PLANT CELL MONOGRAPHS

R. H. Berg
C. G. Taylor
Editors

Cell Biology of Plant Nematode Parasitism

Springer

Plant Cell Monographs
Volume 15

Series Editor: David G. Robinson
Heidelberg, Germany

Plant Cell Monographs

Recently Published Titles

Cell Biology of Plant Nematode Parasitism
Volume Editor: Berg, R. H., Taylor, C. G
Vol. 15, 2009

Functional Organization of the Plant Nucleus
Volume Editor: Meier, I.,
Vol. 14, 2009

The Chloroplast
Interactions with the Environment
Volume Editors: Sandelius, A. S., Aronsson, H.
Vol. 13, 2009

Root Hairs
Volume Editors: Emons, A. M. C., Ketelaar, T.
Vol. 12, 2009

Plant Microtubules
Development and Flexibility
2nd Edition
Volume Editor: Nick, P.
Vol. 11, 2008

Plant Growth Signalling
Volume Editors: Bögre, L., Beemster, G.
Vol. 10, 2008

Cell Division Control in Plants
Volume Editors: Verma, D. P. S., Hong, Z.
Vol. 9, 2008

Endosperm
Volume Editor: Olsen, O.-A.
Vol. 8, 2007

Viral Transport in Plants
Volume Editors: Waigmann, E., Heinlein, M.
Vol. 7, 2007

Nitric Oxide in Plant Growth, Development and Stress Physiology
Volume Editors: Lamattina, L., Polacco, J.
Vol. 6, 2007

The Expanding Cell
Volume Editors: Verbelen, J.-P., Vissenberg, K.
Vol. 5, 2007

The Plant Endoplasmic Reticulum
Volume Editor: Robinson, D. G.
Vol. 4, 2006

The Pollen Tube
A Cellular and Molecular Perspective
Volume Editor: Malhó, R.
Vol. 3, 2006

Somatic Embryogenesis
Volume Editors: Mujib, A., Šamaj, J.
Vol. 2, 2006

Plant Endocytosis
Volume Editors:
Šamaj, J., Baluška, F., Menzel, D.
Vol. 1, 2005

R. Howard Berg • Christopher G. Taylor
Editors

Cell Biology of Plant Nematode Parasitism

Springer

Editors
R. Howard Berg, PhD
Director, Integrated Microscopy Facility
and
Christopher G. Taylor, PhD
Principal Investigator

Donald Danforth Plant Science Center
975 North Warson Road
St. Louis, MO 63132
USA

Series Editor
Prof. Dr. David G. Robinson
Ruprecht-Karls-University of Heidelberg
Heidelberger Institute for Plant Sciences (HIP)
Department Cell Biology
Im Neuenheimer Feld 230
D-69120 Heidelberg
Germany

ISBN: 978-3-540-85213-1 e-ISBN: 978-3-540-85215-5
DOI: 10.1007/978-3-540-85215-5

Library of Congress Control Number: 2008939146

Cover design: WMX Design GmbH, Heidelberg, Germany

Printed on acid-free paper

9 8 7 6 5 4 3 2 1 0

springer.com

Editors

R. Howard Berg is Director of the Integrated Microscopy Facility at the Danforth Plant Science Center, St. Louis, Missouri. He earned his Ph.D. in Agronomy from the University of Florida (1977) and received postdoctoral training at the University of Florida and at Oregon State University. After several years managing the biological electron microscopy facility at the University of Florida, he joined the faculty at the University of Memphis, Tennessee, where he became an Associate Professor. He joined the Danforth Plant Science Center in 2000. His research interests include plant cell interactions with nitrogen-fixing endosymbionts, nematodes, and viruses.

Christopher G. Taylor studied Biochemistry (B.S., 1989) at The Penn State University in State College, Pennsylvania followed by a Ph.D. in Genetics (1995), with an emphasis on gene expression in roots, at North Carolina State University. In 1995, he worked as a Postdoctoral Fellow and Senior Scientist in Nematology at Monsanto Co. in St. Louis, Missouri. In 1999, he became a Senior Scientist and Group Leader of Nematology for Akkadix, Inc. in San Diego, California. From 2001 to the present, he has been a Principal Investigator at the Donald Danforth Plant Science Center in St. Louis, Missouri and Adjunct Assistant Professor at the University of Missouri – Columbia and at the University of Missouri – St. Louis. His major research interests include plant cell biology of root biotic interactions, nematology, microbiology, gene expression analysis and functional genomics.

Preface

Parasites, cysts and root-knot nematodes have evolved sophisticated mechanisms for exploiting plants, with profound agricultural impact. The susceptibility of plants to nematode parasitism has resulted in a significant effort to identify the cellular and molecular mechanisms involved in nematode-induced pathology of plants. We have been fortunate to gather a group of leading scientists who present in this book the current knowledge on nematode parasitism. Plant-nematode interactions are examined from organismal responses down to molecule-specific responses within the nematode and its host plant. In this exciting era of cell biology, computer-enhanced technology, ranging from microscopy to genomic analysis, is bringing us ever closer to using the knowledge generated to reduce the parasitic effects of nematodes on plants.

This book will be a useful reference for advanced undergraduate, graduate and postdoctoral students, as well as senior scientists.

We gratefully acknowledge the help of a number of people in reviewing and editing the manuscript, including Christine Ehret and Marti Shafer of the Danforth Plant Science Center and Jim McCarter, Michelle Hresko, and Bingli Gao of Divergence, Inc.

September 2008

R. Howard Berg
Christopher G. Taylor

Contents

Contributors

Erin Bakker
Laboratory of Nematology, Department of Plant Sciences, Wageningen University, Wageningen, The Netherlands
Erin.Bakker@wur.nl

Thomas J. Baum
Department of Plant Pathology, Iowa State University, Ames, IA 50111, USA

R. Howard Berg
Donald Danforth Plant Science Center, 975 North Warson Road, St. Louis, MO 63132, USA
rhberg@danforthcenter.org

David McK. Bird
Center for the Biology of Nematode Parasitism, North Carolina State University, Raleigh, NC 27695, USA;
Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695, USA
david_bird@ncsu.edu

Eric L. Davis
Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695, USA
eric_davis@ncsu.edu

Thomas Fester
Donald Danforth Plant Science Center, 975 North Warson Road, St. Louis, MO 63132, USA

Godelieve Gheysen
Department of Molecular Biotechnology, Ghent University, Ghent, Belgium
Godelieve.Gheysen@UGent.be

Wladyslaw Golinowski
Department of Botany, Faculty of Agriculture and Biology, Warsaw University of Life Sciences (SGGW), Warsaw, Poland

Aska Goverse
Laboratory of Nematology, Department of Plant Sciences, Wageningen University, Wageningen, The Netherlands

Richard S. Hussey
Department of Plant Pathology, University of Georgia, Athens, GA 30602, USA

Kamila Koropacka
Laboratory of Nematology, Department of Plant Sciences, Wageningen University, Wageningen, The Netherlands

Yuhong Li
Donald Danforth Plant Science Center, St. Louis, MO 63132, USA

James P. McCarter
Divergence Inc., St. Louis, MO 63141, USA;
Genome Sequencing Center, Washington University School of Medicine, St. Louis, MO 63108, USA
mccarter@divergence.com

Melissa G. Mitchum
Division of Plant Sciences and Bond Life Sciences Center, University of Missouri, Columbia, MO 65211, USA
goellnerm@Missouri.edu

Charles H. Opperman
Center for the Biology of Nematode Parasitism, North Carolina State University, Raleigh, NC 27695, USA;
Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695, USA;
Department of Genetics, North Carolina State University, Raleigh, NC 27695, USA
warthog@ncsu.edu

Jennifer E. Schaff
Genomic Sciences Laboratory, North Carolina State University, Raleigh, NC 27695, USA

Geert Smant
Laboratory of Nematology, Department of Plant Sciences, Wageningen University, Wageningen, The Netherlands

Miroslaw Sobczak
Department of Botany, Faculty of Agriculture and Biology, Warsaw University of Life Sciences (SGGW), Warsaw, Poland
miroslaw_sobczak@sggw.pl

Christopher G. Taylor
Donald Danforth Plant Science Center, 975 North Warson Road, St. Louis, MO 63132, USA
ctaylor@danforthcenter.org

Anna Tomczak
Laboratory of Nematology, Department of Plant Sciences, Wageningen University, Wageningen, The Netherlands

Valerie M. Williamson
Department of Nematology, University of California-Davis, Davis, CA 95616, USA

Plant Infection by Root-Knot Nematode

David McK. Bird(✉), Charles H. Opperman, and Valerie M. Williamson

Abstract Plant-parasitic nematodes, particularly the sedentary endoparasitic forms, are cosmopolitan pests, collectively causing over $100 billion in annual crop loss worldwide. In the past decade, significant progress has been made in identifying genes and their products that define key aspects of the host–parasite interface, including enzymes and proteins with direct roles in virulence and resistance. However, little remains known about how a host is identified or how the development of the nematode is coupled to establishment of the parasitic interaction. Here, we consider the role of signaling molecules and their interplay with nematode development from hatch through primary interaction with the plant.

1 A Brief Introduction to Root-Knot Nematode

Although plant-parasitic nematodes are found in three of the five major clades of the phylum Nematoda (Blaxter et al. 1998), much of the damage to crops is caused by the approximately 60-member tylenchid genus, *Meloidogyne* (Sasser and Freckman 1986; Koenning et al. 1999). Reflecting the gross symptoms exhibited by roots infected with these nematodes (Fig. 1), the common name for *Meloidogyne* spp. is "root-knot nematode(s)." More than 2,000 plant species have been designated as hosts to root-knot nematodes, and most cultivated crops are attacked by at least one root-knot nematode species (Sasser 1980). Since its description as a genus (Chitwood 1949), root-knot nematodes have been particularly favored for research by plant nematologists in large measure because of their importance as agricultural parasites. Beyond this, however, the motivation to study root-knot nematode has sprung from scientific curiosity regarding the many intriguing features of their

D.McK. Bird
Center for the Biology of Nematode Parasitism, North Carolina State University, Raleigh, NC 27695, USA and Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695, USA
e-mail: david_bird@ncsu.edu

Plant Cell Monogr, doi:10.1007/7089_2008_31

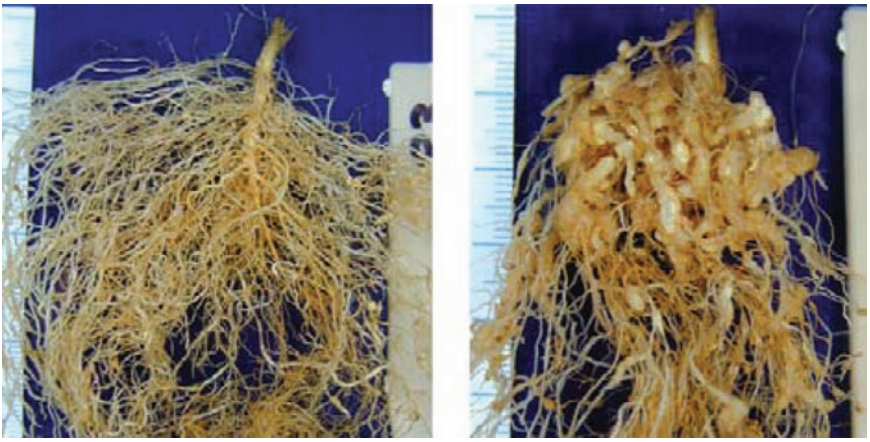


Fig. 1 Symptoms of root-knot nematode infection. Root systems of *Medicago truncatula* plants inoculated with *Meloidogyne incognita*. The plant on the *left* carries a gene conferring resistance to *M. incognita*, whereas the plant on the *right* is susceptible. Characteristic root knots (galls) are evident on the roots of the infected plant

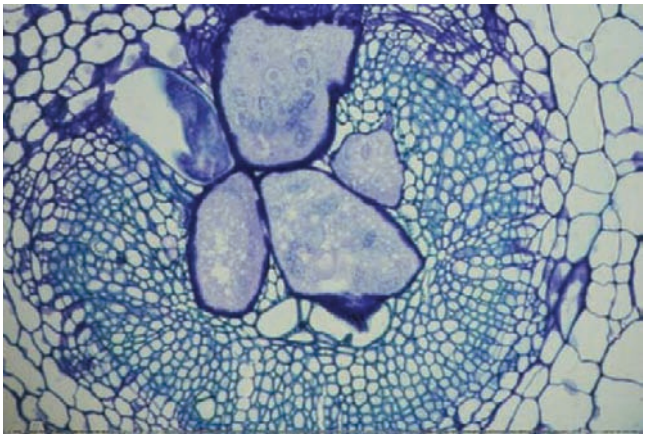


Fig. 2 Transverse section through a mature root gall induced by *Meloidogyne incognita* in tomato, stained with toluidine blue. Four giant cells are evident in the center of the vascular cylinder, surrounded by numerous small cells. The head of the nematode has contracted during fixation, leaving a partially hollow space adjacent to the giant cells

parasitic lifestyle, the most striking of which is the induction of so-called “giant cells” in the host root vasculature (Fig. 2). Induction of giant cells uniquely defines the *Meloidogyne*–host interaction and is central to it because these cells apparently serve as the sole food source for the developing worm.

Very briefly, root-knot nematodes hatch in the soil as motile, vermiform larvae (Fig. 3) able to locate, penetrate and migrate within plant roots (Fig. 4), ultimately

Fig. 3 Newly hatched *Meloidogyne* J2. *Arrows* point to some of the numerous lipid storage vesicles throughout the nematode's body, and the *bar* (S) indicates the retracted stylet

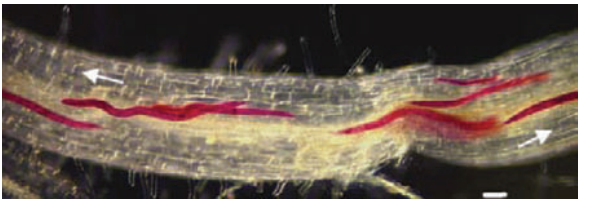


Fig. 4 Root-knot nematode J2 migrating through cleared *Lotus japonicus* roots. The worms were stained with acid fuschin

reaching the developing vascular cylinder where the giant cells are established. Giant cell formation, coupled with expansion and proliferation of nearby pericycle and cortical cells, results in the characteristic root-knot gall. Like many other root-colonizing organisms, root-knot nematodes reside in the apoplast once inside the plant, obtaining nutrition from the symplast through an as yet poorly understood process. Mature females lay eggs out into the soil to complete the lifecycle.

This chapter focuses on the biological events that lead the root-knot nematode to its selection of a host and the irrevocable commitment by the parasite to a sedentary lifestyle. In other words, we consider the events that occur between hatch and the first meal, ending our discussion prior to giant cell ontogeny and operation (Gheysen and Mitchum 2008; Berg et al. 2008). Our focus is on the nematode rather than the host, although in reality both must play equally in the host–parasite interaction.

Our intent is twofold. First, we will discuss events that take place prior to root penetration, arguing that nematode behavior reflects responses to multiple environmental signals. Because little is known yet about the nature of such signals, this will be a short section by necessity. One considerable impediment to progress stems from the fact that the biology prior to host penetration occurs within the complex four-dimensional milieu that is the rhizosphere and surrounding soil. Although some studies attempted to make direct observations of nematodes in the soil (e.g., Pitcher 1967), most of our current understanding comes from analysis of in vitro systems. In the soil, the host for the root-knot nematode is very literally a "moving

target" that is not well modeled in vitro. Similarly, once the nematode has penetrated its host, direct experimental manipulation becomes extremely difficult. Not surprisingly, most of what is known of the biological events associated with root-penetration and subsequent migration comes either from destructive analysis (e.g., following fixation and staining) or from inference based on in vitro experiments.

For at least 40 years (e.g., Bird 1964), a particular focus has been on the proteins secreted by the root-knot nematode second-stage juvenile (J2) during and after migration through the root, and a picture is emerging of the myriad roles played by these proteins (e.g., Baum et al. 2007). We will make no further mention of these proteins in this chapter, not to diminish their importance, but because they are discussed in detail elsewhere in this volume (Davis et al. 2008). Our second goal, therefore, is not to describe the "machinery" deployed by the J2, but rather to frame the events that lead to host selection as a behavioral response by the nematode that culminates in an irrevocable developmental commitment. Despite only a limited data set in this area, a picture is emerging of complex communication between host and parasite that likely influences the behavior of both. Additional signaling between other environmental components, including other rhizosphere organisms, contributes to the complexity. Deciphering these networks may be an important step towards truly understanding plant infection by root-knot nematode.

2 The Root-Knot Nematode Larva at Hatch

Like all nematodes, root-knot nematode embryogenesis/morphogenesis occurs within an environmentally resilient egg, whose shell is principally composed of protein (50%), chitin (30%), and lipid (Bird and McClure 1976). The egg is the most robust life stage of the nematode and precludes passage of even small molecules (such as the fungal toxin α-amanitin) that readily penetrate the cuticle of hatched stages (Rogalski and Riddle 1988). Rendering the egg sensitive to α-amanitin requires the drastic treatment of chitinase digestion followed by mechanical stripping of the vitelline membrane (Edgar et al. 1994). Thus, for root-knot nematodes there is no evidence of the developing larvae perceiving external clues, but it is not inconceivable that such events may take place. Indeed, other tylenchid nematodes, particularly *Globodera* species, almost completely depend on perception of a host-derived signal to induce substantial hatch. On the basis of purification from potato root diffusate, one component of the hatch signal has been proposed to be *trans*-2-(2,13-dihydroxy-9-methoxy-7,7,16-trimethyl-5,10,20-trioxo-19-oxahexacyclo[9.7.0.1^{3},6.0^{3},8.1^{12},15.0^{12},16]-eicosa-1(11),8-dien-15-yl) cyclopropanecarboxylic acid (Mulder et al. 1996).

Immediately prior to hatch, the root-knot nematode eggshell undergoes structural transformation, rendering it permeable to a number of reagents, such as the electron microscopy fixative/stain osmium tetroxide, to which younger eggs are resistant (e.g., Fig. 5 in Bird and Bird 1991). Unlike most nematodes, root-knot nematodes undergo the first of their larval four molts within the egg, thus hatching

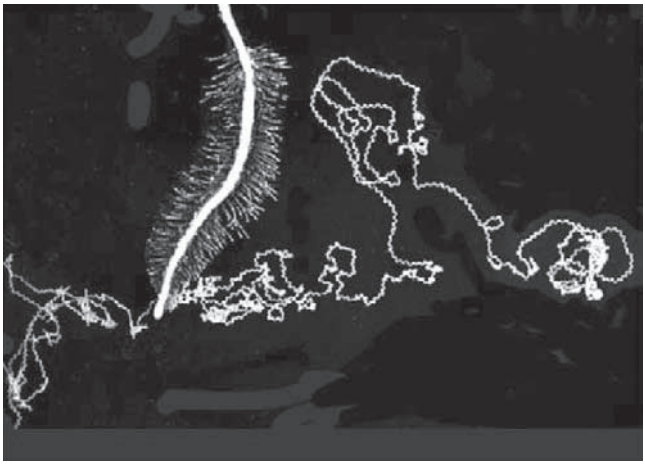


Fig. 5 Root-knot nematode attraction to root. Darkfield image of the track left in an agar surface by two root-knot nematode individuals as they migrated to the root tip

as a J2. Very little is known about the relative importance of eggs and J2s in dormant periods of root-knot nematode such as over wintering or between hosts; however, it is likely that the J2 is the predominant dormant stage because hatch does not require an external cue. Indeed, numerous lines of evidence point to the root-knot nematode (and other tylenchid nematodes) J2 as being analogous to the dauer ("enduring") stage of *Caenorhabditis elegans* (Riddle and Georgi 1990; Bird and Opperman 1998; Opperman and Bird 1998). The dauer was first described as an adaptation by animal-parasitic nematodes (Fuchs 1915), but subsequently appreciated as a phylum-wide phenomenon, extending to plant-associated genera as well (e.g., Fuchs 1937; Bird and Buttrose 1974). Dauers share the properties of arrested development, motility, non-feeding, non-ageing and hence longevity (Cassada and Russell 1975; Klass and Hirsh 1976; Riddle and Albert 1997), attributes that accurately describe root-knot nematode J2s. *C. elegans* dauers also exhibit characteristic morphological features, such as sparse (compared to L3) luminal microvilli, numerous lipid storage vesicles, and a denser cuticle that results in elevated detergent resistance; these features all have been found in tylenchid J2s (Endo 1988; Opperman and Bird 1998) (Fig. 3). A consequence of the suspension of ageing by the root-knot nematode dauer is that the time spent as a J2 largely determines the egg-to-egg time for any given individual.

Dauers have been most extensively studied in *C. elegans*, where they function as facultative, alternative stage-three larvae (L3) and serve as a binary switch to broadly couple larval development to sexual maturity with "boom" (L3) or "bust" (dauer) conditions. On the basis of an elegant amalgam of genetic, biochemical and developmental experiments (reviewed by Riddle and Albert 1997), it was shown that the stage-one larvae (L1) integrates the environmental cues of "dauer-pheromone" and "food signal" (and, to a lesser extent, temperature) to instruct the stage-two larvae (L2) development and the product of the L2 molt (Golden and Riddle 1982).

Dauer-pheromone, which has recently been attributed to three related ascarosides, viz., (-)-6-(3,5-dihydroxy-6-methyltetrahydropyran-2-yloxy) heptanoic acid, 5-O-ascarylosyl-5R-hydroxy-2-hexanone, and an ascaroside derivative of 8R-hydroxy-2E-nonenoic acid (Jeong et al. 2005; Butcher et al. 2007), is constitutively expressed by *C. elegans* and serves as a "quorum sensing" vehicle by which each individual L1 assesses population density. The precise nature of the "food signal" remains obscure but is employed as the numerator for the pheromone denominator; a relative value of >1 says "boom," whereas <1 says "bust."

Unlike in *C. elegans*, the root-knot nematode dauer (J2) stage is obligate. Whether this points to elimination of the inductive pheromone in root-knot nematodes is unknown although, intriguingly, the adaptation of undergoing the first molt within the egg would appear to ensure that the assessed food:pheromone ratio would always be <1. Another difference between *C. elegans* and root-knot nematode dauers is the developmental stage at which they occur (L3 in the former, J2 in the latter). However, as previously discussed (Bird and Opperman 1998), such heterochronic shifts are common across evolutionary space. Indeed, the dauers of *Bursaphelenchus* occur in stage-four larvae (Fuchs 1937). Although any potential role of dauer pheromone in root-knot nematode development is arcane, a role for the food signal seems clear; dauer exit, which involves the simultaneous resumption of development and ageing and a switch in carbon source from internal (i.e., stored lipid) to external, is strictly coupled to the onset of feeding inside the selected host plant (Bird 1996).

Direct biochemical analyses of intermediary metabolism (O'Riordan and Burnell 1989; Wadsworth and Riddle 1989; O'Riordan and Burnell 1990) have revealed that the *C. elegans* dauer larva is metabolically distinct from other stages, presumably reflecting the importance of lipid metabolism for this non-feeding stage. On the basis of the assumption that this biology is likely to be conserved across the phylum, approaches based on data mining (Mitreva et al. 2004) and microarray analysis (Elling et al. 2007) have attempted to compare the dauer transcriptome of the animal-parasitic nematode *Strongyloides stercoralis* and the plant parasite *Heterodera glycines*, respectively, with that of the *C. elegans* dauer. With the strong twin caveats that in each of these experiments (1) only a limited subset of the parasite transcriptome was sampled, and (2) only steady-state dauers were sampled (i.e., the transcriptome was not sampled during dauer entry or exit), both groups concluded that there is no clear evidence for a conserved dauer gene-expression signature across the Nematoda. If this conclusion is correct, it presumably reflects the unique biological adaptations of these diverse nematode species. Whether the same conclusion would be drawn from an experiment comparing the root-knot nematode transcriptome during dauer recovery with gene expression during dauer exit in *C. elegans* (Jones et al. 2001) remains untested.

In contrast to the transcriptome experiments, evidence is accumulating to support the hypothesis that the dauer pathway per se is utilized to regulate dauer entry and exit across the phylum (Blaxter and Bird 1997; Bürglin et al. 1998; Bird and Opperman 1998; Bird et al. 1999). Considerable functional evidence from *C. elegans* points to the dauer pathway as being primarily neuronally mediated, beginning with

perception of the primary effectors (pheromone and food) by the amphids and presumably transmitted downstream by endocrine function. Genetic analysis in *C. elegans* identified 32 genes as dauer affecting (*daf*) (Riddle and Albert 1997), and the molecular nature of 20 of these has been discerned, revealing a signaling pathway that is highly conserved across the animal kingdom, including humans (Wolcow et al. 2002), and which assesses nutrient status and allocates energy resources to development, ageing and fat deposition (i.e., collectively the core of dauer function). *C. briggsae* encodes 19 of the 20 characterized *C. elegans* daf genes but lacks *daf-28*, which encodes the beta-insulin molecule involved in signal transduction. Recent whole genome sequencing of *M. hapla* (Opperman et al. in press; Opperman et al. 2008) has revealed strong orthologs of 14 *C. elegans daf* genes and weak orthologs of three more. Like *C. briggsae*, *M. hapla* lacks an ortholog of *daf-28*. The molecular identities of those genes not found in *M. hapla* appear related to perception of specific cues and, hence, are probably not relevant to the parasitic lifestyle of root-knot nematodes. This demonstrates that, although the basic mechanical aspects of development are conserved, response to environment in parasite versus free-living nematode is substantially distinct, consistent with the transcriptome results (Mitreva et al. 2004; Elling et al. 2007).

3 From Hatch to Root-Penetration

Behaviorally, newly hatched root-knot nematode J2s display random movement when no attractants are present but switch to oriented migration through a concentration gradient and toward a host root tip (Perry and Aumann 1998) (Fig. 5). Although nematode movement is characterized by sinusoidal movements, migrating root-knot nematodes often exhibit quite sharp bends (Fig. 5). Plant-parasitic nematodes are attracted to host roots presumably due to a concentration gradient of substances from the root (Bird 1959; Riddle and Bird 1985), but little is known about the identity of the substances forming these gradients. There are also differences in attraction depending on plant species, the conditions of the assay and, for example, the presence or absence of border cells (Zhao et al. 2000). Although various salts and chemicals have been reported to be attractants, many of these findings have not been substantiated. However, both plant parasitic and free-living nematodes show chemotaxis along gradients of carbon dioxide (Dusenbery 1983; Robinson 1995). Once the J2s reach the root, they accumulate at the zone of elongation. Marked changes in nematode behavior ensue and are characterized by stylet thrusting, probing, and other activities associated with root penetration (Wyss et al. 1992). Aggregation of juveniles occurs, possibly indicating communication between worms by a pheromone (Fig. 6).

Analysis of the cell biology of the response of the host root surface to root-knot nematode exposure also reveals a complex but subtle interaction (Weerasinghe et al. 2005). Briefly, exposure of J2s that have not been exposed to roots since hatching rapidly elicits a wavy root-hair response on the roots of various plant

Fig. 6 Aggregation of root-knot nematodes at the root surface. *Meloidogyne hapla* J2 permitted to migrate to a tomato root in a gel assay accumulate in large masses on the root surface at the zone of elongation. Photos by C. Wang

species tested (including tomato and *Lotus japonicus*). However, repeating this experiment using J2s that have been previously exposed to the host, even for only a brief period (several minutes), elicits striking developmental changes in the root-hair cells, including rapid ionic fluxes, cytoskeletal reorganization, and nuclear relocation (Weerasinghe et al. 2005). Genetic analysis revealed that these host responses require components of the rhizobial Nod-factor perception pathway, with certain mutations in the Nod factor receptor kinases also reducing root-knot nematode infection by tenfold (Weerasinghe et al. 2005). Thus, it appears that components of the host signaling machinery necessary for establishment of mutualistic symbioses are also utilized for the establishment of parasitic symbioses (Lohar and Bird 2003; Bird 2004; Weerasinghe et al. 2005). One hypothesis that stems from this revelation is that evolution of parasitism in root-knot nematodes was accompanied by conscription of the older symbiotic pathways in the plant as a means to enhance the nematode's parasitic ability. Root-knot nematode exudates have also been shown to affect the shape of pea border cells (Zhao et al. 2000).

Collectively, these data also implicate the presence of a root-knot nematode-encoded signal, which has been named NemF (nematode factor). It has been hypothesized that NemF is physically more than one moiety (Weerasinghe et al. 2005). One component (responsible for wavy root-hairs) is produced by root-knot

nematodes prior to root-exposure and may be produced constitutively. Significantly, dead J2s do not produce the effect on plants, nor does the non-plant-parasite *C. elegans*. The second moiety, responsible for more profound developmental changes in the plant (including reorganization of the cytoskeleton and root-hair branching), appears to be produced only in response to the host and possibly reflects the perception by the J2 of a chemical signal from the plant.

Little is known about what changes in gene expression occur in root-knot nematodes between hatching and the onset of feeding in the host. It seems likely that the behavioral changes exhibited by the J2 in response to chemical signals from the host are accompanied by changes in gene expression, potentially including de novo transcription of genes, translation of pre-existing mRNAs, or modification of pre-existing proteins (e.g., by phosphorylation). Upon perception of a host and during movement of the non-feeding larva toward the root, it is reasonable to predict changes in expression of genes regulating metabolic activation, perhaps even similar to those genes up-regulated in *C. elegans* upon dauer exit (Jones et al. 2001). It is likely that genes more strictly involved in parasitism per se are also induced, including those necessary for production of NemF as well as yet to be identified proteins involved with host invasion and suppression of defense responses. Identification of such genes is likely to provide new insights into parasitism.

Studies on the behavior of nematodes once they have penetrated the root and during their migration to their feeding site have been difficult to study in vivo as the nematodes are hidden from view inside the roots. However, video-enhanced contrast light microscopy has been used successfully to visualize invasion of the transparent roots of *Arabidopsis* on agar plates by *M. incognita* (Wyss et al. 1992). J2s were observed destroying epidermal and sub-epidermal cells in the invasion process. This disruption was preceded by lip rubbing and stylet trusts against the cell walls. Once inside the root, the J2s migrated intercellularly between cortical and meristematic cells toward the root tip. After reaching the meristem, the J2s reversed direction, often damaging meristematic cells in the process, and then migrated upwards within the developing vascular cylinder. A feeding site is initiated near the zone of differentiation. Although caution should be used in generalizing the behavior of root-knot nematode in *Arabidopsis* to more typical hosts with more complex root structure, in vitro stained roots of other species are consistent with the same course of migration. The physical and chemical signals from the plant that contribute to the observed migration have not been determined. In addition, multiple J2s frequently invade at the same site and follow the same tract toward the vascular invasion, suggesting that signaling between nematodes may also be involved in the process.

Both mechanical force and enzyme secretions appear to be involved in host penetration and movement to feeding sites, and their relative importance is not known. Some of the numerous cell wall modifying enzymes that are secreted during the infection process are likely to be utilized as aids during invasion (reviewed elsewhere in this volume). Other secretions during invasion may be important in suppressing host defense responses. The intercellular movement, which avoids cell damage during migration to the feeding site, may also be part of the strategy to avoid host recognition as would be expected for an effective biotrophic parasite.

4 Conclusions

There is no doubt that efforts focused on understanding the mechanisms of giant cell formation will continue to be a major and important focus of plant-parasitic nematode research. But this is not the only important question. Careful analysis of the biology of the interaction strongly implies that the key events leading to successful infection by the nematode or successful defense by the host immunity are mediated by host–parasite signaling. In particular, evidence is accumulating that suggests chemical signals are passed between host and nematode prior to penetration (i.e., in the soil; Weerasinghe et al. 2005) and also between individual J2 (as aggregation or quorum sensing pheromones). It has long been appreciated that isolates of plant-parasitic nematodes differ in host range and in attraction to specific hosts, but currently there is little understanding of what host signals are recognized by the nematode and how these signals modify host behavior and gene expression. Such communication represents a particularly attractive target for chemical disruption as a strategy for nematode control as the pre-infective stage is most exposed to the environment (i.e., not protected by the egg or the plant host). A recent publication shows one path for development of novel nematicides based on genomic information about such "linchpins" of nematode biology (McCarter 2004); presumably other paths exist too.

The development of a genetic system for *M. hapla* offers another approach to identifying factors involved in host recognition and infection (Liu et al. 2007). F2 lines have been produced from strains of *M. hapla* that differ in host range and attraction to specific hosts (Liu and Williamson 2006). This resource should allow the mapping of genes that determine these phenotypes. The availability of the genome sequence of *M. hapla* together with the genetic map should lead to the eventual cloning of these traits (Opperman et al. 2008). Use of expression studies and RNAi will help in confirming gene function.

Acknowledgments The Bird, Opperman and Williamson laboratories gratefully acknowledge the financial support of the United States Department of Agriculture and the National Science Foundation.

References

Baum TJ, Hussey RS, Davis EL (2007) Root-knot and cyst nematode parasitism genes: the molecular basis of plant parasitism. Genetic Engineering (NY) 28:17–43

Berg RH, Fester T, Taylor CG (2008) Development of the root-knot nematode feeding cell. Plant Cell Monogr., doi:10.1007/7089_2008_30

Bird AF (1959) The attractiveness of roots to the plant parasitic nematodes *Meloidogyne javanica* and *M. hapla*. Nematologica 4:322–335

Bird AF (1964) Serological studies on the plant parasitic nematode, *Meloidogyne javanica*. Exp Parasitol 15:350–60

Bird AF, Bird J (1991) The structure of nematodes, 2nd edn. Academic Press, San Diego

Bird AF, Buttrose MS (1974) Ultrastructural changes in the nematode *Anguina tritici* associated with anhydrobiosis. J Ultrastruct Res 48:177–189

Bird AF, McClure MA (1976) The tylenchid (Nematoda) egg shell: structure, composition and permeability. Parasitology 72:19–28

Bird DMcK (1996) Manipulation of host gene expression by root-knot nematodes. J Parasitol 82:881–888

Bird DMcK (2004) Signaling between nematodes and plants. Curr Opin Plant Biol 7:372–376

Bird DMcK, Opperman CH (1998) *Caenorhabditis elegans*: a genetic guide to parasitic nematode biology. J Nematol 30:299–308

Bird DM, Opperman CH, Jones SJ, Baillie DL (1999) The *Caenorhabditis elegans* genome: a guide in the post genomics age. Annu Rev Phytopathol 37:247–265

Blaxter M, Bird DMcK (1997) Parasitic nematodes In: Riddle DL, Blumenthal T, Meyer BJ, Priess JR (eds) C. elegans II Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp 851–878

Blaxter ML, DeLey P, Garey J, Liu LX, Scheldeman P, Vierstraete A, Vanfletern J, Mackey LY, Dorris M, Frisse LM, Vida JT, Thomas WK (1998) A molecular evolutionary framework for the phylum Nematoda. Nature 392:71–75

Bürglin TR, Lobos E, Blaxter M (1998) *Caenorhabditis elegans* as a model for parasitic nematodes. Int J Parasitol 28:395–411

Butcher RA, Fujita M, Schroeder FC, Clardy J (2007) Small-molecule pheromones that control dauer development in *Caenorhabditis elegans*. Nat Chem Biol 3:420–422

Cassada RC, Russell RL (1975) The dauer larva, a post-embryonic developmental variant of the nematode *Caenorhabditis elegans*. Dev Biol 46:326–342

Chitwood BG (1949) Root-knot nematodes, Part I. A revision of the genus *Meloidogyne* Goeldi, 1887. Proc Helminth Soc Wash 16:90–104

Davis EL, Hussey RS, Baum TJ (2008) Parasitism genes: what they reveal about parasitism. Plant Cell Monogr., doi:10.1007/7089_2008_29

Dusenbery DB (1983) Chemotactic behavior of nematodes. J Nematol 15:168–173

Edgar LG, Wolf N, Wood WB (1994) Early transcription in *Caenorhabditis elegans* embryos. Development 120:443–451

Elling AE, Mitreva M, Recknor J, Gai X, Martin J, Maier TR, McDermott JP, Hewezi T, Bird DMcK, Davis EL, Hussey RS, McCarter JP, Baum TJ (2007) Divergent evolution of arrested development in the dauer stage of *Caenorhabditis elegans* and the infective stage of *Heterodera glycines*. Genome Biol 8:211

Endo BY (1988) Ultrastructure of the intestine of second and third juvenile stages of the soybean cyst nematode, the *Heterodera glycines*. Proc Helminth Soc Wash 55:117–131

Fuchs AG (1915) Die Naturgeschichte der Nematoden und einiger anderer Parasiten. I. Des *Ips typographus* L. 2. Des *Hylobius abietis* L. Zoologische Jahrbücher, Abteilung für Systematik ökologie und Geographie der Tiere. Jena 38:109–122

Fuchs AG (1937) Neue parasitische und halbparasitische Nematoden bei Borkenkäfern und einige andere Nematoden. I. Teil die Parasiten der Waldgärtner *Myelophilus piniperda* L. und *minor* Hartig und die Genera *Rhabditis* Dujardin, 1845 und *Aphelenchus* Bastian, 1865. Zoologische Jahrbücher, Abteilung für Systematik Ökologie und Geographie der Tiere. Jena 70: 291–380

Gheysen G, Mitchum MG (2008) Molecular insights in the susceptible plant response to nematode infection. Plant Cell Monogr., doi:10.1007/7089_2008_35

Golden JW, Riddle DL (1982) A pheromone influences larval development in the nematode *Caenorhabditis elegans*. Science 218:578–580

Jeong PY, Jung M, Yim YH, Kim H, Park M, Hong E, Lee W, Kim YH, Kim K, Paik YK (2005) Chemical structure and biological activity of the *Caenorhabditis elegans* dauer-inducing pheromone. Nature 433:541–545

Jones SJ, Riddle DL, Pouzyrev AT, Velculescu VE, Hillier L, Eddy SR, Stricklin SL, Baillie DL, Waterston R, Marra MA (2001) Changes in gene expression associated with developmental arrest and longevity in *Caenorhabditis elegans*. Genome Res 11:1346–1352

Klass M, Hirsh D (1976) Non-ageing developmental variant of *Caenorhabditis elegans*. Nature 260:523–525

Koenning SR, Overstreet C, Noling JW, Donald PA, Becker JO, Fortnum BA (1999) Survey of crop losses in response to phytoparasitic nematodes in the United States for 1994. J Nematol 31:587–618

Liu QL, Williamson VM (2006) Host-specific pathogenicity and genome differences between inbred strains of *Meloidoygne hapla*. J Nematol 38:158–164

Liu QL, Thomas VP, Williamson VM (2007) Meiotic parthenogenesis in a root-knot nematode results in rapid genomic homozygosity. Genetics 176:1483–1490

Lohar DP, Bird DMcK (2003) *Lotus japonicus*: A new model to study root-parasitic nematodes. Plant Cell Physiol 44:1176–1184

McCarter JP (2004) Genomic filtering: an approach to discovering novel antiparasitics. Trends Parasitol 20:462–468

Mitreva M, McCarter JP, Martin J, Dante M, Wylie T, Chiapelli B, Pape D, Clifton SW, Nutman TB, Waterston RH (2004) Comparative genomics of gene expression in the parasitic and free-living nematodes *Strongyloides stercoralis* and *Caenorhabditis elegans*. Genome Res 14:209–220

Mulder JG, Diepenhorst P, Plieger P, Bruggemann-Rotgan IEM (1996) Hatching agent for the potato cyst nematode. United States Patent 5585505

Opperman CH, Bird DMcK (1998) The soybean cyst nematode, *Heterodera glycines*: a genetic model system for the study of plant-parasitic nematodes. Cur Opin Plant Biol 1:342–346

Opperman CH, Bird DMcK, Williamson VM, Rohksar DS, Burke M, Cohn J, Cromer J, Diener S, Gajan J, Graham S, Houfek TD, Liu QL, Mitros T, Schaffer R, Scholl E, Sosinski BR, Thomas VP, Windham E (2008) Sequence and genetic map of *Meloidogyne hapla*: A compact nematode genome for plant parasitism. Proc Natl Acad Sci (USA) in press

Opperman CH, Bird DMcK, Schaff JE (2008) Genomic analysis of the root-knot nematode genome. Plant Cell Monogr., doi:10.1007/7089_2008_37

O'Riordan V, Burnell AM (1989) Intermediary metabolism in the dauer larva of the nematode *C. elegans*. I. Glycolysis, gluconeogenesis, oxidate phosphorylation and the tricarboxylic acid cycle. Comp Biochem Physiol 92B:233–238

O'Riordan V, Burnell AM (1990) Intermediary metabolism in the dauer larva. II. The glyoxylate cycle and fatty acid oxidation. Comp Biochem Physiol 95B:125–130

Perry RN, Aumann J (1998) Behavior and sensory responses. In: Perry RN, Wright DJ (eds) The physiology and biochemistry of free-living and plant-parasitic nematodes. CABI Publishing, Wallingford, UK, pp 75–102

Pitcher RS (1967) The host-parasite relations and ecology of *Trichodorus uiruliferus* on apple roots, as observed from an underground laboratory. Nematologica 13:547–557

Riddle DL, Albert PS (1997) Genetic and environmental regulation of dauer larva development. In: Riddle DL, Blumenthal T, Meyer BJ, Priess JR (eds) C. elegans II. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp 739–768.

Riddle DL, Bird AF (1985) Responses of the plant-parasitic nematodes *Rotylenchulus reniformis*, *Anguina agrostis* and *Meloidogyne javanica* to chemical attractants. Parasitology 91:185–195

Riddle DL, Georgi LL (1990) Advances in research on *Caenorhabditis elegans*: application to plant-parasitic nematodes. Ann Rev Phytopathol 28:247–269.

Robinson AF (1995) Optimal release rates for attracting *Meloidogyne incognita*, *Rotylenchulus reniformis*, and other nematodes to carbon dioxide in sand. J Nematol 27:42–50

Rogalski TM, Riddle DL (1988) A *Caenorhabditis elegans* RNA polymerase II gene, *ama-1*, and nearby essential genes. Genetics 118:61–74

Sasser JN (1980) Root-knot nematodes: a global menace to crop production. Plant Dis 64:36–41

Sasser JN, Freckman DW (1986) A world perspective on nematology: The role of society. In: Veech JA, Dickson DW (eds) Vistas of nematology. Society of Nematologists, Inc. Hyattsville, MD

Wadsworth WG, Riddle DL (1989) Developmental regulation of energy metabolism in *Caenorhabditis elegans*. Dev Biol 132:167–173

Weerasinghe RR, Bird DMcK, Allen NS (2005) Root-knot nematodes and bacterial Nod factors elicit common signal transduction events in *Lotus japonicus* root hair cells. Proc Nat Acad Sci USA 102:3147–3152

Wolcow CA, Munoz MJ, Riddle DL, Ruvkun G (2002) IRS and p55 orthologous adapter proteins function in the *C*. elegans daf-2/insulin-like signaling pathway. J Biol Chem 277:41591–41597

Wyss U, Grundler FMW, Munch A (1992) The parasitic behavior of second-stage juveniles of *Meloidogyne incognita* in roots of *Arabidopsis thaliana*. Nematologica 38:98–111

Zhao X, Schmitt M, Hawes MC (2000) Species-dependent effects of border cell and root tip exudates on nematode behavior. Phytopathology 90:1239–1245

Parasitism Genes: What They Reveal about Parasitism

Eric L. Davis(✉), R.S. Hussey, and Thomas J. Baum

Abstract Nematodes are parasites of plants and animals that have evolved diverse and often specific mechanisms to promote a given parasitic lifestyle (Baldwin et al. 2004; Jasmer et al. 2003), including modifications of developmental and reproductive potential, dissemination amongst and location of primary or alternate hosts, and survival strategies in the absence of a suitable host or favorable environment. The genetic pathways underlying these lifecycle adaptations may have parallels with or origins in nonparasitic nematode species that must also adapt to a dynamic or unstable niche. Distinct to the parasites, however, are adaptations to obtain organic nutriment while living in or on another organism. The products of such *parasitism genes* "may be manifested as morphological structures that provide access to parasitism of a particular host (e.g. a nematode stylet) or they may play critical physiological roles in the interaction of the nematode with its host" (Davis et al. 2000).

