* | 0.32 |
| Tobacco budworm moth *Heliothis virescens* | 0.41 |
| Wax moth *Galleria melonella* | 0.50 |
| Silkworm moth *Bombyx mori* | 0.52 |
| Chinese oak silkmoth *Antheraea pernyi* | 1.00 |
| Gypsy moth *Lymantria dispar* | 1.03 |
| Whitefly *Bemisia tabaci* | 1.04 |
| Geometrid moth *Euchlaena irraria* | 1.90 |

the gene density is 1/60,000 bp; this estimation is an average since in these organisms the genes are usually clustered and the repetitive elements are mostly found in the heterochromatin near the centromeres and telomeres. In *Drosophila*, about 15% of the genome is made up of transposons, and more than 30% is satellite DNA, mostly on one chromosome (Dimitri et al. 2005). Finally the genome sequence will allow us to determine the size and the mean number of introns/exons in whitefly genes; for example, in *Drosophila*, there are 2,470 intronless genes. On average, there are about 2.5 introns per gene, compared with 4 in humans, with an average size of ~230 and ~630 bp for exons and introns, respectively (Strachan and Read 2004).

## *Activation and Repression of* B. tabaci *Stress-Response Gene from Insecticide Application, Parasitism by Natural Enemies and High Temperature*

Whiteflies are continuously exposed to biotic (e.g., natural enemies, host plant chemicals, insecticides) and abiotic (climate) stresses. The genes involved in responses to stress are hypothesized to be shared across diverse members of the Animal Kingdom, among the best studied being heat shock proteins (Salvucci et al. 2000). It is likely that stress induced gene activation and repression during the *B. tabaci* parasitization by *E. mundus* is common to other biotic and abiotic stresses. These genes could be targeted once their sequence is known, and their activity could be modulated by gene silencing using siRNA-producing host plants (see below).

### Insecticide Application

The heavy application of insecticides is responsible for the development of resistance (Denholm et al. 1998). Populations of *B. tabaci* Q biotype resistant to pyriproxyfen recently emerged in Israel, displacing the endogenous, less resistant, B biotype (Horowitz et al. 2005). Pyriproxyfen is an insecticide that acts as a juvenile hormone (JH) analogue and disrupts insect development. Gene expression in pyriproxyfen resistant *B. tabaci* Q biotype was studied (Ghanim and Kontsedalov 2007). Upon insecticide treatment of resistant whiteflies, many genes involved in detoxification and oxidative stress were up-regulated.

### Parasitism by Natural Enemies

It is likely that stress induced gene activation and repression during parasitization of *B. tabaci* by natural enemies is common to other biotic and abiotic stresses. Although the whitefly is usually controlled using chemical pesticides, biological control agents constitute an important component in integrated pest management programs (see Arnó et al. Chapter 15; Stansly and Natwick Chapter 17). One of

these agents is the aphelinid wasp *Eretmocerus mundus* (Mercet). *E. mundus* lays its egg on the leaf underneath the *B. tabaci* nymph. The egg hatches and the first instar wasp larva penetrates the host. Initiation of parasitization induces the host to form a cellular capsule around the parasitoid. Around this capsule, epidermal cells multiply and thick layers of cuticle are deposited (Gerling et al. 1990; Gelman et al. 2005). The physiological and molecular processes underlying *B. tabaci-E. mundus* interactions have been investigated using the spotted whitefly cDNA microarray (see below) at two time points of the parasitization process: when the parasitoid larva is at the pre-penetration stage and when it fully penetrates the host (Mahadav et al. 2008). The results clearly indicated that genes known to be part of the defense pathways described in other insects and animals (Barton and Medzhitov 2003) are also involved in the response of *B. tabaci* to parasitization by *E. mundus*. Some of the responses observed included the repression of a serine protease inhibitor (serpin) and the induction of a melanization cascade (Fig. 18.4). A second set of genes that strongly responded to parasitization included bacterial genes encoded by whitefly symbionts. Proliferation of *Rickettsia,* a facultative secondary symbiont, was strongly induced following the initiation of the parasitization process, suggesting that endosymbionts may be involved in the insect host resistance to various environmental stresses.

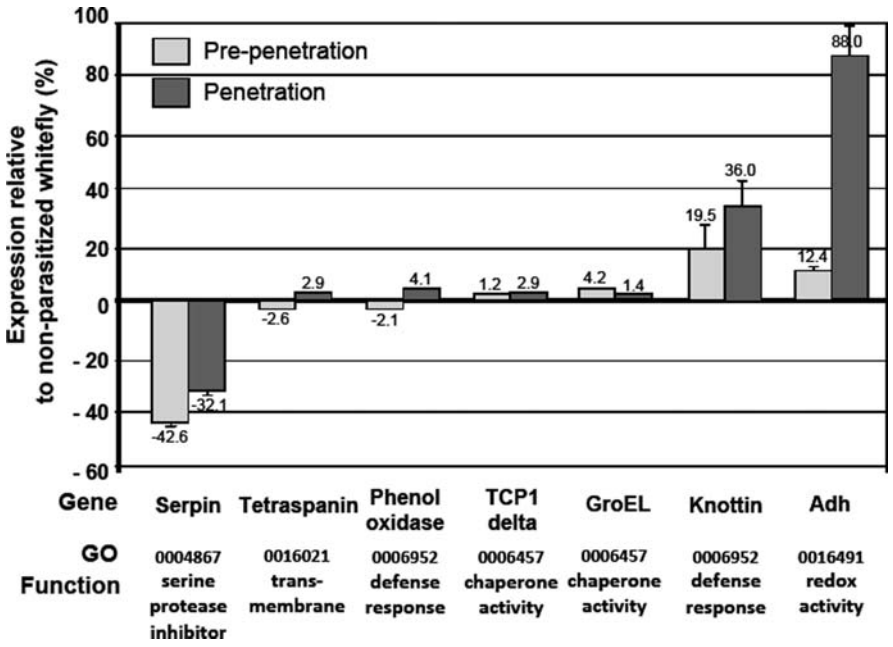



**Fig. 18.4** Quantitative real-time RT-PCR verification of candidate genes induced and repressed following the microarray analyses

### High Temperatures

Gene expression of *B. tabaci* B and Q biotypes was compared at normal (25°C) and high (40°C) temperatures (Mahadav et al. 2009). Gene expression under normal temperature showed clear differences between the two biotypes: At high temperature, B exhibited higher expression of mitochondrial genes, and lower cytoskeleton, heat-shock, and stress-related genes compared to Q. Exposing B biotype whiteflies to heat stress was accompanied by rapid alteration of gene expression.

## *Knowledge Base and Available Tools*

### Colonies of *B. tabaci*

A number of whitefly *B. tabaci* biotypes (A, B, MS, Q, ZHJ1, 2, WAN, others) are maintained in colonies, and/or are available as archived collections in various laboratories in Africa, the Americas, Asia, Australia, Europe, and the Middle East. It was agreed to by the Consortium that the B biotype would be the preferred starting point for the first genome sequence, owing to its widespread distribution, invasiveness, and plethora of available biological information.

### Nuclear Genome Size of *B. tabaci*

The nuclear DNA content of *B. tabaci* B biotype was estimated using flow cytometry. The DNA content males and females was 1.04 and 2.06 pg, respectively (Brown et al. 2005). Results corroborate prior reports based on chromosome counts, indicating that *B. tabaci* males are 1N, and females are 2N. The conversion between DNA content and genome size (1 pg DNA = 980 Mb) indicated that the haploid genome of *B. tabaci* is 1,020 Mb, or about five times that of *D. melanogaster* [Diptera] (Table 18.1). Results provide an important baseline that will facilitate whitefly genome research.