1 Introduction

The stylet (Fig. 1), a protrusible oral spear, is the primary adaptation that allows all plant-parasitic nematodes to breach the plant cell wall to access host cell nutrients, which is essential for nematode growth and reproduction (Hussey 1989). The stylet is a hardened structure of sclerotized cuticle that connects directly to the lumen of the alimentary canal in the nematode esophagus (Bird and Bird 1991), and in all but the trichodorid species, the stylet itself has a hollow lumen with an aperture that provides a continuous channel between the feeding nematode and the parasitized

E.L. Davis
Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695, USA
e-mail: eric_davis@ncsu.edu

Plant Cell Monogr, doi:10.1007/7089_2008_29

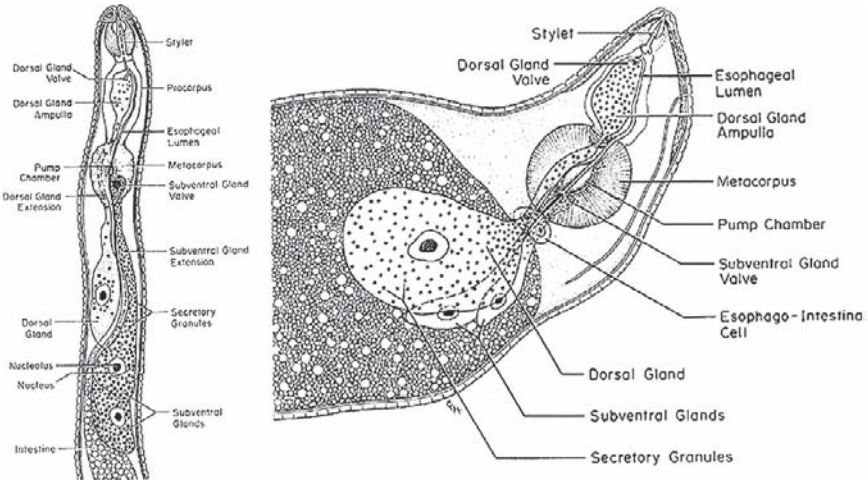


Fig. 1 Illustrations of the anterior portions of the migratory and sedentary stages of endoparasitic nematodes that contain esophageal gland secretory cells associated with the nematode stylet, a hollow oral feeding spear. (**a**) A migratory, pre-infective second-stage juvenile with the two subventral esophageal gland cells packed with secretory granules. (**b**) A swollen female from within infected roots with reduced subventral glands and an enlarged dorsal esophageal gland cell now packed with secretory granules. Reprinted from Hussey (1989) with permission from Annual Reviews

host plant cell. Successful parasitism requires feeding from a living host cell (obligate biotrophy), and for some nematode species, modifications of the host cell are required to promote a sustained feeding relationship.

Detailed investigations summarized in this volume and elsewhere indicate that molecules at the interface of the nematode and host play critical roles in the parasitic process (Hussey 1989; Davis et al. 2004, 2008; Jasmer et al. 2003; Maizels et al. 2004). Potential origins of interacting molecules from the nematode include a dynamic surface coat and natural openings such as the excretory-secretory pore, anal and reproductive openings, chemosensory organs, and the oral aperture. Since modifications of host plant cells for feeding occur at the nematode anterior, molecules secreted from the stylet and amphids emerge as the most likely organs to be directly involved in adaptations for plant parasitism. Most notably, the esophageal gland cells in tylenchid nematodes (Hussey 1989) have evolved into three relatively large secretory cells (one dorsal and two subventral) that are connected to the esophageal lumen and stylet through complex valves (Fig. 1). In the root-knot and cyst nematodes, change in the morphology, activity, and contents of the esophageal gland cells occurs throughout the course of parasitism (Davis et al. 2000, 2004). Activity within the subventral gland cells predominates in the migratory stages of these nematodes while enlargement and activity in the dorsal gland cell is dominant in the subsequent sedentary stages (Hussey 1989). The secreted protein products of parasitism genes expressed in these gland cells have been the subject of intense investigation and form a primary foundation of what we currently know about

nematode parasitism genes (Baum et al. 2006; Davis et al. 2004, 2008; Jasmer et al. 2003; Mitchum et al. 2007; Van Holme et al. 2004).

2 Parasitism Gene Discovery

Early investigations of plant-parasitic nematode secretions were biochemical in nature out of necessity and included elegant experiments that provided insights valuable to this day (Bird 1968; Hussey 1989; Veech et al. 1987). The difficulties in working with microscopic obligate parasites, especially parasitic stages from within plant tissue, remain today and provided a challenge to identify the point of origin of isolated nematode molecules in early investigations. Methods to stimulate stylet secretions from infective larvae – so-called second-stage juveniles (J2) – of the root-knot and cyst nematodes (Fig. 2) in vitro using resorcinol (McClure and von Mende 1987) and 5-methoxy DMT oxalate (Goverse et al. 1994), respectively, provided increased quantities of secretory proteins for direct analyses and antibody production. Relatively recently, secretions collected from nematode J2 stimulated in vitro were subjected to proteomic analyses, and the amino acid sequence generated from the analyses has been used to identify candidate parasitism genes (De Meutter et al. 2001; Jaubert et al. 2002). The adoption of monoclonal antibody technologies to specifically tag and isolate target nematode secretory proteins (Fig. 2) was used to identify discrete secretory proteins in plant nematodes and to monitor their differential synthesis during plant parasitism (Davis et al. 2000, 2004). An amino-terminal sequence of a cyst nematode subventral esophageal gland antigen that was affinity-purified with

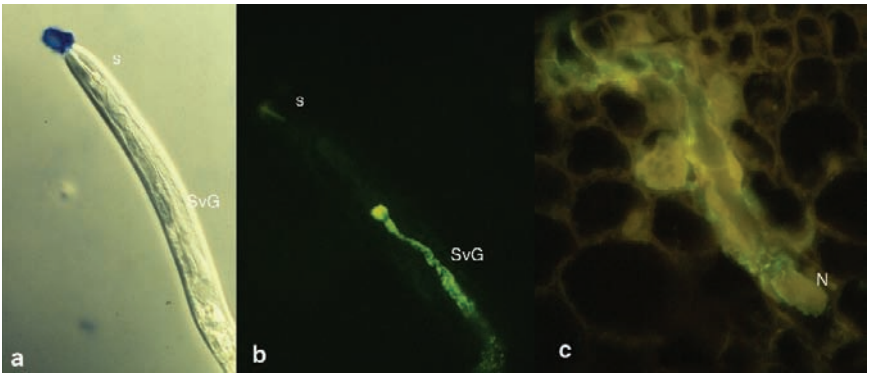


Fig. 2 Proteins produced in the nematode esophageal gland cells and secreted through the nematode stylet. (**a**) Esophageal gland proteins stained blue with Coomassie Briliant Blue are secreted from the stylet of a soybean cyst nematode (SCN) second-stage juvenile (J2) that has been incubated in the serotonin agonist, 5-methoxy-DMT oxalate. (**b**) Fluorescence immunolocalization of the antigen of monoclonal antibody MGR48 (Deboer et al. 1998) in secretory granules synthesized within the subventral esophageal gland cells of a SCN J2. (**c**) Immunolocalization of beta-1,4 endoglucanase (green fluorescence) secreted from an infective SCN J2 along its path of intracellular migration through a soybean root. Reprinted from Wang et al. (1999) with permission from APS Press

monoclonal antibody MGR48 (De Boer et al. 1996) was used to develop PCR primers to obtain the first expressed parasitism genes isolated from plant-parasitic nematodes, beta 1,4-endoglucanases (Smant et al. 1998). This discovery was able to confirm a point of origin of these cell wall-modifying enzymes as suggested in earlier investigations (Deubert and Rohde 1971). The cyst nematode cellulases were the first endoglucanase genes to be cloned from an animal and their striking similarity to bacterial Family 5 glycosyl hydrolases provided some of the first evidence of potential horizontal gene transfer from prokaryotes to eukaryotes (Smant et al. 1998; Hotopp et al. 2007; Ledger et al. 2006; Keen and Roberts 1998). A technique developed for mRNA in situ hybridization in plant nematodes (De Boer et al. 1998) and polyclonal antibodies generated to the recombinant products of the cyst nematode endoglucanase genes confirmed endoglucanase expression exclusively within the subventral esophageal glands (Smant et al. 1998). The anti-endoglucanase sera were subsequently used to confirm for the first time (Wang et al. 1999) the secretion of a nematode esophageal gland protein into plant tissues (Fig. 2).

The rapid advance of techniques in molecular biology, including methods to work with sub-microgram quantities of starting material, ushered in an era of expressed gene characterization in plant-parasitic nematodes. Complimentary DNA (cDNA) amplified from mRNA that was isolated from the dissected anterior and posterior halves of hatched, preparasitic J2 root-knot nematode, *Meloidogyne javanica*, was used to screen cDNA clones derived from the anterior halves (which contained the esophageal glands) of J2 to isolate a gene encoding a secreted chorismate mutase (CM) expressed specifically within the nematode esophageal gland cells (Lambert et al. 1999). Interestingly, the root-knot nematode CM was also most similar to genes in bacteria, and expression of *Mjcm1* in bacteria complemented a CM-deficient mutant (Lambert et al. 1999). Combined with the cyst nematode endoglucanase gene discoveries, these data encouraged early speculation that a number of plant nematode parasitism genes were derived via ancient horizontal gene transfer (Davis et al. 2000). Analyses of the genomic organization of cyst nematode endoglucanase genes have identified differences in intron size with conservation of intron position (Yan et al. 1998) and one endoglucanase gene (*Hg-eng*-5) that lacks any introns (Gao et al. 2004a). Furthermore, multiple endoglucanase genes within close genomic proximity (Yan et al. 2001) suggested the potential for "pathogenicity islands" in plant-parasitic nematodes.

The use of cDNA-AFLP to compare life stages of parasitic nematodes also has been relatively successful for the isolation of potential parasitism genes. The observation (Perry et al. 1989) that secretory granules are synthesized within the subventral esophageal gland cells of *Globodera rostochiensis* J2 within eggs upon hydration, and that subsequent exposure of the same J2 within eggs to potato root diffusate stimulated secretory granule synthesis within the dorsal gland cell (Smant et al. 1997), was exploited to compare cDNA-AFLP profiles derived from nematodes in each treatment (Qin et al. 2000). A number of differential transcript-derived fragments (TDFs) were identified among the different *G. rostochiensis* treatments, and a useful program (GenEST) was designed to cross-reference the TDFs to expressed sequences tags (ESTs) derived from cDNA libraries (Qin et al. 2001). In situ mRNA hybridization was conducted with *G. rostochiensis* clones that were differentially

expressed in cDNA-AFLP, and a number of genes expressed exclusively within the esophageal gland cells were isolated. Similar cDNA-AFLP analyses have been conducted in developmental stages of sugarbeet cyst nematodes (Tytgat et al. 2004) and among root-knot nematode near-isogenic lines (Neveu et al. 2003).

The most powerful and successful approach in identifying parasitism genes has been the direct microaspiration of the esophageal gland contents of multiple parasitic stages of *Heterodera glycines* and *Meloidogyne incognita* dissected from host roots to isolate mRNA and generate cDNA libraries that profiled esophageal gland gene expression throughout the parasitic cycle (Gao et al. 2001a, 2003; Huang et al. 2003, 2004; Wang et al. 2001). Methods of cDNA synthesis that favor inclusion of 5'-end sequence of transcripts were used to construct all gland-cell libraries. A number of cDNA selection procedures including yeast-secretion signal peptide selection (Wang et al. 2001) and subtraction against cDNA derived from nematode intestinal tissues (Gao et al. 2001a; Huang et al. 2004) have been used to identify clones within the gland cell cDNA libraries that encode secreted products that are exclusively expressed within the esophageal gland cells. Expressed sequence tag analyses have also been conducted with relatively complex gland-cell cDNA libraries that also incorporated hybridization with intestinal tissue cDNAs to gland cell macroarrays to enrich subsequent samples for unique ESTs (Gao et al. 2003; Huang et al. 2003). Putative parasitism genes were identified among gland cell cDNA clones using SignalP prediction of a putative secretion signal peptide (Nielsen et al. 1997) and confirmation of expression of the gene within the esophageal gland cells by mRNA in situ hybridization. Using these methods, more than 50 putative parasitism genes developmentally expressed in the esophageal gland cells have now been isolated in both *H. glycines* and *M. incognita*. With the exception of cell wall-modifying enzymes and a few other secreted products, relatively few common parasitism genes exist between *H. glycines* and *M. incognita* (R.S. Hussey, unpublished), and more than 70% of the parasitism gene sequences in both species have no significant database homology (i.e. so-called pioneers), indicating they may be unique to plant-parasitic nematodes.

A large-scale project designed to generate ESTs from multiple species of both plant and mammalian-parasitic nematodes had generated more than 400,000 total ESTs as of 2005 (McCarter et al. 2005; Mitreva et al. 2005a). These EST data are of tremendous significance to our understanding of nematode biology, including the potential discovery of new nematode parasitism genes. The ESTs are derived from mRNA of whole nematodes using several methods of cDNA synthesis, usually representing the life stages(s) that are most readily procured or in highest abundance. Plant-parasitic nematode ESTs (125,412) available (McCarter et al. 2005) by species include *Globodera pallida* (4,378), *Globodera rostochiensis* (5,941), *Heterodera glycines* (24,438), *Heterodera schachtii* (2,818), *Meloidogyne arenaria* (5,108), *Meloidogyne chitwoodi* (12,218), *Meloidogyne hapla* (24,452), *Meloidogyne incognita* (19,934), *Meloidogyne javanica* (7,587), *Meloidogyne paranaensis* (3,710), *Pratylenchus penetrans* (1,928), *Pratylenchus vulnus* (2,485), *Radopholus similes* (1,154), and *Xiphinema index* (9,351). The ESTs from *H. glycines* were generated from discrete, stage-specific cDNA libraries, providing a global

developmental gene expression profile (Elling et al. 2007a). Interestingly, relatively few of the predicted *H. glycines* parasitism genes discovered in gland cell-specific cDNA libraries have been identified among the whole nematode ESTs, highlighting the power of cell-specific isolation of expressed genes. Gene ontology and KEGG analyses of plant nematode EST datasets (McCarter et al. 2005), however, have characterized genes that may play roles in diverse biological functions that could include parasitism. Filters applied to analyze EST data from J2 of *M. incognita* have discovered several other genes that may be candidates of horizontal gene transfer from prokaryotes, including a gene similar to *nodL* that encodes an enzyme in the biosynthetic pathway of rhizobial Nod factor (Scholl et al. 2003). As with the gland cell libraries, the ESTs from whole J2 of *Heterodera schachtii* and *H. glycines* and parasitic stages of root-knot nematodes have been analyzed for the presence of a predicted secretion signal peptide sequence and several new parasitism gene candidates have been identified (Debreuil et al. 2007; Roze et al. 2008; Vanholme et al. 2006; A. Elling and T.J. Baum, unpublished).

Projects are in progress to generate the complete genome sequence of *M. incognita*, *H. glycines,* and *M. hapla* (Abad et al. 2008; K. Lambert, pers. comm.; Opperman et al. 2008) and will undoubtedly identify genes not represented among the available ESTs. Annotation of these genomes will be crucial to identifying genes involved in many biological processes, including parasitism. Development of physical maps and alignment with the genome will provide the roadmap to isolating genes of interest; and having genomes of multiple species available within a genus will accelerate this process and provide the opportunity for comparative genome analyses. Forward genetics are available for species capable of amphimixis, but the development of genetic maps faces many challenges. Controlled crosses are technically challenging, take months to analyze, and the obligate parasitic nature of the nematodes should render genetic mutations in parasitism genes lethal. Forward genetic analyses of plant nematodes have been relegated to identifying nematode lines that vary in virulence on resistant plant cultivars, which led to the genetic identification of virulence loci (Dong and Opperman 1997; Janssen et al. 1991). The developing genome sequence of plant nematodes should be instrumental in identifying candidate virulence genes. AFLP comparisons of isolates of *Meloidogyne* that were near-isogenic for their compatibility on tomato with resistance conditioned by the *Mi* gene have identified a secreted gene product localized to the nematode amphids that was correlated with virulence on *Mi* tomato (Semblat et al. 2001) and a transcript encoding a novel protein only in the avirulent nematode line that could be silenced to revert the line to virulent on *Mi* (Gleason et al. 2008).

3 Functional Analyses

As suggested from the difficulties of forward genetics with plant-parasitic nematodes described above, the functional analyses of putative nematode parasitism genes rely heavily on reverse genetics approaches. Another confounding factor to

the assessment of the roles of nematode genes in parasitism is the current lack of a practical and reproducible transformation system for plant-parasitic nematodes – i.e. no capability exists to introduce a gene construct into a plant nematode for expression or complementation analyses. Transformation of *Caenorhabditis elegans* with the plant nematode gene of interest can generate relevant biological information (Qin et al. 1998), but data pertaining to parasitic ability cannot be derived from this nonparasite. Undoubtedly, the ability to transform plant-parasitic nematodes is critical for widespread functional analysis of parasitism genes. For example, transgenic nematodes with parasitism genes labeled with GFP will assist in determining the destination of the parasitism proteins in host tissues.

Biochemical analyses of candidate parasitism gene products provide a definitive measure of function, and the activity of a number of nematode cell wall-modifying proteins has been assessed accordingly (Bera-Maillet et al. 2000; Gao et al. 2004a; Mitreva-Dautova et al. 2006; Popeijus et al. 2000; Qin et al. 2004). Expression of these genes in plant tissues can present an in vivo measure of function that may be extrapolated to the biological interaction. In fact, expression of any candidate nematode parasitism gene in plant tissues can yield comparable functional information, and examples of such are described in the following section. Since the products of multiple parasitism genes likely act in consort and may interact among themselves, observable plant phenotypes derived from in planta expression of individual parasitism genes may be rare and must be interpreted in this context. Expression constructs for plants often employ the CaMV 35S promoter for near constitutive expression of the transgene (Benfey and Chua 1990), but the use of alternative promoters that provide more controlled expression of the transgene should also be considered. Chemical-inducible promoters (Bohner et al. 1999; Padidam et al. 2003; Zuo et al. 2000) allow both spatial and temporal control of transgene expression provided that the nontarget effects of the inducing chemicals and appropriate controls are considered. Infiltration of plant tissues (usually leaves) with constructs of nematode genes in *Agrobacterium tumefaciens* or in viral expression systems (Chapman et al. 1992) offers a relatively rapid and high-throughput system to assess potential effects on plant tissues prior to whole plant transformation. Transgenes can be expressed and assessed in whole plants such as *Arabidopsis thaliana* and appropriate mutants, or hairy root systems may be adopted for more targeted effects of transgene expression on roots (Cho et al. 2000; Doyle and Lambert 2003; Huang et al. 2006a) given the relative ease of hairy root transformation in some plant species (e.g. soybean). Expression of nematode parasitism genes in any of the plant systems above can also provide substrate to isolate interacting molecules or complexes of host plant origin in co-immunoprecipitation assays that employ antibodies specific for nematode parasitism gene products (Huang et al. 2006a). Similarly, yeast two-hybrid assays have been used to identify an interaction between a nematode parasitism gene product and a specific domain of a host plant intracellular protein (Huang et al. 2006a).

Gene silencing using RNA interference (Fire et al. 1998) technology provides the potential for powerful analyses of parasitism gene function. The discovery that *C. elegans* can ingest double-stranded RNA (dsRNA) which then induces RNAi in

tissues distal to the gut (Timmons and Fire 1998) is particularly appealing for delivery of RNAi technology to plant-parasitic nematodes. The compounds resorcinol and octopamine, respectively, have been used to stimulate hatched J2 root-knot nematodes (Rosso et al. 2005) and cyst nematodes (Urwin et al. 2002) to ingest dsRNA in vitro as a component of "soaking" solutions and induce RNAi of the target nematode gene. RNAi of a gene encoding the major sperm protein of *H. glycines* induced by the soaking method was confirmed, and the RNAi effect was observed in subsequent generations of the nematodes after initial treatment (Urwin et al. 2002). As monitored by mRNA in situ hybridization, soaking J2 stage *M. incognita* in dsRNA of an expressed calreticulin gene induced RNAi within the nematode specimens (Rosso et al. 2005), but this RNAi soaking effect was relatively short-lived (<68 h). A decrease in infectivity of host plant roots and reduced reproductive rate were observed after RNAi soaking of J2 stage *G. rostochiensis* in dsRNA to its expressed endoglucanase gene (Chen et al. 2005) and *H. glycines* in dsRNA to a gene encoding dual oxidase (Bakhetia et al. 2005a). The RNAi soaking assays have demonstrated the ability to specifically silence a target nematode gene, including genes expressed in the esophageal gland cells (Lilley et al. 2007). The RNAi soaking technique requires relatively harsh conditions, including 2–5 mg ml^{-1} of dsRNA, to soak the J2, so potential nonspecific adverse effects on the J2 must be considered in subsequent infection assays. The technology to deliver dsRNA to nematode genes in transformed plant tissues (Wesley et al. 2001) for ingestion by feeding nematodes has the potential to provide a more natural basis for RNAi assays, including the ability to target genes that are expressed exclusively in parasitic stages of nematodes within host tissues (Gheysen and Vanholme 2007; Mitchum et al. 2007). The effects of such in planta RNAi of nematodes can be monitored at the cellular and tissue levels as well as for potential impacts on nematode development and reproduction. Vectors (Wesley et al. 2001) and promoters (Gheysen and Fenoll 2002) are available to express dsRNA directly in plant cells and nematode feeding sites, or alternatively, potential virus-induced gene silencing (Valentine et al. 2004) may be translocated to nematode feeding sites by introducing expression constructs in shoot tissues. Since the size exclusion limit (Urwin et al. 1997) of the feeding tube of sedentary endoparasites (Hussey and Mims 1991) like root-knot and cyst nematodes is less than approximately 40 kDa (~62-bp dsRNA), construct design must consider host-derived molecules of ingestible size (Bakhetia et al. 2005b; Davis et al. 2004). Double-stranded RNA of ingestible size may be produced, however, via the inherent RNAi machinery of host cells that includes a DICER enzyme to digest dsRNA into small interfering RNAs (siRNAs) of 21–23-bp dsRNA, a primary component of the gene silencing process (Novina and Sharp 2004). In this scenario, construct design may span an entire transcript and target members of a gene family, but potential nontarget effects must be considered as the population of siRNAs becomes more complex. The first published report describing successful effects of host-derived RNAi on plant infection by RKN targeted nematode-specific splicing factors and integrase genes essential to nematode cellular and developmental processes (Yadav et al. 2006) was soon followed by host-derived RNAi in soybean that targeted the nematode-specific major sperm protein of the soybean cyst

nematode (Steeves et al. 2006). These encouraging data provide support for the application of similar host-derived RNAi technology to study the function and importance of nematode parasitism genes.

The potential to bioengineer crops that provide RNAi of target nematode parasitism genes and disrupt the parasitic process represents a viable and flexible means to develop novel and durable nematode-resistant crops. Silencing of a root-knot nematode parasitism gene (*16D10*) that encodes a product that interacts with a plant transcription factor (Huang et al. 2006a) by expressing dsRNA in transgenic *Arabidopsis* resulted in transgenic plants that were highly resistant to the four common root-knot nematode species (Huang et al. 2006b). While these results validate the fundamental role of parasitism gene *16D10* in root-knot nematode parasitism of plants, they more significantly provide a "synthetic" resistance gene effective against the world's most damaging plant-parasitic nematodes whose range of resistance is not conditioned by any natural root-knot nematode resistance gene. Therefore, in planta RNAi silencing of *16D10* in root-knot nematodes could lead to the development of transgenic crops with effective broad host resistance to this agriculturally important pathogen and, equally as significant, root-knot nematode resistant crops for which natural resistance genes do not exist.

4 Meet the Candidates

The majority of candidate plant nematode parasitism genes identified to date encode polypeptides that are predicted to be secreted from the nematode esophageal gland cells into plant tissues via the stylet (Baum et al. 2006; Davis et al. 2004; Mitchum et al. 2007; Vanholme et al. 2004) (Fig. 3). As genomic, bioinformatic, and functional analyses progress, the roles of the parasitism genes encoding novel proteins (that now include the majority of candidates) will be elucidated. Our current knowledge of the putative parasitism genes that have database homologues or identifiable functional domains is summarized below.

4.1 Cell Wall-Modifying Proteins

4.1.1 Beta-1,4 Endoglucanases

Early evidence of the production and secretion of cell wall-degrading enzymes by plant-parasitic nematodes (Deubert and Rohde 1971) was confirmed by the first report of expressed beta-1,4 endoglucanase genes in cyst nematodes (Smant et al. 1998). Two members of the cyst nematode endoglucanase gene family that consists of at least six members (Gao et al. 2004a) included *eng-1* which contained a bacterial type II carbohydrate binding domain (CBD) and *eng-2* which did not. The transcripts and translated products of the cyst endoglucanase genes were produced exclusively

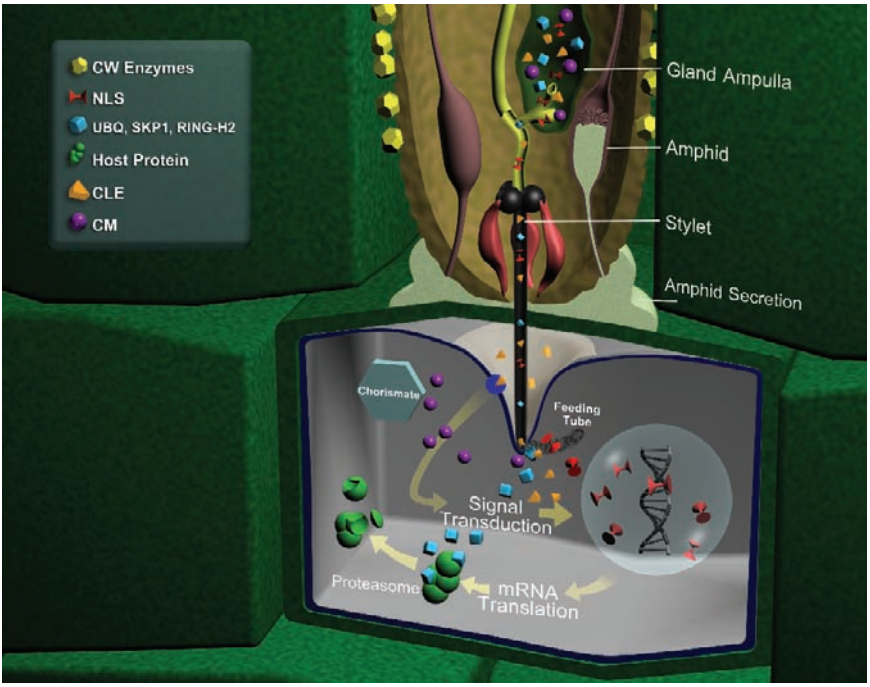


Fig. 3 A model of potential interactions of secreted products of phytonematode parasitism genes with host plant cells. Nematode esophageal gland cell secretions are released through valves within ampulla for transport out of the stylet (feeding spear) into host tissues. Cell wall (CW)-modifying proteins (endoglucanases, pectinases, hemicellulases, and expansin) may be secreted to aid the migration of infective juveniles through host plant tissues. Other nematode gland cell secretions might have multiple roles in the formation of specialized feeding cells by the nematode, including: effects on host cell metabolism by secreted chorismate mutase (CM); signaling by secreted nematode peptides such as homologs to plant CLAVATA/ESR-related (CLE) peptides; selective degradation of host proteins through the ubiquitin (UBQ)-proteosome pathway by UBQ, S-phase kinase associated protein 1 (Skp-1) and RING-H2 secreted from the nematode; and potential effects of secreted nematode proteins that contain nuclear localization signals (NLS) within the host cell nucleus. Figure designed by Bill Baverstock (North Carolina State University Creative Services). Reprinted from Davis et al. (2004) with permission from Elsevier

within the subventral glands as early as the developing J2 stage within the eggshell, and expression persisted until the early J3 stage of cyst nematodes within host roots. Endoglucanase expression was absent in subsequent stages of developing sedentary cyst nematode females, but interestingly, endoglucanase expression resumed during the development of the motile cyst nematode males that exit root tissues (De Boer et al. 1999; Goellner et al. 2000). Secretion of cyst nematode endoglucanase was detected during intracellular migration within host roots (Wang et al. 1999) but not in developing host feeding cells. However, up-regulation of plant endoglucanases was detected subsequently in nematode feeding sites as one (endogenous) component of the extensive cell wall modifications of these cells (Goellner et al. 2001). Expressed endoglucanase

genes were subsequently identified in a number of plant-parasitic nematode species (summarized in Mitchum et al. 2007; Vanholme et al. 2004), and in all cases, the nematode endoglucanase sequence had highest similarity to bacterial or reported nematode endoglucanase genes. Recombinant endoglucanases and induced nematode stylet secretions had the ability to degrade carboxymethylcellulose and a number of other cellulolytic substrates, but digestion of crystalline cellulose by nematode endoglucanases has not been confirmed (Bera-Maillet et al. 2000; Gao et al. 2004a). It is hypothesized that a combination of mechanical force of stylet thrusts and nematode cell wall-digesting enzymes promotes the breach of plant cell walls. Support for this hypothesis was recently provided from experiments in which genes for cell wall-digesting enzymes were silenced by RNAi-soaking techniques and host infectivity of the treated nematodes was reduced (Chen et al. 2005).

4.1.2 Other Hydrolytic Glucanases

In addition to beta-1,4 endoglucanases, other parasitism genes encoding cell wall-modifying proteins produced in the subventral esophageal gland cells of J2 plant-parasitic nematodes have been identified. Expressed pectinase genes encoding pectate lyase and polygalacturonase have been isolated from several nematode species (summarized in Vanholme et al. 2004), and the ability to digest pectolytic substrates was related to the ability to migrate through host root tissues. Expressed beta-1,3-endoglucanase (Kikuchi et al. 2005) and pectate lyase (Kikuchi et al. 2006) were recently reported from the pinewood nematode *Bursaphelenchus xylophilus* and hypothesized as being involved in nematode feeding from plants and co-invading fungal mycelium. The expression of an active chitinase predicted to be secreted from the subventral esophageal glands of the J2 soybean cyst nematode hatched from eggshells is also curious (Gao et al. 2002) and may reflect an adaptation to mitigate concurrent infection by organisms with chitin-containing walls. The first expressed xylanase (Mitreva-Dautova et al. 2006) and beta-galactosidase (Vanholme et al. 2006) genes of animal origin have been reported from root-knot and cyst nematodes, respectively, providing the first evidence of digestion of cell wall hemicellulose as a component of nematode migration through root tissues.

4.1.3 Expansin

The occurrence of several nematode genes encoding an expressed CBD joined with a peptide of nonendoglucnanase origin (Ding et al. 1998; Gao et al. 2004b) is curious since over-expression of a bacterial CBD gene has been reported to increase elongation of plant cells (Shpigel et al. 1998). One gene (*GR-exp1*) expressed in the subventral gland cells of the potato cyst nematode (PCN) that encoded a CBD domain was an expansin-like protein that represented the first confirmed report of such a protein outside the plant kingdom (Qin et al. 2004). Structural analyses derived from the predicted domains of one PCN expansin suggested a best fit with

the three-dimensional structure of extracellular proteins from soil *Actinobacteria* (Kudla et al. 2005). Different than the cell wall-digesting enzymes above, expansins soften cell walls by breaking noncovalent bonds between cell wall fibrils, thereby allowing a sliding of fibrils past each other (Cosgrove 2000). Expansin activity in plant cells was confirmed in proteins derived directly from PCN as well as from the expressed *GR-exp1* product (Qin et al. 2004).

4.2 Cellular Metabolism and Transport

4.2.1 Chorismate Mutase

The esophageal gland cells of both root-knot (Huang et al. 2005; Lambert et al. 1999) and cyst nematodes (Gao et al. 2003; Jones et al. 2003) express parasitism genes encoding chorismate mutase, a pivotal enzyme in the shikimate pathway that converts chorismate to prephenate (Romero et al. 1995). The activity of chorismate mutase is a key regulatory mechanism that determines the cellular balance of the aromatic amino acids phenylalanine, tyrosine, and tryptophan (Romero et al. 1995). The metabolites that have these amino acids as precursors, among which auxin, salicylic acid, and phenylpropanoid derivatives are of particular interest in plant–parasite interactions, would in theory be influenced by introduction of chorismate mutase into host cells by nematodes. Cytoplasmic expression of a root-knot nematode chorismate mutase gene in soybean hairy root cells produced tissues with an auxin-deficient phenotype that could be reversed by the application of exogenous auxin (Doyle and Lambert 2003). A model derived from these results suggests that nematode-secreted chorismate mutases will deplete the cytoplasmic chorismate pool leading to an increased export of chorismate from host cell plastids into the cytoplasm, effectively decreasing synthesis of plastid-produced chorismate-dependent metabolites like auxin or salicylic acid (Doyle and Lambert 2003). Reduction in cellular salicylic acid or phenylpropanoid production in response to the introduction of nematode chorismate mutase in host cell cytoplasm could result in a down-regulation of plant defense against the invading nematode. Consistent with a putative function in defense inactivation, chorismate mutase genes are polymorphic and apparently selected among soybean cyst nematode isolates that differ in capacity to infect soybean genotypes with different sources of resistance (Bekal et al. 2003; Lambert et al. 2005).

4.2.2 Annexin

The dorsal esophageal gland cell of *H. glycines* expresses a gene homologous to annexin that has a predicted signal peptide for secretion (Gao et al. 2003), and another annexin without signal peptide has been isolated from the amphids and other tissues of *Globodera pallida* (Fioretti et al. 2001). Immunodetection of the *G. pallida* annexin was confirmed in nematode excretory-secretory products of J2 stimulated

with 5-methoxy DMT oxalate; however, the *H. glycines* 4F01 annexin (Gao et al. 2003) was immunolocalized exclusively within the dorsal esophageal gland cell (Patel 2008). Annexin genes represent a large family coding for calcium-dependent phospholipid-binding proteins with a wide range of reported functions (Clark et al. 2001), and several known activities of annexin are related to the phenotype of the nematode feeding cells induced in hosts. Some annexins (Clark et al. 2001) are calcium-dependent membrane-binding proteins that may potentially function to regulate ion transport across (feeding) cell membranes and also act to detoxify oxygen radicals that may accumulate across cell membranes (such as those associated with hypersensitive cell death). In plant cells, annexins are associated with Golgi-mediated secretion of new cell membrane and cell wall glucans (Clark et al. 2001). Co-immunoprecipitation of plant annexin with cellulose synthase (CesA) has been reported (Hofmann et al. 2003), suggesting that annexin introduced into host plant cells by nematodes may modulate the extreme modifications of cell walls observed in nematode feeding cells. Sensitivity to osmotic stress demonstrated in the *AnnAt1* mutant of *Arabidopsis thaliana* (Lee et al. 2004) could be complemented by expression of the *H. glycines* 4F01 annexin (Patel 2008), suggesting one potential role for secreted nematode annexin in regulating the osmoticum of host feeding cells.

4.2.3 Calreticulin

Proteomic analysis isolated calreticulin in secretions induced in vitro by J2 root-knot nematode, and the gene encoding this calreticulin was expressed in the nematode's subventral esophageal gland cells (Jaubert et al. 2002). Calreticulins are calcium-binding proteins that are involved in multiple cellular processes including ER-chaperones, nuclear export, mRNA degradation, cell adhesion, and cell calcium homeostasy (Michalak et al. 2002). Although the role(s) of secreted calreticulin in plant–nematode interactions is unclear, secretion of calreticulin in host tissues by several parasites of vertebrates, including nematode parasites (Suchitra and Jochi 2005), suggests a key role for these secretions in parasitism. The secretion of root-knot nematode calreticulin has also been detected within plant tissues, including immunolocalization of secreted calreticulin outside the stylet tip along the host cell wall (Jaubert et al. 2005).

4.3 Cellular Regulation and Targeting

4.3.1 Nuclear Localized Parasitism Proteins

A significant number of the proteins encoded by parasitism genes expressed in plant nematode esophageal gland cells (Gao et al. 2003; Huang et al. 2003) contain both a predicted secretion signal peptide and a motif encoding a putative nuclear localization signal (NLS). These data present the tempting hypothesis that the

secreted products of some nematode parasitism genes become localized to the host cell nucleus. This is not without precedent since antigens of secreted products from the animal-parasitic nematode *Trichinella spiralis* have been immunolocalized to the nucleus of host muscle cells (Jasmer et al. 2003). Expression of GFP and GUS-tagged SCN parasitism proteins in plant cells has demonstrated that some of the predicted NLS domains do indeed function to import products into the plant cell nucleus (Elling et al. 2007b; Tytgat et al. 2004). DNA-binding domains are also predicted in some of the NLS-containing proteins predicted to be secreted by plant-nematodes, suggesting an extraordinary potential for regulatory control within the nucleus of host feeding cells if confirmed.

4.3.2 Ranbpm

Expressed genes encoding secretory proteins with high similarity to proteins that bind to the small G-protein Ran, so-called RanBPMs (Ran-Binding Protein in the Microtubule organizing center), were identified in the dorsal esophageal gland cell of cyst nematodes (Blanchard et al. 2005; Qin et al. 2000). If secreted into plant feeding cells, RanBPM from nematodes could hypothetically interact with the microtubule network involved in spindle formation during host cell mitosis and subsequently affect host cell cycle (Wilde et al. 2001). Altered regulation of host feeding cell cycle is a primary manifestation of plant parasitism by sedentary endoparasitic nematodes (Goverse et al. 2000), but the regulatory foundation of these effects is unknown. Confirmation of secretion of RanBPM from nematodes and functional verification of nematode RanBPM in plant cells will be important steps to understand the potential role of secreted RanBPM in plant–nematode interactions.

4.3.3 Ubiquitination/Proteasome Functions

Several parasitism genes expressed in cyst nematode esophageal gland cells encode secreted isotypes of cytoplasmic proteins involved in the ubiquitination pathway, namely ubiquitin itself, along with proteins (i.e. RING-Zn-Finger-like and Skp1-like proteins) similar to those found in the host E3 ubiquitin protein ligase complex (Gao et al. 2003). Targeted and timed protein degradation (Estelle 2001) may provide a powerful and unique means for regulation of host cell phenotype by nematodes, including potential effects within the host cell nucleus and cytoplasm. Secreted proteasome proteins from cyst nematodes could be involved in polyubiquitination of target host cell proteins for degradation to modulate cellular defense and cell signaling, to influence host cell cycle, or simply to provide a substrate for nutrient uptake by the nematode. Precedent for a potential role in modulating host defense is demonstrated by the activity of a domain of *Pseudomonas syringae* AvrPtoB that functions as a mimic of host plant E3 ubiquitin ligase (Janjusevic et al. 2006). The ubiquitin extension proteins that are predicted to be secreted from cyst nematodes contain a unique nonribosomal extension peptide that is distinct from those of plants

(Gao et al. 2003; Tytgat et al. 2004). The potential exists that ubiquitin functions as a chaperone of this unique extension peptide signal of nematode origin within host cells. Though no functional data on secreted proteasome members from nematodes exists, these putative parasitism gene products are prime candidates for protein interaction studies with host cell components.

4.3.4 The 14-3-3 Protein Family

Two isoforms of a protein identified as a member of the 14-3-3 family were isolated from stylet secretions induced in vitro from J2 root-knot nematode (Jaubert et al. 2004). The expressed genes encoding each 14-3-3 were cloned, and in situ hybridization analysis indicated that one isoform (14-3-3a) was expressed in genital primordia and the other isoform (14-3-3b) was expressed within the dorsal esophageal gland of root-knot nematode juveniles. Expression of a 14-3-3 gene in *H. glycines* has also been localized within genital primordia of infective J2 (E.L. Davis, unpublished). Interestingly, although the 14-3-3b protein was originally isolated from stylet secretions, no signal peptide for secretion was predicted from its coding sequence. Members of the 14-3-3 family are associated with parasites and have diverse roles in primary metabolism, regulation of cellular stress response and defense, organelle trafficking, and the cell cycle in which 14-3-3 often acts as an essential interacting protein or chaperone (Siles-Lucas and Gottstein 2003). All of these potential roles of 14-3-3 are mirrored in observed changes within nematode feeding cells, making the potential of a secreted 14-3-3 (e.g. 14-3-3b) from nematodes an interesting system for functional analyses.

4.4 Mitigating Host Response

4.4.1 Venom Allergen Proteins

The products of the parasitism genes listed above are similar to functionally characterized proteins from other organisms and promote the formulation of defined hypotheses about protein function during parasitism. There are some parasitism protein candidates, however, that are similar to known proteins but whose functions remain obscure. One intriguing group of parasitism proteins contains representatives from root-knot nematodes (Ding et al. 2000) and cyst nematodes (Gao et al. 2001b; Vanholme et al. 2005) that are collectively called venom allergen-like proteins (vaps). Genes encoding vaps are expressed in the subventral esophageal gland cells of these nematodes in the early stages of parasitism, but their secretion in plants and role(s) in parasitism are unclear. Gene sequences for these venom proteins were first described from hymenopteran insects (Fang et al. 1988), and vaps were also identified as secreted proteins (ASP) in the animal-parasitic nematode *Ancylostoma caninum* (Zhan et al. 2003). Genes encoding vaps have since been

found in other nematodes, including parasites as well as the free-living *C. elegans* (Mitreva et al. 2005b). The role of vaps in promoting parasitism is unclear since vaps of excretory-secretory origin have been demonstrated to stimulate mammalian immune response in animal-parasites (Jasmer et al. 2003). The seemingly ubiquitous nature of vaps among nematodes, even nonparasites, suggests a function basic to nematode biology that may have (as of yet, unknown) effects on parasitism.

4.4.2 Surface Defense

The hypodermis is a syncytial cell layer directly beneath the (nonliving) nematode cuticle that forms the new cuticle during molts and secretes a number of molecules for deposition on the cuticle surface of the nematode body (Bird and Bird 1991). Several genes expressed within the hypodermis encode proteins deposited on the cuticle surface that are in direct contact with host cells during nematode invasion of plant tissues. Among the dynamic mixture of proteins at the cuticle surface are proteins with potential roles in mitigating host defense response. Peroxidase genes are expressed in the potato cyst nematode hypodermis (Jones et al. 2004; Robertson et al. 2000), and the peroxidase proteins accumulate on the nematode body surface presumably to detoxify reactive oxygen species generated by the defense response of the host (Waetzig et al. 1999). An antiserum that bound to surface molecules of potato cyst nematode isolated cDNA expressing a homologue to the fatty acid- and retinol-binding (FAR) protein (Prior et al. 2001) family isolated from *C. elegans*. The FAR gene was expressed in the hypodermis of PCN, and its recombinant product demonstrated the ability to bind to retinol and fatty acids of C_{11} to C_{24} length. Recombinant FAR from PCN bound to two plant fatty acids, linolenic and linoleic acid, that are of significance as precursors of plant defense compounds and the jasmonic acid defense signaling pathway. Genes encoding SXP/RAL-2 proteins were expressed in the hypodermis and amphids of the potato cyst nematode (Jones et al. 2000) and exclusively in the subventral gland cells of J2 root-knot nematodes (Tytgat et al. 2005). The SXP-RAL-2 family is limited, to date, to both parasitic and nonparasitic nematodes and, like vaps, may play a fundamental functional role in nematode biology. Immunomodulation of vertebrate hosts has been achieved with SXP-RAL-2 of animal-parasitic nematodes (Wang et al. 1997), providing an undefined function in stimulating host response during parasitism.

4.4.3 Stealth Signals

Several molecules from nematodes with observable effects on plant cells have been reported, but the nature and origins of these molecules from nematodes are unclear. The isolation of secreted cytokinins from root-knot nematode juveniles in the absence of plant hosts (De Meutter et al. 2003) confirmed earlier evidence suggesting that nematodes produce cytokinin endogenously (Dimalla and Van Staden 1977). Since the pathway and tissue localization for cytokinin synthesis could not be

confirmed in the nematode, it was suggested that cytokinins of nematode origin may be an excreted waste product of nucleic acid degradation. This hypothesis relates to the localized hyperplasia that is a hallmark of gall formation, cell cycle regulation in feeding sites, and other cytokinin effects stimulated in roots by root-knot nematodes (Lohar et al. 2004). Incubation of roots of the legume *Lotus japonicus* in the presence of viable juveniles of root-knot nematode prior to infection stimulated identical cytoskeletal activity in root hairs as observed by treatment with rhizobial Nod factors (Weerasinghe et al. 2005). The "NemF" (Nem Factor) from root-knot nematodes also provided a response identical to Nod factor in Nod-receptor mutants, and a similar response to NemF was observed in root hairs of tomato. The identity of NemF and its origins in root-knot nematode have not been reported.

4.5 Bioactive Peptides

Perhaps the most interesting group of parasitism genes are those that encode signaling peptides. Secretions collected and fractionated from hatched juveniles of the potato cyst nematode contained a peptide or peptides of less than 3 kDa that induced mitogenic activity in tobacco leaf protoplasts and human peripheral blood mononuclear cells (Goverse et al. 1999). The nature and origins of this bioactive peptide or peptides from PCN are unknown. Two parasitism genes that encode secreted bioactive peptides produced in the esophageal gland cells of plant nematodes have been the subject of considerable characterization and functional analyses. HG-SYV46, the parasitism gene expressed most strongly in the dorsal gland cell of *H. glycines* during parasitism (Gao et al. 2003), was first isolated from a screen of an expressed *H. glycines* gland cell-specific cDNA library for signal peptides that function in secretion (Wang et al. 2001). Database searches of the complete predicted protein provided no significant homology, but the C-terminus of the SYV46 protein contained the consensus domain of known CLAVATA3/ESR-like (CLE) plant signaling peptides (Olsen and Skriver 2003). Plant CLV3 peptide regulates the balance of stem cell proliferation and differentiation in the shoot meristem through interactions with a CLAVATA1/CLAVATA2 receptor complex to negatively regulate expression of WUSCHEL (Fletcher 2002). Over-expression of HG-CLE in wild-type *Arabidopsis* negatively regulated *wus* expression in the shoot meristem (Mitchum et al. 2008; Wang et al. 2005) and promoted the *wus* phenotype, arrested shoot apical meristem (Fig. 4). Conversely, expression of HG-CLE in a *clv3*-deficient mutant was able to restore the wild-type number and size of floral organs in *Arabidopsis*. The data suggested that secreted HG-CLE may play a role in feeding cell differentiation in roots via direct interaction with a CLV1-like receptor or as a competitive inhibitor of CLE in roots that would augment wild-type differentiation of affected root cells (Davis and Mitchum 2005; Mitchum et al. 2008). Interestingly, the cloned *rhg1* SCN-resistance gene (Lightfoot and Meksem 2002) has homology to the *Xa21* resistance gene and the *CLV1* receptor gene reported in the public database and, like chorismate mutase, polymorphisms of *Hg-cle* correlate to SCN ability to infect soybean genotypes that contain different SCN resistance genes (M.G. Mitchum, pers. comm.).

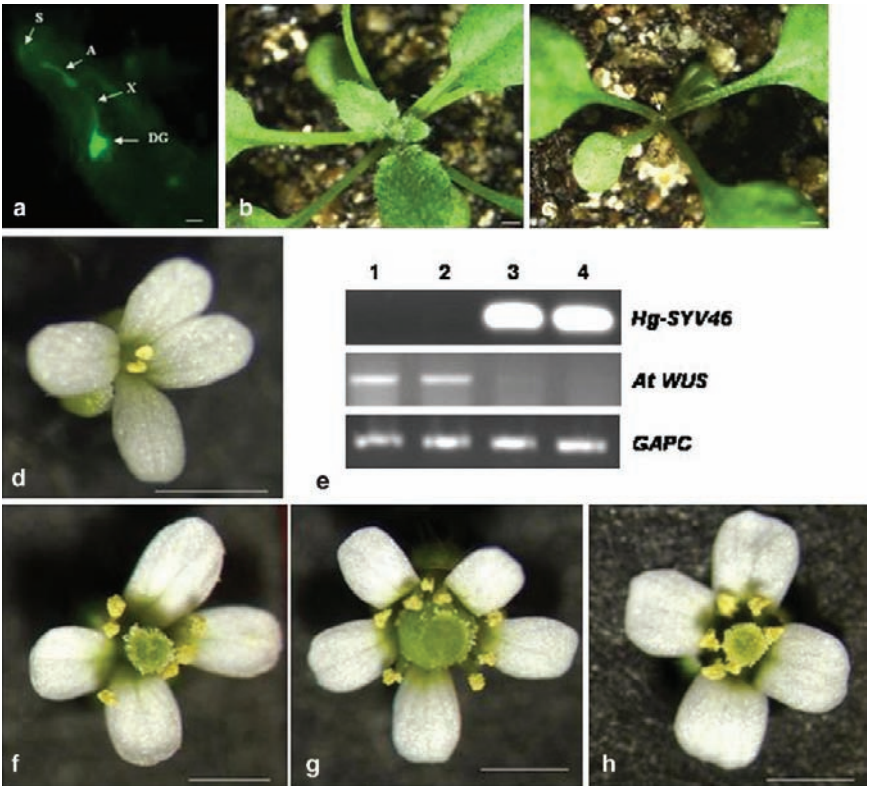


Fig. 4 Localization of HG-SYV46 in *Heterodera glycines* and effects of *Hg-SYV46* expression in transgenic *Arabidopsis thaliana*. (**a**) Polyclonal antibodies localized HG-SYV46 within the dorsal esophageal gland cell, its extension, and the gland cell ampulla at the base of the stylet (feeding spear) of a parasitic stage of *H. Glycines* dissected from a host plant root. (**b**) The developing shoot of a wild-type seedling (ecotype Columbia-0) at 20-days post-germination. (**c**) A *35S::Hg-SYV46* transgenic *Arabidopsis* plant at 20-days post-germination showing an arrested shoot apical meristem (*arrow*). (**d**) Flower of a *35S::Hg-SYV46* transgenic *Arabidopsis* plant showing a decreased number of stamens and missing carpels as compared to wild-type, a phenotype similar to a *wus* flower (Laux et al. 1996). (**e**) RT-PCR analysis of *Hg-SYV46* and *WUS* expression in the shoot apices of *Hg-SYV46* nonexpressed (*promoterless::Hg-SYV46*, lanes 1 & 2) and expressed (*35S::Hg-SYV46*, lanes 3 & 4) individual transgenic *Arabidopsis* lines from 10-day-old seedlings. Expression of the *Arabidopsis gapc* gene was used as an internal control. (**f**) A wild-type *Arabidopsis* flower containing four petals, six stamens, and two fused carpels. (**g**) Flower of an *Arabidopsis clv3-1* mutant (Clark et al. 1995) carrying more floral organs in all whorls than wild-type. (**h**) A fully restored flower of a *clv3-1* mutant with wild-type floral organ size and number obtained from a *35S::Hg-SYV46/clv3-1* transgenic plant. Abbreviations: DG = dorsal gland, X = extension, A = ampulla. *Scale bars* 0.1 mm in **a**, 1.0 mm in **b**–**d** and **f**–**h**. Reprinted from Wang et al. (2005) with permission from Blackwell Publishing Ltd

An expressed parasitism gene encoding a peptide (16D10) with CLE signature was also identified from the subventral esophageal gland cells of root-knot nematode (Huang et al. 2003). The mature 16D10 peptide contained only 13 amino acids and was immunodetected in secretions from the pre-infective juvenile stage of

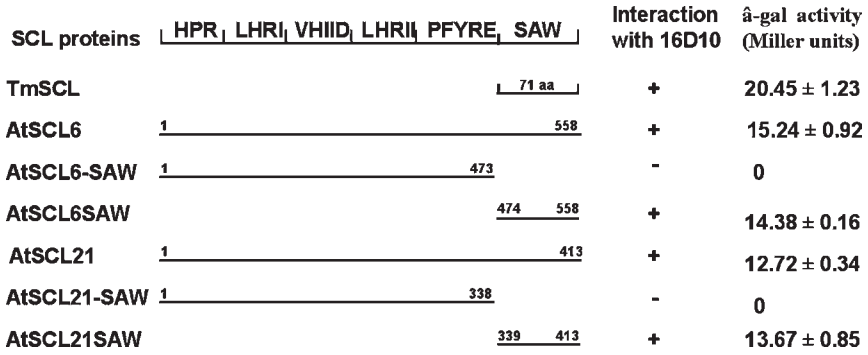


Fig. 5 Interaction of the root-knot nematode 16D10 peptide with plant SCARECROW-like (SCL) transcription factors. The schematic demonstrates the direct interaction of 16D10 with the SAW domain of tomato (tmscl) and *Arabidopsis* (atscl6 and atscl21) proteins in yeast. The specific domains of the SCL proteins represented schematically were tested to interact individually with 16D10. Positive interactions resulting in the activation of HIS3, ADE2, and lacz genes were detected by growth in the absence of histidine and adenine, and the beta-galactosidase activity presented here. Reprinted from Huang et al. (2006a) with permission from APS Press

root-knot nematode. Over-expression of 16D10 was not able to affect the floral phenotype of an *Arabidopsis clv3*-1 mutant as observed for *Hg-cle*, but expression of 16D10 significantly accelerated root growth in both whole *Arabidopsis* plants and tobacco hairy root assays (Huang et al. 2006a). The effects of 16D10 were root-specific and did not result in observable changes in root morphology except for accelerated root proliferation. A yeast two-hybrid screen and co-immunoprecipitation experiments using *Arabidopsis* over-expressing 16D10 demonstrated a specific interaction of the 16D10 peptide with the SAW domain (Fig. 5) of expressed plant SCARECROW-like (SCL) transcription factors. Members of the SCL family are primarily active in plant roots and regulate downstream pathways of root cell growth and differentiation (Pysh et al. 1999). The results of the root-knot nematode 16D10 experiments (Huang et al. 2006a) provide the first evidence that a secreted nematode parasitism gene product may regulate host gene activity via binding to an intracellular plant transcription factor to modulate root cell growth during feeding cell initiation.

5 Perspectives on Nematode Parasitism Genes

Molecules at the interface of the nematode and host are at the front lines of molecular communication and interaction during plant parasitism. Our emerging understanding of the functions of parasitism genes and genetic pathways that promote acquisition of nutriment from the living host suggests that they are diverse and tightly integrated with mechanisms adapted to sustain a parasitic lifestyle. For migratory nematodes that do not engage in a sustained interaction with host plant cells, the adaptations for

nutrient acquisition may be little more than enzymes that promote stylet penetration of host cells and digestion of host-derived nutrients. Migratory parasites that enter plant tissues face additional obstacles to feeding, including migration through different tissues and evasion of host defense (Hussey and Grundler 1998). Emerging information on expressed genes in parasitic stages of migratory endoparasitic nematodes (McCarter et al. 2005), such as *Pratylenchus* and *Bursaphelenchus*, and migratory stages of sedentary endoparasitic nematodes suggests that there are some common adaptations to navigate and survive within the host. Plant nematodes that form a sustained feeding relationship with host cells show another level of adaptive sophistication for parasitism that, at present, appears to be more advanced than the adaptations of migratory parasites (Baldwin et al. 2004; Davis et al. 2004). At the cellular level, this appears to be true even of ectoparasitic nematodes that form sedentary feeding relationships. The feeding of *Criconemella xenoplex* from the same subepidermal cell for days results in unique modifications of neighboring plasmodesmata for solute transport (Hussey et al. 1992a) and likely requires suppression of host defense at some level since plant callose deposition is stimulated around the nematode stylet (Hussey et al. 1992b). Host cellular response to feeding by the sedentary ectoparasite *Xiphinema* has striking similarities with giant-cells formed by *Meloidogyne* (Hussey and Grundler 1998). A number of expressed genes encoding secreted proteins have recently been identified from the pharyngeal gland region of *Xiphinema index* (Furlanetto et al. 2005). As the genomes of sedentary parasites are further explored, "generic" mechanisms underlying the foundations of sustained parasitic relationships may be revealed.

The root-knot and cyst nematodes have emerged as primary models of plant parasitism because of their economic significance (Barker 1998) and the extreme changes in host tissues that they induce (Hussey and Grundler 1998). In both molecular and structural aspects, plant response at these nematode feeding sites has similarities to other plant–microbe interactions (Davis and Mitchum 2005), but the cells induced for feeding within these sites are a unique adaptation to plant infection by nematodes. The growth and development of the sedentary stages of these species must be accommodated and tolerated by the invaded host tissues. Evidence described above suggests that molecules at the nematode body surface that were synthesized and secreted from the hypodermis or released from natural openings of the nematode body may have been adapted to mitigate response of host cells by contact or to mask recognition of the nematode as an invader. Few products of nematode metabolism or secondary pathways that play roles in parasitism have been isolated and characterized, but emerging genomic data and the potential of metabolomics may begin to uncover these potential adaptations. Our knowledge of nematode parasitism genes to date is likely far from exhaustive, and the data derived from plant nematode genome sequencing projects will most certainly illuminate our understanding of adaptations for parasitism. At present, parasitism gene study in nematodes has focused on products encoding expressed secretory proteins that may be direct or processed products of developmentally expressed genes, and the majority of these genes have been identified from the esophageal gland cells of tylenchid plant-parasites that have evolved elaborate and dynamic secretory activity

(Baum et al. 2006; Davis et al. 2004, 2008; Mitchum et al. 2007; Vanholme et al. 2004). The products of these gland cells are directed for secretion from the nematode stylet into the plant tissues in all stages of parasitism, including secretion into cells modified for nutrient ingestion by the nematode. The migratory stages of endoparasitic plant nematodes are dominated by the expression of parasitism genes encoding cell wall-modifying proteins that promote either intercellular or intracellular migration in conjunction with the mechanical force of stylet thrusts. The nematode genes encoding cellulases, pectinases, hemicellulases, and expansin have two primary criteria in common: (1) they are expressed in the subventral esophageal gland cells most active in the early stages of nematode infection and; (2) they all have striking sequence similarity to corresponding enzymes of microbial origin, suggesting horizontal acquisition of genes encoding cell wall-modifying proteins from soil microbes as a major driving force in the evolution of plant parasitism by nematodes (Baldwin et al. 2004; Davis et al. 2000; Jasmer et al. 2003; Ledger et al. 2006).

The transition from the migratory to the sedentary stages of parasitism is a continuum and may be the result of triggering a "developmental switch" akin to emergence from arrested development (dauer) upon environmental cues (Elling et al. 2007a; Mitreva et al. 2004). In some respects, this transition is mirrored in the expression of some parasitism genes within the esophageal gland cells (Davis et al. 2004; Hussey 1989). While the cell wall-modifying genes decrease in activity within the subventral gland cells during this transition, some subventral gland genes continue to be expressed throughout parasitism, and a few are expressed predominantly in the sedentary parasitic stages. Chorismate mutase is expressed in both dorsal and subventral gland cells throughout parasitism (Doyle and Lambert 2003), and the multiple roles of this pivotal metabolic enzyme may change in function as feeding sites are developed. A role for subventral gland parasitism gene products in feeding cell initiation is suggested by the interaction of 16D10 peptides with a plant transcription factor and its effects on root cell growth (Huang et al. 2006a). The activity and nature of multiple parasitism genes expressed in the nematode dorsal esophageal gland cell as feeding sites develop suggests significant and diverse "regulatory" roles for these secretions in parasitism (Gao et al. 2003; Huang et al. 2003). The nature of nematode dorsal gland parasitism gene products suggests multiple mechanisms for the nematode to modulate host feeding cell cycle, metabolism, transport, defense, gene expression, and production of a feeding tube. In contrast to the cell wall-modifying genes, very few of the candidate parasitism genes expressed in the dorsal gland cell have sequence similarity to genes reported from soil microbes. Some of these parasitism genes reflect adaptations of functional domains encoded by endogenous nematode genes for secretion and regulation of parasitism or potential convergent evolution to mimic the products of host plant genes. Polymorphisms observed among some nematode parasitism genes suggest that differential domains of these effectors may have experienced diversifying selection for alleles encoding virulence factors (Birch et al. 2006) that interact directly or indirectly with natural resistance genes. Concerning more global adaptations for parasitism, the observation that diverse suites of novel parasitism genes are expressed by root-knot and cyst nematodes may be reflective of the differential ontogeny of giant-cells and syncytia, respectively

(Davis and Mitchum 2005). The emerging data suggests that nematode parasitism gene products may interact with extracellular and intracellular host cell targets, both of which are accessible to the nematode stylet orifice. How the synthesis and secretion of the different parasitism proteins are regulated spatially and temporally within the same esophageal gland cell is unknown, but it would make a fascinating model for the study of secretory cell biology.

Current in vivo functional analyses of candidate parasitism genes are limited to two primary options, expression of the candidate gene in plants or disruption of the candidate gene in the nematode via gene silencing. Direct expression of candidate nematode genes in whole plants, roots, or cells can, and has, provided biological information relative to the plant–nematode interaction when an observable phenotype is produced (Doyle and Lambert 2003; Huang et al. 2006a; Wang et al. 2005). The activities of multiple members of parasitism gene families and potential interactions of nematode parasitism proteins released concomitantly into the host provide an added level of complexity to assess functional roles in parasitism. The potential to profile host gene expression upon expression of nematode parasitism genes offers the opportunity for comparative analyses to the host gene expression observed in nematode feeding sites. When they can be employed, the use of model plant species and their mutants are of immense benefit to more specifically define parasitism gene function. Although the dissection of individual components facilitates analysis of their function, it stands in contrast to the interaction of multiple components that contributes to the natural infection process. The appeal of gene silencing techniques is the potential to disrupt a single factor (or family) from the interaction and assess potential effects. As with gene expression in hosts, in the absence of high-throughput analyses, the potential success of gene silencing assays are predicated on the generation of an observable phenotype. These are not insurmountable obstacles, however, as the specific effects of some RNAi soaking assays on nematode infectivity of hosts have been observed (Bakhetia et al. 2005a; Chen et al. 2005; Rosso et al. 2005; Urwin et al. 2002). Improved efficiency, delivery, and targeting of gene silencing techniques for plant-parasitic nematodes, including host-derived RNAi (Gheysen and Vanholme 2007; Huang et al. 2006b; Steeves et al. 2006; Yadav et al. 2006), hold great promise for functional analyses of candidate nematode parasitism genes and potential applications for nematode management in crops.

Although the secretion of esophageal gland proteins into plant tissues by nematodes has been demonstrated in video and immunoassays (Doyle and Lambert 2003; Goellner et al. 2001; Jaubert et al. 2005; Wyss and Zunke 1986; Wyss et al. 1992; Wang et al. 1999), the intracellular localization of nematode secretions within feeding cells remains a technical challenge. The demonstration of functional nuclear localization signals (Elling et al. 2007b) and a nematode secretory peptide that can interact with a specific domain of a plant transcription factor (Huang et al. 2006a) have provided tempting models for direct analyses within feeding cells. Application of advanced microscopy that can detect modification or subcellular localization of nematode secretions and their interactions with host molecules within cells lies at the frontier of functional analyses of nematode parasitism. The products of nematode parasitism genes described in this treatise provide a launching pad for such further exploration.

References

Abad P et al. (2008) Genome sequence of the metazoan plant-parasitic nematode *Meloidogyne incognita*. Nature Biotech 26:909–915

Bakhetia M, Charlton W, Atkinson HJ, McPherson MJ (2005a) RNA interference of dual oxidase in the plant nematode *Meloidogyne incognita*. Mol Plant Microbe Interact 18:099–1106

Bakhetia M, Charlton WL, Urwin PE, McPherson MJ, Atkinson HJ (2005b) RNA interference and plant parasitic nematodes. Trends Plant Sci 10:362–367

Baldwin JG, Nadler SA, Adams BJ (2004) Evolution of plant parasitism among nematodes. Annu Rev Phytopathol 42:83–105

Barker KR (1998) Introduction and synopsis of advancements in nematology. In: Barker KR, Pederson GA, Windham GL (eds) Plant nematode interactions. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, pp 1–20

Baum TJ, Davis EL, Hussey RS (2006) Root-knot and cyst nematode parasitism genes: the molecular basis of plant parasitism. In: Setlow J. K. (ed.), Genetic Engineering, Principles and Methods, vol 28, Springer Science + Business Media, Heidelberg, pp. 17–43

Bekal S, Niblack T, Lambert K (2003) A chorismate mutase from the soybean cyst nematode *Heterodera glycines* shows polymorphisms that correlate with virulence. Mol Plant Microbe Interact 16:439–446

Benfey PN, Chua NH (1990) The cauliflower mosaic virus-35S promoter – combinatorial regulation of transcription in plants. Science 25:959–966

Bera-Maillet C, Arthaud L, Abad P, Rosso MN (2000) Biochemical characterization of MI-ENG1, a family 5 endoglucanase secreted by the root-knot nematode *Meloidogyne incognita*. Eur J Biochem 267:3255–3263

Birch PRJ, Rehmany AP, Pritchard L, Kamoun S, Beynon JL (2006) Trafficking arms: oomycete effectors enter host plant cells. Trends Microbiol 14:8–11

Bird AF (1968) Changes associated with parasitism in nematodes. IV. Cytochemical studies on the ampulla of the dorsal gland of Meloidogyne javanica and on exudations from the buccal stylet. J Parasitol 54:879–890

Bird AF, Bird J (1991) The structure of nematodes. Academic Press, San Diego

Blanchard A, Esquibet M, Fouville D, Grenier E (2005) Ranbpm homologue genes characterized in the cyst nematodes *Globodera pallida* and *Globodera mexicana*. Physiol Mol Plant Pathol 67:15–22

Bohner S, Lenk I, Rieping M, Herold M, Gatz C (1999) Transcriptional activator TGV mediates dexamethasone-inducible and tetracycline inactivatable gene expression. Plant J 19:87–95

Chapman S, Kavanagh T, Baulcombe DC (1992) Potato virus X as a vector for gene expression in plants. Plant J 2:549–557

Chen Q, Rehman S, Smant G, Jones JT (2005) Functional analysis of pathogenicity proteins of the potato cyst nematode *Globodera rostochiensis* using RNAi. Mol Plant Microbe Interact 18:621–625

Cho HJ, Farrand SK, Noel GR, Widholm JM (2000) High-efficiency induction of soybean hairy roots and propagation of the soybean cyst nematode. Planta 210:195–204

Clark GB, Sessions A, Eastburn DJ, Roux SJ (2001) Differential expression of members of the annexin multigene family in *Arabidopsis*. Plant Physiol 126:1072–1084

Clark SE, Running MP, Meyerowitz EM (1995) *CLAVAT3* is a specific regulator of shoot and floral meristem development affecting the same process as *CLAVAT1*. Development 121: 2057–2067

Cosgrove DJ (2000) Loosening of plant cell walls by expansins. Nature 407:321–326

Davis EL, Mitchum MG (2005) Nematodes: sophisticated parasites of legumes. Plant Physiol 137:1182–1188

Davis EL, Hussey RS, Baum TJ, Bakker J, Schots A, Rosso MN, Abad P (2000) Nematode parasitism genes. Annu Rev Phytopathol 38:365–396

Davis EL, Hussey RS, Baum TJ (2004) Getting to the roots of parasitism by nematodes. Trends Parasitol 20:134–141

Davis EL, Hussey RS, Mitchum MG, Baum TJ (2008) Parasitism proteins in nematode–plant interactions. Curr Opinion Plant Biol 11:360–366

De Boer JM, Smant G, Goverse A, Davis EL, Overmars HA, Pomp HR, Van Gent Pelzer M, Zilverentant JF, Stokkermans J, Hussey RS, Gommers FJ, Bakker J, Schots A (1996) Secretory granule proteins from the subventral esophageal glands of the potato cyst nematode identified by monoclonal antibodies to a protein fraction from second-stage juveniles. Mol Plant Microbe Interact 9:39–46

DeBoer JM, Yan Y, Bakker J, Davis EL, Baum TJ (1998) *In situ* hybridization to messenger RNA of *Heterodera glycines*. J Nematol 30:309–312

DeBoer JM, Yan Y, Wang X, Smant G, Hussey RS, Davis EL, Baum TJ (1999) Developmental expression of secretory beta-1,4-endoglucanases in the subventral esophageal glands of *Heterodera glycines*. Mol Plant Microbe Interact 12:663–669

De Meutter J, Vanholme B, Bauw G, Tytgat T, Gheysen G, Gheysen G (2001) Preparation and sequencing of secreted proteins from the pharyngeal glands of the plant parasitic nematode *Heterodera schachtii*. Mol Plant Pathol 2:297–301

De Meutter J, Tytgat T, Witters E, Gheysen G, Van Onckelen H, Gheysen G (2003) Identification of cytokinins produced by the plant parasitic nematodes *Heterodera schachtii* and *Meloidogyne incognita*. Mol Plant Pathol 4:271–277

Deubert KH, Rohde RA (1971) Nematode enzymes. In: Zuckerman BM, Mai WF, Rohde RA (eds) Plant parasitic nematodes, vol 2. Academic Press, New York, pp 73–90

Dimalla GG, Van Staden J (1977) Cytokinins in the root-knot nematode, *Meloidogyne incognita*. Plant Sci Lett 10:25–29

Ding X, Shields J, Allen R, Hussey RS (1998) A secretory cellulose-binding protein cDNA cloned from the root-knot nematode (*Meloidogyne incognita*). Mol Plant Microbe Interact 11:952–959

Ding X, Shields J, Allen R, Hussey RS (2000) Molecular cloning and characterization of a venom allergin AG5-like cDNA from *Meloidogyne incognita*. Int J Parasitol 30:77–81

Dong K, Opperman CH (1997) Genetic analysis of parasitism in the soybean cyst nematode *Heterodera glycines*. Genetics 146:1311–18

Doyle EA, Lambert KN (2003) *Meloidogyne javanica* chorismate mutase 1 alters plant cell development. Mol Plant Microbe Interact 16:123–131

Dubreuil G, Magliano M, Deleury E, Abad P, Rosso MN (2007) Transcriptome analysis of root-knot nematode functions induced in the early stages of parasitism. New Phytol 176:426–436

Elling AA, Mitreva M, Recknor J, Gai X, Martin J, Maier TR, McDermott JP, Hewezi T, Bird DM, Davis EL, Hussey RS, Nettleton D, McCarter JP, Baum TJ (2007a) Divergent evolution of arrested development in the dauer stage of *Caenorhabditis elegans* and the infective stage of *Heterodera glycines*. Genome Biol 8:R211

Elling AA, Davis EL, Hussey RS, Baum TJ (2007b) Active uptake of cyst nematode parasitism proteins into the plant cell nucleus. Int J Parasitol 37:1269–1279

Estelle M (2001) Proteases and cellular regulation in plants. Curr Opin Plant Biol 4:254–260

Fang K, Vitale M, Fehlner P, King T (1988) cDNA cloning and primary structure of a white-face hornet venom allergen, antigen 5. Proc Natl Acad Sci (USA) 85:895–899

Fioretti L, Warry A, Porter A, Haydock P, Curtis R (2001) Isolation and localization of an annexin gene (*gp-nex*) from the potato cyst nematode, *Globodera pallida*. Nematology 3:45–54.

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 391:806–11

Fletcher JC (2002) Shoot and floral meristem maintenance in *Arabidopsis*. Annu Rev Plant Biol 53:45–66

Furlanetto C, Cardle L, Brown DJF, Jones JT (2005) Analysis of expressed sequence tags from the ectoparasitic nematode *Xiphinema index*. Nematology 7:95–104

Gao B, Allen R, Maier T, Davis EL, Baum TJ, Hussey RS (2001a) Identification of putative parasitism genes expressed in the esophageal gland cells of the soybean cyst nematode, *Heterodera glycines*. Mol Plant Microbe Interact 14:1247–1254

Gao B, Allen R, Maier T, Davis EL, Baum TJ, Hussey RS (2001b) Molecular characterisation and expression of two venom allergen-like secretory protein genes in *Heterodera glycines*. Int J Parasitol 31:1617–1625

Gao B, Allen R, Maier T, McDermott JP, Davis EL, Baum TJ, Hussey RS (2002) Characterisation and developmental expression of a chitinase gene in *Heterodera glycines*. Int J Parasitol 32:1293–1300

Gao BL, Allen R, Maier T, Davis EL, Baum TJ, Hussey RS (2003) The parasitome of the phytonematode *Heterodera glycines*. Mol Plant Microbe Interact 16:720–726

Gao B, Allen R, Davis EL, Baum TJ, Hussey RS (2004a) Developmental expression and biochemical properties of a ß-1,4-endoglucanase family in the soybean cyst nematode, *Heterodera glycines*. Mol Plant Pathol 5:93–104

Gao B, Allen R, Davis EL, Baum TJ, Hussey RS (2004b) Molecular characterisation and developmental expression of a cellulose-binding protein gene in the soybean cyst nematode *Heterodera glycines*. Int J Parasitol 34:1377–1383

Gheysen G, Fenoll C (2002) Gene expression in nematode feeding sites. Annu Rev Phytopathol 40:191–219

Gheysen G, Vanholme B (2007) RNAi from plants to nematodes. Trends Biotechnol 25:89–92

Gleason CA, Liu QL, Williamson VM (2008) Silencing a candidate nematode effector gene corresponding to the tomato resistance gene *Mi*-1 leads to acquisition of virulence. Mol Plant Microbe Interact 21:576–585

Goellner M, Smant G, DeBoer JM, Baum TJ, Davis EL (2000) Isolation of beta-1,4 endoglucanase genes of *Globodera tabacum* and their expression during parasitism. J Nematol 32:154–165

Goellner M, Wang XH, Davis EL (2001) Endo-beta-1,4-glucanase expression in compatible plant–nematode interactions. Plant Cell 13:2241–2255

Goverse A, Davis EL, Hussey RS (1994) Monoclonal antibodies that bind to the esophageal glands and stylet secretions of *Heterodera glycines*. J Nematol 26:251–259

Goverse A, Rouppe van der Voort J, Roupe van der Voort C, Kavelaars A, Smant G, Schots A, Bakker J, Helder J (1999) Naturally induced secretions of the potato cyst nematode co-stimulate the proliferation of both tobacco leaf protoplasts and human peripheral blood mononuclear cells. Mol Plant Microbe Interact 12:872–881

Goverse A, Engler JD, Verhees J, van der Krol S, Helder J, Gheysen G (2000) Cell cycle activation by plant parasitic nematodes. Plant Mol Biol 43:747–761

Hofmann A, Delmer DP, Wlodawer A (2003) The crystal structure of annexin Gh1 from *Gossypium hirsutum* reveals an unusual S3 cluster. Eur J Biochem 270:2557–2564

Hotopp JC, Clark ME, Oliveira DC, Foster JM, Fischer P, Torres MC, Giebel JD, Kumar N, Ishmael N, Wang S, Ingram J, Nene RV, Shepard J, Tomkins J, Richards S, Spiro DJ, Ghedin E, Slatko BE, Tettelin H, Werren JH (2007) Widespread lateral gene transfer from intracellular bacteria to multicellular eukaryotes. Science 317:1753–1756

Huang G, Gao B, Maier T, Allen R, Davis EL, Baum TJ, Hussey RS (2003) A profile of putative parasitism genes expressed in the esophageal gland cells of the root-knot nematode, *Meloidogyne incognita*. Mol Plant Microbe Interact 16:376–381

Huang G, Dong R, Maier T, Allen R, Davis EL, Baum TJ, Hussey RS (2004) Use of solid-phase subtractive hybridization for the identification of parasitism gene candidates from the root-knot nematode *Meloidogyne incognita*. Mol Plant Pathol 5:217–222

Huang G, Dong R, Allen R, Davis EL, Baum TJ, Hussey RS (2005) Two chorismate mutase genes from the root-knot nematode *Meloidogyne incognita*. Mol Plant Pathol 6:23–30

Huang G, Dong R, Allen R, Davis EL, Baum TJ, Hussey RS (2006a) A root-knot nematode secretory peptide functions as a ligand for a plant transcription factor. Mol Plant-Microbe Interact 19:463–470

Huang G, Allen R, Davis EL, Baum TJ, Hussey RS (2006b) Engineering broad root-knot resistance in transgenic plants by RNAi knockout of a conserved and essential root-knot nematode parasitism gene. Proc Natl Acad Sci USA 103:14302–14306

Hussey RS (1989) Disease-inducing secretions of plant-parasitic nematodes. Annu Rev Phytopathol 27:123–141

Hussey RS, Grundler FM (1998) Nematode parasitism of plants. In: Perry RN, Wright DJ (eds) Physiology and biochemistry of free-living and plant parasitic nematodes. CAB International Press, Wallingford, UK, pp 213–243

Hussey RS, Mims CW (1991) Ultrastructure of feeding tubes formed in giant-cells induced in plants by the root-knot nematode *Meloidogyne incognita*. Protoplasma 162:99–107

Hussey RS, Mims CW, Westcott SW (1992a) Ultrastructure of root-cortical cells parasitized by the ring nematode *Criconemella xenoplax*. Protoplasma 167:55–65

Hussey RS, Mims CW, Westcott SW (1992b) Immunocytochemical localization of callose in root-cortical cells parasitized by the ring nematode *Criconemella xenoplax*. Protoplasma 171:1–6

Janjusevic R, Abramovitch RB, Martin GB, Stebbins CE (2006) A bacterial inhibitor of host programmed cell death defenses is an E3 ubiquitin ligase. Science 311:222–226

Janssen J, Bakker J, Gommers FJG (1991) Mendelian proof for a gene-for-gene relationship between virulence of *Globodera rostochiensis* and the H1 resistance gene in *Solanum tuberosum* ssp. andigena CPC 1673. Rev Nématol 14:207–211

Jasmer DP, Goverse A, Smant G (2003) Parasitic nematode interactions with mammals and plants. Annu Rev Phytopathol 41:245–270

Jaubert S, Ledger TN, Laffaire JB, Piotte C, Abad P, Rosso MN (2002) Direct identification of stylet secreted proteins from root-knot nematodes by a proteomics approach. Mol Biochem Parasitol 121:205–211

Jaubert S, Laffaire JB, Ledger TN, Escoubas P, Amri EZ, Abad P, Rosso MN (2004) Comparative analysis of two 14-3-3 homologues and their expression pattern in the root-knot nematode *Meloidogyne incognita*. Int J Parasitol 34:873–880

Jaubert S, Milac AL, Petrescu AJ, de Almeida-Engler J, Abad P, Rosso MN (2005) In planta secretion of a calreticulin by migratory and sedentary stages of root-knot nematode. Mol Plant Microbe Interact 18:1277–1284

Jones JT, Smant G, Blok VC (2000) SXP/RAL-2 proteins of the potato cyst nematode *Globodera rostochiensis*: secreted proteins of the hypodermis and amphids. Nematology 2:887–893

Jones JT, Furlanetto C, Bakker E, Banks B, Blok V, Chen Q, Phillips M, Prior A (2003) Characterisation of a chorismate mutase from the potato cyst nematode *Globodera pallida*. Mol Plant Pathol 4:43–50

Jones JT, Reavy B, Smant G, Prior A (2004) Glutathione peroxidases of the potato cyst nematode *Globodera rostochiensis*. Gene 324:47–54

Keen NT, Roberts PA (1998) Plant parasitic nematodes: digesting a page from the microbe book. Proc Natl Acad Sci (USA) 95:4789–4790

Kikuchi T, Shibuya H, Jones JT (2005) Molecular and biochemical characterization of an endo-beta-1,3-glucanase from the pinewood nematode *Bursaphelenchus xylophilus* acquired by horizontal gene transfer from bacteria. Biochem J 389:117–125

Kikuchi T, Shibuya H, Aikawa T, Jones JT (2006) Cloning and characterization of pectate lyases expressed in the esophageal gland of the pinewood nematode *Bursaphelenchus xylophilus*. Mol Plant Microbe Interact 19:280–287

Kudla U, Qin L, Milac A, Kielak A, Maissen C, Overmars H, Popeijus H, Roze E, Petrescu A, Smant G, Bakker J, Helder J (2005) Origin, distribution and 3D-modeling of Gr-EXPB1, and expansin from the potato cyst nematode *Globodera rostochiensis*. FEBS Lett 579:2451–2457

Lambert KN, Allen KD, Sussex IM (1999) Cloning and characterization of an esophageal gland specific chorismate mutase from the phytoparasitic nematode *Meloidogyne javanica*. Mol Plant Microbe Interact 12:328–335

Lambert KN, Bekal S, Domier LL, Niblack TL, Noel GR, Smyth CA (2005) Selection of *Heterodera glycines chorismate mutase-1* alleles on nematode-resistant soybean. Mol Plant Microbe Interact 18:593–601

Laux T, Mayer KFX, Berger J, Jurgens G (1996) The *WUSCHEL* gene is required for shoot and floral meristem integrity in *Arabidopsis*. Development 122:87–96

Ledger TN, Jaubert S, Bosselut N, Abad P, Rosso MN (2006) Characterization of a new b-1,4 endoglucanase gene from the root-knot nematode *Meloidogyne incognita* and evolutionary scheme for phytonematode family-5 glycosyl hydrolases. Gene 382:121–128

Lee S, Lee EJ, Yang EJ, Lee JE, Park AR, Song WH, Park OK (2004) Proteomic identification of annexins, calcium-dependant membrane binding proteins that mediate osmotic stress and abscissic acid signal transduction in Arabidopsis. Plant Cell 16:1378–1391

Lightfoot D, Meksem K (2002) Isolated polynucleotides and polypeptides relating to loci underlying resistance to soybean cyst nematode and soybean sudden death syndrome and methods employing the same. US Pat Application Publ #2002144310

Lilley CJ, Bakhetia M, Charlton WL, Urwin PE (2007) Recent progress in the development of RNA interference for plant-parasitic nematodes. Mol Plant Pathol 8:701–711

Lohar DP, Schaff JE, Laskey JG, Kieber JJ, Bilyeu KD, Bird DM (2004) Cytokinins play opposite roles in lateral root formation, and nematode and Rhizobial symbioses. Plant J 38:203–214

Maizels RM, Balic A, Gomez-Escobar N, nair M, Taylor MD, Allen JE (2004) Helminth parasites – masters of regulation. Immunol Rev 201:89–116

McCarter JP, Bird DM, Mitreva M (2005) Nematode gene sequences: update for December 2005. J Nematol 37:417–421

McClure MA, von Mende N (1987) Induced salivation in plant-parasitic nematodes. Phytopathol 77:1463–1469

Michalak M, Robert Parker JM, Opas M (2002) Ca2+ signaling and calcium binding chaperones of the endoplasmic reticulum. Cell Calcium 32:269–278

Mitchum MG, Hussey RS, Davis EL, Baum TJ (2007) Application of biotechnology to understand pathogenesis of nematode plant pathogens. In: Punja ZK, DeBoer S, Sanfacon H (eds) Biotechnology & plant disease management. CABI International, Wallingford, UK, pp 58–86

Mitchum MG, Wang X, Davis EL (2008) Diverse and conserved roles of CLE peptides. Curr Opinion Plant Biol 11:75–81

Mitreva M, McCarter JP, martin J, Dante M, Wylie T, Chiapelli B, Pape D, Clifton SW, Nutman TB, Waterson RH (2004) Comparative genomics of gene expression in the parasitic and free-living nematodes *Strongyloides stercoralis* and *Caenorhabditis elegans*. Genome Res 14:209–220

Mitreva M, Blaxter ML, Bird DM, McCarter JP (2005a) Comparative genomics of nematodes. Trends Genet 21:573–581

Mitreva M, McCarter JP, Arasu P, Hawdon J, Martin J, Dante M, Wylie T, Xu J, Stajich JE, Kapulkin V, Clifton SW, Waterson RH, Wilson R (2005b) Investigating hookworm genomes by comparative analysis of two Ancylostoma species. BMC Genomics 6:58

Mitreva-Dautova M, Roze E, Overmars H, De Graff L, Schots A, Helder J, Goverse A, Bakker J, Smant G (2006) A symbiont-independent endo-1,4-beta-xylanase from the plant-parasitic nematode *Meloidogyne incognita*. Mol Plant Microbe Interact 19:521–529

Neveu C, Jaubert S, Abad P, Castagnone-Sereno P (2003) A set of genes differentially expressed between avirulent and virulent *Meloidogyne incognita* near-isogenic lines encode secreted proteins. Mol Plant Microbe Interact 16:1077–1084

Nielsen H, Engelbrecht J, Brunak S, von Heijne G (1997) Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. Protein Eng 10:1–6

Novina CD, Sharp PA (2004) The RNAi revolution. Nature 430:161–164

Olsen AN, Skriver K (2003) Ligand mimicry? A plant-parasitic nematode polypeptide with similarity to CLAVATA3. Trends Plant Sci 8:55–57

Opperman CH, Bird DMcK, Schaff JE (2008) Genomic analysis of the root-knot nematode genome. Plant Cell Monogr., doi:10.1007/7089_2008_37