### Expressed Sequence Tags

To address the general shortage of genomic sequence information, three cDNA libraries have been constructed: one from non-viruliferous whiteflies (eggs, immature instars, and adults) and two from adult insects that fed on tomato plants infected by two geminiviruses: TYLCV and ToMoV (Leshkowitz et al. 2006). In total, a sequence of 18,976 clones was determined. After quality control and removal of clones of mitochondrial origin, 9,110 sequences remained, which included 3,843 singletons and 1,017 contigs. The number of sequences from the libraries that were assembled into contigs and singletons were: eggs (201), instars (1,816), non-viruliferous adults (2,093), TYLCV-viruliferous adults (2,704), and ToMoV viruliferous adults (2,296).

In addition, approximately 1,000 bases aligned with the genome of the *B. tabaci* endosymbiotic bacterium *Candidatus* Portiera aleyrodidarum, originating primarily from the egg and nymphal instar libraries. Genes were identified representing important biological processes, including membrane transport, sub-cellular trafficking, protein translation, innate immunity, development and growth, and abiotic and biotic stresses.

The sequences have been posted in GenBank. They can be found in the site of the "Whitefly Genome Project": URL http://www.ncbi.nlm.nih.gov/sites/entrez?Db=genomeprj& cmd=ShowDetailView&TermToSearch=18077 (Fig. 18.5). The accession numbers are as follows: from egg library EE602518 to 602718, from nymphal instar library EE602719 to EE604534, from non-viruliferous adults EE595518 to EE597607, from ToMoV viruliferous adults EE597608 to EE599906, and from TYLCV viruliferous adults EE599905 to EE602517. More information on these sequences can be found in three files:

1. Sequences and the contigs they assembled:
   www.biomedcentral.com/content/supplementary/1471-2164-7-79-S1.xl
2. Contigs and singletons information (names, library count, length, etc.):
   www.biomedcentral.com/content/supplementary/1471-2164-7-79-S2.xl
3. Top BLAST hit for each contig and singleton against the databases searched:
   www.biomedcentral.com/content/supplementary/1471-2164-7-79-S3.xl

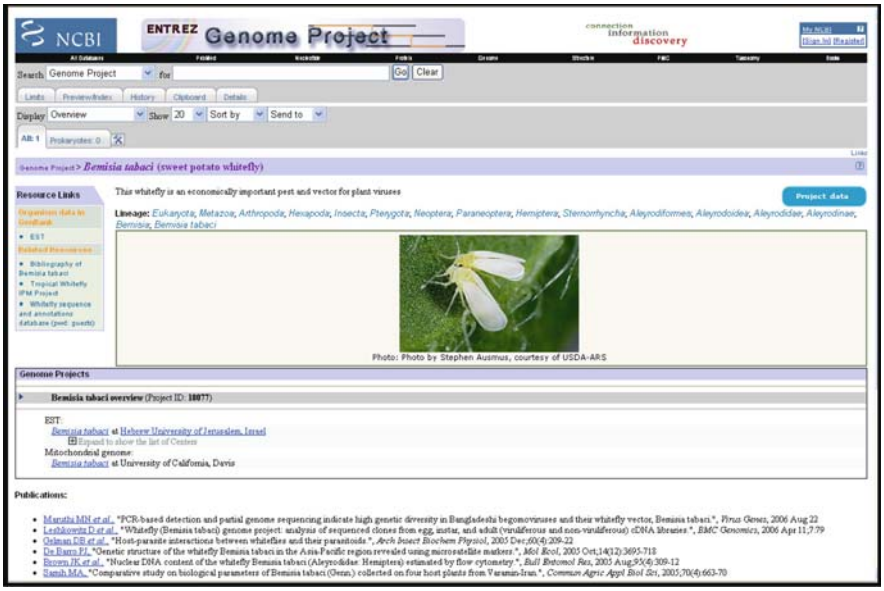



**Fig. 18.5** Home page of *The Whitefly Genome Project* at NCBI. URL: http://www.ncbi.nlm.nih.gov/sites/entrez?Db=genomeprj&cmd=ShowDetailView&TermToSearch=18077

Recently normalized adult and gut EST libraries have been constructed for the B biotype and 454 sequencing (454 Life Sciences, Branford CN) is presently underway. These new data are expected to substantially enrich the EST resources by providing the first near-complete transcriptome for *B. tabaci* (Brown et al. unpublished data). This partial transcriptome will serve as a useful tool for whole genome annotation. Thus, we anticipate that these past and present projects will lead to the full genome sequencing of the first tropical homopteran.

## Spotted cDNA Microarrays – Functional Genomics

Based on the sequence of the ESTs (see above), a first microarray has been designed that contains 6,000 entries and includes all contigs and singletons (Fig. 18.6). This microarray has proven to be an excellent tool to study gene expression during whitefly development, circulative transmission of TYLCV, comparison between *B. tabaci* biotypes, and parasitization by natural enemies (Mahadav et al. 2008, 2009).

Additional investigations will lead to an improved understanding of virus/vector relationships by identifying all proteins that are involved in and essential to *B. tabaci*-mediated virus transmission. Identifying key salivary gland and gut proteins will position us for future studies of functionally relevant proteins, namely cloning, in situ localization, over-expression, antibody production, biochemical analysis to determine function, and protein-protein interactions (yeast-two hybrid, pull-down and other functional analyses). The complete *B. tabaci* genome sequence will provide an essential tool for this effort. Knowledge of the conserved cellular and molecular phenomena that underlie *B. tabaci*-mediated virus transmission will offer promising approaches for the disruption of specific targets at critical and vulnerable points in the pathway (Cicero et al. 1995; Brown and Czosnek 2002; Ghanim et al. 2001a, b; Hunter et al. 1998; Rosell et al. 1999; 2003).

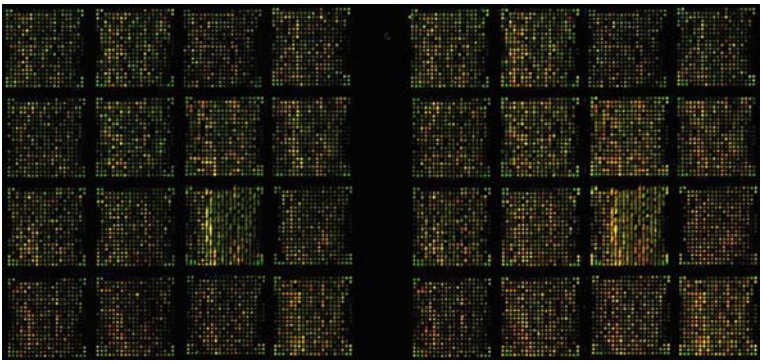


**Fig. 18.6** Spotted cDNA representing 6,000 clones from the whitefly *B. tabaci* B biotype. The figure shows hybridization of transcripts from the B and Q biotype, using the B biotype array

### Gene Silencing

The function of *B. tabaci* genes can be determined by a reverse genetics approach. Introduction of double-stranded RNA (dsRNA) into living cells or organisms can cause silencing of specific genes and disruption of protein expression, as first demonstrated in the nematode *Caenorhabditis elegans* (Fire et al. 1998). In insects, significant reduction in gene and protein expression was demonstrated in the fruitfly *Drosophila melanogaster*, the mosquito *Anopheles gambiae*, the caterpillar *Manduca sexta*, the milkweed bug *Oncopeltus fasciatus*, the cockroach *Periplaneta Americana*, the triatomine bug *Rhodnius prolixus*, the light apple brown moth *Epiphyas postvittana*, the ladybird beetle *Harmonia axyridis* and the pea aphid *Acyrthosiphon pisum* (see references in Ghanim et al. 2007a). Gene silencing was achieved in *B. tabaci* following injection of dsRNA between the mesothorax and the metathorax of newly emerged adults. The dsRNA molecules were based on exonic sequences derived from genes specifically expressed in *B. tabaci* organs. The treatment resulted in specific and significant decrease in gene expression. Furthermore, injection of dsRNA targeting the *Drosophila* chickadee gene (which encodes a homolog of profilin, a small actin binding protein) caused severe disruption of normal *B. tabaci* oocyte development, suggesting a similar function in the two insects.

These experiments indicated that it is possible to silence *B. tabaci* genes by microinjection of dsRNA. It might be possible to achieve gene silencing by expressing the siRNA in host plants on which whiteflies are feeding, as shown for several other insects.

## *Top Candidate Biotypes for Genome-Transcriptome Sequencing*

### B Biotype

The worldwide introduction of the B biotype was the single most significant impetus behind the recent molecular phylogenetic and population genetics studies carried out to investigate the extent of variation for this little known "cryptic species." The B biotype is the most aggressive *B. tabaci* to date and represents extreme polyphagy, is widely distributed, and the best known as an important pest and virus vector. Additionally, it is the only widespread *B. tabaci* biotype to cause physiological disorders such as squash silverleaf and tomato irregular ripening. The B biotype has all of the hallmarks of an invasive species; propensity for developing insecticide resistance, high fecundity, and capacity to disperse long distance.

### A Biotype

During the 1980s the polyphagous A biotype reached extreme pest status in cotton and vegetable growing areas in the Southwestern USA deserts, and was responsible for the first outbreaks of begomovirus and crinivirus-incited diseases in vegetable crops in the region. The A type shares many of the same traits with B type but

exhibits lower fecundity, more moderate polyphagy with a distinct host range, is not resistant to the same classes of insecticides, originated in the New World, and does not disperse long-distance. The A and B biotypes have identical primary symbionts (Zchori-Fein and Brown 2002). However, the A biotype is infected by *Ca*. Cardinium, a putative cytoplasmic incompatibility-inducer, whereas, the B biotype is not (Caballero et al. 2001). In terms of other endosymbionts, the A and B biotypes each harbor a distinct suite of secondary symbionts (Zchori-Fein and Brown 2002) and so these additional genomes will be made available if de novo methods are used.

### Q Biotype

The Q-biotype is thought to have originated from the Mediterranean region, and has been associated with insecticide resistance there. It exhibits resistance to pyriproxyfen (Horowitz et al. 2003) and buprofezin, and reduced susceptibility to the neonicotinoid insecticides imidicloprid, acetamiprid and thiamethoxam. While the B biotype is defined by high fecundity, aggressiveness and wide host range, the Q biotype is known to develop greater resistance to insecticides (Horowitz et al. 2003; 2005). It also harbors *Ca*. P. aleyrodidarum, the obligatory primary symbiotic bacterium of whiteflies, as well as several secondary symbionts including *Rickettsia, Hamiltonella, Wolbachia, Arsenophonus, Cardinium* and *Fritschea* (Baumann 2005; Gottlieb et al. 2006). The relative abundance of secondary symbionts was recently determined in populations of *B. tabaci* from Israel (Cheil et al. 2007). *Hamiltonella* was detected only in the B biotype, while *Wolbachia* and *Arsenophonus* were found in the Q biotype. *Rickettsia* was abundant in both biotypes, while *Cardinium* and *Fritschea* were not found in any of the populations. The association found between whitefly biotypes and secondary symbionts suggests a possible contribution of these bacteria to host characteristics such as insecticide resistance, and virus transmission ability (Cheil et al. 2007). In Spain, the Q biotype transmits TYLCV more efficiently than the B biotype does (Sanchez-Campos et al. 1999; Jiang et al. 2004).

### Others

Additional viable genomes to target and that would permit a succinct comparison of monophagy with polyphagy and contrast invasiveness with more benign or less invasive behaviors that are known among a growing number of biological types. Such candidates include the monophagous Ug2, the invasive Cassava mosaic disease vector associated with the CMD pandemic threatening eastern Africa, and which may be monophagous to cassava, the monophagous Benin (ASA) biotype of *Asystasia* spp., the MS (non-B) relative of the B biotype (different sister clades), native to the Reunion Islands, the prospectively monophaghous T biotype from Italy/Mediterranean, the S biotype that occurs in Spain and west Africa, the EAN or WAN from Australia, ZHJ1 or ZHJ2 from China, and endemic populations to South America and the Caribbean region, among others. In addition the sweet potato-associated *B. tabaci* in Uganda is an interesting candidate. It is polyphagous but

does not colonize cassava (J. Legg personal communication) and may possibly be a member of the *B. tabaci* species complex instead of the *B. tabaci* sibling species group (see Gill and Brown Chapter 1).

## *Available Annotation Tools*

### Model Insects

The haploid genome of *B. tabaci* is estimated to be approximately 1,020 Mbp, or 5 times the size of the *D. melanogaster* genome. The closest relatives to *B. tabaci* for which genome sequences are being worked out are the, pea aphid (furthest advanced), the green peach aphid, and a cereal aphid (in progress), which are hompterans (genome size range for Insecta = 0.18–0.89 pg), but represent a different family (Aphididae vs Aleyrodidae) and regional origin (temporate vs. tropical). These aphid genomes together with *Drosophila, Anopheles*, and others will aid significantly in the bioinformatic annotation.

### *B. tabaci* Transcriptome

Additional resources available or under construction for investigating the functional genomics of *B. tabaci* and also as a scaffold for annotation of the genome include:

1. EST libraries (contigs available presently number >10,000) generated by The Hebrew University of Jerusalem, The Volcani Center, USDA-ARS, Florida, and The University of Arizona (supported by funding from The United States-Israel Binational Agricultural Research and Development and the Israel Science Foundation).
2. The transcriptome (subtracted EST library) of the B biotype using 454 sequencing, underway at The University of Arizona (supported by funding from The United States-Israel Binational Agricultural Research and Development, and The University of Arizona ARL Biotechnology Core Facility http://biotech.arl.arizona.edu/index.php/core-facilities.html).
3. Microarray resources developed by The Hebrew University and The Volcani Center in Israel that are presently being employed toward management of the whitefly as an important agricultural pest.

## *Sequencing and* De Novo *Assembly of Whitefly Endosymbiont Genomes – The Microbiome*

These undertakings will provide new information concerning all three symbiont genomes – potential interactions and biological significance – for which no genome information is available, including the first *Ca.* Cardinium sequence. Unraveling genetic/genomic relationships between the endosymbiont genes/genomes will allow

elucidatation of putative whitefly-endosymbiont genome interactions that are hypothesized to drive whitefly biotype formation, influence phenotypic plasticity, and select for invasiveness, monophagy, and begomovirus specificity, among others.

The smallest bacterial genome reported thus far comes from *Mycoplasma genitalium*, an intracellular parasite of human epithelial cells, and comprises a circular chromosome of 580 kb with only 470 coding genes. *Ca*. Buchnera aphidicola is the primary endosymbiont of the aphid, *A. pisum*. The genome size is extremely reduced (one circular chromosome of 641 kb, with only 564 coding genes and two plasmids), compared to its free-living counterparts, and the genome of the primary whitefly *B. tabaci* endosymbiont *Ca*. P. aleyrodidarum is similar in size (Thao and Baumann 2004; Pérez-Brocal et al. 2006).