Padidam M, Gore M, Lu DL, Smirnova O (2003) Chemical-inducible, ecdysone receptor-based gene expression system for plants. Transgenic Res 12:101–109

Patel N (2008) Functional analyses of cyst nematode parasitism genes. PhD Dissertation, North Carolina State University

Perry RN, Zunke U, Wyss U (1989) Observations on the response of the dorsal and subventral oesophageal glands of *Globodera rostochiensis* to hatching stimulation. Rev Nematol 12:91–96

Popeijus H, Overmars H, Jones J, Blok V, Goverse A, Helder J, Schots A, Bakker J, Smant G (2000) Degradation of plant cell walls by a nematode. Nature 406:36–37

Prior A, Jones JT, Blok VC, Beauchamp J, McDermott L, Cooper A, Kennedy MW (2001) A surface-associated retinol- and fatty acid-binding protein (Gp-FAR-1) from the potato cyst nematode *Globodera pallida*: lipid binding activities, structural analysis and expression pattern. Biochem J 356:387–394

Pysh LD, Wysocka-Diller JW, Cmilleri C, Bouchez D, Benfey PN (1999) The *GRAS* gene family in *Arabidopsis*: sequence characterization and basic expression analysis of the *SCARECROW-LIKE* genes. Plant J 18:111–119

Qin L, Smant G, STokkermans J, Bakker J, Schots A, Helder J (1998) Cloning of a trans-spliced glyceraldehydes-3-phosphate-dehydrogenase gene from the potato cyst nematode *Globodera rostochiensis* and expression of its putative promoter region in *Caenorhabditis elegans*. Mol Biochem Parasitol 96:59–67

Qin L, Overmars H, Popeijus H, Roupe Van Der Voort JNAM, Groenink W, van Koert P, Schots A, Bakker J, Smant G (2000) An efficient cDNA-AFLP-based strategy for the identification of putative pathogenicity factors from the potato cyst nematode *Globodera rostochiensis*. Mol Plant Microbe Interact 13:830–836

Qin L, Prins P, Jones JT, Popeijus H, Smant G, Bakker J, Helder J (2001) GenEST, a powerful bi-directional link between cDNA sequence data and gene expression profiles generated by cDNA-AFLP. Nucleic Acids Res 29:1616–1622

Qin L, Kudla U, Roze EHA, Goverse A, Popeijus H, Nieuwland J, Overmars H, Jones JT, Schots A, Smant G, Bakker J, Helder J (2004) Plant degradation: A nematode expansin acting on plants. Nature 427:30

Robertson L, Robertson W, Sobczak M, Helder J, Tetaud E, Ariyanayagam M, Ferguson M, Fairlamp A, Jones JT (2000) Cloning, expression and functional characterization of a peroxiredoxin from the potato cyst nematode *Globodera rostochiensis*. Mol Biochem Parasitol 111:41–49

Romero RM, Roberts MF, Phillipson JD (1995) Chorismate mutase in microorganisms and plants. Phytochem 40:1015–1025

Rosso MN, Dubrana MP, Cimbolini N, Jaubert S, Abad P (2005) Application of RNA interference to root-knot nematode genes encoding esophageal gland proteins. Mol Plant Microbe Interact 18:615–620

Roze E, Hanse B, Mitreva M, Vanholme B, Bakker J, Smant G (2008) Mining the secretome of the root-knot nematode *Meloidogyne chitwoodi* for candidate parasitism genes. Mol Plant Pathol 9:1–10

Scholl EH, Thorne JL, McCarter JP, Bird DM (2003) Horizontally transferred genes in plant-parasitic nematodes: a high-throughput genomic approach. Genome Biol 4:R39

Semblat JP, Rosso MN, Hussey RS, Abad P, Castagnone-Sereno P (2001) Molecular cloning of a cDNA encoding an amphid-secreted putative avirulence protein from the root-knot nematode *Meloidogyne incognita*. Mol Plant Microbe Interact 14:72–79

Shpigel E, Roiz L, Goren R, Shoseyov O (1998) Bacterial cellulose-binding domain modulates in vitro elongation of different plant cells. Plant Physiol 117:1185–94

Siles-Lucas M, Gottstein B (2003) The 14-3-3 protein: a key molecule in parasites as in other organisms. Trends Parasitol 19:575–581

Smant G, Goverse A, Stokkermans JPWG, De Boer JM, Pomp H, Zilverentant JF, Overmars HA, Helder J, Schots A, Bakker J (1997) Potato root difusate-induced secretion of soluble, basic proteins originating from the subventral oesophageal glands of potato cyst nematodes. Phtyopathol 87:839–845

Smant G, Stokkermans JPWG, Yan YT, de Boer JM, Baum TJ, Wang XH, Hussey RS, Gommers FJ, Henrissat B, Davis EL, Helder J, Schots A, Bakker J (1998) Endogenous cellulases in animals: Isolation of beta-1,4-endoglucanase genes from two species of plant-parasitic cyst nematodes. Proc Natl Acad Sci USA 95:4906–4911

Steeves RM, Todd TC, Essig JS, Trick HN (2006) Transgenic soybeans expressing siRNAs specific to a major sperm protein gene suppress *Heterodera glycines* reproduction. Funct Plant Biol 33:991–999

Suchitra S, Jochi P (2005) Characterization of *Haemonchus contortus* calreticulin suggests its role in feeding and immune evasion by the parasite. Biochim Biophys Acta 1722:293–303

Timmons L, Fire A (1998) Specific interference by ingested dsRNA. Nature 395:854

Tytgat T, Vanholme B, De Meutter J, Claeys M, Couvreur M, Vanhoutte I, Gheysen G, Van Criekinge W, Borgonie G, Coomans A, Gheysen G (2004) A new class of ubiquitin extension proteins secreted by the dorsal pharyngeal gland in plant parasitic cyst nematodes. Mol Plant Microbe Interact 17:846–852

Tytgat T, Vercauteren I, Vanholme B, De Meutter J, Vanhoutte I, Gheysen G, Borgonie G, Coomans A, Gheysen G (2005) An SXP/RAL-2 protein produced by the subventral pharyngeal glands in the plant parasitic root-knot nematode *Meloidogyne incognita*. Parasitol Res 95:50–54

Urwin PE, Moller SG, Lilley CJ, McPherson MJ, Atkinson HJ (1997) Continual green-fluorescent protein monitoring of cauliflower mosaic virus 35S promoter activity in nematode-induced feeding cells in *Arabidopsis thaliana*. Mol Plant Microbe Interact 10:394–400

Urwin PE, Lilley CJ, Atkinson HJ (2002) Ingestion of double-stranded RNA by preparasitic juvenile cyst nematodes leads to RNA interference. Mol Plant Microbe Interact 15:747–752

Valentine T, Shaw J, Blok VC, Phillips MS, Oparka KJ, Lacomme C (2004) Efficient virus-induced gene silencing in roots using a modified tobacco rattle virus vector. Plant Physiol 136:3999–4009

Vanholme B, De Meutter J, Tytgat T, Van Montagu M, Coomans A, Gheysen G (2004) Secretions of plant-parasitic nematodes: a molecular update. Gene 332:13–27

Vanholme B, Mitreva MD, Van Criekinge W, Logghe M, Bird D, McCarter JP, Gheysen G (2005) Detection of putative secreted proteins in the plant-parasitic nematode *Heterodera schachtii*. Parasitol Res 98:414–424

Vanholme B, Mitreva M, Van Criekinge W, Logghe M, Bird D, McCarter JP, Gheysen G (2006) Detection of putative secreted proteins in the plant-parasitic nematode *Heterodera schachtii*. Parasitol Res 98:414–424

Veech JA, Starr JL, Nordgren RM (1987) Production and partial characterization of stylet exudates from adult females of *Meloidogyne incognita*. J Nematol 19:463–468

Waetzig GH, Sobczak M, Grundler FMW (1999) Localization of hydrogen peroxide during the defense response of *Arabidopsis thaliana* against the plant-parasitic nematode *Heterodra glycines*. Nematology 1:681–686

Wang SH, Zheng HJ, Dissanayake S, Chen WF, Tao ZH, Lin SZ, Piessens WF (1997) Evaluation of recombinant chitinase and SXP1 antigens as antimicrofilarial vaccines. Am J Trop Med Hyg 56:474–481

Wang X, Meyers DM, Baum TJ, Smant G, Hussey RS, Davis EL (1999) In planta localization of a ß-1,4-endoglucanse secreted by *Heterodera glycines*. Mol Plant Microbe Interact 12:64–67

Wang X, Allen R, Ding XF, Goellner M, Maier T, de Boer JM, Baum TJ, Hussey RS, Davis EL (2001) Signal peptide-selection of cDNA cloned directly from the esophageal gland cells of the soybean cyst nematode *Heterodera glycines*. Mol Plant Microbe Interact 14:536–544

Wang X, Mitchum MG, Gao BL, Li C, Diab H, Baum TJ, Hussey RS, Davis EL (2005) A parasitism gene from a plant-parasitic nematode with function similar to *CLAVATA3/ESR (CLE)* of *Arabidopsis thaliana*. Mol Plant Pathol 6:187–191

Weerasinghe R, Bird DM, Allen NS (2005) Root-knot nematodes and bacterial Nod factors elicit common signal transduction events in *Lotus japonicus*. Proc Natl Acad Sci (USA) 102:3147–3152

Wesley SV, Helliwell CA, Smith NA, Wang M, Rouse DT, Liu Q, Gooding PS, Singh SP, Abbott D, Stoutjesdijk PA, Robinson SP, Gleave AP, Green AG, Waterhouse PM (2001) Construct design for efficient, effective and high-throughput gene silencing in plants. Plant J 27:581–590

Wilde A, Lizaragga SB, Zhang L, Wiese C, Gliksman NR, Walczak CE, Zheng Y (2001) Ran stimulates spindle assembly by altering microtubule dynamics and the balance of motor activities. Nature Cell Biol 3:221–227

Wyss U, Zunke U (1986) Observations on the behaviour of second stage juveniles of *Heterodera schachtii* inside host roots. Rev Nematol 9:153–165

Wyss U, Grundler FMW, Munch A (1992) The parasitic behaviour of second stage juveniles of *Meloidogyne incognita* in roots of *Arabidopsis thaliana*. Nematologica 38:98–111

Yadav BC, Veluthambi K, Subramaniam K (2006) Host-generated double stranded RNA induces RNAi in plant-parasitic nematodes and protects the host from infection. Mol Biochem Parasitol 148:219–222

Yan YT, Smant G, Stokkermans J, Qin L, Helder J, Baum T, Schots A, Davis E (1998) Genomic organization of four beta-1,4-endoglucanase genes in plant-parasitic cyst nematodes and its evolutionary implications. Gene 220:61–70

Yan Y, Smant G, Davis EL (2001) Functional screening yields a new beta-1,4 endoglucanase gene from *Heterodera glycines* that may be the product of a recent gene duplication. Mol Plant Microbe Interact 14:63–71

Zhan B, Liu Y, Badamchian M, Williamson A, Feng J, Loukas A, Hawdon JM, Hotez PJ (2003) Molecular characterization of the *Ancylostoma*-secreted protein family from the adult stage of *Ancylostoma caninum*. Int J Parasitol 33:897–907

Zuo J, Niu QW, Chua NH (2000) An estrogen-receptor-based transactivator XVE mediates highly inducible gene expression in transgenic plants. Plant J 24:265–273

Molecular Insights in the Susceptible Plant Response to Nematode Infection

Godelieve Gheysen(✉) and Melissa G. Mitchum

Abstract Sedentary endoparasitic nematodes have evolved sophisticated strategies to form permanent feeding sites within host plant roots to ensure their survival. The process of feeding site formation entails an elaborate transformation of normal root cells into enlarged, multinucleate, and metabolically active cell types to supply the nutritional needs of the nematode. The signal-exchange that occurs between nematodes and their hosts to trigger the chain of molecular events associated with feeding cell formation has not been resolved. Presumably, the signals for the induction of feeding cells come from the nematode; thus, secretions originating in the esophageal gland cells and directly injected through the stylet into host tissues during parasitism have been implicated as key molecules. It is evident from the distinct morphological features of feeding sites that the nematode signals likely interfere with fundamental aspects of plant cell biology and differentiation. Molecular studies have shown that these changes are accompanied by extensive alterations in plant gene expression. Researchers have taken advantage of a wide array of methodologies to catalogue the genes as either up- or down-regulated in nematode feeding sites, and technological advances are now enabling feeding cell-specific analyses. As comprehensive profiles of genes expressed in feeding sites are generated, it has become increasingly important to determine which genes play essential roles in their formation. In the future, the true challenge will be to integrate our knowledge of nematode signals with host cell responses to elucidate a complete picture of feeding site formation.

1 Introduction

Plants respond to nematode infection in a variety of ways, mainly depending on the type and pathotype of the nematode. Plant-parasitic nematodes can be ecto- or endoparasites and either sedentary or roaming. In the latter case, the nematodes feed upon and quickly destroy plant cells, primarily provoking a plant stress

G. Gheysen
Department of Molecular Biotechnology, Ghent University, Ghent, Belgium
e-mail: Godelieve.Gheysen@UGent.be

Plant Cell Monogr, doi:10.1007/7089_2008_35

response. At the other end of the spectrum, root-knot and cyst nematodes establish a complex and long-lasting relationship with their host plants, inducing the redifferentiation of selected root cells into large multinucleate nematode feeding sites (NFS). Because detailed molecular analysis of the plant response has only been performed on root-knot and cyst nematode infected roots, the data presented in this chapter has largely been derived from these systems. However, the prevalence of certain features such as enlarged nuclei and cell wall changes in very diverse feeding cells (Wyss 2002) indicates that the molecular insights gained in these well-studied systems may, to some extent, be extrapolated to other plant–nematode interactions.

This chapter will primarily deal with publications since 2002 and general insights from preceding studies. For a more thorough overview of the earlier gene expression analyses, we refer to Gheysen and Fenoll (2002). Particulars of the life cycles are presented in Bird et al. (2008) and details of the cellular changes elicited by nematode infection are reviewed by Endo (1984) and can be found in Berg et al. (2008) and Sobczak and Golinowski (2008). To facilitate the comprehension of this chapter, we will encapsulate the main features as highlighted in the following paragraphs, beginning with a brief review of the infection process.

The nematode developing inside the egg undergoes a first molt before hatching. The resulting second-stage juvenile is the infective stage that penetrates the plant root and migrates through root tissues to a suitable location near the vascular tissue. During migration, the subventral esophageal glands of the nematode are very active and secrete a mixture of cell-wall modifying enzymes to assist with burrowing. It is generally believed that secretions from the esophageal glands are also major determinants of feeding cell initiation. For more details on nematode secretions, we refer readers to several recent reviews (Davis et al. 2004; Vanholme et al. 2004; Davis et al. 2008).

Feeding cell formation is a complex process associated with dramatic changes in plant gene expression. Cyst nematodes select a single cell, often in the vascular parenchyma, to induce a feeding cell called a syncytium (Fig. 1). The selected cell responds to the nematode stimulus by gradually widening plasmodesmata to neighboring cells (Grundler et al. 1998), followed by the fusion of the protoplasts of the adjacent cells. At later stages, cell wall openings are formed de novo and the syncytium progressively expands by incorporating hundreds of adjacent cells.

Unlike cyst nematodes, root-knot nematodes induce the development of three to six giant-cells (Fig. 1) to feed from. The first sign of giant-cell induction is the formation of binucleate cells (Jones and Payne 1978). The cell plate vesicles line up between the two daughter nuclei but are then dispersed, disrupting cell plate formation. Additional mitoses uncoupled from cell division generate multinucleate, hypertrophied cells up to 100-times the size of normal root vascular parenchyma cells. In most plant hosts, the surrounding pericycle and cortical cells divide to form the typical gall or root-knot.

Feeding cells function as transfer cells to tap nutrients and solutes from the vascular tissues and provide them to the developing nematodes. Typical for transfer cells, the cell wall alongside the xylem forms finger-like projections

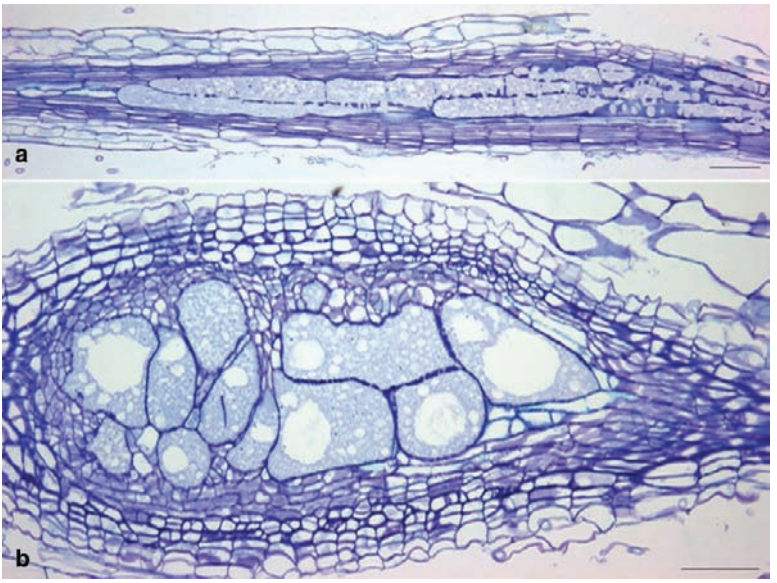


Fig. 1 Nematode feeding sites induced by cyst and root-knot nematodes. **a** A syncytium induced by *Heterodera schachtii* in Arabidopsis roots 7 days after infection. **b** Giant-cells induced by *Meloidogyne incognita* in Arabidopsis roots 10 days after infection. Bars – 100 μm

lined with plasma membrane, which facilitates water transport from the xylem into the feeding cell (Jones and Northcote 1972). The dense granular cytoplasm of feeding cells resembles that of meristematic cells: many ribosomes, organelles, and small vacuoles. High metabolic activity and constant withdrawal of cytoplasm by the nematodes convert the feeding cells into metabolic sinks for the host plant.

A variety of approaches have been used to identify gene expression changes occurring specifically within NFS including promoter–reporter assays and in situ hybridization (Fig. 2). To assess their role in NFS formation, hence gaining molecular insight into NFS development, functional analysis of these plant genes is crucial. The consequence of knocking out a gene (or over-expressing it) on NFS formation, however, has only been investigated in a few instances (Table 1). For reasons related to the amount of genomic information and the availability of mutant and marker lines, changes in gene expression of plants infected by nematodes have preferably been studied using the model plant, *Arabidopsis thaliana*. However, the use of crop plants such as soybean (*Glycine max*) and tomato (*Solanum lycopersicum*) for molecular studies on plant–nematode interactions is gaining in importance as genomic data and microarray platforms become increasingly available. Microarray analyses (Alkharouf et al. 2006; Bar-Or et al. 2005; Hammes et al. 2005; Ithal et al. 2007a,b; Jammes et al. 2005; Khan et al. 2004; Klink et al. 2005; Puthoff et al. 2003; Ramsay et al.

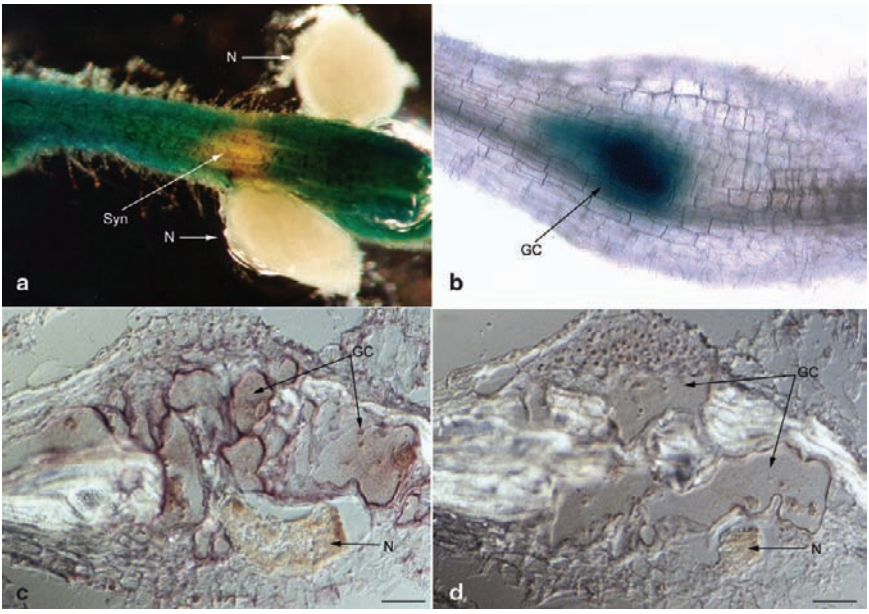


Fig. 2 Gene expression changes occurring specifically within nematode feeding sites detected using promoter–reporter assays and *in situ* hybridization. **a** Activity of the *NOS* promoter (pNOS) in nematode-infected sugarbeet hairy roots. A sugarbeet hairy root with the *pNOS-GUS* construct at 20 days after inoculation with *H. schachtii*. The blue color demonstrates very strong promoter activity outside the syncytium, but down-regulation of the *NOS* promoter inside the syncytium. In sections this down-regulation is visible as early as 4 days after inoculation. **b** Activity of the *AtCel1* promoter in nematode-infected tobacco roots. Transgenic tobacco with the *Cel1-GUS* construct at 7 days after inoculation with *Meloidogyne incognita*. The blue color demonstrates very strong promoter activity within the developing galls. In sections, *Cel1-GUS* expression was localized to developing giant-cells (Mitchum et al. 2004). **c**, **d** Sections of nematode-infected tobacco roots. Two-week-old galls induced by *Meloidogyne incognita* on tobacco roots were sectioned and probed with a digoxigenin-labeled antisense (**c**) or sense (**d**) probe corresponding to the tobacco endo-β-1,4-glucanase 7 (*Cel7*) gene (Goellner et al. 2001). The purple color demonstrates very strong expression of *Cel7* within giant-cells. No staining was observed with the sense probe control. GC, giant-cell; N, nematode; Syn, syncytium; *NOS*, nopaline synthase. Bars – 100 μm

2004; Wang et al. 2003) have significantly enlarged the list of nematode-responsive genes (Li et al. 2008) and confirmed previous analyses that the down-regulation of genes is as relevant to NFS formation as gene induction (Gheysen and Fenoll 2002). In the following sections, we highlight recent molecular insights into the susceptible plant response to nematode infection, particularly with regard to the plant genes involved, and how these genes may be contributing to the induction and function of NFS.

Table 1 Changes in nematode infections on transgenic and mutant plants compared to the control

Gene name	Characteristics	Plant	Root-knot	Cyst	Reference
dgt	Auxin-insensitive mutant	*Solanum lycopersicum*	↘	↘	Richardson and Price (1984), Goverse et al. (2000)
axr2	Auxin-insensitive mutant	*Arabidopsis thaliana*	n.t.	↘	Goverse et al. (2000), Wubben et al. (2001)
pin1/1-ttg1	Auxin transport mutant	*Arabidopsis thaliana*	n.t.	↘	Goverse et al. (2000)
pin2/eir1-1	Auxin transport mutant	*Arabidopsis thaliana*	n.t.	↘	Goverse et al. (2000), Wubben et al. (2001)
rhd1	Hypersensitive ethylene mutant	*Arabidopsis thaliana*	n.t.	↗	Wubben et al. (2004)
eto1, eto2, eto3	Ethylene over-producing mutants	*Arabidopsis thaliana*	n.t.	↗	Goverse et al. (2000), Wubben et al. (2001)
etr1	Ethylene receptor mutant	*Arabidopsis thaliana*	n.t.	↘	Wubben et al. (2001)
ein2, ein3	Ethylene insensitive mutants	*Arabidopsis thaliana*	n.t.	↘	Wubben et al. (2001)
har1	CLAVATA1-like receptor kinase mutant	*Lotus japonicus*	↗	n.t.	Lohar and Bird (2003)
AtCKX3, ZmCKX1	Transgenics with lower sensitivity to cytokinin	*Lotus japonicus*	↘	n.t.	Lohar et al. (2004)
sid2-1, pad4-1	Salicylic acid deficient mutants	*Arabidopsis thaliana*	n.t.	↗	Wubben et al. (2008)
NAHG	Salicylic acid deficient transgenic	*Arabidopsis thaliana*	n.t.	↗	Wubben et al. (2008)
npr1-2, npr1-3	Salicylic acid insensitive mutants	*Arabidopsis thaliana*	n.t.	↗	Wubben et al. (2008)
sni1	*NPR1*-suppressor mutant	*Arabidopsis thaliana*	n.t.	↘	Wubben et al. (2008)
NAHG	Salicylic acid deficient transgenic	*Solanum lycopersicum*	n.s.d.	n.t.	Bhattarai et al. (2008)
jai1	JA insensitive mutant	*Arabidopsis thaliana*	↘	n.t.	Bhattarai et al. (2008)
def1	JA deficient mutant	*Arabidopsis thaliana*	n.s.d.	n.t.	Bhattarai et al. (2008)

(continued)

Table 1 (continued)

Gene name	Characteristics	Plant	Root-knot	Cyst	Reference
TOBRB7 antisense	Aquaporin down-regulated	*Nicotiana tabacum*	↘	n.t.	Opperman and Conkling (1996)
rpe	T-DNA tagged mutant in *RPE*	*Arabidopsis thaliana*	↘	n.s.d.	Favery et al. (1998)
PHAN antisense	Phantastica down-regulated	Unpubl.	↘	n.t.	Koltai et al. (2001)
ENOD40 overexpression	Early nodulin gene up-regulated	*Medicago truncatula*	↗	n.t.	Favery et al. (2002)
nfr1 and *nfr5*	Nod factor receptor mutants	*Lotus japonicus*	↘	n.t.	Weerasinghe et al. (2005)
WRKY23 RNAi	*WRKY23* down-regulated	*Arabidopsis thaliana*	n.t.	↘	Grunewald et al. (2008) and Gheysen (unpublished)
CDKA;1 cosuppression	*CDKA;1* down-regulated	*Arabidopsis thaliana*	↘	↘	Van de Cappelle et al. (2008)
CCS52A RNAi	*CCS52A2* down-regulated	*Arabidopsis thaliana*	n.t.	↘	Van de Cappelle, de Almeida-Engler and Gheysen (unpublished)
EXPA5 antisense	Hairy roots with expansin down-regulated	*Solanum lycopersicum*	↘	n.t.	Gal et al. (2005)
map65-3	T-DNA tagged mutant in *MAP65-3*	*Arabidopsis thaliana*	↘	n.t.	Caillaud et al. (2008)
cel2	Endo-1,4-β-glucanase mutant	*Arabidopsis thaliana*	n.t	↘	Wieczorek et al. (2008)
kor3	Endo-1,4-β-glucanase mutant	*Arabidopsis thaliana*	n.t	↘	Wieczorek et al. (2008)
CEL7 RNAi	*CEL7* endo-1,4-β-glucanase silenced	*Solanum tuberosum*	n.t	↘	Karczmarek et al. (2008)
CEL9C1 RNAi	*CEL9C1* endo-1, 4-β-glucanase silenced	*Solanum tuberosum*	n.t	↘	Karczmarek et al. (2008)

Direction of arrow indicates if infection is higher or lower on this plant line than on the corresponding wild type. n.t. not tested; n.s.d. not significantly different

2 Activation of Plant Defense Responses During a Compatible Interaction

From the moment a nematode penetrates the plant root, a molecular dialogue is set up resulting in chemical warfare. Defense responses include the production of harmful oxygen radicals and systemic signaling compounds as well as the activation of defense genes that lead to the generation of structural barriers, pathogenesis-related proteins and toxins (such as phytoalexins) that debilitate the invader. It is not surprising that this also happens in the compatible interaction, but then the nematode can either evade or overcome the plant defense. The protein surface coat of plant-parasitic nematodes contains peroxiredoxin and lipid-binding proteins that might respectively break down hydrogen peroxide and inhibit lipoxygenase activity, an important enzyme in the jasmonic acid signaling pathway (reviewed in Vanholme et al. 2004).

Plant genes known to be involved in wound and defense response are up-regulated during infection by nematodes (reviewed in Gheysen and Fenoll 2002). Quite a few of the up-regulated genes are also involved in cell wall modifications or in the biosynthesis of secondary metabolites that could be crucial in establishing the parasitic interaction. Cell wall fortification is an important induced plant defense response for confining the pathogen, and many of the genes implicated in this process such as extensin, lignin biosynthesis genes, and peroxidase are up-regulated upon nematode infection. However, extensive cell wall modifications are also fundamental to feeding site structure (for details, see Sect. 5). Similarly, the accumulation of flavonoids, isoflavonoids, and other phenolics is a typical plant defense response. On the other hand, flavonoids inhibit auxin transport and their local production could trigger the accumulation of auxin, an outstanding candidate implicated in early feeding site development (see Sect. 9). There are also indications that specific plant defense pathways may be locally down-regulated within the feeding sites. For example, several genes encoding key enzymes in jasmonic acid biosynthesis, an important defense signaling molecule, were found to be down-regulated in syncytia of soybean induced by *H. glycines* (Ithal et al. 2007b).

3 Polyploidy in NFS Is Correlated with Activation of Cell-Cycle Genes

From initial microscopy analyses (Endo 1984), it was evident that characteristic cytological changes take place in the nuclei of developing feeding cells (Berg et al. 2008; Sobczak and Golinowski 2008). Syncytia, as well as giant-cells, contain multiple large and amoeboid nuclei. These nuclear responses are among the first visible changes in developing feeding cells. Cytological observations suggest that nuclei in giant-cells divide through successive mitoses without intermittent cytokinesis (acytokinetic mitosis) while the multinucleate state in syncytia is attained by amalgamation of

many uninucleate root cells (Gheysen et al. 1997). Although mitosis takes place in the surrounding dividing cells, it has never been observed inside the syncytium. Enlargement of nuclei is generally caused by endoreduplication, a term used for an abnormal cell cycle that repeatedly goes through the DNA synthesis-phase but skips mitosis (Gheysen and Fenoll 2002). There is little doubt that endoreduplication occurs in syncytia; in contrast, the enlargement of giant-cell nuclei could also be explained by the fusion of nuclei, possibly due to entanglement of the abundant cellular mitotic apparatus.

More recently, the molecular mechanisms underlying the cell cycle events in syncytia and giant-cells have been explored in detail. Initial studies on the expression of a few core cell cycle genes in *A. thaliana* revealed their up-regulation within the first hours after the nematode has selected a cell to initiate a NFS. Two cyclin-dependent kinases (*CDKA;1* and *CDKB1;1*), an A-type cyclin (*Arath;CycA2;1*) and a mitotic cyclin (*Arath;CycB1;1*), typical for the late G2 until M phase, were found to be expressed in giant-cells as well as syncytia (Niebel et al. 1996). It was therefore concluded that the cell cycle proceeds at least until the late G2 phase in both types of feeding cells. This has also been recently reported for endoreplicating trichome-neighboring cells (Weinl et al. 2005). During later stages of syncytium development [around 10–14 days after infection (dai)], the cell cycle genes were still expressed at the growing edges to probably allow syncytia to reach a critical size necessary for the cyst nematodes to properly complete their life cycle.

To analyze the importance of cell cycle activation for the development of feeding cells, cell cycle-inhibiting drugs have been used (de Almeida-Engler et al. 1999). Upon hydroxyurea treatment (which prevents DNA synthesis), early giant-cell and syncytium development was blocked in Arabidopsis. This demonstrates that genome multiplication is essential for the formation of both types of feeding cells. However, the application of hydroxyurea at later stages resulted in normal development of the feeding sites. Upon early application (1 and 3 dai) of oryzalin (which arrests cells in mitosis), root-knot nematode development in Arabidopsis was completely inhibited (de Almeida-Engler et al. 1999; Wiggers et al. 2002). The formation of giant-cells was initiated but their development severely hampered. In contrast, when oryzalin was applied at later stages (9 dai), the majority of the root-knot nematodes could complete their life cycle. This indicates that mitosis is mainly required for early giant-cell differentiation and initial expansion. Mitosis can occur up to 21 dai in *Pisum sativum* infected by *M. incognita*, probably supporting an optimal but nonessential expansion of the feeding cell (Wiggers et al. 2002). Syncytia arise by fusion of adjacent cells; therefore, one would assume that mitosis is not involved in the formation of the multinucleate syncytium, and thus oryzalin should not affect cyst nematode development. Application of oryzalin at 1 dai, however, resulted in the complete inhibition of syncytium development and no cysts were formed on these plants. When oryzalin was applied at later stages (3 and 9 dai), an increasing number of the infective juveniles developed into cysts. These data indicate that mitotic activity is also required for proper syncytium development. It was observed that oryzalin inhibits the mitotic activity in cells prior to syncytium incorporation and, as a consequence, syncytium expansion is restricted

(de Almeida-Engler et al. 1999). Recently, it was demonstrated that silencing *AtCDKA;1*, an essential cell cycle gene, using a NFS-promoter results in significant reduction of nematode development on *A. thaliana* (Van de Cappelle et al. 2008).

These studies do not distinguish cell cycle events in giant-cells versus syncytia, particularly with regard to the importance of endoreduplication. Endoreduplication is a very important process in plant cell differentiation and therefore has received a substantial amount of scientific attention. The control of endoreduplication is not yet fully understood, but several key proteins have been identified, mainly in *A. thaliana* (Fig. 3). The heterodimeric transcription factor E2Fa-DPa activates the G1/S transition in both the mitotic and endoreduplication cycles. The transition into the mitotic cycle was postulated to depend on the presence of the MIF (mitosis-inducing factor) (De Veylder et al. 2002) and the activity of specific inhibitors of the endocycle (DEL1) (Vlieghe et al. 2005). MIF might be a complex of a cyclin-dependent-kinase B (CDKB1;1) (Boudolf et al. 2004) and an A2 class cyclin (Imai et al. 2006). The switch between mitosis and the endocycle almost certainly occurs as a result of specific premature destruction of mitotic cyclins by a CCS52 (cell cycle switch) complex (Cebolla et al. 1999). *A. thaliana* contains three *CCS52* genes. CCS52 defines the activity and substrate specificity of the anaphase-promoting complex (APC), a multi-component ubiquitin ligase that plays a fundamental role in the metaphase–anaphase transition and exit from mitosis by targeting specific cell cycle proteins for degradation by the ubiquitin-proteasome pathway (Kondorosi and Kondorosi 2004).

If feeding cells endoreduplicate, one would expect low expression of *CDKB1;1*, *Cyclin A2*, and *DEL1* and a high level of expression of *CCS52. CCS52* was first discovered in *Medicago truncatula*, where it was highly expressed in endoreduplicating nodule tissues (Cebolla et al. 1999), and its expression was also analyzed in galls (Favery et al. 2002; Koltai et al. 2001). *CCS52* expression was found inside the giant-cells and in the surrounding cells, indicating the occurrence of endoreduplication. In the same experiments however, high *CCS52*

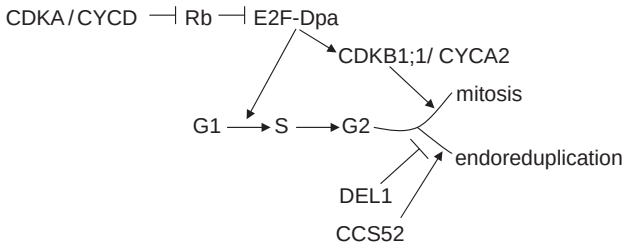


Fig. 3 E2Fa-DPa activates the G1/S transition in both the mitotic and endoreduplication cycles. The transition into the mitotic cycle was postulated to depend on the presence of MIF (mitosis-inducing factor) and the activity of DEL1. MIF might be a complex of a cyclin-dependent-kinase B (CDKB1;1) and an A2 class cyclin. The switch between mitosis and the endocycle almost certainly occurs as a result of specific premature destruction of mitotic cyclins by a CCS52 (cell cycle switch) complex. Scheme modified from Boudolf et al. (2004), with information incorporated from Vlieghe et al. (2005) and Imai et al. (2006)

expression was also observed in lateral root meristems, challenging the model that *CCS52* is typically expressed in endoreduplicating tissues. Nevertheless, up-regulation of *CCS52* clearly results in higher ploidy (Cebolla et al. 1999; Van de Cappelle, de Almeida-Engler and Gheysen, unpublished results) and down-regulation of *CCS52* in lower ploidy (Vinardell et al. 2003). Transgenic *M. truncatula* plants with lower *CCS52* expression showed severe inhibition of nodule development (Vinardell et al. 2003), and preliminary experiments also indicate inhibition of nematode development (Van de Cappelle, de Almeida-Engler and Gheysen, unpublished results).

According to Roudier et al. (2003) *MtCycA2;2*, shown to exclusively play a role in mitotic cycles and not be expressed during endoreduplication, is repressed during nodule and gall development in *M. truncatula*, indicating endoreduplication in both systems (symbiosis and nematode parasitism). Unfortunately, no information is available on the expression of this gene in syncytia.

These results so far would indicate the occurrence of endoreduplication in giant-cells as well as syncytia. In giant-cells, endoreduplication is probably preceded by or even alternates with mitosis. Indeed, it was recently shown that endoreduplicated cells can re-enter the mitotic cell cycle (Weinl et al. 2005). Another study by Huang et al. (2003b) renders the story even more complicated. The *A. thaliana Prolifera* gene (*PRL*), which is normally expressed in dividing cells and not in endoreduplicating tissues, is up-regulated in giant-cells and syncytia but not in the surrounding, dividing cells. This indicates that our assessment of cell cycle expression is not yet precise enough to predict or explain how the aberrant cycles in NFS are controlled. It has been shown that a particular expression level often determines the outcome of the cell cycle for certain genes. For example, moderate over-expression of *DEL1* inhibits the endocycle whereas higher levels also block mitosis (Vlieghe et al. 2005). Similarly, a moderate level of *KRP2* (a cell cycle inhibitor) over-expression increases endoreduplication whereas higher levels decrease endoreduplication (Verkest et al. 2005). On top of all of this, the importance of posttranscriptional regulation and protein activity changes makes it clear that the more we learn about cell cycle, the more complicated the picture becomes.

4 Cytoskeletal Rearrangements Within NFS

Numerous studies have demonstrated the importance of plant cytoskeletal rearrangements upon pathogen attack (Takemoto et al. 2003 and references therein). The actin filaments are focally reorganized towards sites of penetration and, in the case of a biotrophic fungus, actin cables are targeted to the fungal feeding organs (haustoria). Sedentary nematodes also induce long-term rearrangements of the cytoskeleton. Actin genes are highly expressed in NFS as would be expected in large expanding cells that need extensive internal transport (de Almeida-Engler et al. 2004). In *A. thaliana*, tubulin genes are moderately up-regulated in syncytia and at higher levels in giant-cells, probably reflecting the need of a mitotic

cytoskeleton for the rapidly dividing nuclei in the latter. RT-PCR experiments using RNA extracted from laser-captured soybean syncytia induced by *H. glycines* revealed a strong induction of β-tubulin and a weaker up-regulation of α-tubulin as compared to samples extracted from uninfected roots (Klink et al. 2005). Analysis of the cytoskeleton in *A. thaliana* by immunolocalization of the actin and tubulin proteins and by the use of *GFP*-fusions showed that the cytoskeleton was strongly disrupted in syncytia (de Almeida-Engler et al. 2004). The same diffuse or clumped tubulin labeling was seen in soybean syncytia (Klink et al. 2005). In giant-cells, although disturbed compared to normal root cells, actin and microtubular fibers are visible with abnormally thick actin cables present in the cell cortex. A functional mitotic apparatus, consisting of microtubule-forming spindles (for separating the chromosomes during nuclear division) and phragmoplasts (initiating the cell plate), is present in the developing giant-cells (de Almeida-Engler et al. 2004).

Depolymerization of the actin and microtubular cytoskeleton with cytochalasin D or oryzalin, respectively, only inhibited feeding cell and nematode development when applied at early stages of infection (3 dai). In contrast, stabilization of the microtubular cytoskeleton with taxol at later stages (14 dai) resulted in normally developed feeding cells but interfered with nematode development. As nematode feeding involves the retrieval of large volumes of cytoplasm, a degree of cytoskeleton fragmentation may decrease cytoplasm viscosity and therefore facilitate uptake during nematode feeding.

It is not known how nematode infection brings about the observed cytoskeletal changes. A possible player from the plant side is the actin-nucleating protein, formin. Formins are cytoskeleton-organizing proteins involved in the establishment of cell polarity and growth. From the 21 predicted *A. thaliana* genes, three (*AtFH1, AtFH6*, and *AtFH10*) were shown to be up-regulated in giant-cells at 7 and 14 dai (Favery et al. 2004). *AtFH6* was expressed as early as 2 dai and the protein was uniformly distributed throughout the plasma membrane. It was therefore suggested that AtFH6 distribution could be involved with the isotropic growth of the giant-cells.

Another cytoskeletal protein, microtubule-associated protein 65-3 (MAP-65-3), that is essential for cytokinesis in somatic plant cells, is also required for giant-cell ontogenesis (Caillaud et al. 2008). The protein colocalizes to the mitotic microtubular arrays and the cell plate in mitotic cells. The corresponding gene is up-regulated in giant-cells and the protein can be found on the aborted cell plates. Knocking-out the gene results in dwarf plants with defects in cell division and upon nematode infection, giant-cells are initiated but cannot develop normally, resulting in the death of the nematode (Caillaud et al. 2008).

5 Cell Wall Architectural Modifications in NFS

Plant cell walls are composed of cellulose microfibrils embedded in a matrix of hemicellulose and pectin polymers. The cell wall undergoes pronounced changes throughout plant development by recruiting several different classes of cell-wall

modifying proteins (CWMPs) and enzymes to the extracellular space. The plant cell wall also represents a structural barrier to infection by a wide range of plant pathogens. Sedentary endoparasitic cyst and root-knot nematodes have evolved sophisticated mechanisms to breach the structural barrier of the plant cell wall during the penetration and migration phase of the life cycle yet fine-tuned their manipulation of the cell wall for the establishment of feeding sites.

Cyst and root-knot nematodes secrete a battery of cell-wall modifying proteins to facilitate penetration and migration, albeit by different modes (i.e., intracellular vs. intercellular, respectively) through host root tissues. CWMPs are encoded by nematode parasitism genes, the details of which are covered in Davis et al. (2008). A large number of genes encoding CWMPs have been identified in cyst and root-knot nematodes, yet there is very little evidence to support a role during the induction and formation of syncytia and giant-cells. Accumulating evidence suggests that the CWMPs involved in the extensive wall modifications within feeding cells are of plant origin (Goellner et al. 2001; Karczmarek et al. 2008; Mitchum et al. 2004; Vercauteren et al. 2002; Wang et al. 2007; Wieczorek et al. 2006, 2008).