The genome sizes of the cytoplasmic incompatibility-inducing bacteria *Wolbachia* and *Cardinium*, vary depending on the host and bacterium. *Wolbachia* is closely related to other *Rickettsia* and so is expected to be small and within a manageable range. Only several *Ca*. Cardinium spp. are known and promise to be unique, in that they are divergent from *Wolbachia* (16S rRNA gene) but possibly have evolved a convergent cytoplasmic incompatibility mechanism (or have acquired genes similar to *Wolbachia* spp. by lateral transfer), both intriguing possibilities.

## Synergy – Other Genome Projects and User Communities

### *The User Community is Global*

A particularly exciting aspect of this work is the opportunity to expand the reach of interrelated genomics projects for arthropods, and most specifically other insects, that may shed new light on all of the organisms, owing to a comparative approach between closely and distantly related species. Eventually, the combination of tools and informatics developed by collective members of the Whitefly Genome Consortium can be extended to other clade-specific arthropod genome projects. Thus, an important synergy has been already formed with the establishment of the *Whitefly Genome Consortium*, which consists of a large number of members from over 12 countries with an enthusiastic interest in this undertaking and its future goals.

Letters and/or email messages in support of this *Whitefly Genome Project* have been received from many individuals, already committed members of the Whitefly Consortium, and other interested members of a broad user community.

- In total, over 48 supporting communications were received from industry (Syngenta, Dupont, Bayer Crop Science, HM-Vilmorin and Seminis-Monsanto vegetable seed, KeyGene, Cotton Incorporated, Arizona Cotton Growers).
- A broad representation among researchers, systematists, virologists, entomologists, producers, and industry partners, indicated enthusiastic support, and

included individuals from Australia, Brazil, France, Germany, Japan, China, Israel, Egypt, South Africa, Morocco, Taiwan, Uganda, Spain, Mexico, United Kingdom, and the United States (represented by members from Arizona, California, Florida, and Texas).

### *Insect Genome Projects*

- http://www.ncbi.nlm.nih.gov/sites/entrez?cmd=Search&db=genomeprj&term="Hexapoda"%5BOrganism%5D: Examples of genomes and/or transcriptomes (among 51 current entries) in progress or completed include: *Aedes aegypti, A. albopictus, Anopheles gambiae str. PEST, Apis mellifera, B. tabaci, Bombyx mori, Drosophila melanogaster, D. persimilis, D. pseudoobscura, D. sechellia, D. simulans, D. yakuba, Helicoverpa armigera*, *Toxoptera citricida* (brown citrus aphid), *Homalodisca vitripennis* (glassy-winged sharpshooter), *A. pisum* (pea aphid), *Rhodnius prolixus* (vector of Chagas' disease), *Nasonia vitripennis*, *N. giraulti, N. longicornis*, *Tribolium castaneum* (red flour beetle).

### *Some Relevant Websites*

- European Whitefly Studies Network http://ec.europa.eu/research/agro/fair/en/uk4303.html
- Global Invasive Species Database http://www.issg.org/database/welcome/
- Whitefly Taxonomy and Ecology http://www.sel.barc.usda.gov/whitefly/wfframe.htm
- Whitefly Knowledgebase-USDA & University of Florida http://entnemdept.ufl.edu/fasulo/whiteflies/wfly0002.htm
- Whitefly IPM Project http://www.tropicalwhiteflyipmproject.cgiar.org/project-results.jsp;
- Crop Loss-Cotton http://www.entomology.msstate.edu/resources/tips/cotton-losses/data/
- Tropical whiteflies http://www.spipm.cgiar.org/;
- Whiteflies in the News http://www.ars.usda.gov/is/AR/archive/jul98/soft0798.htm
- *Bemisia* Global Literature Database http://www.ars.usda.gov/Services/docs.htm?docid=10916;
- Whitefly Management http://www.ipm.ucdavis.edu/PMG/PESTNOTES/pn7401.html;
- Comparative Toxicogenomics Database http://ctd.mdibl.org/voc.go;

## Conclusions

Collectively, genomics, proteomics and functional genomics efforts will initiate further local, regional, national and international partners to expand present and future efforts aimed at determining the *B. tabaci* genome and proceed to undertake

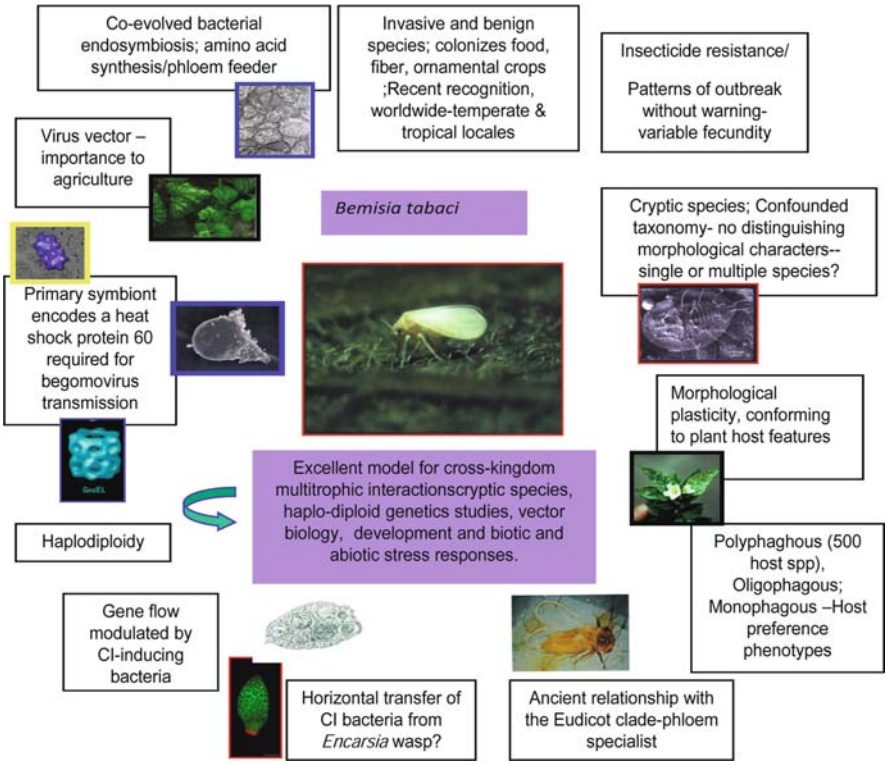



**Fig. 18.7** *Bemisia tabaci* is an ancient tropical haplodiploid, homopteran whose importance as a novel model arthropod has become recognized. *B. tabaci* is a cryptic sibling species group having a confounded taxonomic status owing to the absence of morphological characters that permit differentiation between behaviorally distinct haplotypes. It is a phloem-feeder, and an important vector of emerging plant virus groups. It harbors varied endosymbionts, which may modulate gene flow and perhaps speciation. Damage attributed to *B. tabaci* exceeds 5 billion dollars per year globally (Courtesy, J.K. Brown)

functional genomics aspects that are of high interest amongst a broad user community, but for which sufficient tools are yet unavailable or inaccessible. This will truly be a case where homopterans, as a diverse group of organisms, can be exploited to extend both fundamental and application-based knowledge (Fig. 18.7). The exciting possibility that commercial, academic, and outreach efforts can benefit from one another is an excellent example of how internationally conducted genomics projects are able to cross-feed and create more powerful resources than any one project could accomplish on its own.