Despite the conserved function of syncytia and giant-cells as nutrients sinks, their ontogeny particularly differs with regard to alterations in cell wall architecture. Giant-cells are formed by hypertrophy of single vascular parenchyma cells up to 100x their normal size. In contrast, syncytia form by progressive cell wall dissolution and fusion of adjacent cells. Although giant-cells and syncytia differ in the extent of cell wall degradation, the walls of both types of feeding structures require loosening for expansion and elongation. Additional characteristic cell wall modifications shared by both giant-cells and outer walls of syncytia include thickening and formation of finger-like ingrowths adjacent to xylem vessels which increase the plasmalemma surface area to support increased solute uptake. Although the nature of the thickened walls is not clear, it may involve callose or lignin deposition (Grundler et al. 1998; Jones and Northcote 1972) to strengthen the walls so that the NFS can withstand the increasing turgor pressure. The extensive cell wall architectural modifications, namely loosening, dissolution, growth, and thickening, appear to be mediated by a tightly regulated cohort of CWMPs.

Detailed molecular studies to characterize host gene expression changes specifically occurring within syncytia and giant-cells have implicated an important role for several different classes of plant CWMPs in the observed cell-wall architectural modifications (Table 2). The first molecular study to characterize cell wall changes induced in nematode feeding cells was conducted on a tobacco extensin (EXT) (Niebel et al. 1993). Extensins are hydroxyproline-rich glycoproteins (HRGPs) and the most abundant structural protein family of the plant cell wall. Expression of a tobacco extensin was shown to increase in roots infected with root-knot nematodes but not cyst nematodes. The extensin promoter was active in the center of initiating galls as early as 2 dai and peaked at 7 dai. After one week, expression was restricted to the cortex, endodermal and pericycle layers that divide to form the gall. It remains to be shown whether the expression of extensin during the early stages of the root-knot nematode host interaction is important for giant-cell formation or whether this is a reflection of a general defense response elicited by the plant in an

Table 2 Nematode-responsive plant genes encoding cell wall modifying proteins

Gene name	Putative function	AGI or accession #	Cyst	RKN	References
Endo-β-1,4-glucanases (EGases)					
NtCEL2	Endo-β-1,4-glucanase	AF362948	Up-regulated 7-9 dai IR[a,e]	Up-regulated 7-14 dai NFS[e]	Goellner et al. (2001)
NtCEL4	Endo-β-1,4-glucanase	AF362950	Up-regulated 7-9 dai IR[e]	Up-regulated 7-9 dai IR[e]	Goellner et al. (2001)
NtCEL5	Endo-β-1,4-glucanase	AF362951	Up-regulated 7-9 dai IR[e]	Up-regulated 7-9 dai IR[e]	Goellner et al. (2001)
NtCEL7	Endo-β-1,4-glucanase	AF362947; DQ156498 promoter	Up-regulated 7 dai NFS[a,b,e]	Up-regulated 7-14 dai NFS[a,b,e]	Goellner et al. (2001), Wang et al. (2007)
NtCEL8	Endo-β-1,4-glucanase	AF362949	Up-regulated 7 dai NFS[a,b,e]	Up-regulated 7-14 dai NFS[a,e]	Goellner et al. (2001)
AtCEL1	Endo-β-1,4-glucanase	At1g70710; X98543 promoter	ND NFS[b,d]	Up-regulated 3-13 dai NFS[b]	Mitchum et al. (2004), Wieczorek et al. (2008)
AtCEL2	Endo-β-1,4-glucanase	At1g02800	Up-regulated 5-7 dai NFS[d] and 10 dai NFS[a]	N.I.	Wieczorek et al. (2008)
AtCEL3	Endo-β-1,4-glucanase	At1g71380	Up-regulated 5-7 dai NFS[d], up-regulated 5-15 dai IR[e]	N.I.	Wieczorek et al. (2008)
AtCEL5	Endo-β-1,4-glucanase	At1g22880	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. (2008)
KOR	Endo-β-1,4-glucanase	At5g49720	Up-regulated 5-7 dai NFS[d]	N.I.	Wieczorek et al. (2008)
KOR2	Endo-β-1,4-glucanase	At1g65610	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. (2008)
KOR3	Endo-β-1,4-glucanase	At4g24260	Up-regulated 5-7 dai NFS[d] and 10 dai NFS[a]	N.I.	Wieczorek et al. (2008)
AtCEL	Endo-β-1,4-glucanase	At1g19940	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. (2008)
AtCEL	Endo-β-1,4-glucanase	At1g23210	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. (2008)
AtCEL	Endo-β-1,4-glucanase	At1g48930	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. (2008)

(continued)

Table 1.2 (continued)

Gene name	Putative function	AGI or accession #	Cyst	RKN	References
AtCEL	Endo-β-1,4-glucanase	At1g64390	Up-regulated 5-7 dai NFS[d], up-regulated 5-15 dai IR[e]	N.I.	Wieczorek et al. (2008)
AtCEL	Endo-β-1,4-glucanase	At1g75680	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. (2008)
AtCEL	Endo-β-1,4-glucanase	At2g32990	Up-regulated 5-7 dai NFS[d], up-regulated 5-15 dai IR[e]	N.I.	Wieczorek et al. (2008)
AtCEL	Endo-β-1,4-glucanase	At2g44540	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. (2008)
AtCEL	Endo-β-1,4-glucanase	At2g44550	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. (2008)
AtCEL	Endo-β-1,4-glucanase	At2g45560	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. (2008)
AtCEL	Endo-β-1,4-glucanase	At2g44570	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. (2008)
AtCEL	Endo-β-1,4-glucanase	At3g43860	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. (2008)
AtCEL	Endo-β-1,4-glucanase	At4g02290	Up-regulated 5-7 dai NFS[d], up-regulated 5-15 dai IR[e]	N.I.	Wieczorek et al. (2008)
AtCEL	Endo-β-1,4-glucanase	At4g09740	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. (2008)
AtCEL	Endo-β-1,4-glucanase	At4g11050	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. (2008)
AtCEL	Endo-β-1,4-glucanase	At4g38990	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. (2008)
AtCEL	Endo-β–1,4-glucanase	At4g39000	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. (2008)
AtCEL	Endo-β-1,4-glucanase	At4g39010	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. (2008)
AtCEL	Endo-β-1,4-glucanase	At4g23560	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. (2008)
SlCel1	Endo-β-1,4-glucanase	U13054	ND 7 dai IR[e]	N.I.	Karczmarek et al. (2008)
SlCel2	Endo-β-1,4-glucanase	U13055	ND 7 dai IR[e]	N.I.	Karczmarek et al. (2008)
SlCel3	Endo-β-1,4-glucanase	U78526	ND 7 dai IR[e]	N.I.	Karczmarek et al. (2008)
SlCel4	Endo-β-1,4-glucanase	U20590	ND 7 dai IR[e]	N.I.	Karczmarek et al. (2008)

(continued)

Table 1.2 (continued)

Gene name	Putative function	AGI or accession #	Cyst	RKN	References
SlCel5	Endo-β-1,4-glucanase	AF077339	ND 7 dai IR[e]	N.I.	Karczmarek et al. (2008)
SlCel7	Endo-β-1,4-glucanase	Y11268	Up-regulated 7 dai IR[e], up-regulated 2-10 dai NFS[a,c]	N.I.	Karczmarek et al. (2008)
SlCel9C1	Endo-β-1,4-glucanase	AF098292	Up-regulated 7 dai IR[e], up-regulated 5-10 dai NFS[a,c]	N.I.	Karczmarek et al. (2008)
Extensins (EXT)					
NpEXT	Extensin	M34371	Up-regulated during migration[a,b]	Up-regulated 2-7 dai NFS; 7+ dai galls[a,b,c]	Niebel et al. (1993)
GmEXTL	Extensin-like	BU577532	Up-regulated 2-10 dai IR[h]	N.I.	Ithal et al. (2007a)
GmEXTL	Extensin-like	BI971744	Up-regulated 2-10 dai IR; up-regulated 2dai NFS[g,h]	N.I.	Ithal et al. (2007a)
GmEXTL	Extensin-like	CK606454	Up-regulated 2-10 dai IR[h]	N.I.	Ithal et al. (2007a)
GmEXTL	Extensin-like	CF807746	Up-regulated 2-10 dai IR; up-regulated 2dai NFS[g,h]	N.I.	Ithal et al. (2007a)
GmEXT	Extensin	BM139486	Up-regulated 2 dai IR[h]	N.I.	Khan et al. (2004)
GmEXTL	Extensin-like	BU577532	Up-regulated 2 dai NFS[g]	N.I.	Ithal et al. (2007b)
GmEXTL	Extensin-like	AW348859	Up-regulated 2 dai NFS[g]	N.I.	Ithal et al. (2007b)
GmEXTL	Extensin-like	AW307368	Up-regulated 2 dai NFS[g]	N.I.	Ithal et al. (2007b)
GmEXTL	Extensin-like	BQ454193	Up-regulated 2 dai NFS[g]	N.I.	Ithal et al. (2007b)
GmEXT	Extensin	AW757140	Down-regulated 2-10 dai IR[h]	N.I.	Ithal et al. (2007a)
GmEXTL	Extensin-like	AW185750	Down-regulated 2 dai NFS[g]	N.I.	Ithal et al. (2007b)
GmEXTL	Extensin-like	BQ453262	Down-regulated 2 dai NFS[g]	N.I.	Ithal et al. (2007b)
GmEXTL	Extensin-like	BI497973	Down-regulated 2 dai NFS[g]	N.I.	Ithal et al. (2007b)

(continued)

Table 1.2 (continued)

Gene name	Putative function	AGI or accession #	Cyst	RKN	References
GmEXTL	Extensin-like	CF807748	Down-regulated 2 dai NFS[g]	N.I.	Ithal et al. (2007b)
GmEXTL	Extensin-like	BI974583	Down-regulated 2 dai NFS[g]	N.I.	Ithal et al. (2007b)
Expansins (EXP)					
LeEXPA5	Expansin	AF059489	N.I.	ND 4 or 10 dai NFS[a,h]	Gal et al. 2005
AtEXPA1	Expansin	At1g69530	upregulated 5-7 dai NFS[b,d]	upregulated 7-14dai galls[h]	Jammes et al. 2005; Wieczorek et al. 2006
AtEXPA2	Expansin	At5g05290	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. 2006
AtEXPA3	Expansin	At2g37640	upregulated 5-7 dai NFS[a,b,d]	N.I.	Wieczorek et al. 2006
AtEXPA4	Expansin	At2g39700	upregulated 5-7 dai NFS[b,d]	N.I.	Wieczorek et al. 2006
AtEXPA5	Expansin	At3g29030	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. 2006
AtEXPA6	Expansin	At2g28950	upregulated 3-15 dai NFS[a,b,d]	upregulated 14-21 dai galls[h]	Jammes et al. 2005; Wieczorek et al. 2006
AtEXPA7	Expansin	At1g12560	ND NFS 5-7 dai[d]	upregulated 14-21 dai galls[h]	Jammes et al. 2005; Wieczorek et al. 2006
AtEXPA8	Expansin	At2g40610	upregulated 5-7 dai NFS[a,d]	N.I.	Wieczorek et al. 2006
AtEXPA9	Expansin	At5g02260	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. 2006
AtEXPA10	Expansin	At1g26770	upregulated 5-7 dai NFS[a,b,d]	upregulated 14-21 dai galls[h]	Jammes et al. 2005; Wieczorek et al. 2006
AtEXPA11	Expansin	At1g20190	ND NFS 5-7 dai[d]	upregulated 14-21 dai galls[h]	Jammes et al. 2005; Wieczorek et al. 2006
AtEXPA12	Expansin	At3g15370	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. 2006
AtEXPA13	Expansin	At3g03220	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. 2006
AtEXPA14	Expansin	At5g56320	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. 2006
AtEXPA15	Expansin	At2g03090	upregulated 5-7 dai NFS[b,d]	upregulated 7-14dai galls[h]	Jammes et al. 2005; Wieczorek et al. 2006

(continued)

Table 1.2 (continued)

Gene name	Putative function	AGI or accession #	Cyst	RKN	References
AtEXPA16	Expansin	At3g55500	upregulated 5-7 dai NFS[a,b,d]	upregulated 14-21 dai galls[h]	Jammes et al. 2005; Wieczorek et al. 2006
AtEXPA17	Expansin	At4g01630	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. 2006
AtEXPA18	Expansin	At1g62980	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. 2006
AtEXPA19	Expansin	At3g29365	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. 2006
AtEXPA20	Expansin	At4g38210	upregulated 5-7 dai NFS[d]	N.I.	Wieczorek et al. 2006
AtEXPA21	Expansin	At5g39260	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. 2006
AtEXPA22	Expansin	At5g39270	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. 2006
AtEXPA23	Expansin	At5g39280	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. 2006
AtEXPA24	Expansin	Atg39310	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. 2006
AtEXPA25	Expansin	At5g39300	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. 2006
AtEXPA26	Expansin	At5g39290	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. 2006
AtEXPB1	Expansin	At2g20750	ND NFS 5-7 dai[d]; down-regulated 3 dai IR[h]	upregulated 7-14dai galls[h]	Puthoff et al. 2003; Jammes et al. 2005; Wieczorek et al. 2006
AtEXPB2	Expansin	At1g65680	ND NFS 5-7 dai[d]; upregulated 3 dai IR[h]	N.I.	Puthoff et al. 2003; Wieczorek et al. 2006
AtEXPB3	Expansin	At4g28250	upregulated 5-7 dai NFS[d]	upregulated 7-14dai galls[h]	Jammes et al. 2005; Wieczorek et al. 2006
GmEXPA3	Expansin	BM091956	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmEXPA4	Expansin	AF516880, CF805822	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmEXPA5	Expansin	AW509184	upregulated 2-10 dai IR[h]; upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007a,b
GmEXPA6	Expansin	CA785167	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmEXPA8	Expansin	AI759701, CF805734	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b

(continued)

Table 1.2 (continued)

Gene name	Putative function	AGI or accession #	Cyst	RKN	References
GmEXPA10	Expansin	CD394837	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmEXPA15	Expansin	CD417217	upregulated 2-10 dai IR[h]; upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007a,b
GmEXPL	Expansin-related	BQ742395	upregulated 2-10 dai IR[h]	N.I.	Ithal et al. 2007a
Xyloglucan Endotransglycosylase (XET)					
AtXET	Xyloglucan endotransglycosylase	At4g14130	downregulated 3 dai IR[h]	N.I.	Puthoff et al. 2003
AtXET	Xyloglucan endotransglycosylase	At4g30290	N.I.	downregulated 7-21 dai galls[h]	Jammes et al. 2005
AtXET	Xyloglucan endotransglycosylase	At3g48580	N.I.	upregulated 7 dai galls[h]; downregulated 21 dai galls[h]	Jammes et al. 2005
GmXET	Xyloglucan endotransglycosylase	AW310549	downregulated 2-10 dai IR[h]	N.I.	Ithal et al. 2007a
GmXET	Xyloglucan endotransglycosylase	BM568229	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmXET	Xyloglucan endotransglycosylase	BQ298739	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmXET	Xyloglucan endotransglycosylase	AW707175	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmXET	Xyloglucan endotransglycosylase	CD414740	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmXET	Xyloglucan endotransglycosylase	BG363116	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmXET	Xyloglucan endotransglycosylase	CK605938	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
Polygalacturonase (PG)					
GmPG1	Polygalacturonase	AF128266	upregulated 1-3 dai IR[i,j]	N.I.	Malalingam et al. 1999
GmPG2	Polygalacturonase	AF128267	upregulated 1-3 dai IR[i,j]	N.I.	Malalingam et al. 1999
AtPG	Polygalacturonase	At2g41850	upregulated 3 dai IR[h]	N.I.	Puthoff et al. 2003

(continued)

Table 1.2 (continued)

Gene name	Putative function	AGI or accession #	Cyst	RKN	References
AtPG	Polygalacturonase	At1g05660	downregulated 3 dai IR[h]	N.I.	Puthoff et al. 2003
Pectinacetylesterase (PAE)					
AtPAE	Pectinacetylesterase	AY050847	upregulated 3 dai NFS; ND NFS 7 dai[a,c]	upregulated 1-5 dai NFS[a,c]	Vercauteren et al. 2002
AtPE	Pectinesterase	At2g45220	downregulated 3 dai IR[h]	N.I.	Puthoff et al. 2003
AtPE	Pectinesterase	At1g53830	downregulated 3 dai IR[h]	N.I.	Puthoff et al. 2003
GmPE	Pectinesterase	BE822184	upregulated 2-10 dai IR[h]	N.I.	Ithal et al. 2007a
GmPE	Pectinesterase	BI967224	upregulated 2-5 dai IR[h]	N.I.	Ithal et al. 2007a
GmPE	Pectinesterase	BF066411	upregulated 2-5 dai IR[h]	N.I.	Ithal et al. 2007a
GmPE	Pectinesterase	BI970277	downregulated 2-10 dai IR[h]	N.I.	Ithal et al. 2007a
GmPE	Pectinesterase	BE821923	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmPE	Pectinesterase	AW309342	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmPE	Pectinesterase	BE475550	downregulated 2dai NFS[g]	N.I.	Ithal et al. 2007b
Pectate Lyase (PEL)					
AtPEL	Pectate lyase	At1g67750	N.I.	upregulated 21 dai galls[h]	Jammes et al. 2005
AtPEL	Pectate lyase	At4g24780	N.I.	upregulated 7-14 dai galls[h]	Jammes et al. 2005
AtPEL	Pectate lyase	At1g04680	N.I.	upregulated 14-21 dai galls[h]	Jammes et al. 2005
AtPEL	Pectate lyase	At3g27400	N.I.	upregulated 14-21 dai galls[h]	Jammes et al. 2005
AtPEL	Pectate lyase	At3g09540	N.I.	upregulated 14-21 dai galls[h]	Jammes et al. 2005
Glycosyl Hydrolase					
AtGH	Glycosyl hydrolase family 9 protein	At1g71380	upregulated 3 dai IR[h]	N.I.	Puthoff et al. 2003
AtGH	Glycosyl hydrolase family 1 protein	At3g60140	upregulated 3 dai IR[h]	N.I.	Puthoff et al. 2003

(continued)

Table 1.2 (continued)

Gene name	Putative function	AGI or accession #	Cyst	RKN	References
AtGH	Glycosyl hydrolase family 3 protein	At5g49360	N.I.	downregulated 7-21 dai galls[h]	Jammes et al. 2005
AtGH	Glycosyl hydrolase family 28 protein	At3g15720	N.I.	upregulated 7-21 dai galls[h]	Jammes et al. 2005
GmGH	Glycosyl hydrolase family 28 protein	BQ612383	upregulated 2-10 dai IR[h]	N.I.	Ithal et al. 2007a
GmGH	Glycosyl hydrolase family 1 protein	BQ080473	upregulated 2-10 dai IR[h]	N.I.	Ithal et al. 2007a
GmGH	Glycosyl hydrolase family 1 protein	BG507836	upregulated 2-10 dai IR[h]	N.I.	Ithal et al. 2007a
GmGH	Glycosyl hydrolase family 17 protein	CA819291	upregulated 2-10 dai IR[h]	N.I.	Ithal et al. 2007a
GmGH	Glycosyl hydrolase family 1 protein	BU926932	upregulated 5-10 dai IR[h]	N.I.	Ithal et al. 2007a
GmGH	Glycosyl hydrolase family 8 protein	CD407063	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmGH	Glycosyl hydrolase family 8 protein	AI416659	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmGH	Glycosyl hydrolase family 8 protein	BE658250	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmGH	Glycosyl hydrolase family 3 protein	BI971040	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmGH	Glycosyl hydrolase family 17 protein	BI969847	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b

(continued)

Table 1.2 (continued)

Gene name	Putative function	AGI or accession #	Cyst	RKN	References
GmGH	Glycosyl hydrolase family 17 protein	CD396678	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmGH	Glycosyl hydrolase family 1 protein	AW309536	downregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmGH	Glycosyl hydrolase family 1 protein	BI785739	downregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmGH	Glycosyl hydrolase family 31 protein	CA799910	downregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmGH	Glycosyl hydrolase family 3 protein	AW705239	downregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmGH	Glycosyl hydrolase family 1 protein	BU551231	downregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmGH	Glycosyl hydrolase family 1 protein	BQ612438	downregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmGH	Glycosyl hydrolase family 3 protein	BU549884	downregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmGH	Glycosyl hydrolase family 20 protein	CD416483	downregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b

Abbreviations: At = Arabidopsis thaliana; dai = days after infection; Gm = Glycine max; IR = infected roots; ND = not detected; NFhS = nematode feeding site; N.I. = no information; Np = Nicotiana plumbaginifolia; Nt = Nicotiana tabacum; Sl= Solanum lycopersicum

[a] *in situ* hybridization
[b] promoter-GUS
[c] antibody
[d] feeding cell specific cDNA library
[e] RT-PCR infected root segments
[f] RT-PCR LCM feeding cells
[g] microarray LCM feeding cells
[h] microarray; infected root segments
[i] differential display; infected root segments
[j] Northern blot; infected root segments

attempt to ward off an invading pathogen by strengthening the cell walls. It has been suggested that extensin expression in the gall pericycle may be contributing to a need for new structural cell wall components in dividing cells. Furthermore, expression in the gall cortex may be required to increase the rigidity of the walls in response to mechanical pressure created by the swelling of the central cylinder (Niebel et al. 1993). More recent microarray studies have shown that extensins are also up-regulated during cyst-nematode plant interactions (Ithal et al. 2007b; Khan et al. 2004; Puthoff et al. 2003). Similarly, up-regulation of peroxidases, enzymes that play an important role in strengthening of the cell wall through rapid cross-linking of cellulose, HRGPs, and lignin, has been observed. Increased expression of structural cell wall proteins and peroxidases in nematode-infected roots (Puthoff et al. 2003; Ithal et al. 2007b) may be one mechanism used to maintain a balance between cell wall strengthening and loosening during feeding cell formation.

In contrast to cell wall strengthening, cell wall loosening for expansion, elongation, and dissolution requires the activity of different classes of hydrolytic enzymes, and several have been implicated in NFS formation (Goellner et al. 2001; Mitchum et al. 2004; Vercauteren et al. 2002). Beta-1,4-endoglucanase (EGases) are hydrolytic enzymes that cleave beta-1,4-glucosidic linkages, and there is considerable evidence supporting a role for these enzymes in a wide range of plant developmental processes involving changes in cell wall architecture. EGase genes are up-regulated in both root-knot and cyst nematode infected root tissues compared to uninfected roots, and their expression has been localized within developing giant-cells and syncytia (Goellner et al. 2001; Karczmarek et al. 2008; Wieczorek et al. 2008). Five tobacco EGases and two of at least eight tomato EGases were found to be up-regulated by the tobacco cyst nematode and potato cyst nematode, respectively, in nematode-infected roots (Goellner et al. 2001; Karczmarek et al. 2008). Seven of the 25 members of the Arabidopsis EGase gene family are up-regulated in syncytia induced by the beet cyst nematode. Two of these family members, *AtCEL2* and *AtKOR3*, are exclusively expressed in shoot tissues during normal plant growth and development but are strongly up-regulated in developing syncytia. A reduction in female development on *cel2* and *kor3* mutants indicates that these two EGases play an important role in nematode feeding site formation. Similarly, growth and development of potato cyst nematodes was severely hampered on potato (*Solanum tuberosum*) plants silenced for *StCEL7* or *StCEL9C1* (Karczmarek et al. 2008). An Arabidopsis pectin acetylesterase (*PAE*) gene was also shown to be up-regulated in initiating giant-cells, syncytia, and cells surrounding the nematode (Vercauteren et al. 2002). The level of *PAE* declined in giant-cells at later time-points but was still detectable in parenchyma, endodermis, and pericycle cells of the root gall. PAEs catalyze the deacetylation of esterified pectin thereby increasing the accessibility of pectin to degrading enzymes. The expression of the same EGase and PAE genes in both types of feeding cells suggests that similar mechanisms for cell growth involving loosening of the primary cell wall and middle lamella contributes to their formation. On the other hand, distinct expression of individual gene family members in either giant-cells or syncytia may contribute to observed differences in the extent of cell wall dissolution for each cell type. This is true for the promoter

of the Arabidopsis elongation-specific EGase gene, *AtCel1*, which was shown to be expressed in developing giant-cells but not syncytia (Mitchum et al. 2004; Wieczorek et al. 2008), suggesting that this EGase plays a unique role in giant-cell formation.

More recently, the differential expression of plant expansins has been observed in cyst and root-knot nematode infected roots (Gal et al. 2005; Jammes et al. 2005; Puthoff et al. 2003; Wieczorek et al. 2006). Expansins are cell-wall loosening proteins that function by disrupting noncovalent bonds between cellulose and hemicellulose polymers to assist in the elongation and expansion of cell walls. In galls induced by root-knot nematodes on tomato roots, *LeEXPA5* transcript was detected in gall cells surrounding the giant-cells, but not within giant-cells at either 4 or 10 dai (Gal et al. 2005). The use of an antisense construct to disrupt expansin expression in tomato roots resulted in reduced galling upon infection by root-knot nematodes suggesting that expansins play an important role in gall formation. Similarly, an Arabidopsis gene expression profiling experiment comparing gall tissue to uninfected root tissues detected up-regulation of seven *AtEXPA* genes, two *AtEXPB* genes, and two expansin-like (*AtEXL*) genes in developing galls (Jammes et al. 2005). It remains to be shown whether these expansins are specifically expressed within developing giant-cells to contribute to their formation. On the contrary, Wieczorek et al. (2006) have characterized the expression of the Arabidopsis α- and β-expansin gene families in cyst nematode-infected roots and demonstrated the expression of several different expansins within developing syncytia. Of the 26 member Arabidopsis α-expansin gene family, nine members were up-regulated and two members were down-regulated within developing syncytia. Of the three β-expansin members tested, only *AtEXPB3* was shown to be up-regulated in the syncytium. Interestingly, two of the α-expansins (*AtEXPA3* and *AtEXPA16*) up-regulated in the syncytium were found to be shoot-specific. Similarly, laser-capture microdissection coupled with microarray profiling has also identified the specific up-regulation of seven soybean α-expansins (*GmEXPA*) in syncytia induced by soybean-cyst nematode (Ithal et al. 2007b).

More recent microarray analyses in Arabidopsis and soybean (Jammes et al. 2005; Puthoff et al. 2003; Ithal et al. 2007a,b) have identified the differential regulation of several additional classes of CWMPs, including xyloglucan endotransglycosylases (XETs), β-xylosidases, reversibly glycosylated polypeptides (RGPs), and pectate lyases (see Li et al. 2008). From these studies it is evident that feeding cell formation is a complex process that likely requires the synergistic activity and coordinated regulation of several different types of plant CWMPs, as well as the differential expression of individual gene family members. Moreover, the processes that give rise to feeding cells involve recruitment of plant genes that are not necessarily expressed in roots during their growth and development. The regulation of genes encoding CWMPs within developing feeding cells also appears to be conserved among diverse plant species which is consistent with the idea that nematodes interfere with fundamental aspects of plant development. Conserved activity of tobacco EGase promoters in nematode feeding sites induced in Arabidopsis, tomato, and soybean supports this idea (Mitchum et al. 2004; Wang et al. 2007). Phytohormones function as key regulators

of plant hydrolytic enzymes and the altered phytohormone balance within developing feeding cells (see Sect. 9) may trigger a developmental cascade leading to the controlled regulation of genes encoding CWMPs. Future exploitation of genomic information for the detailed characterization of entire gene families will aid in determining subsets of gene family members that are either specifically expressed or suppressed within feeding cells. This information can be compared to gene expression in other tissues and developmental processes to provide important insight into their formation. In addition, gene knock-out approaches will allow the role of individual family members in feeding cell formation to be assessed.

6 Transport Routes of Nutrients and Water into NFS

Sedentary nematodes continuously withdraw nutrients from metabolically active feeding sites as they develop. Therefore, these NFS represent major sinks for metabolites such as sugars and amino acids imported from the phloem. This occurs symplastically through plasmodesmata or apoplastically via transmembrane transporter proteins. Plasmodesmata connecting the syncytium with neighboring cells were assumed to be nonfunctional because of cell wall deposits, and this was supported by the fact that fluorescent dyes injected into syncytia were unable to move into neighboring cells (Bockenhoff and Grundler 1994; Bockenhoff et al. 1996). These data led to the widely accepted concept that transport from the phloem into *H. schachtii*-induced syncytia is apoplastic, involving transmembrane transporters. However, Hoth et al. (2005) challenged this concept by demonstrating the trafficking of GFP from unloading phloem into syncytia. Recent grafting experiments, using transgenic *A. thaliana* scions expressing GFP in the phloem along with wild-type rootstocks, unequivocally confirmed the movement of GFP from the phloem into syncytia and corroborated the existence of a symplastic route (Hofmann and Grundler 2006; Hofmann et al. 2007). In tomato, nematode-induced galls are also connected to the phloem by plasmodesmata (Dorhout et al. 1993).

Nevertheless, several studies have shown up-regulation of transporter proteins in NFS which would indicate the importance of an apoplastic route. *A. thaliana* genes encoding sucrose transporters were expressed higher in NFS than in uninfected roots [shown for *AtSUC2* and *AtSUC4* in syncytia (Hofmann et al. 2007) and for *AtSUC1* in giant-cells (Hammes et al. 2005)]. The role of these transporters was validated via competitive inhibition using maltose in the plant growth medium which inhibits SUC activity. Nematode development was significantly reduced in comparison to sucrose or glucose although root growth was not affected (Hofmann et al. 2007). A comprehensive microarray analysis was performed on the expression of 634 transport proteins represented in the Arabidopsis Membrane Protein Library; 30 were found to be up-regulated and 20 down-regulated at several time-points after infection with *Meloidogyne* (Hammes et al. 2005). Expression of different members of a gene family is often divergently affected. Functional and protein localization studies will be needed to determine the potential role of these transporters in plant–nematode interactions.

The current model for solute transport from the phloem into NFS (based on syncytium research) takes into account both apoplastic and symplastic transport. In initial feeding cells, plasmodesmatal connections with surrounding cells close in response to the infecting nematode, except at sites of cell fusion. In the following days, the turgor pressure increases in the syncytium, and increased expression of transporter proteins facilitates nutrient supply from the phloem (at 7 dai 80% of the studied syncytia are still symplastically isolated and thus dependent on transporter-based solute import) (Hofmann et al. 2007). As syncytia approach their final stage of expansion, plasmodesmata reopen (around 12 dai).

Sijmons et al. (1991) estimated that developing juveniles of *H. schachtii* withdraw an amount of solute equivalent to four times the syncytial volume every 24 h (Sijmons et al. 1991). The water necessary for replenishing this comes from the xylem, with the invaginations of cell wall and plasma membrane facilitating this drain. Genes encoding water channel proteins (aquaporins) are highly up-regulated in NFS to support this massive water transport. For example, the *TobRB7* gene from tobacco is induced in giant-cells from 4 dai until 35 dai (Opperman et al. 1994), and the corresponding gene in tomato was also shown to be highly up-regulated in giant-cells (He et al. 2005). *TobRB7* was not up-regulated in tobacco syncytia, but the soybean aquaporin *GmPIP2.2* showed a sevenfold enhanced expression in soybean syncytia formed by *H. glycines* (Klink et al. 2005). This apparent contradiction can again be explained by dissimilar expression patterns of different genes from the same family as clearly illustrated by two *A. thaliana* aquaporin genes. The expression of *AtPIP2.5* was induced 17-fold in galls compared to control root tissue while *AtPIP2.6* was expressed at similar levels in both tissues (Hammes et al. 2005).

7 Increased Metabolic Activity of NFS

Metabolic reprogramming is a key feature of nematode feeding cells. Consistent with their role as nutrient sinks, elevated sugar levels and increased rates of metabolism through the glycolytic and pentose phosphate pathways have been observed in both syncytia and giant-cells. Early physiological studies detected high levels of glucose-6-phosphate and 6-phosphogluconate dehydrogenase (G6PDH) activity in histochemical preparations of nematode feeding cells (Endo and Veech 1968), hinting at an increased flux through these pathways and the simultaneous production of a wide array of intermediates for use in other metabolic processes. Glucose-6-phosphate serves as a precursor for glycolysis and the pentose phosphate pathway (PPP). In glycolysis, glucose is oxidized to pyruvic acid, a process that generates energy in the form of ATP, NADH, and three-carbon and six-carbon intermediates for other biosynthetic pathways. G6PDH is the first step in the oxidative pentose phosphate pathway catalyzing the conversion of glucose-6-phosphate to 6-phosphogluconate. More recent molecular studies are beginning to confirm some of these earlier observations. For example, an Arabidopsis phosphoglycerate mutase/biphosphoglycerate mutase (*PGM/bPGM*) gene was up-regulated early in both giant-cells and syncytia, and its expression

increased during feeding cell development (Mazarei et al. 2003). PGM catalyzes the reversible interconversion of 3-phosphoglycerate and 2-phosphoglycerate, a key step in sugar metabolism during glycolysis. In addition, microarray analysis of laser-captured syncytia induced in soybean identified the up-regulation of genes encoding enzymes at each step of the glycolytic and PP pathways (Ithal et al. 2007b). The PPP plays several roles in plant metabolism. Cells utilize the PPP to maintain a pool of reducing molecules in the form of NADPH to protect against oxidative stress. During the nonoxidative phase of the PPP, carbohydrate intermediates are produced. Carbohydrate intermediates such as ribose-5-phosphate serve as the building blocks of ribose and deoxyribose for the synthesis of nucleic acids. Consistent with this, the promoter of a soybean phosphoribosylformyl-glycinamidine (FGAM) synthase, an enzyme of the de novo purine biosynthetic pathway, was shown to be activated during syncytia formation (Vaghchhipawala et al. 2004). Erythrose-4-phosphate, another product of the PPP, serves as a precursor to the shikimate pathway for the production of plant phenolic compounds including aromatic amino acids for protein and IAA synthesis, flavonoids, alkaloids, lignin, and other secondary metabolites. An essential role for the pentose phosphate pathway in feeding cell maintenance is supported by a study of the Arabidopsis *RPE* gene. *RPE* encodes ribulose-phosphate 3-epimerase (*RPE*), an enzyme that catalyzes the reversible conversion of ribulose-5-phosphate and xylulose-5-phosphate. *RPE* is up-regulated in giant-cells and during the late stages of syncytium development, suggesting increased flux through the PPP (Favery et al. 1998). Mutations in *RPE* result in a lethal phenotype due to the inability of the plant to maintain sugar phosphate levels; however, partial rescue can be established with exogenous sucrose to produce dwarf plants with light green leaves and reduced root systems that eventually die. Upon root-knot infection, rescued *rpe* dwarfs do not support gall development, highlighting the importance of the PPP in this process. In contrast, cyst nematode development was not impaired on *rpe* mutants (Favery et al. 1998). Currently, there is not enough data to explain the observed differences in the requirement of the PPP pathway for giant-cell but not syncytia formation.

8 Signal Transduction Pathways and Transcription Factors

Transcription factors and protein kinases involved in signal transduction pathways are crucial in cell differentiation processes. Those found to be up- or down-regulated in NFS, therefore, merit particular attention. For example, Puthoff et al. (2003) found genes encoding serine-threonine kinases, a calmodulin-related protein and a calcium-dependent protein kinase to be down-regulated in cyst nematode-infected roots of *Arabidopsis thaliana*. More recent microarray analyses have added several genes potentially important in NFS signaling (Jammes et al. 2005; Khan et al. 2004; Ithal et al. 2007b). Proteins encoded by gene families are often involved in complex pathways; therefore, their specific

roles in NFS formation are difficult to grasp without detailed functional studies. Some transcription factors, however, have been analyzed more thoroughly. For example, the Mt-KNOX transcription factor is highly expressed in giant-cells while its role in uninfected plants is to maintain meristem function and develop lateral organs (Koltai et al. 2001). Disruption of auxin transport was found to mimic KNOX over-expression, and a strict correlation between KNOX expression and elevated cytokinin levels has been observed. The importance of auxin and cytokinin in NFS development is discussed below. KNOX expression is in turn regulated by the transcription factor PHAN, which is also highly expressed in giant-cells (Koltai et al. 2001). Another transcription factor highly expressed in both early giant-cells and syncytia is *AtWRKY23*. This gene is induced by auxin and is important for primary root development and nematode infection (Grunewald et al. 2008; Gheysen, unpublished results). Another transcription factor, AtABI3, is up-regulated in early syncytia but not giant-cells (De Meutter et al., 2005). ABI3 was originally identified as a central signaling factor in desiccation during embryogenesis, but further analyses have indicated an important role for this protein in cell differentiation and meristem quiescence (Rohde et al. 2000). An EREBP-domain protein (EREBP; ethylene-responsive element-binding protein) is a transcription factor down-regulated in the compatible soybean–*H. glycines* interaction but up-regulated in the incompatible interaction (Mazarei et al. 2002). Overexpression of this transcription factor results in the up-regulation of several PR-proteins suggesting that its down-regulation in the compatible interaction may interfere with the plant defense response to facilitate successful nematode infection (Mazarei et al. 2007).

The only plant receptor kinases in the plant–nematode interaction that have been studied in some detail are part of a remarkable common signal transduction pathway for root-knot nematodes and rhizobial Nod factors in *Lotus japonicus* (Weerasinghe et al. 2005). Root-knot nematodes invoke root-hair waviness and branching in Lotus root hairs similar to the effect of Nod factors. Furthermore, the ability of root-knot nematodes to establish feeding sites and reproduce was notably reduced in mutant lines defective in the Nod factor receptor genes *NFR1*, *NFR5*, and *SYMRK* (Table 1) (Weerasinghe et al. 2005).

9 Developmental Reprogramming for NFS Formation

Comparisons with better understood systems of plant cell differentiation and organ formation may be helpful for our understanding of NFS development. It was postulated by Nutman (1948) that microbes can tap into plant developmental pathways and, more specifically, that nodulation was related to lateral root formation. In addition, root-knot nematodes and endosymbiotic rhizobia induce similar structures within plant roots (galls and nodules, respectively) and this has inspired studies comparing gene expression between these two processes (reviewed in Davis and

Mitchum 2005). The genes encoding the transcription factors PHAN and KNOX, the early nodulin gene *ENOD40*, and the cell cycle gene *CCS52* are highly expressed in nodules, giant-cells, and lateral root initials (Koltai et al. 2001). Similarly, *MtENOD11*, encoding a cell wall proline-rich protein, is up-regulated by *Rhizobium* and *Meloidogyne* infection (Boisson-Dernier et al. 2005). However, a larger-scale comparison (Favery et al. 2002) revealed that similarities in gene expression between nodules and galls may be limited. Only 2 of 192 genes expressed in nodules in *Medicago truncatula* were up-regulated upon root-knot nematode infection in their experimental set-up.

The correlation of genes expressed in NFS and initiation sites of lateral roots is striking. In a tagging experiment where 103 lines were first selected for their expression in NFS, 38% of these lines also displayed expression at the base of developing lateral roots (Barthels et al. 1997). Among those studied in more detail were Att0001 and Att0728 (Barthels et al. 1997) in which the *AtWRKY23* and *TIFY10a* genes were tagged, respectively. This former gene encodes a WRKY transcription factor involved in root development (Grunewald and Gheysen, unpublished results) while the latter is a member of the JAZ protein family. The similarity in the expression pattern of these genes is not only in their early expression in NFS (giant-cells and syncytia) but also their up-regulation upon auxin treatment (Grunewald and Gheysen, unpublished results). Other auxin-regulated genes involved in feeding cell formation include cell cycle genes, expansins, and cell-wall hydrolyzing enzymes.

Auxin has long been suspected to play a central role in NFS formation. Auxin also seems to be a common factor in root developmental events such as lateral root initiation, symbiotic nodules, root-knot galls, and syncytia (Mathesius 2003). Many of the cellular changes occurring when nematodes become sedentary can be partially mimicked by application of indole-3-acetic acid (IAA), i.e., endoreduplication (Valente et al. 1998), acytokinetic mitosis (Naylor et al. 1954), cell enlargement (Jones et al. 1998), cell-wall breakdown (Fan and Maclachlan 1967), and lateral root formation (Torrey 1950). Resistant peach rootstocks are susceptible to *M. javanica* following auxin treatment (Kochba and Samish 1971), and the auxin-insensitive tomato mutant *diageotropica* is resistant to *M. incognita* (Richardson and Price 1984). Ever since, evidence for the role of auxin in NFS formation has continuously accumulated. The auxin-responsive promoter *GH3* is rapidly and transiently activated in initiating rhizobial nodules and during root gall initiation by *Meloidogyne* in white clover (Hutangura et al. 1999). Auxin appeared to accumulate basipetal (above) and was reduced acropetal (below) to the forming gall, indicating a block in auxin transport at the infection site. *DR5* is an artificial promoter with auxin-responsive elements derived from the *GH3* promoter and it is often used as a sensitive and specific indicator of the presence of auxin in plant tissues. Strong and local DR5::GUS activation was visible in *A. thaliana* roots infected by *M. incognita* or *H. schachtii* (Karczmarek et al. 2004) at 18 h after infection (the earliest tested time-point). Although the possible secretion of auxin-like compounds by the nematode or increased plant auxin biosynthesis or sensitivity cannot be excluded, the most likely explanation is an accumulation of auxin due to disruption of polar auxin transport. Hutangura et al. (1999) demonstrated a

local accumulation of intracellular flavonoids in initiating root galls of white clover roots infected by *M. javanica*. Several flavonoids are known to be potent auxin transport inhibitors. Disturbance of auxin gradients in the plant medium by addition of N-(1-naphtyl)phtalamic acid (NPA), a polar auxin transport inhibitor, results in abnormal feeding cells, and auxin-insensitive mutants hardly support cyst nematode reproduction (Goverse et al. 2000) (Table 1). These experiments demonstrate that auxin is an essential plant hormone for successful root-knot and cyst nematode infection.

For cytokinins, on the other hand, there is only support for a role in root-knot nematode infection. High cytokinin levels, either endogenous or applied, correlate with plant susceptibility to root-knot nematodes, and biologically active cytokinins have been shown to be produced by *Meloidogyne* spp. (Bird and Loveys 1980; De Meutter et al. 2003; Dropkin et al. 1969; Kochba and Samish 1971; Kochba and Samish 1972). The cytokinin-responsive promoter *ARR5* is up-regulated in young galls induced by *M. incognita*, at the base of lateral root primordia and in rhizobial-induced nodule primordia (Lohar et al. 2004), again illustrating similarities between these processes. Nevertheless, in roots expressing cytokinin oxidase (causing a decrease in cytokinin levels), lateral root formation is greater while the numbers of both nodules and galls are decreased compared to wild-type roots (Lohar et al. 2004).

A third plant hormone which has emerged as a player in plant–cyst nematode interactions is ethylene. *A. thaliana* ethylene-insensitive mutants are less susceptible to *H. schachtii* compared to wild-type plants (Table 1) (Wubben et al. 2001), while ethylene-overproducing *A. thaliana* mutants attract more juveniles and result in larger syncytia and females (Table 1) (Goverse et al. 2000). Enhanced syncytial cell wall dissolution and expansion in the latter mutants suggests that ethylene-induced cell wall degradation is involved in syncytium formation (Goverse et al. 2000). More recent experiments on a different mutant with increased ethylene susceptibility and hypersusceptibility to *H. schachtii* confirms the importance of ethylene for cyst nematode infection (Table 1) (Wubben et al. 2004).

Concerning the hormones that have a distinguished role in plant defense signaling after pathogen or insect attack, namely salicylic acid (SA) and jasmonic acid (JA), conflicting results have apparently been obtained (Bhattarai et al. 2008; Cooper et al. 2005; Wubben et al. 2008). More research is certainly needed to understand the role of these hormones in plant–nematode interactions.

10 Initiation of NFS

The identification of the nematode signal(s) triggering NFS development also promises to provide new opportunities for unraveling the infection process. In contrast to nodulation by Rhizobia, where Nod-factors are the key molecules in nodule initiation, the nematode almost certainly uses a medley of different enzymatic and regulatory molecules for establishing a successful parasitic interaction. At least,

this is what can be deduced from the variety of proteins that are secreted from the esophageal glands into the plant roots (De Meutter et al. 2001; Gao et al. 2003; Huang et al. 2003a; Jaubert et al. 2002). For more details, see Davis et al. (2008); however, we will discuss several "parasitism proteins" to illustrate the range of possibilities for their role in NFS formation.

Cyst nematodes and root-knot nematodes both secrete chorismate mutases (Jones et al. 2003; Lambert et al. 1999). Chorismate mutase converts chorismate to prephenate in the shikimate pathway. Compounds derived from chorismate include auxin and salicylic acid, while a variety of secondary metabolites are derived from prephenate, including flavonoids which can act as auxin transport inhibitors. It is not known whether this nematode-secreted enzyme interferes with auxin biosynthesis or transport, plant defense, or other metabolic processes of host cells.

Nematode-secreted peptides increasingly demand attention as possible actors in NFS establishment. A secreted peptide from *H. glycines* containing a conserved CLAVATA3/ESR (CLE) motif characteristic of plant CLE peptides (Olsen and Skriver 2003) was shown to partially rescue the *A. thaliana clv3-1* mutant phenotype, and its expression in wild-type plants caused a wuschel-like phenotype typical for CLV3 overexpression (Wang et al. 2005). These results suggest that cyst nematode-secreted CLE peptides may function as ligand mimics of host plant CLEs for the redifferentiation of root cells for syncytium formation (reviewed in Mitchum et al. 2008). A secreted peptide from *Meloidogyne incognita* that is essential for parasitism (Huang et al. 2006b) may also interfere with root cell differentiation for giant-cell formation because it has been shown to interact with the SAW domain of SCL6 and SCL21, two Scarecrow-Like (SCL) transcription factors (Huang et al. 2006a) which belong to the GRAS protein family (Bolle 2004). Although the functions of SCL6 and SCL21 are unknown, several other members of the GRAS protein family have been shown to play important roles in plant development and signaling (Bolle 2004).

The ubiquitination pathway plays a prominent role in many plant regulatory pathways: as part of hormone signaling, for cell cycle regulation, during rhizobial nodulation, and pathogen infection (Zeng et al. 2006). There is mounting evidence that cyst nematodes exploit the host ubiquitination pathway in order to manipulate the plant response. Several cyst nematodes secrete an ubiquitin-extension protein from their dorsal gland cells (Tytgat et al. 2004). Other proteins similar to those involved in ubiquitination pathways are also expressed in their dorsal gland including S-phase kinase associated proteins (Skp-1) and RING-H2 proteins, which are subunits of the complex that transfers ubiquitin tags to target proteins (Gao et al. 2003). Although understanding the role of the ubiquitination pathway in syncytium development still requires more research, it is intriguing to note that changes in ubiquitination pathways can affect cell cycle regulation in some organisms and give rise to cells that go through multiple S phases with no intervening mitosis (Lammer et al. 1998). This results in hypertrophic nuclei similar to those found in syncytia.

11 Conclusions

The mechanisms used by phytonematodes to redifferentiate plant cells into morphologically distinct cell types with ontogenies unique to each plant–nematode combination have long fascinated researchers. Although our knowledge of the complex molecular mechanisms underlying feeding site formation still remains fragmentary, substantial progress to characterize gene expression in these cells over the last decade has established the framework necessary to begin to unravel the genetic networks directly involved in their formation. Despite the fact that significant progress has been made in cataloguing plant and nematode genes involved in the compatible interaction, the ultimate question of how sedentary nematodes trigger feeding site development remains a puzzle with many missing pieces. Thus, an exciting and challenging road lies ahead and promises to unveil new molecular insights into our understanding of nematode feeding sites.

Acknowledgments We are grateful to our colleagues for sharing their unpublished results for inclusion in this review. We also thank Wim Grunewald and Nagabhushana Ithal for critically reading the manuscript. We acknowledge the support for funding provided in part by the United States Department of Agriculture-National Research Initiative Competitive Grants Program (nos. 2005-35604-15434; 2006-35607-16601; 2007-35607-17790), Missouri Soybean Merchandising Council, and the University of Missouri Agriculture Experiment Station to M.G.M and by the Fund for Scientific Research-Flanders (3G003108) and Ghent University (BOF-01G00805) to G.G.

References

Alkharouf NW, Klink VP, Chouikha IB, Beard HS, MacDonald MH, Meyer A, Knap HT, Khan R, Matthews BF (2006) Time course microarray analyses reveal global changes in gene expression of susceptible *Glycine max* (soybean) roots during infection by *Heterodera glycines* (soybean cyst nematode). Planta 224:838–852

Bar-Or C, Kapulnik Y, Koltai H (2005) A broad characterization of the transcriptional profile of the compatible tomato response to the plant parasitic root-knot nematode *Meloidogyne javanica*. Eur J Plant Path 111:181–192

Barthels N, van der Lee FM, Klap J, Goddijn OJM, Karimi M, Puzio P, Grundler FMW, Ohl SA, Lindsey K, Robertson L, Robertson WM, Van Montagua M, Gheysen G, Sijmons PC (1997) Regulatory sequences of Arabidopsis drive reporter gene expression in nematode feeding structures. Plant Cell 9:2119–2134

Berg RH, Fester T, Taylor CG (2008) Development of the root-knot nematode feeding cell. Plant Cell Monogr., doi:10.1007/7089_2008_30

Bhattarai KK, Xie Q-G, Mantelin S, Bishnoi U, Girke T, Navarre DA, Kaloshian I (2008) Tomato susceptibility to root-knot nematodes requires an intact JA signaling pathway. Mol Plant Microbe Interact 21:1205–1214

Bird AF, Loveys BR (1980) The involvement of cytokinins in a host–parasite relationship between the tomato (*Lycopersicon esculentum*) and a nematode (*Meloidogyne incognita*). Parasitology 80:497–505

Bird DMcK, Opperman CH, Williamson VM (2008) Plant infection by root-knot nematode. Plant Cell Monogr., doi:10.1007/7089_2008_31

Bockenhoff A, Grundler FMW (1994) Studies on the nutrient-uptake by the beet cyst–nematode *Heterodera schachtii* by in situ microinjection of fluorescent probes into the feeding structures in *Arabidopsis thaliana*. Parasitology 109:249–255

Bockenhoff A, Prior DAM, Grundler FMW, Oparka KJ (1996) Induction of phloem unloading in *Arabidopsis thaliana* roots by the parasitic nematode *Heterodera schachtii*. Plant Physiol 112:1421–1427

Boisson-Dernier A, Andriankaja A, Chabaud M, Niebel A, Journet EP, Barker DG, de Carvalho-Niebel F (2005) *MtENOD11* gene activation during rhizobial infection and mycorrhizal arbuscule development requires a common AT-rich-containing regulatory sequence. Mol Plant Microbe Interact 18:1269–1276

Bolle C (2004) The role of GRAS proteins in plant signal transduction and development. Planta 218:683–692

Boudolf V, Vlieghe K, Beemster GTS, Magyar Z, Acosta JAT, Maes S, Van Der Schueren E, Inze D, De Veylder L (2004) The plant-specific cyclin-dependent kinase CDKB1;1 and transcription factor E2Fa-DPa control the balance of mitotically dividing and endoreduplicating cells in Arabidopsis. Plant Cell 16:2683–2692

Caillaud M-C, Lecomte P, Jammes F, Quentin M, Pagnotta S, Andrio E, de Almeida-Engler J, Marfaing N, Gounon P, Abad P, Favery B (2008) MAP65-3 microtubule-associate protein is essential for nematode-induced giant-cell ontogenesis in Arabidopsis. Plant Cell 20:423–437

Cebolla A, Vinardell JM, Kiss E, Olah B, Roudier F, Kondorosi A, Kondorosi E (1999) The mitotic inhibitor ccs52 is required for endoreduplication and ploidy-dependent cell enlargement in plants. EMBO J 18:4476–4484

Cooper WL, Jia L, Goggin L (2005) Effect of jasmonate-induced defenses on root-knot nematode infection of resistant and susceptible tomato cultivars. J Chem Ecol 31:1953–1967

Davis EL, Mitchum MG (2005) Nematodes: sophisticated parasites of legumes. Plant Physiol 137:1182–1188

Davis EL, Hussey RS, Baum TJ (2004) Getting to the roots of parasitism by nematodes. Trends Parasitol 20:134–141

Davis EL, Hussey RS, Baum TJ (2008) Parasitism genes: what they reveal about parasitism. Plant Cell Monogr., doi:10.1007/7089_2008_29

De Almeida-Engler J, De Vleesschauwer V, Celenza JR, Inzé D, Van Montagu M, Engler G, Gheysen G (1999) The use of molecular markers and cell cycle inhibitors to analyze cell cycle progression in nematode-induced galls and syncytia. Plant Cell 11:793–807

De Almeida-Engler J, Van Poucke K, Karimi M, De Groodt R, Gheysen G, Engler G, Gheysen G (2004) Dynamic cytoskeleton rearrangements in giant-cells and syncytia of nematode-infected roots. Plant J 38:12–26

De Meutter J, Vanholme B, Bauw G, Tytgat T, Gheysen GDC, Gheysen GDR (2001) Preparation and sequencing of secreted proteins from the pharyngeal glands of the plant parasitic nematode *Heterodera schachtii*. Mol Plant Pathol 2:297–301

De Meutter J, Tytgat T, Witters E, Gheysen G, Van Onckelen H, Gheysen G (2003) Identification of cytokinins produced by the plant parasitic nematodes *Heterodera schachtii* and *Meloidogyne incognita*. Mol Plant Pathol 4:271–277

De Meutter J, Robertson L, Parcy F, Mena M, Fenoll C, Gheysen G (2005) Differential activation of *ABI3* and *LEA* genes upon plant parasitic nematode infection. Mol Plant Pathol 6:321–325

De Veylder L, Beeckman T, Beemster GTS, de Almeida-Engler J, Ormenese S, Maes S, Naudts M, Van der Schueren E, Jacqmard A, Engler G, Inze D (2002) Control of proliferation, endoreduplication and differentiation by the Arabidopsis E2Fa-DPa transcription factor. EMBO J 21:1360–1368

Dorhout R, Gommers FJ, Kolloffel C (1993) Phloem transport of carboxyfluorescein through tomato roots infected with *Meloidogyne incognita*. Physiol Mol Plant Pathol 43:1–10

Dropkin VH, Helgeson JP, Upper CD (1969) The hypersensitivity reaction of tomatoes resistant to *Meloidogyne incognita*: reversal by cytokinins. J Nematol 1:55–61

Endo BY (1984) Histopathology and ultrastruture of crops invaded by certain sedentary endoparasitic nematodes. In: Veech JA, Dickson DW (eds) Vistas on nematology. Society of Nematology, Hyattsville, pp 196–210

Endo BY, Veech JA (1968) The histochemical localization of oxidoreductive enzymes of soybeans infected with the root-knot nematode *Meloidogyne incognita* acrita. Phytopathology 59:418–425

Fan D-F, Maclachlan GA (1967) Massive synthesis of ribonucleic acid and cellulase in the pea epicotyl in response to indoleacetic acid, with and without concurrent cell division. Plant Physiol 42:1114–1122

Favery B, Lecomte P, Gil N, Bechtold N, Bouchez D, Dalmasso A, Abad P (1998) *RPE*, a plant gene involved in early developmental steps of nematode feeding cells. EMBO J 17:6799–6811

Favery B, Complainville A, Vinardell JM, Lecomte P, Vaubert D, Mergaert P, Kondorosi A, Kondorosi E, Crespi M, Abad P (2002) The endosymbiosis-induced genes *ENOD40* and *CCS52a* are involved in endoparasitic-nematode interactions in *Medicago truncatula*. Mol Plant Microbe Interact 15:1008–1013

Favery B, Chelysheva LA, Lebris M, Jammes F, Marmagne A, de Almeida-Engler J, Lecomte P, Vaury C, Arkowitz RA, Abad P (2004) Arabidopsis formin AtFH6 is a plasma membrane-associated protein upregulated in giant-cells induced by parasitic nematodes. Plant Cell 16:2529–2540

Gal TZ, Aussenberg ER, Burdman S, Kapulnik Y, Koltai H (2005) Expression of a plant expansin is involved in the establishment of root-knot nematode parasitism in tomato. Planta 224:155–162

Gao BL, Allen R, Maier T, Davis EL, Baum TJ, Hussey RS (2003) The parasitome of the phytonematode *Heterodera glycines*. Mol Plant Microbe Interact 16:720–726

Gheysen G, Fenoll C (2002) Gene expression in nematode feeding sites. Ann Rev Phytopathol 40:191–219

Gheysen G, De Almeida Engler J, Van Montagu M (1997) Cell cycle regulation in nematode feeding sites. In: Fenoll C, Grundler FMW, Ohl SA (eds) Cellular and molecular aspects of plant nematode interactions, vol 10. Kluwer, Dordrecht, pp 120–132

Goellner M, Wang XH, Davis EL (2001) Endo-β-1,4-glucanase expression in compatible plant-nematode interactions. Plant Cell 13:2241–2255

Goverse A, Overmars H, Engelbertink J, Schots A, Bakker J, Helder J (2000) Both induction and morphogenesis of cyst nematode feeding cells are mediated by auxin. Mol Plant Microbe Interact 13:1121–1129

Grundler FMW, Sobczak M, Golinowski W (1998) Formation of wall openings in root cells of *Arabidopsis thaliana* following infection by the plant-parasitic nematode *Heterodera schachtii*. Eur J Plant Pathol 104:545–551

Grunewald W, Karimi M, Wieczorek K, Van de Cappelle E, Grundler F, Beeckman T, Inzé D, Gheysen G (2008) A role for AtWRKY23 in feeding site establishment of plant-parasitic nematodes. Plant Physiology 148:358–368

Hammes UZ, Schachtman DP, Berg RH, Nielsen E, Koch W, McIntyre LM, Taylor CG (2005) Nematode-induced changes of transporter gene expression in Arabidopsis roots. Mol Plant Microbe Interact 18:1247–1257

He B, Magill C, Starr JL (2005) Laser capture microdissection and real-time PCR for measure mRNA in giant-cells induced by *Meloidogyne javanica*. J Nematol 37:308–312

Hofmann J, Grundler FMW (2006) Females and males of root-parasitic cyst nematodes induce different symplasmic connections between their syncytial feeding cells and the phloem in *Arabidopsis thaliana*. Plant Physiol Biochem 44:430–433

Hofmann J, Wieczorek K, Blochl A, Grundler FMW (2007) Sucrose supply to nematode-induced syncytia depends on the apoplasmic and symplasmic pathway. J Exp Bot 58:1591–1601

Hoth S, Schneidereit A, Lauterbach C, Scholz-Starke J, Sauer N (2005) Nematode infection triggers the de novo formation of unloading phloem that allows macromolecular trafficking of green fluorescent protein into syncytia. Plant Physiol 138:383–392

Huang GZ, Gao BL, Maier T, Allen R, Davis EL, Baum TJ, Hussey RS (2003a) A profile of putative parasitism genes expressed in the esophageal gland cells of the root-knot nematode *Meloidogyne incognita*. Mol Plant Microbe Interact 16:376–381

Huang X, Springer PS, Kaloshian I (2003b) Expression of the Arabidopsis *MCM* gene *PROLIFERA* during root-knot and cyst nematode infection. Phytopathology 93:35–41

Huang G, Dong R, Allen R, Davis EL, Baum TJ, Hussey RS (2006a) A root-knot nematode secretory peptide functions as a ligand for a plant transcription factor. Mol Plant Microbe Interact 19:463–470

Huang G, Allen R, Davis EL, Baum TJ, Hussey RS (2006b) Engineering broad root-knot resistance in transgenic plants by RNAi silencing of a conserved and essential root-knot nematode parasitism gene. Proc Natl Acad Sci USA 103:14302–14306

Hutangura P, Mathesius U, Jones MGK, Rolfe BG (1999) Auxin induction is a trigger for root gall formation caused by root-knot nematodes in white clover and is associated with the activation of the flavonoid pathway. Aust J Plant Physiol 26:221–231

Imai KK, Ohashi Y, Tsuge T, Yoshizumi T, Matsui M, Oka A, Aoyama T (2006) The A-type cyclin CYCA2;3 is a key regulator of ploidy levels in Arabidopsis endoreduplication. Plant Cell 18:382–396

Ithal N, Recknor J, Nettleton D, Hearne L, Maier T, Baum TJ, Mitchum MG (2007a) Parallel genome-wide expression profiling of host and pathogen during soybean cyst nematode infection of soybean. Mol Plant Microbe Interact 20:293–305

Ithal N, Recknor J, Nettleton D, Maier T, Baum TJ, Mitchum MG (2007b) Developmental transcript profiling of cyst nematode feeding cells in soybean roots. Mol Plant Microbe Interact 20:510–525

Jammes F, Lecomte P, Almeida-Engler J, Bitton F, Martin-Magniette ML, Renou JP, Abad P, Favery B (2005) Genome-wide expression profiling of the host response to root-knot nematode infection in Arabidopsis. Plant J 44:447–458

Jaubert S, Ledger TN, Laffaire JB, Piotte C, Abad P, Rosso MN (2002) Direct identification of stylet secreted proteins from root-knot nematodes by a proteomic approach. Mol Biochem Parasitol 121:205–211

Jones MGK, Northcote DH (1972) Nematode-induced syncytium – multinucleate transfer cell. J Cell Sci 10:789–809

Jones MGK, Payne HL (1978) Early stages of nematode-induced giant-cell formation in roots of Impatiens-Balsamina. J Nematol 10:70–84

Jones AM, Im KH, Savka MA, Wu MJ, DeWitt NG, Shillito R, Binns AN (1998) Auxin-dependent cell expansion mediated by overexpressed auxin-binding protein 1. Science 282:1114–1117

Jones JT, Furlanetto C, Bakker E, Banks B, Blok V, Chen Q, Phillips M, Prior A (2003) Characterization of a chorismate mutase from the potato cyst nematode *Globodera pallida*. Mol Plant Pathol 4:43–50

Karczmarek A, Overmars H, Helder J, Goverse A (2004) Feeding cell development by cyst and root-knot nematodes involves a similar early, local and transient activation of a specific auxin-inducible promoter element. Mol Plant Pathol 5:343–346

Karczmarek A, Fudall S, Lichocka M, Sobczak M, Kurek W, Janakowski S, Roosien J, Golinowski W, Bakker J, Goverse A, Helder J (2008) Expression of two functionally distinct plant endo-β-1,4-glucanases is essential for the compatible interaction between potato cyst nematode and its hosts. Mol Plant Microbe Interact 21:791–798

Khan R, Alkharouf N, Beard H, MacDonald M, Chouikha I, Meyer S, Grefenstette J, Knap H, Matthews B (2004) Microarray analysis of gene expression in soybean roots susceptible to the soybean cyst nematode two days post invasion. J Nematol 36:241–248

Klink VP, Alkharouf N, MacDonald M, Matthews B (2005) Laser capture microdissection (LCM) and expression analyses of *Glycine max* (soybean) syncytium containing root regions formed by the plant pathogen *Heterodera glycines* (soybean cyst nematode). Plant Mol Biol 59:965–979

Kochba J, Samish RM (1971) Effect of kinetin and 1-naphthylacetic acid on root-knot nematodes in resistant and susceptible peach root-stocks. J Amer Soc Hort Sci 96:458–461

Kochba J, Samish RM (1972) Level of endogenous cytokinins and auxin in roots of nematode-resistant and susceptible peach rootstocks. J Amer Soc Hort Sci 97:115–119

Koltai H, Dhandaydham M, Opperman C, Thomas J, Bird D (2001) Overlapping plant signal transduction pathways induced by a parasitic nematode and a rhizobial endosymbiont. Mol Plant Microbe Interact 14:1168–1177

Kondorosi E, Kondorosi A (2004) Endoreduplication and activation of the anaphase-promoting complex during symbiotic cell development. FEBS Lett 567:152–157

Lambert KN, Allen KD, Sussex IM (1999) Cloning and characterization of an esophageal-gland-specific chorismate mutase from the phytoparasitic nematode *Meloidogyne javanica*. Mol Plant Microbe Interact 12:328–336

Lammer D, Mathias N, Laplaza JM, Jiang WD, Liu Y, Callis J, Goebl M, Estelle M (1998) Modification of yeast Cdc53p by the ubiquitin-related protein Rub1p affects function of the SCFCdc4 complex. Genes Dev 12:914–926

Li L, Fester T, Taylor CG (2008) Transcriptomic analysis of nematode infestation. Plant Cell Monogr., doi: 10.1007/7089_2008_36

Lohar DP, Bird DM (2003) *Lotus japonicus*: A new model to study root-parasitic nematodes. Plant and Cell Physiology 44:1176–1184

Lohar DP, Schaff JE, Laskey JG, Kieber JJ, Bilyeu KD, Bird DM (2004) Cytokinins play opposite roles in lateral root formation, and nematode and Rhizobial symbioses. Plant J 38:203–214

Mathesius U (2003) Conservation and divergence of signalling pathways between roots and soil microbes – the *Rhizobium*-legume symbiosis compared to the development of lateral roots, mycorrhizal interactions and nematode-induced galls. Plant Soil 255:105–119

Mazarei M, Puthoff DP, Hart JK, Rodermel SR, Baum TJ (2002) Identification and characterization of a soybean ethylene-responsive element-binding protein gene whose mRNA expression changes during soybean cyst nematode infection. Mol Plant Microbe Interact 15:577–586

Mazarei M, Lennon KA, Puthoff DP, Rodermel SR, Baum TJ (2003) Expression of an Arabidopsis phosphoglycerate mutase homologue is localized to apical meristems, regulated by hormones, and induced by sedentary plant-parasitic nematodes. Plant Mol Biol 53:513–530

Mazarei M, Elling A, Maier T, Puthoff D, Baum TJ (2007) GmEREBP1 is a transcription factor activating defense genes in soybean and Arabidopsis. Mol Plant Microbe Interact 20:107–119

Mitchum MG, Sukno S, Wang XH, Shani Z, Tsabary G, Shoseyov O, Davis EL (2004) The promoter of the *Arabidopsis thaliana Cel1* endo-1,4-β-glucanase gene is differentially expressed in plant feeding cells induced by root-knot and cyst nematodes. Mol Plant Pathol 5:175–181

Mitchum MG, Wang X, Davis EL (2008) Diverse and conserved roles of CLE peptides. Curr Opin Plant Biol 11:75–81

Naylor A, Sander G, Skoog F (1954) Mitosis and cell enlargement without cell division in excised tobacco pith tissue. Physiol Plant 7:25–29

Niebel A, de Almeida-Engler J, Tire C, Engler G, Vanmontagu M, Gheysen G (1993) Induction patterns of an extensin gene in tobacco upon nematode infection. Plant Cell 5:1697–1710

Niebel A, de Almeida-Engler J, Hemerly A, Ferreira P, Inze D, VanMontagu M, Gheysen G (1996) Induction of *cdc2a* and *cyc1At* expression in *Arabidopsis thaliana* during early phases of nematode-induced feeding cell formation. Plant J 10:1037–1043

Nutman PS (1948) Physiological studies on nodule formation. Part I. The relation between nodulation and lateral root formation in red clover. Ann Bot 12:81–94

Olsen AN, Skriver K (2003) Ligand mimicry? Plant-parasitic nematode polypeptide with similarity to CLAVATA3. Trends Plant Sci 8:55–57

Opperman CH, Taylor CG, Conkling MA (1994) Root-knot nematode-directed expression of a plant root-specific gene. Science 263:221–223

Opperman CH, Conkling MA (1996) Root-knot nematode induced *TobRB7* expression and antisense transgenic resistance strategies. In Biology of plant-Microbe Interactions, ed. Stacey G, Mullin B, Gresshoff PM. pp 521–526. St. Paul: Int. Soc. Mol. Plant-Microbe Interact

Puthoff DP, Nettleton D, Rodermel SR, Baum TJ (2003) Arabidopsis gene expression changes during cyst nematode parasitism revealed by statistical analyses of microarray expression profiles. Plant J 33:911–921

Ramsay K, Wang ZH, Jones MGK (2004) Using laser capture microdissection to study gene expression in early stages of giant-cells induced by root-knot nematodes. Mol Plant Pathol 5:587–592

Richardson L, Price NS (1984) Observations on the biology of *Meloidogyne incognita* and the *diageotropica* tomato mutant. Rev Nematol 7:64–69

Rohde A, Kurup S, Holdsworth M (2000) ABI3 emerges from the seed. Trends Plant Sci 5:418–419

Roudier F, Fedorova E, Lebris M, Lecomte P, Gyorgyey J, Vaubert D, Horvath G, Abad P, Kondorosi A, Kondorosi E (2003) The *Medicago* species A2-type cyclin is auxin regulated and involved in meristem formation but dispensable for endoreduplication-associated developmental programs. Plant Physiol 131:1091–1103

Sijmons PC, Grundler FMW, Vonmende N, Burrows PR, Wyss U (1991) *Arabidopsis thaliana* as a new model host for plant parasitic nematodes. Plant J 1:245–254

Sobczak M, Golinowski W (2008) Structure of cyst nematode feeding sites. Plant Cell Monogr., doi:10.1007/7089_2008_38

Takemoto D, Jones DA, Hardham AR (2003) GFP-tagging of cell components reveals the dynamics of subcellular re-organization in response to infection of Arabidopsis by oomycete pathogens. Plant J 33:775–792

Torrey JG (1950) The induction of lateral roots by indoleacetic acid and root decapitation. Am J Bot 37:257–264

Tytgat T, Vanholme B, De Meutter J, Claeys M, Couvreur M, Vanhoutte I, Gheysen G, Van Criekinge W, Borgonie G, Coomans A, Gheysen G (2004) A new class of ubiquitin extension proteins secreted by the dorsal pharyngeal gland in plant parasitic cyst nematodes. Mol Plant Microbe Interact 17:846–852

Vaghchhipawala ZE, Schlueter JA, Shoemaker RC, Mackenzie SA (2004) Soybean FGAM synthase promoters direct ectopic nematode feeding site activity. Genome 47:404–413

Valente P, Tao WH, Verbelen JP (1998) Auxins and cytokinins control DNA endoreduplication and deduplication in single cells of tobacco. Plant Sci 134:207–215

Van de Cappelle E, Plovie E, Kyndt T, Grunewald W, Cannoot B, Gheysen G (2008) *AtCDKA;1* silencing in *Arabidopsis thaliana* reduces reproduction of sedentary plant-parasitic nematodes. Plant Biotechnol J 6:749–757

Vanholme B, De Meutter J, Tytgat T, Van Montagu M, Coomans A, Gheysen G (2004) Secretions of plant-parasitic nematodes: a molecular update. Gene 332:13–27

Vercauteren I, de Almeida-Engler J, De Groodt R, Gheysen G (2002) An *Arabidopsis thaliana* pectin acetylesterase gene is upregulated in nematode feeding sites induced by root-knot and cyst nematodes. Mol Plant Microbe Interact 15:404–407

Verkest A, Manes CLD, Vercruysse S, Maes S, Van der Schueren E, Beeckman T, Genschik P, Kuiper M, Inze D, De Veylder L (2005) The cyclin-dependent kinase inhibitor KRP2 controls the onset of the endoreduplication cycle during Arabidopsis leaf development through inhibition of mitotic CDKA;1 kinase complexes. Plant Cell 17:1723–1736

Vinardell JM, Fedorova E, Cebolla A, Kevei Z, Horvath G, Kelemen Z, Tarayre S, Roudier F, Mergaert P, Kondorosi A, Kondorosi E (2003) Endoreduplication mediated by the anaphase-promoting complex activator CCS52A is required for symbiotic cell differentiation in *Medicago truncatula* nodules. Plant Cell 15:2093–2105

Vlieghe K, Boudolf V, Beemster GTS, Maes S, Magyar Z, Atanassova A, de Almeida-Engler J, De Groodt R, Inze D, De Veylder L (2005) The DP-E2F-like gene *DEL1* controls the endocycle in *Arabidopsis thaliana*. Curr Biol 15:59–63

Wang ZH, Potter RH, Jones MGK (2003) Differential display analysis of gene expression in the cytoplasm of giant-cells induced in tomato roots by *Meloidogyne javanica*. Mol Plant Pathol 4:361–371

Wang X, Mitchum MG, Gao BL, Li CY, Diab H, Baum TJ, Hussey RS, Davis EL (2005) A parasitism gene from a plant-parasitic nematode with function similar to CLAVATA3/ESR (CLE) of *Arabidopsis thaliana*. Mol Plant Pathol 6:187–191

Wang X, Replogle A, Davis EL, Mitchum MG (2007) The tobacco *Cel7* gene promoter is auxin-responsive and locally induced in nematode feeding sites of heterologous plants. Mol Plant Pathol 8:423–436

Weerasinghe RR, Bird DM, Allen NS (2005) Root-knot nematodes and bacterial Nod factors elicit common signal transduction events in *Lotus japonicus*. Proc Natl Acad Sci USA 102:3147–3152

Weinl C, Marquardt S, Kuijt SJH, Nowack MK, Jakoby MJ, Hulskamp M, Schnittger A (2005) Novel functions of plant cyclin-dependent kinase inhibitors, ICK1/KRP1, can act non-cell-autonomously and inhibit entry into mitosis. Plant Cell 17:1704–1722

Wieczorek K, Golecki B, Gerdes L, Heinen P, Szakasits D, Durachko DM, Cosgrove DJ, Kreil DP, Puzio PS, Bohlmann H, Grundler FMW (2006) Expansins are involved in the formation of nematode-induced syncytia in roots of *Arabidopsis thaliana*. Plant J 48:98–112

Wieczorek K, Hofmann J, Biochl A, Szakasits D, Bohlmann H, Grundler FMW (2008) Arabidopsis endo-1,4-β-glucanases are involved in the formation of root syncytia induced by *Heterodera schachtii*. Plant J 53:336–351

Wiggers RJ, Thornton NT, Starr JL (2002) The effects of colchicine on number of giant-cell nuclei and nematode development in *Pisum sativum* infected by *Meloidogyne incognita*. Nematol 4:107–109

Wubben MJE, Su H, Rodermel SR, Baum TJ (2001) Susceptibility to the sugar beet cyst nematode is modulated by ethylene signal transduction in *Arabidopsis thaliana*. Mol Plant Microbe Interact 14:1206–1212

Wubben MJE, Rodermel SR, Baum TJ (2004) Mutation of a UDP-glucose-4-epimerase alters nematode susceptibility and ethylene responses in Arabidopsis roots. Plant J 40:712–724

Wubben MJE, Jing J, Baum TJ (2008) Cyst nematode parasitism of *Arabidopsis thaliana* is inhibited by salicylic acid (SA) and elicits uncoupled SA-independent pathogenesis-related gene expression in roots. Mol Plant Microbe Interact 21:424–432

Wyss U (2002) Feeding behaviour of plant parasitic nematodes. In: Lee DL (ed) The biology of nematodes. Taylor and Francis, London, pp 233–259

Zeng LR, Vega-Sanchez ME, Zhu T, Wang GL (2006) Ubiquitination-mediated protein degradation and modification: an emerging theme in plant microbe interactions. Cell Res 16:413–426

Resistant Plant Responses

Anna Tomczak, **Kamila Koropacka, Geert Smant, Aska Goverse, and Erin Bakker(✉)**

Abstract Resistance to nematodes takes place at different functional and morphological levels. The first level of resistance is the so-called pre-infectional resistance and occurs before the nematode has had a chance to enter the plant. Against pathogens that can overcome this first level of resistance, plants have evolved a second level of basic resistance, called nonhost immunity. The nonhost immune system has many similarities to the innate immune system of animals. Host resistance, however, is only effective against particular (sub)populations of the pathogen, mostly within a species. Identification of genes underlying quantitative and qualitative nematode disease resistance is the first step to increase our knowledge of the different resistance gene mechanisms. Ultimately, understanding the mechanisms underlying the co-evolution between host plant resistance and nematode (a)virulence is essential for the development of durable crop protection strategies.

1 Introduction

Plants are constantly under attack from a wide range of pathogens and pests including bacteria, viruses, fungi, oomycetes, insects, and nematodes. Fortunately, the majority of plant–pathogen interactions are incompatible due to the failure of the pathogen to localize a potential host plant or to recruit the appropriate battery of modifying enzymes needed for parasitism. In addition, co-evolution between plants and pathogens resulted in the development of an immune system which, in contrast to animals that have both an adaptive and an innate immune system, is completely innate (Zipfel and Felix 2005). This defense system is composed of overlapping layers including nonhost immunity (e.g. specific recognition of nonspecific

E. Bakker
Laboratory of Nematology, Department of Plant Sciences, Wageningen University
Wageningen, Netherlands
email: erin.bakker@wur.nl

Plant Cell Monogr, doi:10.1007/7089_2008_39

pathogen-derived components), host resistance, which is only effective against a specific pathogen race or population, and induced systemic resistance (ISR).

The development of molecular techniques has made it possible to gradually uncover the mechanisms underlying the different layers of disease resistance in plants. In the last decade an increasing number of defense-related genes involved in resistance to various pathogens have been isolated from different plant species, and several elicitors of defense responses were identified from a wide range of pathogens (reviewed by Bonas and Lahaye 2002). However, the molecular mechanisms underlying resistant plant responses to parasitic nematodes are still largely unknown.

Cyst and root-knot nematodes are major pathogens of a number of agronomically important crops such as cereals, soybean, potato, tomato, and sugar beet. The lack of natural enemies and the shortage of adequate resistance genes in crop plants are factors that underlie the very substantial damage caused by these organisms. The estimated worldwide losses caused by plant-parasitic nematodes are about US $ 125 billion annually (Chitwood 2003). One way to control them is the use of nematode resistant cultivars. To that end a broad range of resistances to either cyst or root-knot nematode species has been identified over the years in several crop species in order to develop durable crop protection strategies (Williamson and Hussey 1996; Williamson 1998; Jung and Wyss 1999; Williamson 1999; Bakker 2002).

To date, five genes conferring resistance to cyst and root-knot nematodes have been isolated from beet (Cai et al. 1997), potato (Van der Vossen et al. 2000; Paal et al. 2004), and tomato (Milligan et al. 1998; Vos et al. 1998; Ernst et al. 2002), which allow structural and functional analyses to unravel their role in nematode recognition and the activation of a disease resistance response. The corresponding nematode-derived elicitors, however, are not yet identified. Many aspects of disease resistance signaling in response to plant-parasitic nematodes are thought to resemble the mechanisms underlying the defense responses to other plant pathogens, which are often better characterized. Therefore, the aim of this chapter is not only to present a comprehensive overview of the molecular genetic aspects involved in plant resistance responses to cyst and root-knot nematodes, but also to place this in the context of what is currently known in the field of disease resistance in general.

2 Pre-Infectional Resistance

Before a pre-parasitic juvenile from cyst and root-knot nematodes is able to parasitize a plant species, it has to hatch from the egg, become attracted to the plant roots and penetrate the plant tissue. If the infective juvenile is blocked at any of these stages, the plant apparently is not a suitable host for this particular nematode and therefore de facto resistant. Because this resistance occurs before the pre-parasitic nematode has had a chance to enter the plant, we will refer to this type of resistance as pre-infectional resistance.

Eggs of sedentary endoparasitic nematodes hatch under the influence of plant components released by the plant into the soil. In 1953, Jones and Winslow noticed that pre-parasitic juveniles from beet, potato, and carrot cyst nematodes hatch when soaked in root diffusates of their respective hosts but not when soaked in root diffusates of nonhosts. However, sometimes infective juveniles do hatch in the presence of certain plant species that cannot be successfully parasitized by the nematode. Apparently, nematode infection is then blocked at a later stage of the infection process. Such phenomena can be of great agronomical value because these plants can be used as so-called trap crops to reduce the number of cysts in the soil. For instance, the density of cysts from the soybean cyst nematode *Heterodera glycines* was reduced between 70 and 90% after cultivation of the nonhost species *Crotalaria juncea* and *C. spectabilis* when compared to fallow (Kushida et al. 2003). Interestingly, it was also noticed that the number of juveniles entering the *C. juncea* and *C. spectabilis* roots did not differ significantly from those entering the susceptible soybean roots.

Plant cells are protected by the presence of a rigid cell wall to ward off foreign invaders. However, based on several studies regarding nematode infection of resistant and susceptible cultivars as well as host and nonhost plants (reviewed by Kaplan and Keen 1980) it can be concluded that nematodes freely penetrate roots of host and nonhost alike and that these mechanical barriers rarely appear to be effective against plant-parasitic nematodes. The presence of a typical robust hollow spear (stylet) located in the head region of the nematode enables the nematode to overcome this major barrier. The action of stylet thrusting combined with the release of cell-wall degrading enzymes via this stylet facilitate the penetration of the root and subsequent migration of endoparasitic nematodes to the appropriate feeding site (for details see Chap. 2 on parasitism genes and the review by Davis et al. 2004).

In some cases, plants can prevent nematode invasion with a chemical barrier by releasing naturally occurring nematicides or repellants. For instance, the ornamental grass species *Eragrostis curvula* has a high concentration of pyrocatechol in its roots, preventing *Meloidogyne* species from entering (Scheffer et al. 1962) and cucumbers harboring the dominant *Bi* (bitter) locus attract far fewer *M. incognita* juveniles than near isogenic cucumbers that lack this locus (Da Costa 1971)

3 Nonhost Immunity to Nematodes

Against pathogens that can overcome constitutive barriers as mentioned in the previous section, plants evolved a so-called nonhost immune system that has many similarities to the innate immune system of animals (Zipfel and Felix 2005). This type of resistance is based on the recognition of nonspecific factors, which can be wound- and injury-related structures indirectly derived from pathogens upon infection (Matzinger 2002) or pathogen-associated molecular patterns (PAMPs) directly derived from pathogens themselves (Janeway Jr and Medzhitov 2002). PAMPs have to be functionally important components because they are shared between all members of a certain pathogen group (Chisholm et al. 2006). To date, a number of

PAMPs have been identified including flagellin from bacteria, and xylanase and chitin from fungi (reviewed by Nürnberger and Lipka 2005).

Currently, two plant receptors have been identified that are involved in the recognition of nonspecific elicitors, i.e. FLS2 from *A. thaliana*, which recognizes flagellin (Gomez-Gomez and Boller 2000) and LeEIX from tomato, which recognizes xylanase (Ron and Avni 2004). Interestingly, FLS2 consists of a protein-kinase, a trans-membrane domain, and an extracellular leucine-rich repeat and thereby resembles the XA21 protein from rice that confers host-specific resistance to *Xanthomonas oryzae* (Song et al. 1995). The LeEIX receptor also has a trans-membrane domain and an extracellular leucine-rich repeat but lacks the protein-kinase. This receptor structurally resembles the tomato race-specific resistance proteins Cf-2, Cf-4, Cf-5, and Cf-9 that are protective against *Cladosporium fulvum* (Jones et al. 1994; Dixon et al. 1996, 1998; Thomas 1997). These findings contribute to the assumption that similar pathways are induced in nonhost immunity and host-specific resistance, which is supported by the activation of a mutual defense cascade involving Mitogen-Activated Protein Kinases (MAPKs) (Zhang and Klessig 2001).

Nonhost immunity in plants manifests itself by local changes and enforcement of the plant's basal defense system due to the enhanced production of various defense compounds like waxes, cutin, suberin, lignin, and callose, or the production of oxidative reaction elements like reactive oxygen species (ROS), free radicals, and peroxidases (Kawalleck et al. 1995). In response to nematode infection, plants were shown to reinforce the cell wall by depositing callose at the site of stylet penetration (Grundler et al. 1997). Additionally, the protease inhibitor LeMir was found to be induced early in the interaction between root-knot nematodes and tomato as well as after wounding of the roots. LeMir shows similarity to the soybean trypsin-inhibitor family and can be secreted outside the roots, which suggest its interaction with soil-born microorganisms like nematodes (Brenner et al. 1998).

In several studies, oxidative defense responses to nematodes were shown. Upon infection of the nonhost *Arabidopsis thaliana* with the soybean cyst nematode *H. glycines*, a local cell death response was observed (Grundler et al. 1997) and the production ROS found (Waetzig et al. 1999). An induction of ascorbate free radical (AFR) reductase was shown to occur during the incompatible interaction between tomato and root-knot nematodes as well as after wounding (Lambert et al. 1999). In the same study the up-regulation of genes with a similarity to a wide variety of peroxidases was found.

These results suggest the induction of a nonhost immune response upon cyst and root-knot nematode infection, although nothing is known yet about potential factors that could be recognized by the plant. Considering the fact that nematodes use a whole battery of plant cell-wall degrading enzymes to facilitate penetration of and migration through the root, it could be proposed that plant-derived components resulting from cell wall degradation play a role as elicitors in nonhost immunity to nematodes.

4 Host-Specific Disease Resistance to Nematodes

4.1 Quantitative and Qualitative Disease Resistance

In contrast to nonhost immunity, host resistance is only effective against particular subpopulations of the pathogen, usually within a species. Host-specific resistance is called gene-for-gene resistance if it requires the presence of both a race-specific avirulence (Avr) gene in the pathogen and a corresponding cultivar-specific single dominant resistance (R) gene in the host plant (Flor 1942). The biochemical interpretation of this concept is a receptor–ligand model in which plants activate a defense mechanism upon R protein-mediated recognition of a pathogen-derived AVR product (Van Der Biezen and Jones 1998). Although several pairs of cognate *R* and *Avr* genes are identified, direct interaction could only be proven in four cases (Jia et al. 2000; Deslandes L et al. 2003; Dodds et al. 2006; Ueda et al. 2006). Alternatively, other plant components were shown to interact with avirulence proteins (reviewed in Bogdanove 2002) supporting the so-called guard model (Dangl and Jones 2001) in which AVR protein-induced modifications of host products are recognized by R proteins that "guard" these host products.

Not all cases of host-specific resistance comply with either the gene-for-gene or the guard model. It is possible that intermediate resistant phenotypes occur which can be an indication that resistance is controlled by multiple resistance loci. These resistance phenotypes tend to be measured quantitatively, so they are known as quantitative resistance characters, and the genetic loci associated with them are called quantitative trait loci (QTL). Structure and function of the proteins encoded by QTL are unknown, but quantitative resistance is assumed to be more durable than resistance conferred by a single *R* gene (Parlevliet 2002).