**Acknowledgement** The *2006 "White Paper" Committee* **:** Scott Atkins, Judith K. Brown (Co-Chair), Laura Boykin, Henryk Czosnek (Chair), Paul De Barro, Don Frohlich, Murad Ghanim, Margarita Hadjistylli, Abdel Hanafi, Moshe Lapidot, Cindy McKenzie, Shai Morin, Jane Polston, Rosemarie Rosell, Robert Shatters (Co-Chair), Xiomara Sinisterra, and William Wintermantel. Supported by The United States-Israel Binational Agricultural Research and Development (awards IS-3479-03 and IS-4062-07), The Israel Science Foundation (award 884/07) and by The University of Arizona ARL Biotechnology Core Facility.

## Literature Cited

Barton GM, Medzhitov R. 2003. Toll-like receptor signaling pathways. *Science* 300:1524–1525.

Baumann P. 2005. Biology of bacteriocyte-associated endosymbionts of plant sap-sucking insects. *Annu. Rev. Microbiol.* 59:155–189.

Baumann P, Munson MA, Lai C-Y, Clark MA, Baumann L, Moran NA, Campbell BC. 1993. Origin and properties of bacterial endosymbionts of aphids, whiteflies, and mealybugs. *ASM News* 5:21–24.

Bejarano ER, Khashoggi A, Witty M, Lichtenstein CP. 1996. Integration of multiple repeats of geminiviral DNA into the nuclear genome of tobacco during evolution. *Proc. Natl. Acad. Sci. USA* 93:759–764.

Bedford ID, Briddon RW, Brown JK, Rosell RC, Markham PG. 1994. Geminivirus transmision and biological characterisation of *Bemisia tabaci* (Gennadius) biotypes from different geographic regions. *Ann. Appl. Biol.* 125:311–325.

Berry S, Rey MEC, Rogan D, Fondong VN, Fauquet CM, Brown JK. 2004. Molecular evidence for distinct *Bemisia tabaci* geographically genotypes from cassava in Africa. *Ann. Entomol. Soc. Am.* 97:852–859.

Bird J. 1957. A whitefly-transmitted mosaic of *Jatropha gossypifolia*. *Technical Paper, Agricultural Experimental Station of Puerto Rico* 22:1–35.

Blackman RL, Cahill M. 1998. The karyotype of *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Bull. Entomol. Res.* 88:213–215.

Blair CD, Adleman ZN, Olsen KE. 2000. Molecular strategies for interrupting arthropod-borne virus transmission by mosquitoes. *Clin. Microbiol. Rev.* 13:651–661.

Bradeen JM, Timmermans MCP, Messing J. 1997. Dynamic genome organization and gene evolution by positive selection in geminiviruses (*Geminiviridae*). *Mol. Biol. Evol.* 14:1114–1124.

Brown JK. 2007. The *Bemisia tabaci* complex: genetic and phenotypic variation and relevance to TYLCV-vector interactions. In *The Tomato Yellow Leaf Curl Virus Disease: Management, Molecular Biology and Breeding for Resistance*, ed. H Czosnek, pp. 25–56. The Netherlands: Springer.

Brown JK, Frohlich DR, Rosell RC. 1995. The sweetpotato/silverleaf whiteflies: biotypes of *Bemisia tabaci* (Genn.), or a species complex? *Annu. Rev. Entomol.* 40:511–534.

Brown JK, Idris AM. 2005. Genetic differentiation of the whitefly *Bemisia tabaci* (Genn.) mitochondrial COI and geographic congruence with the coat protein of the plant virus genus: *Begomovirus*. *Ann. Entomol. Soc. Am.* 98:827–837.

Brown JK. 2000. Molecular markers for the identification and global tracking of whitefly vector-begomovirus complexes. *Virus Res.* 71:233–260.

Brown JK. 2001. The molecular epidemiology of begomoviruses. In *Trends in Plant Virology*, ed. JA Khan, J Dykstra, pp. 279–316. New York: The Haworth Press, Inc.

Brown JK, Czosnek H. 2002. Whitefly transmitted viruses. In *Advances in Botanical Research*, ed. RT Plumb, Vol. 36, pp. 65–100. New York: Academic Press.

Brown JK, Lambert GM, Ghanim M, Czosnek H, Galbraith DW. 2005. Nuclear DNA content of the whitefly *Bemisia tabaci* (Genn.) (Aleyrodidae: Homoptera/Hemiptera) estimated by flow cytometry. *Bull. Entomol. Res.* 95:309–312.

Buchner P. 1965. *Endosymbiosis of Animals with Plant Microorganisms*. New York: John Wiley and Sons.

Burban C, Fishpool LDC, Fauquet C, Fargette D, Thouvenel J-C. 1992. Host-associated biotypes within West African populations of the whitefly *Bemisia tabaci* (Genn.), Homoptera: Aleyrodidae. *J. Appl. Entomol.* 113:416–423.

Butlin R. 1995. Genetic variation in mating signals and responses. In *Speciation and the Recognition Concept, Theory and Application*, ed. DM Lambert, HG Spencer, pp. 327–366. Baltimore, MD: The Johns Hopkins University Press.

Byrne DN, Bellows TS, Jr, Parrella MP. 1990. Whiteflies in agricultural systems. In *Whiteflies: their Bionomics, Pest Status, and Management*, ed. D Gerling, pp. 227–261. Andover, Hants, UK: Intercept Ltd.

Byrne FJ, Cahill M, Denholm I, Devonshire AL. 1995. Biochemical identification of interbreeding between B-type and non B-type strains of the tobacco whitefly *Bemisia tabaci*. *Biochem. Genet.* 33:1–23.

Caballero R, Brown JK. 2008. Positive evidence for interbreeding and differential gene flow between three well characterized biotypes of the *Bemisia tabaci* complex (Gennadius) (Hemiptera: Aleyrodidae) excludes geographic and host barriers as isolating factors. *Fourth International Bemisia WorkshopInternational Whitefly Genomics Workshop* Duck Key, Florida, USA ed. PA Stansly, CL McKenzie. *J. Insect Sci. 8*:(4), http://www.insectscience.org/8.04/ref/abstract16.html

Caballero R, Torres-Jerez I, Brown JK. 2001. Two distinct *Wolbachia* identified in *Bemisia tabaci* (Genn.) are associated with uni-directional cytoplasmic incompatibility in infected and uninfected biotypes. In *Proceedings of The 6th International Whitefly and Geminivirus Workshop*, Ragusa, Sicily, February 27–March 2, 2001.

Caciagli P, Bosco D, Al-Bitar L. 1995. Relationships of the Sardinian isolate of tomato yellow leaf curl geminivirus with its whitefly vector *Bemisia tabaci* Gen. *Eur. J. Plant Pathol.* 101:163–170.

Cahill M, Byrne FJ, German K, Denholm I, Devonshire AL. 1995. Pyrethroid and organophosphate resistance in the tobacco whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae). *Bull. Entomol. Res.* 85:181–187.

Campbell BC. 1993. Congruent evolution between whiteflies (Homoptera: Aleyrodidaw) and their bacterial endosymbionts based on respective 18S and 16S rDNAs. *Curr. Microbiol.* 26:129–132.

Campbell BC, Steffen-Campbell JD, Gill RJ. 1996. Origin and radiation of whiteflies: an initial molecular phylogenetic assessment. In Bemisia *1995: Taxonomy, Biology, Damage, Control, and Management*, ed. D Gerling, RT Mayer, pp. 29–51. Andover, Hants, UK: Intercept Ltd.

Cheil E, Gottlieb Y, Zchori-Fein E, Mozes-Daube N, Katzir N, Inbar M, Ghanim M. 2007. Biotype-dependent secondary symbiont communities in sympatric populations of *Bemisia tabaci*. *Bull. Entomol. Res.* 97:1–7.