R gene-mediated resistance has several attractive features for disease control. In many cases, a single *R* gene can provide complete resistance to a particular population, strain, or certain species of pathogen when present in an otherwise susceptible plant. Monogenic resistances are desirable for breeding purposes because of their simplicity in being introgressed. The plant response is also usually very fast and local, which restricts the collateral damages in the plant caused by a pathogen infection. Unfortunately, in many plant–pathogen interactions, this type of resistance can be broken down relatively fast due to alterations in the coevolving pathogen.

Plant breeders were using disease resistance genes to control plant disease long before they were identified and analyzed. Over the years, various nematode resistances have been mapped and some of the underlying *R* genes have now been cloned (Table 1). This work has been extensively reviewed (Williamson 1999; Bakker et al. 2006; Williamson and Kumar 2006) and therefore, we will only focus on new reports on cyst and root-knot nematode resistance in major crops that have appeared since the last review paper.

Table 1 Overview of loci that confer resistance to root-knot and cyst nematodes in major crop species

Gene	Pathogen	SD[a] or QTL[b]	Origin	Crop	Cloned	Chromo-some	References
Gro1.4	*Globodera rostochiensis*	QTL	*Solanum spegazzinnii*	Potato	no	III	Kreike et al. (1996)
H1	*G. rostochiensis*	SD	*S. tuberosum ssp. andigena*	Potato	No	V	Kreike et al. (1993), Pineda et al. (1993), Bakker et al. (2004)
GroVI	*G. rostochiensis*	SD	*S. vernei*	Potato	No	V	Jacobs et al. (1996)
Gro1	*G. rostochiensis*	SD	*S. spegazzinnii*	Potato	Yes	VII	Barone et al. (1990) Ballvora et al. (1995), Paal et al. (2004)
Gro1.2	*G. rostochiensis*	QTL	*S. spegazzinnii*	Potato	No	X	Kreike et al. (1996)
Gro1.3	*G. rostochiensis*	QTL	*S. spegazzinnii*	Potato	No	XI	Kreike et al. (1996)
Hero A	*G. rostochiensis*	SD	*Lycopersicon pimpernel-lifolium*	Tomato	Yes	11	Ganal et al. 1995; Ernst et al. (2002)
Grp1	*G. pal/G. ros*	QTL	*Solanum spp.c*	Potato	No	V	Rouppe Van Der Voort et al. (1998)
Gpa	*G. pallida*	QTL	*S. spegazzinnii*	Potato	no	V	Kreike et al. (1994)
Gpa2	*G. pallida*	SD	*S. tuberosum ssp. andigena*	Potato	Yes	XII	Rouppe Van Der Voort et al. (1997), Van der Vossen et al. (2000)
Gpa3	*G. pallida*	SD	*S. tarijense*	Potato	No	XI	Wolters et al. (1998)
Gpa4	*G. pallida*	QTL	*S. tuberosum ssp. tuberosum*	Potato	No	IV	Bradshaw et al. (1998), reviewed by Gebhardt and Valkonen (2001)
Gpa5	*G. pallida*	QTL	*Solanum spp.c*	Potato	No	V	Rouppe van der Voort et al. (2000)
Gpa6	*G. pallida*	QTL	*Solanum spp.c*	Potato	No	IX	Rouppe van der Voort et al. (2000)
GpaVSspl	*G. pallida*	QTL	*S. sparsipilum*	Potato	No	V	Caromel et al. (2005)
GpaXISspl	*G. pallida*	QTL	*S. sparsipilum*	Potato	No	XI	Caromel et al. (2005)
GpaM1	*G. pallida*	QTL	*S. spegazzinnii*	Potato	No	V	Caromel et al. (2003)
GpaM2	*G. pallida*	QTL	*S. spegazzinnii*	Potato	No	VI	Caromel et al. (2003)
GpaM3	*G. pallida*	QTL	*S. spegazzinnii*	Potato	No	XII	Caromel et al. (2003)
Rmc1	*Meloidogyne chitwoodi*	SD	*S. bulbocastanum*	Potato	No	XI	Brown et al. (1996), Rouppe Van Der Voort et al. (1999)
RMc1-hou	*M. chitwoodi*	SD	*S. hougasi*	Potato	No	XI	Draaistra (2006)
RMc1-fen	*M. chitwoodi/M. fallax*	SD	*S.fendleri*	Potato	No	XI	Draaistra (2006)
MfaXII spl.	*M. fallax*	Major QTL	*S. sparsipilum*	Potato	No	XII	Kouassi et al. (2006)
RMf-chc	*M. fallax*	QTL	*S. chacoense*	Potato	No	?	Draaistra (2006)
RMh-chcA, RMh-chcB	*M. hapla*	QTL	*S. chacoense*	Potato	No	?	Draaistra (2006)

RMh-tar	*M. hapla*	QTL	*S. tarijense*	Potato	No	VII	Draaistra (2006)
Mi-3	*M. inc/M. jav*	SD	*L. peruvianum*	Tomato	No	12	Yaghoobi et al. (1995), Yaghoobi et al. (2005)
Mi-1	*M. spp.*	SD	*L. peruvianum*	Tomato	Yes	6	Aarts et al. (1998), Milligan et al. (1998), Vos et al. (1998)
Mi-5 *Mi-LA2157*	*M. incognita/M. javanica/M. arenaria*	SD	*L. peruvianum*	Tomato	No	6	Veremis et al. (1999)
Mi-9	*M. incognita/M. javanica/M. arenaria*		*L. peruvianum*	Tomato	No	6	Ammiraju et al. (2003)
Hsa-1Og	*Heterodera sacchari*	codominat	*Oryza glaberrima*	Rice	No	11	Lorieux et al. (2003)
Rhg1	*H. glycines*	bigenic	*Glycine max*	Soybean	No	G	Concibido et al. (1997), Ruben et al. (2006)
Rhg4	*H. glycines*	bigenic	*G. max*	Soybean	No	A	Meksem et al. (2001a)
Hs1pro1	*H. schachtii*	SD	*Beta procumbens*	Sugar beet	Yes	I	Lange et al. (1993; Cai et al. (1997)
Hs1pat1	*H. schachtii*	SD	*B. patellaris*	Sugar beet	No	I	Lange et al. (1993)
Hs1web1	*H. schachtii*	SD	*B. webbiana*	Sugar beet	No	I	Kleine et al. (1998)
Hs2web7	*H. schachtii*	SD	*B. webbiana*	Sugar beet	No	VII	Kleine et al. (1998)
Hs2pro7	*H. schachtii*	SD	*B. procumbens*	Sugar beet	No	VII	Lange et al. (1993)
Hs3web8	*H. schachtii*	SD	*B. webbiana*	Sugar beet	No	VIII	Kleine et al. (1998)
Ha1	*H. avenae*	SD	*Hordeum vulgare*	Barley	No	2	Barr et al. (1998)
Ha2	*H. avenae*	SD	*H. vulgare*	Barley	No	2	Kretschmer et al. (1997)
Ha3	*H. avenae*	SD	*H. vulgare*	Barley	No	2	Barr et al. (1998)
Ha4	*H. avenae*	SD	*H. vulgare*	Barley	No	5	Barr et al. (1998)
Cre1	*H. avenae*	SD	*Triticum aestivum*	Wheat	No	2B	De Majnik et al. (2003)
Cre3	*H. avenae*	SD	*T. aestivum*	Wheat	No	2D	Eastwood et al. (1994), De Majnik et al. (2003)
Cre5(=CreX)	*H. avenae*	QTL	*Ae. ventricosa/T. ventricosum*	Wheat	No	2AS	Jahier et al. (2001)
Cre6	*H. avenae*	SD	*Ae. ventricosa/T. ventricosum*	Wheat	No	$5N^{v}$	Ogbonnaya et al. (2001)
CreF	*H. avenae*	SD	*T. aestivum*	Wheat	No	7HL	Paull et al. (1998)
CreR	*H. avenae*	SD	*Secale cereale*	Rye	No	6R	Asiedu et al. (1990)

[a]SD = single dominant *R* gene

[b]QTL = quantitative trait locus

[c]Mapped in (tetraploid) clone that is an interspecific hybrid between *S. tuberosum* and several wild potato species including *S. vernei*, *S. vernei* ssp. *ballsii*, *S. oplocense*, and *S. tuberosum* ssp. a*ndigena*

Recently, the first nematode resistance gene was found in rice. The mapping of genes and QTL for nematode resistance in rice was initiated with the recent discovery of a natural source of resistance against the cyst nematode *Heterodera sacchari*, which is harmful for sugar cane and rice, in the African *Oryza* spp. germplasm (Lorieux et al. 2003). By means of linkage mapping, using segregating populations derived from *O. sativa* and *O. glabberrima*, the *Hsa-1*^{Og} was mapped to chromosome 11 in rice. *Hsa-1*^{Og} is a major gene controlling the resistance to *H. sacchari* and exhibits codominant inheritance.

In potato, five additional QTL have been identified against different populations of the potato cyst nematode *Globodera pallida*. Three QTL named *GpaM1*, *GpaM2*, and *GpaM3* were found in the wild potato species *Solanum spegazzinnii* and mapped on chromosome V, VI, and XII, respectively (Caromel et al. 2003). These three could explain about 72% of the total variation. Moreover, two QTL originating from the wild species *S. sparsipilum*, namely *GpaV*^{s}*spl* and *GpaXI*^{s}*spl*, were proven to explain 89% of phenotypic variation and mapped to chromosome V (major effect) and chromosome XI (minor effect) (Caromel et al. 2005).

Several resistance loci against root-knot nematode populations that represent an asexual mode of reproduction, and are therefore less variable than populations in outcrossing species, have been found in potato and proven to follow a quantitative mode of action. Three QTL encoding resistance to *Meloidogyne fallax* ($R_{Mf\text{-}chc}$) and *M. hapla* ($R_{Mh\text{-}chc}A$, $R_{Mh\text{-}chc}B$) were mapped in an *S. chacoense* hybrid (Draaistra 2006). However, due to the large differences in AFLP patterns between *S. chacoense* and *S. tuberosum*, the identification of the linkage group could not be established. Another quantitative resistance locus against *M. hapla* was mapped in an *S. tarijense* hybrid (Draaistra 2006). $R_{Mh\text{-}tar}$ could be placed on the distal end of the short arm of chromosome VII. In addition, the two single dominant loci $R_{Mc1\text{-}hou}$ and $R_{Mc1\text{-}fen}$ derived from *S. hougasi* and *S. fendleri*, respectively, were mapped on chromosome XI (Draaistra 2006). They underlie resistance against *M. chitwoodi*, but interestingly, $R_{Mc1\text{-}fen}$ was also proven to be effective against *M. fallax.*

In soybean, the digenic resistance to the soybean cyst nematode *Heterodera glycines* is controlled by two loci *Rhg1* and *Rhg 4*, which map on the linkage groups G and A2, respectively (Meksem et al. 2001a). Recently, an integrated physical and genetic map of the 0.2-cM interval that encompasses the *Rhg1* locus was constructed and the molecular characterization of the protein encoded by a candidate resistance gene was described (Ruben et al. 2006).

4.2 Identification and Characterization of Nematode R Genes

R genes encode for proteins with a modular structure and they can be classified in different groups based on the specific combination of functional domains of which they are composed (Fig. 1). The majority of *R* genes belong to the super family of nucleotide-binding (NB) – leucine-rich repeat (LRR) genes (Ellis and Jones 1998; Meyers et al. 1999). This class of genes is very abundantly present in plant species

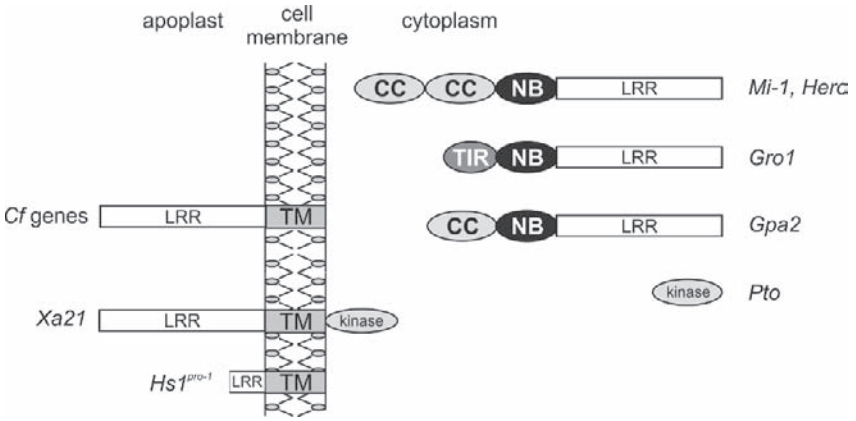


Fig. 1 Schematic drawing of the modular structure and the predicted localization of different types of disease resistance genes. The products encoded by the nematode resistance genes *Mi-1*, *Hero*, *Gpa2*, *Gro1*, and *Hs1pro-1* as well as the bacterial resistance gene *Pto* are located in the cytoplasm, while the *Cf* and *Xa21* gene products have an extracellular LRR domain as they contain a transmembrane domain

and encodes large proteins ranging from 860 to about 1,900 amino-acids. In *Arabidopsis*, it is estimated that at least 200 different NB-LRR genes exist compromising up to 1% of the genome (Meyers et al. 1999). On the basis of the N-terminal part, the NB-LRR proteins can be further subdivided into two classes containing either a coiled-coil (CC) domain or a Toll-Interleukin receptor (TIR) homology domain. The CC-NB-LRR proteins are present in both monocots and dicots, whereas monocots are lacking the TIR-NB-LRR proteins. A current overview of plant R protein structure and function can be found in the review written by McHale et al. (2006).

In 1997, the first nematode resistance gene was cloned from sugar beet by Cai et al. (1997). The gene $Hs1^{pro\text{-}1}$ confers resistance to the beet cyst nematode *Heterodera schachtii*. Sequence comparison revealed that it encodes an R protein, which shows no homology with any known *R* gene although it contains an atypical LRR domain of only 146 amino acids and a putative transmembrane domain. Investigation of gene expression patterns under biotic and abiotic stresses by means of a promoter: reporter gene fusion showed that $Hs1^{pro\text{-}1}$ is up-regulated only during the incompatible plant–nematode interaction and its promoter activates a feeding site-specific gene expression pattern (Thurau et al. 2003).

The identification and characterization of four other nematode resistance genes (*Mi-1*, *Gpa2*, *Hero*, *Gro1*) from potato and tomato showed that they do belong to the super family of NB-LRR resistance genes. *Gro1* distinguishes itself from the other three by having a TIR domain, whereas the others have a CC domain. Despite the structural similarities, some differences in function do occur. The two genes identified in potato, *Gpa2* (Van der Vossen et al. 2000) and *Gro1* (Paal et al. 2004), confer resistance against specific populations of the potato cyst nematodes *G. pallida* and *G. rostochiensis*, respectively: whereas the tomato gene *Hero* (Ernst et al. 2002)

recognizes a broad spectrum of potato cyst nematode species and populations. It confers resistance to all economically important pathotypes of both *G. rostochiensis* and *G. pallida* (Sobczak et al. 2005). A similar broad spectrum resistance is mediated by the tomato gene *Mi-1* (Milligan et al. 1998; Vos et al. 1998), which is effective against the three major root-knot nematode species: *M. incognita*, *M. javanica*, and *M. arenaria*. Additionally, the *Mi-1* gene confers resistance to piercing/sucking insects, i.e. the potato aphid *Macrosiphum euphorbiae* (Vos et al. 1998) and the whitefly *Bemisia tabaci* (Nombela et al. 2003), which suggests multiple recognition specificities.

Interestingly, both the Mi-1 protein and the HERO protein harbor an unusual N-terminal domain. Although in both proteins two CC regions are predicted (Williamson et al. 2000; Ernst et al. 2002), the two domains do not show any significant sequence or structural similarity (Ernst et al. 2002). The N-terminal domain of Mi-1 resembles that of the late blight resistance protein Rpi-blb2 (Van der Vossen et al. 2005), which is positioned in a homologues region in potato and has an overall amino acid identity of 82%. Apart from that, like the N-terminal domain of HERO, it has no significant similarity to other sequences. Interestingly, preliminary data from sequence analysis of the *H1* locus in potato, conferring resistance to the potato cyst nematode *G. rostochiensis*, revealed the presence of resistance gene candidates with a similar N-terminal domain (amino acid homology with both *Mi-1* and *Rpi-blb2* is around 50%) (Tomczak et al., unpublished data).

Recently, an *R* gene candidate gene was identified in soybean at the *Rhg1* locus, which is involved in resistance against the soybean cyst nematode *H. glycines* (Ruben et al. 2006). Sequence comparison revealed that this putative resistance gene belongs to a distinct class of *R* genes consisting of three functional domains including an LRR domain composed of 12 extracellular repeats, a trans-membrane domain, and a kinase domain. The encoding protein shows high homology to the bacterial resistance gene *Xa21* from rice and an *Arabidopsis* receptor-like kinase gene family.

4.3 Activation of R Gene-Mediated Nematode Resistance

Mapping and cloning of *R* genes conferring resistance to endoparasitic nematodes is a major contribution to the elucidation of the genetic and molecular mechanisms underlying nematode resistance. Resistance to cyst and root-knot nematodes is characterized by an arrest in feeding cell induction and development often as the result of a local hypersensitive response (HR) at the infection site. The HR is a form of programmed cell death (Greenberg 1997; Morel and Dangl 1997; Pontier et al. 1998), and shares several features with apoptosis in mammalian cells (Lamb and Dixon 1997; Mittler et al. 1997). A number of nematode-resistance phenotypes have been described for both cyst and root-knot nematodes (Bakker et al. 2006). Responses range from the complete abolishment of nematode development when the establishment of the feeding site is arrested in an early stage of infection, to a

significant reduction of the number of fully developed adult females and cysts when feeding cell development is blocked in a later stage. The structure and histology of feeding cell formation in resistant plants is extensively described for root-knot nematodes in Chap. 5 and for cyst nematodes in Chap. 6.

The modular structure of the encoding R proteins allows the study of their separate roles in nematode recognition and the induction of a defense response that leads to nematode resistance. Unfortunately, these structure-function studies are seriously hampered by the fact that the corresponding elicitors from the nematodes are still unknown. However, an elicitor-independent hypersensitive response for the *Mi-1* gene was obtained in an agroinfiltration assay in *Nicotiana benthamiana* leaves upon expression of a chimeric construct that consisted of the N-terminal domain from *Mi-1.1*, a nonfunctional homologue that is 91% identical to *Mi-1.2*, the functional *Mi-1* gene. For the same chimeric *R* gene, no transgenic tomato plants could be recovered (Hwang et al. 2000), suggesting that an HR reaction takes place in cells that express this gene. In this paper, it was shown that a six-amino acid region in the LRR in *Mi-1* is required but not sufficient for resistance. In a follow-up study, the amino acids that are essential for nematode resistance were determined (Hwang and Williamson 2003) by introducing each of the 40 amino acid differences between the LRR of *Mi-1.2* and *Mi-1.1* into *Mi-1.2*. They found 24 amino acids that appeared to be required for signaling and three consecutive amino acids that may be involved in nematode recognition. Apparently, the N-terminal part 1 (NT-1), which consists of the first 161 amino acids, was able to repress the transmission of a signal by the LRR domain that leads to an HR and a model was proposed in which this negative regulation was compromised in the presence of a root-knot nematode elicitor (Hwang and Williamson 2003).

Similar results were obtained in a structure-function study of the potato resistance gene *Rx1*, which is a close relative (93% nucleotide identity) of the nematode resistance gene *Gpa2*. Physical interactions were observed between the N-terminal CC domain and the NB-LRR domains or between the CC-NBS domains and the LRR domain in the absence of the elicitor, the coat protein from the potato virus X. However, those interactions were disrupted in the presence of the avirulent coat protein, suggesting the activation of Rx-mediated signaling by relieving the negative intramolecular regulation of the NBS domain (Moffett et al. 2002). This domain, also called the NB-ARC (nucleotide binding adaptor shared by NOD-LRR proteins, APAF1, R proteins, and CED4) domain (McHale et al. 2006), seems to be involved in specific binding and hydrolysis of ATP as was shown for the two tomato resistance genes *Mi-1* and *I2* (Tameling et al. 2002). ATP hydrolysis is thought to result in conformational changes that regulate downstream signaling.

High sequence homology between the closely related viral resistance gene *Rx1* and the nematode resistance gene *Gpa2* suggests that a similar model might be applicable for the activation of a resistance response to the potato cyst nematode. To study the mechanisms underlying Gpa2-mediated resistance in potato, a series of domain swap constructs between different domains from Rx1 and Gpa2 are currently under investigation using agroinfiltration assays and nematode resistance tests. Preliminary data show that nematode specificity resides in the LRR domain

of Gpa2 as a chimeric construct between the CC-NBS domain of Rx1 and the LRR domain of Gpa2 is able to confer nematode resistance in a gene-for-gene specific manner (Koropacka et al., unpublished data).

4.4 R Gene-Mediated Defense Responses to Nematodes

Plant responses to pathogens are associated with massive changes in gene expression. For example, in *Arabidopsis*, a change in the gene expression levels of more than 2,000 genes has been observed within 9 h upon inoculation with the bacterial pathogen *Pseudomonas syringe* (Tao et al. 2003). It is thought that early activation of genes involved in phytohormone biosynthesis modifies the hormonal balance of the host plant, leading to the appropriate transcriptome changes. Gene expression studies of several plant–nematode interactions showed that different defense-related genes are up-regulated upon infection of both susceptible and resistant plants, including genes encoding peroxidase, chitinase, lipoxygenase, extensin, and proteinase inhibitors (reviewed in Williamson and Hussey 1996; Gheysen and Fenoll 2002). Furthermore, genes encoding enzymes involved in biosynthetic pathways are induced early during infection. For example, glyceollin in soybean appears to be involved in phytoalexin biosynthesis after *M. incognita* infection (Kaplan et al. 1980) and chalcone synthase is produced in white clover upon infection with *M. javanica* (Hutangura et al. 1999).

Expression of these defense-related genes in both the compatible and the incompatible interaction suggests a role in basal resistance. However, it is hypothesized that the defense response is only strong and quick enough to prevent successful nematode infection in the presence of a functional R protein that can recognize the appropriate AVR protein from the nematode. The induction of toxins, PR-genes and the hairpin-induced *hin1*-like gene during the compatible interaction between root-knot nematodes and tomato suggests that the nematodes are identified as pathogens (Bar-Or et al. 2005). However, in the absence of components necessary for a host-specific defense reaction (like a functional *R* gene), no HR is elicited and the defense response is not fully effective.

There is now a significant amount of evidence pointing to specific MAPKs as fundamental components of defense pathways that play a role in both basal defense and in more specific interactions involving *R* gene-mediated resistance (Pedley and Martin 2005). They are involved in the generation of ROS (Kovtun et al. 2000; Ren et al. 2002), the induction of PR protein expression and in gene transcription (Ahlfors et al. 2004; Kim and Zhang 2004; Lee et al. 2004). Although there is no direct evidence yet for the role of MAPKs in nematode resistance, it was recently shown that *Mi-1*-mediated aphid resistance was abolished in tomato when *Le*MKK2, *Le*MPK2, *Le*MPK1, or *Le*MPK3 were silenced (Li et al. 2006). It will be interesting to see whether MAPKs play a similar role in *Mi-1*-mediated nematode resistance.

An oxidative burst, Ca^{2+} uptake, and phosphorylation changes are among the earliest responses associated with a host-specific resistance response. Rapid production of ROS, some of which may be generated by a multi-subunit NADPH oxidase complex in the plasma membrane (Doke et al. 1996; Xing et al. 1997), is often associated with cell death. Recent research has implicated nitric oxide (NO), together with ROS, in the induction of a HR during plant–pathogen interactions (Shapiro 2005). Generation of elevated levels of NO was shown in tomato plants in response to avirulent root-knot nematodes (Melillo et al. 2006b).

Two key components in *R* gene-mediated resistance signaling are SGT1 and RAR1 (Austin et al. 2002; Azevedo et al. 2002; Liu et al. 2002; Peart et al. 2002). In yeast, SGT1 is a component of the SCF (SKP cullin F-box) complex, which is an integral part of protein ubiquitination (Kitagawa et al. 1999). This suggests that protein degradation is implicated in resistance signaling, which is supported by the observation that the *Arabidopsis* R protein RPM1 is degraded when the elicitor (AvrRpm1 or AvrB) is present (Boyes et al. 1998). Another important protein involved in *R* gene-mediated signaling is HSP90 (heat shock protein 90) (Liu et al. 2004) which directly interacts with SGT1 and RAR1 (Holt et al. 2003; Shirasu and Schulze-Lefert 2003). RAR1, SGT1, and HSP90 are suggested to form a chaperone complex mediating the folding of R proteins and their incorporation into functional complexes (Shirasu and Schulze-Lefert 2003). For *Mi-1*-mediated resistance to aphids and nematodes, it appears that HSP90 is required while RAR-1 is not for either resistance (Kaloshian et al., unpublished results). It was reported that different R proteins varied in their requirement for SGT1 and RAR1 to function. Whether SGT-1 is involved in *Mi-1*-mediated resistance remains to be seen.

Another gene specifically required for *Mi-1*-mediated resistance is *Rme-1*, which is unlinked to *Mi-1* and not required for the functioning of other resistance genes like *Pto*. *Rme-1* acts early in the *Mi-1* pathway, either at the same step as the *Mi-1* product or upstream of *Mi-1* (Martinez De Ilarduya et al. 2004). Interestingly, the *rme-1* mutant also compromised the *Mi-1*-mediated aphid resistance (De Ilarduya et al. 2001). The structure and function of *Rme-1* has to be investigated, including the possibility that Rme-1 is a potential virulence target for nematodes and aphids, guarded by the Mi-1 protein.

Considering the practical applications for isolated resistance genes, it is important to know if they can be transferred to a range of economically important crops where similar resistance is not available. So far, there has been limited success in transferring functional *R* genes to other species (Williamson and Kumar 2006). For example, the *Mi-1* gene confers effective resistance against root-knot nematodes and the potato aphid when transferred into susceptible tomato. When introduced into tobacco or *Arabidopsis*, however, it does not confer any of these resistance specificities (Williamson et al., unpublished). On the other hand, a heterologous expression of the *Mi-1* gene in eggplant caused resistance to root-knot nematodes but no longer resistance to the potato aphid (Goggin et al. 2006). The tomato gene *Hero*, which confers resistance to potato cyst nematodes (PCN), was not effective in potato according to another report (Sobczak et al. 2005).

Thus, the influence of the plant's genetic background can be proposed as a factor determining the heterologous gene functionality, probably through the presence of other gene components necessary for the resistance response (Williamson and Kumar 2006). It was shown that even within cultivated tomato; genotype differences were influencing the efficacy of *Mi-1* resistance (Jacquet et al. 2005). Understanding this phenomenon will be a challenge but it seems to be necessary for a successful transfer of nematode resistance to a new species. It might also provide insight into host factors that mediate specificity of recognition and signaling (Williamson and Kumar 2006).

5 Evolution of Nematode Resistance Specificity

5.1 Genomic Organization and Molecular Evolution of Nematode R Gene Clusters

Genome-wide sequence analysis and genetic mapping of *R* gene candidates have shown that disease resistance genes are often located in clusters of homologous *R* genes spread throughout the plant genome (reviewed by Gebhardt and Valkonen 2001). *R* gene clusters from different genotypes and even related species are often located in the same chromosomal region. These regions are therefore called "hot-spots of resistance." Remarkably, in potato, QTL conferring resistance to the potato cyst nematode often co-localize with hot-spots of single dominant resistance genes, suggesting that they may contribute to partial resistance to nematodes. Another option suggests that quantitative resistance is mediated by an *R* gene but that the potato cyst nematode populations used to screen for resistance consist of a mixture of virulent and avirulent genotypes. Most of the cyst nematodes reproduce by obligate outcrossing, and there is generally great variation in host range and response to specific resistance genes between and within field populations (Bakker et al. 1993).

The multigenic nature of most resistance loci may facilitate meiotic instability in a heterozygous state. Unequal crossing-over and gene conversion have been suggested to play a role in the generation of new *R* gene specificities (Hammond-Kosack and Jones 1997; Hulbert 1997; Parniske et al. 1997). However, since these processes tend to homogenize the paralogs, divergent evolution must be strong enough to counteract the homogenization process.

With the exception of the beet cyst nematode resistance gene $Hs1^{pro\text{-}1}$ (Cai et al. 1997), all nematode resistance genes cloned to date reside in complex loci harboring tandemly repeated *R* gene homologues. The root-knot nematode gene *Mi-1* (Milligan et al. 1998; Vos et al. 1998) is located in a cluster of seven homologous *R* genes on chromosome VI of tomato, whereas the potato cyst nematode resistance gene *Hero* (Ernst et al. 2002) is located in a genomic region containing at least 14 homologous genes on chromosome IV of tomato. The gene *Gro1* (Paal et al. 2004) is also member of a large cluster containing 13 *R* gene homologues located on

chromosome VII of potato, whereas the potato gene *Gpa2* (Van der Vossen et al. 2000) is present in a relatively small cluster of four highly homologous genes on chromosome XII.

The specificities of the other members of these nematode *R* gene clusters are unknown except for the *Gpa2* locus in potato. For bacterial and fungal resistance loci, members of an *R* gene cluster often confer resistance to different isolates or strains from the same pathogen species. Interestingly, the *Gpa2* cluster also harbors the resistance gene *Rx1* (Bendahmane et al. 1999), which confers extreme disease resistance to a completely unrelated pathogen, namely the potato virus X. The fact that these two highly homologous resistance genes (88% amino acid identity) reside in the same cluster and on the same haplotype of the diploid potato clone SH83-92-488 (Rouppe Van Der Voort et al. 1997) strongly suggest that unequal crossing-over and gene conversion play a role in the evolution of the two specificities. The other two members of this cluster are a pseudogene and a putative resistance gene of unknown specificity.

In plants, the LRR domain likely acts as the receptor for pathogen-generated elicitors. Each LRR comprises a core of about 26 amino acids containing an LxxLxxLxLxxN/C motif, which forms a -sheet. The LRR domain is involved in *R* gene specificity; therefore, to study the mechanisms underlying the evolutionary dynamics of this *R* gene cluster, the LRR domain of three additional resistance gene homologues with a high sequence similarity to *Gpa2* and *Rx1* has been isolated from the susceptible haplotype of the resistant diploid potato clone SH83-92-488 and of another six homologues from the susceptible diploid potato clone RH89-039-16 (Bakker et al. 2003). Sequence comparison of these 13 homologous LRRs shows a patchwork of sequence similarities, indicating mosaic evolution. Interestingly, breakpoints often coincide with hypervariable amino acid positions present in the LRR domain (as defined by Parniske et al. 1997), suggesting a role for unequal crossing-over and gene conversion in the generation of new *R* gene specificities in the *Gpa2/Rx1* cluster (Bakker 2003).

The ratio of nucleotide changes that result in asynonymous amino acids (K_a) and those that result in synonymous amino acids (K_s) can be a way to identify the evolutionary pressure on a stretch of coding sequences (Parniske et al. 1997). When the evolutionary pressure is neutral, the amount of changes resulting in the same amino acid should equal the amount of changes resulting in different amino acids with an end result of $K_a/K_s = 1$. When $K_a/K_s < 1$, the changes are not favored and the evolutionary pressure is towards conservation. The third possibility is $K_a/K_s > 1$. In that case, changes in the amino acid composition are favored and the evolutionary pressure is towards diversification. Analysis of the nucleotide sequence of the LRR domain of the *Gpa2/Rx1* homologues revealed a mean K_a/K_s ratio of 2.69. This ratio is well above 1 and therefore, the LRR domain of the *Gpa2/Rx1* homologues is under positive selection pressure. Analyses of the LRR -strand/ -turn motifs, which harbor the solvent-exposed residues thought to be involved in protein–protein interaction, resulted in a mean K_a/K_s ratio of 3.33. These findings support the idea that the LRR domain and more precisely the solvent-exposed residues of the LRR -strand/ -turn motifs are involved in *R* gene specificity. The strong positive selection

pressure that acts on these regions is an indication that diversifying selection also plays a role in the development of new *R* gene specificities (Bakker 2003).

In contrast to the *Gpa2/Rx1* cluster, sequence analysis of the *Mi-1* locus in tomato did not point at a role for unequal crossing-over and gene conversion (Seah et al. 2004). The *Mi-1* gene is introgressed from the wild relative and supposedly ancestral progenitor L*ycopersicon peruvianum.* Although evidence has been found for an inversion of this locus between the two species, the copy numbers of the homologues in each of the two clusters is conserved.

5.2 The Genetic Basis of Nematode Virulence

The gene-for-gene hypothesis assumes that host resistance is determined by complementary pairs of pathogen-encoded avirulence genes (*Avr* gene) which are recognized by products encoded with plant resistance genes (*R* gene). The evolution of such a gene-for-gene interaction is often compared with a co-evolutionary "arms race" (Dawkins and Krebs 1979; Michelmore and Meyers 1998). This theory assumes that the parasite is always capable of overcoming resistance by developing a new way to circumvent recognition by the host. This results in elevated selection pressure on the host population for new resistance that matches the virulent pathotype while the overcome resistance gene declines. However, it has been suggested that a "trench warfare" model assuming repeated advances and retreats of resistance and virulence alleles may be more appropriate (Frank 1992; Stahl et al. 1999).

Only the interaction between the potato cyst nematode *G. rostochiensis* and potato has demonstrated Mendelian proof of a gene-for-gene interaction (Janssen et al. 1991). Selection of pure parasitic and nonparasitic lines of *G. rostochiensis* and subsequent reciprocal crosses using these lines have shown that parasitism is recessively inherited at a single locus and that the inheritance is not sex-linked (Janssen et al. 1990, 1991). A dominant locus *H1* present in resistant potato cultivars was demonstrated as only being effective against certain pathotypes of *G. rostochiensis*, while nematodes carrying recessive parasitism alleles could reproduce normally on these plants. Because of a segregation pattern of 3:1 nonparasitic to parasitic, which is typical for single gene inheritance and a proven dominant nature of the *H1* resistance, it has been proposed that this interaction classifies as a gene-for-gene type of mechanism (Janssen et al. 1991).

For a limited number of other incompatible plant–nematode interactions, the genetic basis of nematode virulence was investigated. Studies using three highly homozygous inbred lines were performed for the soybean cyst nematode *H. glycines* (Dong and Opperman 1997). The inbred line crosses clearly demonstrated that parasitic ability is inherited in a Mendelian fashion. Both dominant and recessive genes were found and proven by linkage analysis to be unlinked loci (Dong 1998). In the case of root-knot nematodes, analysis of virulence segregation in progeny of a controlled cross of *M. hapla* indicated that virulence in the nematode is inherited as a single recessive trait, and that the nematode–bean interaction

might be classified as a gene-for-gene interaction (Chen and Roberts 2003). Aiming to facilitate map-based cloning of genes that mediate plant–nematode interactions, a genetic map has been constructed for the potato cyst nematode *G. rostochiensis* (Rouppe van der Voort et al. 1999). Because of the outcrossing nature of *G. rostochiensis* and technical limitations in using individual offspring genotypes for map construction, this map was made with a bulked offspring population. To our knowledge, this map has not yet been used to map parasitism or avirulence genes.

5.3 Nematode Avirulence Genes

To establish a parasitic relationship with their host plants, cyst and root-knot nematodes have to overcome multiple layers of defense responses to modify host root cells into beneficial feeding structures. Several cyst and root-knot nematode genes encoding secreted proteins produced in the esophageal glands were shown to have the features of pathogenicity factors (reviewed in Vanholme et al. 2004; Davis et al. 2004). Avr proteins are generally considered to be virulence factors required for successful infection of the host plant and as such, these nematode parasitism genes are good candidates to encode avirulence proteins. However, proof has not been obtained yet for any of them to act as a resistance gene-dependent plant defense elicitor.

In an attempt to identify avirulence gene products involved in *Mi*-mediated resistance in tomato, cDNA-AFLP fingerprinting was used for pairwise comparison of the expression profiles of nearly isogenic lines from the root-knot nematode *M. incognita*, avirulent and virulent on *Mi*-resistant plants (Semblat et al. 2001). This resulted in the identification of several differentially expressed genes including *map-1*, which was shown to encode for a protein containing a predictive signal peptide for secretion and two classes of repetitive motives. Immunolocalization experiments confirmed that the MAP-1 protein is secreted by the amphids, which are the principal chemosensory organs of the nematode. The role of *map-1* in avirulence, however, has never been demonstrated. In addition, a transcript present in avirulent but absent in virulent lines of *M. javanica* has also been identified. Curiously, this gene does not resemble *map-1*, suggesting that there may be more than one gene that can mediate nematode recognition in tomato plants with the *Mi-1* gene (Williamson and Gleason 2003).

The amount and variability of putative nematode pathogenicity factors and limited knowledge about nematode resistance mechanisms make it very difficult to predict which proteins secreted by nematodes can play a role in avirulence. Besides secreting a mixture of cell-wall degrading enzymes that act on the plant cell walls and facilitate migration, nematodes also release secretions into the feeding site initiation cell (Williamson and Hussey 1996). Most of the proteins have predicted signal peptides and are proven to be produced exclusively in esophageal glands, although some proteins lack such a signal and have been shown to be secreted by potato cyst nematode *Globodera* spp. as well (Robertson et al. 2000; Fioretti et al. 2001). The predicted

cytoplasmic localization of nematode resistance genes and the secretion of signaling molecules into the plant cell suggest that these pathogenicity factors are potential candidates for nematode avirulence products.

6 Induced Resistance to Nematodes

In 1960, Ross observed that tobacco plants challenged with tobacco mosaic virus (TMV) subsequently developed increased resistance to a secondary infection in distal tissues (Ross 1961). For this phenomenon he coined the term systemic acquired resistance (SAR). Kuc et al. (1959) observed a similar type of induced resistance against scab disease in apple that was chemically induced. They introduced the term induced systemic resistance (ISR). Later, it was proved that this effective defense response occurs in various plant species against the whole spectrum of pathogens (Kuc 1982; Ryals et al. 1994; Sticher et al. 1997). A historic account of the use of the SAR and ISR terms is extensively given by Tuzun (2006). He proposes to name all actively induced systemic defense mechanisms as ISR, regardless of the inducer (pathogenic, nonpathogenic, or chemical).

ISR can be induced by certain strains of nonpathogenic rhizobacteria (e.g. *Pseudomonas* spp.), by Arbuscular Mycorrhizal fungi, or by the application of certain chemical compounds (e.g. salicylic acid and benzothiadiazole) (Van Peer et al. 1991; Wei et al. 1991). ISR to nematodes induced by rhizobacteria has been observed in potato against *G. pallida* (Hasky-Günther et al. 1998), in tomato against the root-knot nematodes *M. incognita* and *M. arenaria* (Sikora 1992; Santhi and Sivakumar 1997), and in white clover against the clover cyst nematode *H. trifoli* (Kempster et al. 2001). Colonization by the Arbuscular Mycorrhizal fungus *Glomus versiforme* was proven to be effective against *M. incognita* in grapevine and seemed to involve a transcriptional activation of the Class III Chitinase Gene *VCH3* (Li et al. 2006). Finally, several chemical elicitors of induced resistance were tested on wheat and barley for their ability to reduce the number of *H. avenae* and *H. latipons* cysts. Only the application of DL-*B*-amino-*n*-butyric acid (BABA) induced effective resistance (Oka and Cohen 2001).

Interestingly, bacterial components such as the lipopolysaccharides extracted from the surface of the Gram negative *Rhizobium etli*, and more specifically the O-antigen, were observed to play an important role in ISR (Van Peer and Schippers 1992; Leeman et al. 1995; Van Wees et al. 1997). However, it is not the O-antigen but the oligosaccharides of the core-region of LPS and to a lesser extent the A-fraction that induce systemic resistance to the potato cyst nematode *G. pallida* (Reitz et al. 2002).

SA is assumed to be a key molecule required for ISR activation because an exogenous application of salicylic acid (SA) induces systemic resistance to TMV in tobacco (White 1979). However, there is evidence that this process is more complex and that many other signal molecules are involved. Two other signal molecules (i.e. jasmonic acid and ethylene) have also been identified as playing a role in ISR. Usually SA-dependent pathways are jasmonic acid (JA) and ethylene (ET)

independent and vice versa (Penninckx et al. 1996; Dong 1998; Pieterse and Van Loon 1999). Moreover, SA-dependent signaling and JA-dependent signaling can inhibit each other (Gupta et al. 2000). In addition, the accumulation of pathogenesis-related (PR) proteins is thought to contribute to resistance and the proteins are often treated as markers for the enhanced resistance state mediated by ISR (Kessmann et al. 1994; Ryals et al. 1994).

ISR has also been observed in incompatible nematode–plant interaction. Systemic changes in gene expression in resistant potato plants carrying the *H1* gene following root infection with the cyst nematode *G. rostochiensis* were found (Hammond-Kosack et al. 1989). The changes involved the disappearance of innate proteins and the accumulation of novel gene products. Because SA treatment showed a similar gene expression pattern, it can be assumed that a SA-dependent pathway was induced. Follow-up studies documented the changes in β-1,3-glucanases activity in leaves after infecting potato roots of cultivars carrying different resistance specificities with four potato cyst nematode populations. The resulting range of compatible and incompatible interactions elicited various classes of β-1,3-glucanases, both extra- and intracellularly targeted (Rahimi et al. 1996). One of them, β-D-glucosidase, was demonstrated to be significantly more active in the resistant cultivar carrying the *H1* gene infected with an avirulent potato cyst nematode population. The same effect could not be induced by the application of silver nitrate. These observations suggest that β-D-glucosidase is specifically up-regulated in this type of resistance response. The same potato cultivars were later tested for increased chitinase activity after *Globodera* spp. infection. The intercellular fluid of the leaves of all the nematode-infected plants tested showed significant increases in exochitinase activity (Rahimi et al. 1998). Endochitinase activity was up-regulated exclusively in roots of a *S. vernei*-derived resistant clone after infection with three out of four tested potato cyst nematode populations. Therefore, it can be concluded that although a lot of pathogenesis-related genes are induced unspecifically, some of them can be linked to certain types of host resistance or host–parasite combinations. In addition, PR-protein accumulation has been reported for a few incompatible plant–nematode interactions, suggesting the activation of ISR. Strong induction of PR-1 transcription and slight induction of PR-5 expression was observed for tomato roots carrying the nematode resistance gene *Hero* after infection with the potato cyst nematode *G. pallida* (Sobczak et al. 2005).

SA, JA, and ET do not seem to solely play a role in ISR. It has been shown that SA plays a crucial role in defense responses mediated by the *Mi-1* root-knot nematode resistance gene. Tomato plants carrying the *Mi-1* gene and expressing the *NahG* gene, which encodes salicylate hydroxylase, an enzyme that degrades salicylic acid to catechol, partly lost the resistance to root-knot nematodes (Branch et al. 2004) and aphids (Li et al. 2006). Benzothiadiazole, an analog of SA, completely restores nematode resistance in *Mi-1* resistant roots transformed with *NahG* but does not confer resistance in susceptible tomato roots (Branch et al. 2004).