Cicero JM, Hiebert E, Webb SE. 1995. The alimentary canal of *Bemisia tabaci* and *Trialeurodes abutilonea* (Homoptera, Sternorrhyncha): histology, ultrastructure and correlations to function. *Zoomorphology* 115:31–39.

Clark MA, Baumann L, Munson MA, Baumann P, Campbell BC, Duffus JE, Osborne LS, Moran NA. 1992. The eubacterial endosymbionts of whiteflies (Hemiptera: Aleyrodidae) constitute a lineage separate from the endosymbionts of aphids and mealybugs. *Curr. Microbiol.* 25:119–123.

Cock MJW. 1993. Bemisia tabaci, *An Update 1986–1992 on the Cotton Whitefly with an Annotated Bibliography*, 78pp. Silwood Park, UK: CAB IIBC.

Cohen S. 1967. The occurrence in the body of *Bemisia tabaci* of a factor apparently related to the phenomenon of "periodic acquisition" of tomato yellow leaf curl virus. *Virology* 31:180–183.

Cohen S, Duffus JE, Liu HY. 1989. Acquisition, interference, and retention of cucurbit leaf curl viruses in whiteflies. *Phytopathology* 79:109–113.

Cohen S, Kern J, Harpaz I, Ben-Joseph R. 1988. Epidemiological studies of the tomato yellow leaf curl virus (TYLCV) in the Jordan Valley, Israel. *Phytoparasitica* 16:259–270.

Cohen S, Marco S. 1970. Periodic occurrence of an anti-TMV factor in the body of whiteflies carrying the tomato yellow leaf curl virus (TYLCV). *Virology* 40:363–368.

Costa AS, Russell LM. 1975. Failure of *Bemisia tabaci* to breed on cassava plants in Brazil (Homoptera: Aleyrodidae). *Cienc. Cult.* 27:388–390.

Costa HS, Westcot DM, Ullman DE, Rosell RC, Brown JK, Johnson MW. 1995. Morphological variation in *Bemisia* endosymbionts. *Protoplasma* 189:194–202.

Costa HS, Brown JK. 1991. Variation in biological characteristics and in esterase patterns among populations of *Bemisia tabaci* (Genn.) and the association of one population with silverleaf symptom development. *Entomol. Exp. Appl.* 61:211–219.

Costa HS, Brown JK, Sivasupramanium S, Bird J. 1993. Regional distribution, insecticide resistance and reciprocal crosses between the A and type B of *Bemisia tabaci*. *Insect Sci. Appl.* 14:255–266.

Czosnek H. 2007. Interactions of Tomato yellow leaf curl virus with its insect vector. In *The Tomato Yellow Leaf Curl Virus Disease: Management, Molecular Biology And Breeding For Resistance*, ed. H Czosnek, pp 157–170. The Netherlands: Springer.

Czosnek H, Ghanim M, Ghanim M. 2002. Circulative pathway of begomoviruses in the whitefly vector *Bemisia tabaci* – insights from studies with *Tomato yellow leaf curl virus*. *Ann. Appl. Biol.* 140:215–231.

De Barro PJ. 2005. Genetic structure of the whitefly *Bemisia tabaci* in the Asia-Pacific region revealed using microsatellite markers. *Mol. Ecol.* 14:3695–3718.

De Barro PJ, Hart PJ. 2000. Mating interactions between two biotypes of the whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Australia. *Bull. Entomol. Res.* 90:103–112.

De Barro PJ, Driver F, Trueman JWH, Curran J. 2000. Phylogenetic relationships of world populations of *Bemisia tabaci* (Gennadius) using ribosomal ITS1. *Mol. Phylogenet. Evol.* 16:29–36.

Denholm I, Cahill M, Dennehy TJ, Horowitz AR. 1998. Challenges with managing insecticide resistance in agricultural pests, exemplified by the whitefly *Bemisia tabaci*. *Phil. Trans. R. Soc. Lond. B* 353:1757–1767.

DeRisi JL, Kyer VR, Brown PO. 1997. Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* 278:680–686.

Dimitri P, Corradini N, Rossi F, Mei E, Zhimulev IF, Vernì F. 2005. Transposable elements as artisans of the heterochromatic genome in *Drosophila melanogaster Cytogenet*. *Genome Res.* 110:165–172.

Dobson SL, Bourtzis K, Braig HR, Jones BF, Zhou W, Rousset F, O'Neill SL. 1999. *Wolbachia* infections are distributed throughout insect somatic and germ line tissues. *Insect Biochem. Mol. Biol.* 29:153–160.

Douglas AE. 1998. Nutritional interactions in insect-microbial symbioses: aphids and their symbiotic bacteria *Buchnera*. *Annu. Rev. Entomol.* 43:17–37.

Eisen MB, Spellman PT, Brown PO, Botstein D. 1998. Cluster analysis and display of genome-wide expression patterns. *Proc. Natl. Acad. Sci. USA* 95:14863–14868.

Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. 1998. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* 391: 806–811.

Frohlich DR, Brown JK, Bedford I, Markham P. 1996. Mitochondrial 16S ribosomal subunit as a molecular marker in *Bemisia*, and implications for population variability. In Bemisia *1995: Taxonomy, Biology, Damage, Control and Management*, ed. D Gerling, RT Mayer, pp. 143–145. Andover, Hants, UK: Intercept Ltd.

Frohlich D, Torres-Jerez I, Bedford ID, Markham PG, Brown JK. 1999. A phylogeographic analysis of the *Bemisia tabaci* species complex based on mitochondrial DNA markers. *Mol. Ecol.* 8:1593–1602.

Gawel NJ, Bartlett AC. 1993. Characterization of differences between whiteflies using RAPD-PCR. *Insect Mol. Biol.* 2:33–38.

Gelman DB, Gerling D, Blackburn MA. 2005. Host-parasitoid interactions relating to penetration of the whitefly, *Bemisia tabaci,* by the parasitoid wasp, *Eretmocerus mundus*. *J. Insect Sci.* 5:46.

Gennadius P. 1889. Disease of tobacco plantations in the Trikonia. The aleurodid of tobacco [in Greek]. *Ellenike Georgia* 5:1–3.

Gerling D, Mayers R (eds.). 1996. Bemisia *1995: Taxonomy, Biology, Damage, Control and Management*. Andover, Hants, UK: Intercept.

Gerling D, Orion T, Delarea Y. 1990. *Eretmocerus* penetration and immature development: a novel approach to overcome host immunity. *Arch. Insect Biochem. Physiol.* 13:247–253.

Ghanim M, Czosnek H. 2000. Tomato yellow leaf curl geminivirus (TYLCV-Is) is transmitted among whiteflies (*Bemisia tabaci*) in a sex-related manner. *J. Virol.* 74:4738–4745.

Ghanim M, Kontsedalov S. 2007. Gene expression in pyriproxyfen resistant *Bemisia tabaci* Q biotype. *Pest Manag. Sci.* 63:776–783.

Ghanim M, Morin S, Czosnek H. 2001a. Rate of *Tomato Yellow Leaf Curl Virus* (TYLCV) translocation in the circulative transmission pathway of its vector, the whitefly *Bemisia tabaci*. *Phytopathology* 91:188–196.

Ghanim M, Rosell RC, Campbell LR, Czosnek H, Brown JK, Ullman DE. 2001b. Microscopic analysis of the digestive, salivary and reproductive organs of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) biotype B. *J. Morphol.* 248:22–40.

Ghanim M, Kontsedalov S, Czosnek H. 2007a. Tissue-specific gene silencing by RNA interference in the whitefly *Bemisia tabaci* (Gennadius). *Insect Biochem. Mol. Biol.* 37:732–738.

Ghanim M, Sobol I, Ghanim M, Czosnek H. 2007b. Horizontal transmission of begomoviruses between *Bemisia tabaci* biotypes. *Arthropod-Plant Inter.* 1:195–204.

Gill RJ. 1990. *The morphology of whiteflies*. In *Whiteflies, Their Bionomics, Pest Status and Management*, ed. D Gerling, pp. 13–46. Andover, Hants, UK: Intercept Ltd.

Gillespie JP, Kanost MR, Trenczek T. 1997. Biological mediators of insect immunity. *Annu. Rev. Entomol.* 42:611–643.

Girardin SE, Sansonetti PJ, Philpott DJ. 2002. Intracellular vs extracellular recognition of pathogens-common concepts in mammals and flies. *Trends Microbiol.* 10:193–199.

Gottlieb Y, Ghanim M, Chiel E, Gerling D, Portnoy V, Steinberg S, Tzuri G, Horowitz AR, Belausov E, Mozes-Daube N, Kontsedalov S, Gershon M, Gal S, Katzir N, Zchori-Fein E. 2006. Identification and localization of a *Rickettsia* sp in *Bemisia tabaci* (Homoptera: Aleyrodidae). *Appl. Environ. Microbiol.* 72:3646–3652.

Gupta RS. 1995. Evolution of the chaperonin families (Hsp60, Hsp10 and Tep-1) of proteins and the origin of eukaryotic cells. *Mol. Microbiol.* 15:1–11.

Gunning RV, Byrne FJ, Devonshire AL. 1997. Electrophoretic analysis of non-B and B-biotype *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) in Australia. *Aust. J. Entomol.* 36:245–249.

Holt RA, Subramanian GM, Halpern A et al. 2002. The genome sequence of the malaria mosquito *Anopheles gambiae*. *Science* 298:129–149.

Horowitz AR, Denholm I, Gorman K, Cenis JL, Kontsedalov S, Ishaaya I. 2003. Biotype Q of *Bemisia tabaci* identified in Israel. *Phytoparasitica* 31:94–98.

Horowitz AR, Kontsedalov S, Khasdan V, Ishaaya I. 2005. Biotypes B and Q of *Bemisia tabaci* and their relevance to neonicotinoid and pyriproxyfen resistance. *Arch. Insect Biochem. Physiol.* 58:216–225.

Hunter WB, Hiebert E, Webb SE, Tsai JK, Polston JE. 1998. Location of geminiviruses in the whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae). *Plant Dis.* 82:1147–1151.

Jiang YX, de Blas C, Barrios L, Fereres A. 2000. Correlation between whitefly (Homoptera: Aleyrodidae) feeding behavior and transmission of *Tomato yellow leaf curl virus*. *Ann. Entomol. Soc. Am.* 93:573–579.

Jiang YX, de Blas C, Bedford ID, Nombela G, Muniz M. 2004. Effects of *Bemisia tabaci* biotype on the transmission of tomato yellow leaf curl Sardinia virus (TYLCSV-ES) between tomato common weeds. *Spanish J. Agri. Res.* 2:115–119.

Jiu M, Zhou X-P, Tong L, Xu J, Yang X, Wan F-H, Liu S-S. 2007. Vector-virus mutualism accelerates population increase of an invasive whitefly. *PloS ONE* 1:e182.

Kirkpatrick M, Ravigné V. 2002. Speciation by natural and sexual selection: models and experiments. *Am. Naturalist* 159:S22–S35.

Kunik T, Gafni Y, Czosnek H, Citovsky V. 1997. Transgenic tomato plants expressing TYLCV capsid protein are resistant to the virus: the role of nuclear localization signal (NLS) in the resistance. *Acta Horticult.* 447:387–391.

Leshkowitz D, Gazit S, Reuveni E, Ghanim M, Czosnek H, McKenzie C, Shatters RG, Jr, Brown JK. 2006. Whitefly (*Bemisia tabaci*) genome project: analysis of sequenced clones from egg, instar, and adult (viruliferous and non-viruliferous) cDNA libraries. *BMC Genomics* 7:79.

Liu SS, DeBarro PJ, Xu J, Luan JB, Zang LS, Ruan YM, Wan FH. 2007. Asymmetric mating interactions drive widespread invasion and displacement in a whitefly. *Science* 318: 1769–1772.

Ma D, Gorman K, Devine G, Luo W, Denholm I. 2007. The biotype and insecticide-resistance status of whiteflies, *Bemisia tabaci* (Hemiptera: Aleyrodidae), invading cropping systems in Xinjiang Uygur autonomous region of northwestern China. *Crop Prot.* 26:612–617.

Mahadav A, Gerling D, Gottlieb Y, Czosnek H, Ghanim M. 2008. Gene expression in the whitefly *Bemisia tabaci* pupae in response to parasitization by the wasp *Eretmocerus mundus*. *BMC Genomics* 9:342.

Mahadav A, Kontsedalov S, Czosnek H, Ghanim M. 2009. Thermotolerance and gene expression following heat stress in the whitefly *Bemisia tabaci* B and Q biotypes. *Insect Biochem. Mol. Biol.* 39:668–676.

Martin JH. 2003. Whiteflies (Hemiptera: Aleyrodidae) – their systematic history and the resulting problems of conventional taxonomy, with special reference to descriptions of *Aleyrodes proletella* (Linnaeus, 1758) and *Bemisia tabaci* (Gennadius, 1889). *Entomologist's Gazette* 54:125–136.

Maruthi MN, Colvin J, Thwaites RM, Banks GK, Gibson G, Seal SE. 2004. Reproductive incompatibility and cytochrome oxidase I gene sequence variability amongst host-adapted and geographically separate *Bemisia tabaci* populations (Hemiptera: Aleyrodidae). *Syst. Entomol.* 29:560–568.

McKenzie CL. 2002. Effect of *Tomato mottle virus* (ToMoV) on *Bemisia tabaci* biotype B (Homoptera: Aleyrodidae) oviposition and adult survivorship on healthy tomato. *Fla. Entomol.* 85:367–368.

Moran NA, Telang A. 1998. Bacteriocyte-associated symbionts of insects. *Bioscience* 48: 295–304.

Morin S, Ghanim M, Sobol I, Czosnek H. 2000. The GroEL protein of the whitefly *Bemisia tabaci* interacts with the coat protein of transmissible and non-transmissible begomoviruses in the yeast two-hybrid system. *Virology* 276:404–416.

Morin S, Ghanim M, Zeidan M, Czonek H, Verbeek M, van den Heuvel FJM. 1999. A GroEL homologue from endosymbiotic bacteria of the whitefly *Bemisia tabaci* is implicated in the circulative transmission of tomato yellow leaf curl virus. *Virology* 256:75–84.

Morin S, Williamson MS, Goodson SJ, Brown JK, Tabashnik BE, Dennehy TJ. 2002. Mutations in the *Bemisia tabaci* sodium channel gene associated with resistance to a pyrethroid plus organophosphate mixture. *Insect Biochem. Mol. Biol.* 32:1781–1791.

Mound LA. 1963. Host-correlated variation in *Bemisia tabaci* (Gennadius)(Homoptera: Aleyrodidae). *Proc. Royal Entomol. Soc. Lond. A* 38:171–180.

Mound LA, Halsey SH. 1978. *Whitefly of the World: A Systematic Catalogue of the Aleyrodidae (Homoptera) with Host Plant and Natural Enemy Data*. British Museum, 340pp.

Munson MA, Baumann P, Clark MA, Baumann L, Moran NA, Voegtlin DJ, Campbell BC. 1991. Evidence for the establishment of aphid-eubacterium endosymbiosis in an ancestor of four aphid families. *J. Bacteriol.* 173:6321–6324.

Pérez-Brocal V, Gil R, Ramos S, Lamelas A, Postigo M, Michelena J, Silva F, Moya A, Latorre A. 2006. A small microbial genome: the end of a long symbiotic relationship? *Science* 314:312–313.

Perring TM, Cooper D, Rodriguez RJ, Farrar CA, Bellows TS, Jr. 1993. Identification of a whitefly species by genomic and behavioral studies. *Science* 259(74):77.

Perring TM, Symmes EJ. 2006. Courtship behavior of *Bemisia argentifolii* (Hemiptera: Aleyrodidae) and whitefly mate recognition. *Ann. Entomol. Soc. Am.* 99:598–606.

Pimentel D, Lach L, Zuniga R, Morrison D. 2000. Environmental and economic costs of nonindigenous species in the United States. *Bioscience* 50:53–65.

Provencher LM, Morse GE, Weeks AR, Normark BB. 2005. Parthenogenesis in the *Aspidiotus nerii* Complex (Hemiptera: Diaspididae): a single origin of a worldwide, polyphagous lineage associated with *Cardinium* bacteria. *Ann. Entomol. Soc. Am.* 98:629–635.

Ronda M, Adán A, Beitia DF, Cifuentes D, Cenis JL. 2000. Interbreeding between biotypes of *Bemisia tabaci*. European Whitefly Studies Network Newsletter No. 3, http://www.whitefly.org/EWSN-NewDownLds-pdf/EWSN_Newsletter03.pdf

Rosell RC, Bedford ID, Frohlich DR, Gill RJ, Brown JK, Markham PG. 1997. Analysis of morphological variation in distinct populations of *Bemisia tabaci* (Homoptera: Aleyrodidae). *Ann. Entomol. Soc. Am.* 90:575–589.

Rosell R, Lichty JE, Brown JK. 1995. Ultrastructure of the mouthparts of adult sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae). *Int. J. Insect Morphol. Embryol.* 24:297–306.

Rosell RC, Torres-Jerez I, Brown JK. 1999. Temporal pathway of geminivirus in whitefly extracts, saliva, hemolymph and honeydew. *Phytopathology* 89:239–246.

Rosell RC, Davidson EW, Jancovich JK, Hendrix DL, Brown JK. 2003. Size limitations in the filter chamber and digestive tract of nymphal and adult *Bemisia tabaci* whiteflies (Hemiptera: Aleyrodidae). *Ann. Entomol. Soc. Am.* 96:544–552.

Rubinstein G, Czosnek H. 1997. Long-term association of tomato yellow leaf curl virus (TYLCV) with its whitefly vector *Bemisia tabaci*: effect on the insect transmission capacity, longevity and fecundity. *J. Gen. Virol.* 78:2683–2689.

Russell LM. 1957. Synonyms of *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae). *Bull. Brooklyn Ent. Soc.* 52:122–123.

Sanchez-Campos S, Navas-Castillo J, Camero R, Soria C, Diaz JA, Moriones E. 1999. Displacement of Tomato yellow leaf curl virus (TYLCV)-Sr by TYLCV-Is in tomato epidemics in Spain. *Phytopathology* 89:1038–1043.

Sakharkar MK, Kangueane P. 2004. Genome SEGE: A database for 'intronless' genes in eukaryotic genomes. *BMC Genomics* 5:67.

Salvucci ME, Stecher DS, Henneberry TJ. 2000. Heat shock proteins in whitefies, an insect that accumulates sorbitol in response to heat stress. *J. Therm. Biol.* 25:363–371.

Schlee D. 1970. Verwandtschaftsforschung an fossilen und rezenten Aleyrodina (Insectas, Hemiptera). *Stuttg. Beitr. Nat.kd. A Biol.* 213:1–74.

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Ann. Entomol. Soc. Am.* 87:651–701.

Sinisterra XH, McKenzie CL, Hunter WB, Powell CA, Shatters RG, Jr. 2005. Differential transcriptional activity of plant pathogenic begomoviruses in their whitefly vector. *J. Gen. Virol.* 86:1525–1532.

Southamer R, Breeuwer JAJ, Hurst GDD. 1999. *Wolbachia pipientis*: microbial manipulator of arthropod reproduction. *Annu. Rev. Microbiol.* 53:71–102.

Sseruwagi P, Legg JP, Maruthi MN, Colvin J, Rey MEC, Brown JK. 2005. Genetic diversity of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) populations and presence of the B and a non-B biotype that is capable of inducing silverleaf symptoms in squash, in Uganda. *Ann. App. Biol.* 147:253–265.

Strachan T, Read AP. 2004. *Human Molecular Genetics*, 3rd Edition. London and New York: Garland Science.

Thao ML, Baumann L. 2004. Evolutionary relationships of primary prokaryotic and endosymbionts of whiteflies and their hosts. *Appl. Environm. Microbiol.* 70:3401–3406.

Tram U, Sullivan W. 2002. Role of delayed nuclear envelope breakdown and mitosis in *Wolbachia*-induced cytoplasmic incompatibility. *Science* 296:1124–1126.

van den Heuvel JFJM, Verbeek M, van der Wilk F. 1994. Endosymbiotic bacteria associated with circulative transmission of potato leafroll virus by *Myzus persicae*. *J. Gen. Virol.* 75: 2559–2569.

Viscarret MM, Torres-Jerez I, Agostini de Manero E, López SN, Botto EE, Brown JK. 2003. Mitochondrial DNA evidence for a distinct clade of New World *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae) from Argentina and Bolivia, and presence of the Old World B biotype in Argentina. *Ann. Entomol. Soc. Am.* 96:65–72.

Weeks AR, Breeuwer JAJ. 2003. A new bacterium from the Cytophaga-Flavobacterium-Bacteroides phylum that causes sex-ratio distortion. In *Insect Symbiosis*, ed. K Bourtzis, A Miller, pp. 165–176. Boca Raton, Florida: CRC Press LLC.

Weeks AR, Velten R, Stouthamer R. 2003. Incidence of a new sex-ratio-distorting endosymbiotic bacterium among arthropods. *Proc. R. Soc. Lond., B, Biol. Sci.* 270:1857–1865.

Werling D, Jungi TW. 2003. Toll-like receptors linking innate and adaptive immune response. *Vet. Immunol. Immunopathol.* 91:1–12.

Werren JH. 1997. Biology of *Wolbachia*. *Annu. Rev. Entomol.* 42:587–609.

Werren JH. 1998. *Wolbachia* and speciation. In *Endless Forms: Species and Speciation*, ed. D Howard, S Berlocher, pp. 245–260. Oxford: Oxford University Press.

Wool D, Gerling D, Bellotti AC, Morales FJ. 1993. Esterase electrophoretic variation in *Bemisia tabaci* (Gennadius) (Hem., Aleyrodidae) among host plants and localities in Israel. *J. Appl. Entomol.* 115:185–196.

Zchori-Fein E, Brown JK. 2002. Diversity of prokaryotes associated with *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae). *Ann. Entomol. Soc. Am.* 95:711–718.

Zchori-Fein E, Perlman SJ. 2004. Distribution of the bacterial symbiont *Cardinium* in arthropods. *Mol. Ecol.* 13:2009–2016.

# Index



P.A. Stansly, S.E. Naranjo (eds.), *Bemisia*: Bionomics and Management of a Global Pest,
DOI 10.1007/978-90-481-2460-2, © Springer Science+Business Media B.V. 2010