To study the role of JA in both basal and *Mi-1*-mediated root-knot nematode resistance, the *jai-1* mutant compromised in JA perception and wild-type parents was used for infection studies. Nematodes reproduced significantly lower on *jai-1*

plants compared to the wild-type parents. Knowing that SA is required for root-knot nematode resistance, the lower nematode reproduction on *jai-1* plants suggests the existence of cross talk between SA and JA signaling in nematode resistance. However, the introduction of the *jai-1* mutation in the *Mi-1* background showed no role for JA in *Mi-1*-mediated resistance (Kaloshian et al., unpublished data).

7 Perspectives

Co-evolution between parasitic nematodes and their host plants has resulted in the development of a gamut of nematode resistances in the center of origin of different crop plants. Resistance to cyst and root-knot nematodes provides an important durable crop protection strategy and as such, breeders have put much effort into the identification of resistance sources from various wild relatives. However, just a small portion of the genetic diversity has been explored to date. Recently, a small number of genes underlying nematode resistance have been characterized, but it is anticipated that this number will increase in the near future as results of the ongoing plant genome sequencing efforts and high-throughput analysis methods become available. Meanwhile, sequence information related to nematode resistance genes is being used to speed up the breeding process through marker-assisted selection.

The identification of nematode *R* genes has shown that they share structural and functional similarities to other plant pathogen disease resistance genes. Therefore, data derived from model systems like the potato virus X resistance gene *Rx1* from potato can be used to study the mechanisms underlying the resistance responses induced by different types of nematode *R* genes. However, structural and functional analyses of nematode resistance genes are still hampered by laborious and time-consuming nematode resistance assays on host plant roots. We expect that once a nematode avirulence gene has been identified, these studies will advance much faster as they allow the use of elegant in planta systems such as agroinfiltration assays (ATTA).

A major challenge in the field of nematode resistance is the identification of the first avirulence gene which is able to induce a resistance response in a gene-for-gene specific manner in the presence of the corresponding *R* gene. Most likely the encoding avirulence product will normally play a role in nematode virulence and as such, it is anticipated that candidate elicitors are already present in the large set of parasitism genes known to date. It will be interesting to see how these nematode-derived molecules will be – directly or indirectly – recognized by the resistance genes and how they interact as virulence factors with other plant components during the establishment of a parasitic relationship.

To discover a nematode *Avr* gene, *Mi1* virulent and avirulent near isogenic lines (NILs) of *M. incognita* were compared using the AFLP display technique (Semblat et al. 2001). Comparing 25,000 fragments resulted in 30 bands that were present only in avirulent NILs. One of these bands was studied in more detail and cDNA analysis revealed a putative protein of 458 amino acids containing a predicted N-terminal signal peptide. Immunolocalization in second-stage juveniles showed that the protein

was present in the amphidial secretions of the nematode. Later, a similar approach was used, this time using cDNA-AFLP, still in pursuit of *AvrMi1* (Neveu et al. 2003). Comparison of 24,025 bands resulted in 22 differential transcript-derived fragments that were present in the avirulent NILs and absent in the virulent NILs. The differential expression of nine genes was confirmed with reverse transcription and in situ hybridization of five of these sequences showed that two were specific for the intestinal cells, one for the subventral and two for the dorsal esophageal gland. Recently, in another attempt to discover *AvrMi1*, a virulent *M. javanica* strain was obtained from an avirulent *M. javanica* strain after selection on resistant tomato plants harboring the *Mi1* gene (Gleason et al. 2008). Comparing the virulent with the avirulent strain using cDNA-AFLP resulted in one fragment, *Cg-1*, that was present in the avirulent strain and absent in the virulent strain. Subsequent silencing of *Cg-1* in avirulent *M. javanica* by soaking J2 juveniles in dsRNA corresponding to part of the predicted transcript of *Cg-1* (RNAi) resulted in a gain of virulence.

A completely different approach starts with a known parasitism gene. Chorismate mutase is a secreted enzyme produced in the esophageal glands of *H. glycines* (Bekal et al. 2003) that shows different forms in lines that are either virulent and avirulent to soybean plants harboring a *H. glycines* resistance. It was also shown that after selection on three different resistant soybean plants, one chorismate mutase allele dropped significantly in frequency on one of the three resistant plants, while the allele frequency on the other two resistant plants remained the same (Lambert et al. 2005). This result suggests that one of the chorismate mutase alleles is specifically unfavorable for one type of resistance and might indicate a correlation with a resistance-specific (a)virulence.

One of the most intriguing questions for further exploration is how cyst and root-knot nematodes are able to modulate or circumvent the host defense system. A common mechanism, which is shared by both plant and mammalian parasitic nematodes, is the capacity to neutralize host-generated reactive oxygen species (ROS) by the presence of antioxidants at the cuticular surface of (pre)parasitic juveniles (reviewed by Jasmer et al. 2003). Another mechanism is the suppression of defense responses by for example fatty acid- and retinol-binding proteins, which were shown to inhibit precursors of plant defense compounds and systemic jasmonic acid signaling (Prior et al. 2001). To avoid recognition by disease resistance genes, cyst and root-knot nematodes are expected to have evolved mechanisms that allow diversification of avirulence genes. Understanding the mechanisms underlying the co-evolution between host plant resistance and nematode (a)virulence will be essential for the development of durable crop protection strategies.

References

Aarts MGM, Hekkert BTL, Holub EB, Beynon JL, Stiekema WJ, Pereira A (1998) Identification of R-gene homologous DNA fragments genetically linked to disease resistance loci in *Arabidopsis thaliana*. Mol Plant Microbe Interact 11:251–258

Ahlfors R, Macioszek V, Rudd J, Brosche M, Schlichting R, Scheel D, Kangasjärvi J (2004) Stress hormone-independent activation and nuclear translocation of mitogen-activated protein kinases in *Arabidopsis thaliana* during ozone exposure. Plant J 40:512–522

Ammiraju JSS, Veremis JC, Huang X, Roberts PA, Kaloshian I (2003) The heat-stable root-knot nematode resistance gene *Mi-9* from *Lycopersicon peruvianum* is localized on the short arm of chromosome 6. Theor Appl Genet 106:478–484

Asiedu R, Fisher JM, Driscoll CJ (1990) Resistance to *Heterodera avenue* in the rye genome of triticale. Theor Appl Genet 79:331–336

Austin MJ, Muskett P, Kahn K, Feys BJ, Jones JDG, Parker JE (2002) Regulatory role of SGT1 in early R gene-mediated plant defenses. Science 295:2077

Azevedo C, Sadanandom A, Kitagawa K, Freialdenhoven A, Shirasu K, Schulze-Lefert P (2002) The RAR1 interactor SGT1, an essential component of R gene-triggered disease resistance. Science 295:2073–2076

Bakker E (2003) Molecular and genetic analyses of potato cyst nematode resistance loci. Lab Nematol, Dept Plant Sciences. Wageningen Univ, PhD thesis

Bakker E, Achenbach U, Bakker J, Van Vliet J, Peleman J, Segers B, Van Der Heijden S, Van Der Linde P, Graveland R, Hutten R, Van Eck H, Coppoolse E, Van Der Vossen E, Bakker J, Goverse A (2004) A high-resolution map of the *H1* locus harbouring resistance to the potato cyst nematode *Globodera rostochiensis*. Theor Appl Genet 109:146–152

Bakker E, Butterbach P, Rouppe Van Der Voort J, Van Der Vossen E, Van Vliet J, Bakker J, Goverse A (2003) Genetic and physical mapping of homologues of the virus resistance gene *Rx1* and the cyst nematode resistance gene *Gpa2* in potato. Theor Appl Genet 106:1524–1531

Bakker E, Dees R, Bakker J, Goverse A (2006) Mechanisms involved in plant resistance to nematodes. In: Tuzum S, Bent E (eds) Multigenic and induced systemic resistance in plants. Springer, Berlin, pp 314–334

Bakker J (2002) Durability of resistance against potato cyst nematodes. Euphytica 124:157–162

Bakker J, Folkertsma RT, Rouppe van der Voort JNAM, de Boer JM, Gommers FJ (1993) Changing concepts and molecular approaches in the management of virulence genes in potato cyst nematodes. Annu Rev Phytopathol 31:169–190

Ballvora A, Hesselbach J, Niewohner J, Leister D, Salamini F, Gebhardt C (1995) Marker enrichment and high-resolution map of the segment of potato chromosome VII harboring the nematode resistance gene *Gro1*. Mol Gen Genet 249:82–90

Bar-Or C, Kapulnik Y, Koltai H (2005) A broad characterization of the transcriptional profile of the compatible tomato response to the plant parasitic root-knot nematode *Meloidogyne javanica*. Eur J Plant Pathol 111:181–192

Barone A, Ritter E, Schachtschabel U, Debener T, Salamini F, Gebhardt C (1990) Localization by restriction fragment length polymorphism mapping in potato of a major dominant gene conferring resistance to the potato cyst nematode *Globodera rostochiensis*. Mol Gen Genet 224:177–182

Barr AR, Chalmers KJ, Karakousis A, Kretschmer JM, Manning S, Lance RCM, Lewis J, Jeffries SP, Langridge P (1998) RFLP mapping of a new cereal cyst nematode resistance locus in barley. Plant Breed 117:185–187

Bekal S, Niblack TL, Lambert KN (2003) A chorismate mutase from the soybean cyst nematode *Heterodera glycines* shows polymorphisms that correlate with virulence. Mol Plant Microbe Interact 16:439–446

Bendahmane A, Kanyuka K, Baulcombe DC (1999) The *Rx* gene from potato controls separate virus resistance and cell death responses. Plant Cell 11:781–792

Bogdanove AJ (2002) Protein-protein interactions in pathogen recognition by plants. Plant Mol Biol 50:981–989

Bonas U, Lahaye T (2002) Plant disease resistance triggered by pathogen-derived molecules: Refined models of specific recognition. Curr Opin Microbiol 5:44–50

Boyes DC, Nam J, Dangl JL (1998) The *Arabidopsis thaliana RPM1* disease resistance gene product is a peripheral plasma membrane protein that is degraded coincident with the hypersensitive response. Proc Natl Acad Sci USA 95:15849

Bradshaw JE, Hackett CA, Meyer RC, Milbourne D, McNicol JW, Phillips MS, Waugh R (1998) Identification of AFLP and SSR markers associated with quantitative resistance to *Globodera pallida* (Stone) in tetraploid potato (*Solanum tuberosum* subsp. *tuberosum*) with a view to marker-assisted selection. Theorl Appl Genet 97:202–210

Branch C, Hwang CF, Navarre DA, Williamson VM (2004) Salicylic acid is part of the *Mi-1*-mediated defense response to root-knot nematode in tomato. Mol Plant Microbe Interact 17:351–356

Brenner ED, Lambert KN, Kaloshian I, Williamson VM (1998) Characterization of *LeMir*, a root-knot nematode-induced gene in tomato with an encoded product secreted from the root. Plant Physiol 118:237–247

Brown CR, Yang CP, Mojtahedi H, Santo GS, Masuelli R (1996) RFLP analysis of resistance to Columbia root-knot nematode derived from *Solanum bulbocastanum* in a BC2 population. Theor Appl Genet 92:572–576

Cai D, Kleine M, Kifle S, Harloff HJ, Sandal NN, Marcker KA, Klein-Lankhorst RM, Salentijn EMJ, Lange W, Stiekema WJ, Wyss U, Grundler FMW, Jung C (1997) Positional cloning of a gene for nematode resistance in sugar beet. Science 275:832–834

Caromel B, Mugniery D, Kerlan MC, Andrzejewski S, Palloix A, Ellisseche D, Rousselle-Bourgeois F, Lefebvre V (2005) Resistance quantitative trait loci originating from *Solanum sparsipilum* act independently on the sex ratio of *Globodera pallida* and together for developing a necrotic reaction. Mol PlantMicrobe Interact 18:1186–1194

Caromel B, Mugniery D, Lefebvre V, Andrzejewski S, Ellisseche D, Kerlan MC, Rousselle P, Rousselle-Bourgeois F (2003) Mapping QTLs for resistance against *Globodera pallida* (Stone) Pa2/3 in a diploid potato progeny originating from *Solanum spegazzinii*. Theor Appl Genet 106:1517–1523

Chen P, Roberts PA (2003) Genetic analysis of (a)virulence in *Meloidogyne hapla* to resistance in bean (*Phaseolus vulgaris*). Nematology 5:687–697

Chisholm ST, Coaker G, Day B, Staskawicz BJ (2006) Host-microbe interactions: Shaping the evolution of the plant immune response. Cell 124:803–814

Chitwood DJ (2003) Research on plant-parasitic nematode biology conducted by the United States Department of Agriculture – Agricultural Research Service. Pest Manage Sci 59:748–753

Concibido VC, Lange DA, Denny RL, Orf JH, Young ND (1997) Genome mapping of soybean cyst nematode resistance genes in 'Peking', PI 90763, and PI 88788 using DNA markers. Crop Sci 37:258–264

Da Costa CP, Jones CM (1971) Resistance in cucumber, *Cucumis sativus* L. to three species of cucumber beetles. Hort Sci 6:340–343

Dangl JL, Jones JDG (2001) Plant pathogens and integrated defence responses to infection. Nature 411:826–833

Davis EL, Hussey RS, Baum TJ (2004) Getting to the roots of parasitism by nematodes. Trends Parasitol 20:134–141

Dawkins R, Krebs JR (1979) Arms races between and within species. Proc R Soc Lond Biol Sci 205:489–511

De Ilarduya OM, 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

De Majnik J, Ogbonnaya FC, Moullet O, Lagudah ES (2003) The *Cre1* and *Cre3* nematode resistance genes are located at homeologous loci in the wheat genome. Mol Plant Microbe Interact 16:1129–1134

Deslandes LOJ, Peeters N, Feng DX, Khounlotham M, Boucher C, Somssich I, Genin S, Marco Y (2003) Physical interaction between RRS1-R, a protein conferring resistance to bacterial wilt, and PopP2, a type III effector targeted to the plant nucleus. Proc Natl Acad Sci USA 100:8024–8029

Dixon MS, Hatzixanthis K, Jones DA, Harrison K, Jones JDG (1998) The tomato *Cf-5* disease resistance gene and six homologs show pronounced allelic variation in leucine-rich repeat copy number. Plant Cell 10:1915–1925

Dixon MS, Jones DA, Keddie JS, Thomas CM, Harrison K, Jones JDG (1996) The tomato *Cf-2* disease resistance locus comprises two functional genes encoding leucine-rich repeat proteins. Cell 84:451–459

Dodds PN, Lawrence GJ, Catanzariti AM, Teh T, Wang CIA, Ayliffe MA, Kobe B, Ellis JG (2006) Direct protein interaction underlies gene-for-gene specificity and coevolution of the flax resistance genes and flax rust avirulence genes. Proc Natl Acad Sci USA 103:8888–8893

Doke N, Miura Y, Sanchez LM, Park HJ, Noritake T, Yoshioka H, Kawakita K (1996) The oxidative burst protects plants against pathogen attack: Mechanism and role as an emergency signal for plant bio-defence – a review. Gene 179:45–51

Dong K, Opperman CH (1997) Genetic analysis of parasitism in the soybean cyst nematode *Heterodera glycines*. Genetics 146:1311–1318

Dong X (1998) SA, JA, ethylene, and disease resistance in plants. Curr Opin Plant Biol 1:316–323

Draaistra J (2006) Genetic analyses of root-knot nematode resistance in potato. Lab Plant Breed, Dept Plant Sci, Wageningen Univ. PhD thesis

Eastwood RF, Lagudah ES, Appels R (1994) A directed search for DNA sequences tightly linked to cereal cyst nematode resistance genes in *Triticum tauschii*. Genome 37:311–319

Ellis J, Jones D (1998) Structure and function of proteins controlling strain-specific pathogen resistance in plants. Curr Opin Plant Biol 1:288–293

Ernst K, Kumar A, Kriseleit D, Kloos DU, Phillips MS, Ganal MW (2002) The broad-spectrum potato cyst nematode resistance gene (*Hero*) from tomato is the only member of a large gene family of NBS-LRR genes with an unusual amino acid repeat in the LRR region. Plant J 31:127–136

Fioretti L, Warry A, Porter A, Haydock P, Curtis R (2001) Isolation and localisation of an annexin gene (*gp-nex*) from the potato cyst nematode, *Globodera pallida*. Nematology 3:45–54

Flor HH (1942) Inheritance of pathogenicity in *Metampsora tini*. Phytopathology 32:653–669

Frank SA (1992) Models of plant-pathogen coevolution. Trends Genet 8:213–219

Ganal MW, Simon R, Brommonschenkel S, Arndt M, Phillips MS, Tanksley SD, Kumar A (1995) Genetic mapping of a wide spectrum nematode resistance gene (*Hero*) against *Globodera rostochiensis* in tomato. Mol Plant Microbe Interact 8:886–891

Gebhardt C, Valkonen JPT (2001) Organization of genes controlling disease resistance in the potato genome. Annu Rev Phytopathol 39:79–102

Gheysen G, Fenoll C (2002) Gene expression in nematode feeding sites. Annu Rev Phytopathol 40:191–219

Gleason AC, Liu QL, Williamson VM (2008) Silencing a candidate nematode effector gene corresponding to the tomato resistance gene *Mi-1* leads to acquisition of virulence. Mol Plant Microbe Interact 21:576–585

Goggin FL, Jia L, Shah G, Hebert S, Williamson VM, Ullman DE (2006) Heterologous expression of the *Mi-1.2* gene from tomato confers resistance against nematodes but not aphids in eggplant. Mol Plant Microbe Interact 19:383–388

Gomez-Gomez L, Boller T (2000) FLS2: An LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in *Arabidopsis*. Mol Cell 5:1003–1011

Greenberg JT (1997) Programmed cell death in plant-pathogen interactions. Annu Rev Plant Physiol Plant Mol Biol 48:525–545

Grundler FMW, Sobczak M, Lange S (1997) Defence responses of *Arabidopsis thaliana* during invasion and feeding site induction by the plant-parasitic nematode *Heterodera glycines*. Physiol Mol Plant Pathol 50:419–429

Gupta V, Willits MG, Glazebrook J (2000) *Arabidopsis thaliana* EDS4 contributes to salicylic acid (SA)-dependent expression of defense responses: Evidence for inhibition of jasmonic acid signaling by SA. Mol Plant Microbe Interact 13:503–511

Hammond-Kosack KE, Atkinson HJ, Bowles DJ (1989) Systemic accumulation of novel proteins in the apoplast of the leaves of potato plants following root invasion by the cyst-nematode *Globodera rostochiensis*. Physiol Mol Plant Pathol 35:495–506

Hammond-Kosack KE, Jones JDG (1997) Plant disease resistance genes. Annu Rev Plant Biol 48:575–607

Hasky-Günther K, Hoffmann-Hergarten S, Sikora RA (1998) Resistance against the potato cyst nematode *Globodera pallida* systemically induced by the rhizobacteria *Agrobacterium radiobacter* (G12) and *Bacillus sphaericus* (B43). Fundam Appl Nematol 21:511–517

Holt BF, Hubert DA, Dangl JL (2003) Resistance gene signaling in plants – complex similarities to animal innate immunity. Curr Opin Immunol 15:20–25

Hulbert SH (1997) Structure and evolution of the *rp1* complex conferring rust resistance in maize. Annul Rev Phytopathol 35:293–310

Hutangura P, Mathesius U, Jones MGK, Rolfe BG (1999) Auxin induction is a trigger for root gall formation caused by root-knot nematodes in white clover and is associated with the activation of the flavonoid pathway. Aust J Plant Physiol 26:221–231

Hwang CF, Bhakta AV, Truesdell GM, Pudlo WM, Williamson VM (2000) Evidence for a role of the N terminus and leucine-rich repeat region of the *Mi* gene product in regulation of localized cell death. Plant Cell 12:1319–1329

Hwang CF, Williamson VM (2003) Leucine-rich repeat-mediated intramolecular interactions in nematode recognition and cell death signaling by the tomato resistance protein Mi. Plant J 34:585–593

Jacobs JME, Van Eck HJ, Horsman, Arens PFP, Verkerk-Bakker B, Jacobsen E, Pereira A, Stiekema WJ (1996) Mapping of resistance to the potato cyst nematode *Globodera rostochiensis* from the wild potato species *Solanum vernei*. Mol Breed 2:51–60

Jacquet M, Bongiovanni M, Martinez M, Verschave P, Wajnberg E, Castagnone-Sereno P (2005) Variation in resistance to the root-knot nematode *Meloidogyne incognita* in tomato genotypes bearing the *Mi* gene. Plant Pathol 54:93–99

Jahier J, Abelard P, Tanguy AM, Dedryver F, Rivoal R, Khatkar S, Bariana HS (2001) The *Aegilops ventricosa* segment on chromosome 2AS of the wheat cultivar 'VPM1' carries the cereal cyst nematode resistance gene *Cre5*. Plant Breed 120:125–128

Janeway Jr CA, Medzhitov R (2002) Innate immune recognition. Annu Rev Immunol 20:197–216

Janssen R, Bakker J, Gommers FJ (1991) Mendelian proof for a gene-for-gene relationship between virulence of *Globodera rostochiensis* and the *H1* resistance gene in *Solanum tuberosum* ssp. *andigena* CPC 1673. Rev Nematol 14:213–219

Janssen R, Bakker J, Gommers FJ (1990) Selection of virulent and avirulent lines of *Globodera rostochiensis* for the *H1* resistance gene in *Solanum tuberosum* ssp. *andigena* CPC 1673. Rev Nematol 13:265–268

Jasmer DP, Goverse A, Smant G (2003) Parasitic nematode interactions with mammals and plants. Annu Rev Phytopathol 41:245–270

Jia Y, McAdams SA, Bryan GT, Hershey HP, Valent B (2000) Direct interaction of resistance gene and avirulence gene products confers rice blast resistance. EMBO J 19:4004–4014

Jones DA, Thomas CM, Hammond-Kosack KE, Balint-Kurti PJ, Jonest JDG (1994) Isolation of the tomato *Cf-9* gene for resistance to *Cladosporium fulvum* by transposon tagging. Science 266:789–793

Jones FGW, Winslow RD (1953) Hatching responses in root eelworms (*Heterodera* spp.). Nature 171:478–479

Jung C, Wyss U (1999) New approaches to control plant parasitic nematodes. Appl Microbiol Biotechnol 51:439–446

Kaplan D, Keen N, Thomason I (1980) Studies on the mode of action of glyceollin in soybean incompatibility to the root-knot nematode, *Meloidogyne incognita*. Physiol Plant Pathol 16:319–325

Kaplan DT, Keen NT (1980) Mechanisms conferring plant incompatibility to nematodes. Rev Nematol 3:123–134

Kawalleck P, Schmelzer E, Hahlbrock K, Somssich IE (1995) Two pathogen-responsive genes in parsley encode a tyrosine-rich hydroxyproline-rich glycoprotein (hrgp) and an anionic peroxidase. Mol Gen Genet 247:444–452

Kempster VN, DaviesK A, Scott ES (2001) Chemical and biological induction of resistance to the clover cyst nematode (*Heterodera trifolii*) in white clover (*Trifolium repens*). Nematol 3:35–43

Kessmann H, Staub T, Ligon J, Oostendorp M, Ryals J (1994) Activation of systemic acquired disease resistance in plants. Eur J Plant Pathol 100:359–369

Kim CY, Zhang S (2004) Activation of a mitogen-activated protein kinase cascade induces WRKY family of transcription factors and defense genes in tobacco. Plant J 38:142–151

Kitagawa K, Skowyra D, Elledge SJ, Harper JW, Hieter P (1999) SGT1 encodes an essential component of the yeast kinetochore assembly pathway and a novel subunit of the SCF ubiquitin ligase complex. Mol Cell 4:21–33

Kleine M, Voss H, Cai D, Jung C (1998) Evaluation of nematode-resistant sugar beet (*Beta vulgaris* L.) lines by molecular analysis. Theor Appl Genet 97:896–904

Kouassi AB, Kerlan M-C, Caromel B, Dantec J-P, Fouville D, Manzanares–Dauleux M, Ellissèche D, Mugniéry D (2006) A major gene mapped on chromosome XII is the main factor of a quantitatively inherited resistance to *Meloidogyne fallax* in *Solanum sparsipilum*. Theor Appl Genet 112:699–707

Kovtun Y, Chiu WL, Tena G, Sheen J (2000) Functional analysis of oxidative stress-activated mitogen-activated protein kinase cascade in plants. Proc Natl Acad Sci USA 97:2940–2945

Kreike CM, De Koning JRA, Vinke JH, Van Ooijen JW, Gebhardt C (1993) Mapping of loci involved in quantitatively inherited resistance to the potato cyst-nematode *Globodera rostochiensis* pathotype Ro1. Theor Appl Genet 87:464–470

Kreike CM, de Koning JRA, Vinke JH, van Ooijen JW, Stiekema WJ (1994) Quantitatively-inherited resistance to *Globodera pallida* is dominated by one major locus in *Solanum spegazzinii*. Theor Appl Genet 88:764–769

Kreike CM, Kok-Westeneng AA, Vinke JH, Stiekema WJ (1996) Mapping of QTLs involved in nematode resistance, tuber yield and root development in *Solanum* sp. Theor Appl Genet 92:463–470

Kretschmer JM, Chalmers KJ, Manning S, Karakousis A, Barr AR, Islam AKMR, Logue SJ, Choe YW, Barker SJ, Lance RCM, Langridge P (1997) RFLP mapping of the *Ha2* cereal cyst nematode resistance gene in barley. Theor Appl Genet 94:1060–1064

Kuc J (1982) Induced immunity to plant disease. Bioscience 32:854–860

Kuc J, Barnes E, Daftsios A, Williams E (1959) The effect of amino acids on susceptibility of apple varieties to scab. Phytopathology 49:313–315

Kushida A, Suwa N, Ueda Y, Momota Y (2003) Effects of *Crotalaria juncea* and *C. spectabilis* on hatching and population density of the soybean cyst nematode, *Heterodera glycines* (Tylenchida: Heteroderidae). Appl Entom Zool 38:393–399

Lamb C, Dixon RA (1997) The oxidative burst in plant disease resistance. Annu Rev Plant Biol 48:251–275

Lambert KN, Ferrie BJ, Nombela G, Brenner ED, Williamson VM (1999) Identification of genes whose transcripts accumulate rapidly in tomato after root-knot nematode infection. Physiol Mol Plant Pathol 55:341–348

Lambert KN, Bekal S, Domier LL, Niblack TL, Noel GR, Smyth CA (2005) Selection of *Heterodera glycines* chorismate mutase-1 alleles on nematode-resistant soybean. Mol Plant Microbe Interact 18:593–601

Lange W, Muller J, Debock TSM (1993) Virulence in the beet cyst-nematode (*Heterodera schachtii*) versus some alien genes for resistance in beet. Fundam Appl Nematol 16:447–454

Lee J, Rudd JJ, Macioszek VK, Scheel D (2004) Dynamic changes in the localization of MAPK cascade components controlling pathogenesis-related (PR) gene expression during innate immunity in parsley. J Biol Chem 279:22440–22448

Leeman M, Van Pelt JA, Den Ouden FM, Heinsbroek M, Bakker PA, Schippers B (1995) Induction of systemic resistance against fusarium wilt of radish by lipopolysaccharides of *Pseudomonas fluorescens*. Phytopathology 85:1021–1027

Li HY, Yang GD, Shu HR, Yang YT, Ye BX, Nishida I, Zheng CC (2006) Colonization by the arbuscular mycorrhizal fungus *Glomus versiforme* induces a defense response against the root-knot

nematode *Meloidogyne incognita* in the grapevine (*Vitis amurensis* Rupr.), which includes transcriptional activation of the class III chitinase gene VCH3. Plant Cell Physiol 47:154–163

Li Q, Xie QG, Smith-Becker J, Navarre DA, Kaloshian I (2006) *Mi-1*-mediated aphid resistance involves salicylic acid and mitogen-activated protein kinase signaling cascades. Mol Plant Microbe Interact 19:655–664

Liu Y, Burch-Smith T, Schiff M, Feng S, Dinesh-Kumar SP (2004) Molecular chaperone Hsp90 associates with resistance protein N and its signaling proteins SGT1 and Rar1 to modulate an innate immune response in plants. J Biol Chem 279:2101–2108

Liu Y, Schiff M, Serino G, Deng XW, Dinesh-Kumar SP (2002) Role of SCF ubiquitin-ligase and the COP9 signalosome in the N gene-mediated resistance response to Tobacco mosaic virus. Plant Cell 14:1483–1496

Lorieux M, Reversat G, Garcia Diaz SX, Denance C, Jouvenet N, Orieux Y, Bourger N, Pando-Bahuon A, Ghesquière A (2003) Linkage mapping of *Hsa-10g*, a resistance gene of African rice to the cyst nematode, *Heterodera sacchari*. Theor Appl Genet 107:691–696

Martinez De Ilarduya O, Nombela G, Hwang CF, Williamson VM, Muniz 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

Matzinger P (2002) The danger model: A renewed sense of self. Science 296:301–305

McHale L, Tan X, Koehl P, Michelmore R (2006) Plant NBS-LRR proteins: Adaptable guards. Genome Biol 7:212

Meksem K, Pantazopoulos P, Njiti VN, Hyten LD, Arelli PR, Lightfoot DA (2001a) 'Forrest' resistance to the soybean cyst nematode is bigenic: Saturation mapping of the *Rhg1* and *Rhg4* loci. Theor Appl Genet 103:710–717

Melillo MT, Leonetti P, Leone A, Veronico P, Bleve-Zacheo T (2006b) Plants say no to root-knot nematodes. XXVIII Int Sympos Eur Soc Nematol, 5–9 June, 2006. Blagoevgrad, Bulgaria.

Meyers BC, Dickerman AW, Michelmore RW, Sivaramakrishnan S, Sobral BW, Young ND (1999) Plant disease resistance genes encode members of an ancient and diverse protein family within the nucleotide-binding superfamily. Plant J 20:317–332

Michelmore RW, Meyers BC (1998) Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process. Genome Res 8:1113–1130

Milligan SB, Bodeau J, Yaghoobi J, Kaloshian I, Zabel P, Williamson VM (1998) The root-knot nematode resistance gene *Mi* from tomato is a member of the leucine zipper, nucleotide binding, leucine-rich repeat family of plant genes. Plant Cell 10:1307–1319

Mittler R, Del Pozo O, Meisel L, Lam E (1997) Pathogen-induced programmed cell death in plants, a possible defense mechanism. Dev Genet 21:279–289

Moffett P, Farnham G, Peart J, Baulcombe DC (2002) Interaction between domains of a plant NBS-LRR protein in disease resistance-related cell death. EMBO J 21:4511–4519

Morel JB, Dangl JL (1997) The hypersensitive response and the induction of cell death in plants. Cell Death Differ 4:671–683

Neveu C, Jaubert S, Abad P, Castagnone-Sereno P (2003) A set of genes differentially expressed between avirulent and virulent *Meloidogyne incognita* near-isogenic lines encode secreted proteins. Mol Plant Microbe Interact 16:1077–1084

Nombela G, Williamson VM, Muniz 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

Nürnberger T, Lipka V (2005) Non-host resistance in plants: New insights into an old phenomenon. Mol Plant Pathol 6:335–345

Ogbonnaya FC, Seah S, Delibes A, Jahier J, Lopez-Brana I, Eastwood RF, Lagudah ES (2001) Molecular-genetic characterisation of a new nematode resistance gene in wheat. Theor Appl Genet 102:623–629

Oka Y, Cohen Y (2001) Induced resistance to cyst and root-knot nematodes in cereals by dl-beta-amino-n-butyric acid. Eur J Plant Pathol 107:219–227

Paal J, Henselewski H, Muth J, Meksem K, Menendez CM, Salamini F, Ballvora A, Gebhardt C (2004) Molecular cloning of the potato *Gro1-4* gene conferring resistance to pathotype Ro1 of the root cyst nematode *Globodera rostochiensis*, based on a candidate gene approach. Plant J 38:285–297

Parlevliet JE (2002) Durability of resistance against fungal, bacterial and viral pathogens; present situation. Euphytica 124:147–156

Parniske M, Hammond-Kosack KE, Golstein C, Thomas CM, Jones DA, Harrison K, Wulff BBH, Jones JDG (1997) Novel disease resistance specificities result from sequence exchange between tandemly repeated genes at the *Cf-4/9* locus of tomato. Cell 91:821–832

Paull JG, Chalmers KJ, Karakousis A, Kretschmer JM, Manning S, Langridge P (1998) Genetic diversity in Australian wheat varieties and breeding material based on RFLP data. Theor Appl Genet 96:435–446

Peart JR, Lu R, Sadanandom A, Malcuit I, Moffett P, Brice DC, Schauser L, Jaggard DAW, Xiao S, Coleman MJ, Dow M, Jones JDG, Shirasu K, Baulcombe DC (2002) Ubiquitin ligase-associated protein SGT1 is required for host and nonhost disease resistance in plants. Proc Natl Acad Sci USA 99:10865–10869

Pedley KF, Martin GB (2005) Role of mitogen-activated protein kinases in plant immunity. Curr Opin Plant Biol 8:541–547

Penninckx I, Eggermont K, Terras FRG, Thomma B, Samblanx GWD, Buchala A, Metraux JP, Manners JM, Broekaert WF (1996) Pathogen-induced systemic activation of a plant defensin gene in *Arabidopsis* follows a salicylic acid-independent pathway. Plant Cell 8:2309–2323

Pieterse CMJ, Van Loon LC (1999) Salicylic acid-independent plant defence pathways. Trends in Plant Sci 4:52–58

Pineda O, Bonierbale MW, Plaisted RL, Brodie BB, Tanksley SD (1993) Identification of RFLP markers linked to the *H1* gene conferring resistance to the potato cyst nematode *Globodera rostochiensis*. Genome 36:152–156

Pontier D, Balague C, Roby D (1998) The hypersensitive response. A programmed cell death associated with plant resistance. C R Acad Sci Ser III 321:721–734

Prior A, Jones JT, Blok VC, Beauchamp J, McDermott L, Cooper A, Kennedy MW (2001) A surface-associated retinol- and fatty acid-binding protein (Gp-FAR-1) from the potato cyst nematode G*lobodera pallida*: lipid binding activities, structural analysis and expression pattern. BiochemJ 356:387–394

Rahimi S, Perry RN, Wright DJ (1996) Identification of pathogenesis-related proteins induced in leaves of potato plants infected with potato cyst nematodes, *Globodera* species. Physiol Mol Plant Pathol 49:49–59

Rahimi S, Perry RN, Wright DJ (1998) Detection of chitinases in potato plants following infection with the potato cyst nematodes, *Globodera rostochiensis* and *G. Pallida*. Nematologica 44:181–193

Reitz M, Oger P, Meyer A, Niehaus K, Farrand SK, Hallman J, Sikora RA (2002) Importance of the O-antigen, core-region and lipid A of rhizobial lipopolysaccharides for the induction of systemic resistance in potato to *Globodera pallida*. Nematology 4:73–79

Ren D, Yang H, Zhang S (2002) Cell death mediated by MAPK is associated with hydrogen peroxide production in *Arabidopsis*. J Biol Chem 277:559–565

Robertson L, Robertson WM, Sobczak M, Helder J, Tetaud E, Ariyanayagam MR, Ferguson MAJ, Fairlamb A, Jones JT (2000) Cloning, expression and functional characterisation of a peroxiredoxin from the potato cyst nematode *Globodera rostochiensis*. Mol Biochem Parasitol 111:41–49

Ron M, Avni A (2004) The receptor for the fungal elicitor ethylene-inducing xylanase is a member of a resistance-like gene family in tomato. Plant Cell 16:1604–1615

Ross AF (1961) Localized acquired resistance to plant virus infection in hypersensitive hosts. Virology 14:329–339

Rouppe van Der Voort J, Lindeman W, Folkertsma R, Hutten R, Overmars H, van Der Vossen E, Jacobsen E, Bakker J (1998) A QTL for broad-spectrum resistance to cyst nematode species

(*Globodera* spp.) maps to a resistance to gene cluster in potato. Theor Appl Genet 96:654–661

Rouppe van der Voort J, van der Vossen E, Bakker E, Overmars H, van Zandvoort P, Hutten R, Klein Lankhorst RK, Bakker J (2000) Two additive QTLs conferring broad-spectrum resistance in potato to *Globodera pallida* are localized on resistance gene clusters. Theor Appl Genet 101:1122–1130

Rouppe van Der Voort J, Janssen GJW, Overmars H, van Zandvoort PM, van Norel A, Scholten OE, Janssen R, Bakker J (1999) Development of a PCR-based selection assay for root-knot nematode resistance (*Rmc1*) by a comparative analysis of the *Solanum bulbocastanum* and *S. tuberosum* genome. Euphytica 106:187–195

Rouppe van der Voort JN, van Eck HJ, van Zandvoort PM, Overmars H, Helder J, Bakker J (1999) Linkage analysis by genotyping of sibling populations: A genetic map for the potato cyst nematode constructed using a 'pseudo-F2' mapping strategy. Mol Gen Genet 261:1021–1031

Rouppe van Der Voort JN, van Zandvoort P, van Eck HJ, Folkertsma RT, Hutten RCB, Draaistra J, Gommers FJ, Jacobsen E, Helder J, Bakker J (1997) Use of allele specificity of comigrating AFLP markers to align genetic maps from different potato genotypes. Mol Gen Genet 255:438–447

Ruben E, Jamai A, Afzal J, Njiti VN, Triwitayakorn K, Iqbal MJ, Yaegashi S, Bashir R, Kazi S, Arelli P, Town C, Meksem K, Lightfoot DA (2006) Genomic analysis of the *rhg1* locus: Candidate genes that underlie soybean resistance to the cyst nematode. Mol Genet Genomics 276:503–516

Ryals J, Uknes S, Ward E (1994) Systemic acquired resistance. Plant Physiol 104:1109–1112

Santhi A, Sivakumar CV (1997) Biocontrol potential of *Pseudomonas fluorescens* (Migula) against root-knot nematode, *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1994 on tomato. J Biol Control 9:113–115

Scheffer F, Kikuth R, Visser JH (1962) Die Wurzelausscheidun-gen von Eragrostis curvula (Schrad.) Nees und ihr Einfluss auf Wurzelknoten-Nematoden. Z fur Pflanznhr Bodenkd 98:114–120

Seah S, Yaghoobi J, Rossi M, Gleason CA, Williamson VM (2004) The nematode-resistance gene, *Mi-1*, is associated with an inverted chromosomal segment in susceptible compared to resistant tomato. Theor Appl Genet 108:1635–1642

Semblat JP, Rosso MN, Hussey RS, Abad P, Castagnone-Sereno P (2001) Molecular cloning of a cDNA encoding an amphid-secreted putative avirulence protein from the root-knot nematode *Meloidogyne incognita*. Mol Plant Microbe Interact 14:72–79

Shapiro AD (2005) Nitric oxide signaling in plants. Vitam Horm 72:339–398

Shirasu K, Schulze-Lefert P (2003) Complex formation, promiscuity and multi-functionality: protein interactions in disease-resistance pathways. Trends Plant Sci 8:252–258

Sikora RA (1992) Management of the antagonistic potential in agricultural ecosystems for the biological control of plant parasitic nematodes. Annu Rev Phytopathol 30:245–270

Sobczak M, Avrova A, Jupowicz J, Phillips MS, Ernst K, Kumar A (2005) Characterization of susceptibility and resistance responses to potato cyst nematode (*Globodera* spp.) infection of tomato lines in the absence and presence of the broad-spectrum nematode resistance *Hero* gene. Mol Plant Microbe Interact 18:158–168

Song WY, Wang GL, Chen LL, Kim HS, Pi LY, Holsten T, Gardner J, Wang B, Zhai WX, Zhu LH, Fauquet C, Ronald P (1995) A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. Science 270:1804–1806

Stahl EA, Dwyer G, Mauricio R, Kreitman M, Bergelson J (1999) Dynamics of disease resistance polymorphism at the *Rpm1* locus of Arabidopsis. Nature 400:667–671

Sticher L, Mauch-Mani B, Métraux JP (1997) Systemic acquired resistance. Annu Rev Phytopathol 35:235–270

Tameling WIL, Elzinga SDJ, Darmin PS, Vossen JH, Takken FLW, Haring MA, Cornelissen BJC (2002) The tomato R gene products *I-2* and *Mi-1* are functional ATP binding proteins with ATPase activity. Plant Cell 14:2929–2939

Tao Y, Xie Z, Chen W, Glazebrook J, Chang HS, Han B, Zhu T, Zou G, Katagiri F (2003) Quantitative nature of *Arabidopsis* responses during compatible and incompatible interactions with the bacterial pathogen *Pseudomonas syringae*. Plant Cell 15:317–330

Thomas CM (1997) Characterization of the tomato *Cf-4* gene for resistance to *Cladosporium fulvum* identifies sequences that determine recognitional specificity in Cf-4 and Cf-9. Plant Cell 9:2209–2224

Thurau T, Kifle S, Jung C, Cai D (2003) The promoter of the nematode resistance gene *Hs1pro-1* activates a nematode-responsive and feeding site-specific gene expression in sugar beet (*Beta vulgaris* L.) and *Arabidopsis thaliana*. Plant Mol Biol 52:643–660

Tuzun S (2006) Terminology related to induced systemic resistance: Incorrect use of synonyms may lead to a scientific dilemma by misleading interpretation of results. In: Tunzin S, Bent E (eds) Multigenic and induced systemic resistance in plants. Springer, Berlin, pp 1–8

Ueda H, Yamaguchi Y, Sano H (2006) Direct interaction between the Tobacco mosaic virus helicase domain and the ATP-bound resistance protein, N factor during the hypersensitive response in tobacco plants. Plant Mol Biol 61:31–45

Van der Biezen EA, Jones JDG (1998) Plant disease-resistance proteins and the gene-for-gene concept. Trends Biochem Sci 23:454–456

Van der Vossen EA, Gros J, Sikkema A, Muskens M, Wouters D, Wolters P, Pereira A, Allefs S (2005) The *Rpi-blb2* gene from *Solanum bulbocastanum* is an *Mi-1* gene homolog conferring broad-spectrum late blight resistance in potato. Plant J 44:208–222

Van der Vossen EAG, Rouppe van der Voort JNAM, Kanyuka K, Bendahmane A, Sandbrink H, Baulcombe DC, Bakker J, Stiekema WJ, Klein-Lankhorst RM (2000) Homologues of a single resistance-gene cluster in potato confer resistance to distinct pathogens: A virus and a nematode. Plant J 23:567–576

Van Peer R, Niemann GJ, Schippers B (1991) Induced resistance and phytoalexin accumulation in biological control of fusarium wilt of carnation by *Pseudomonas* sp. strain WCS417r. Phytopathol 91:728–734

Van Peer R, Schippers B (1992) Lipopolysaccharides of plant-growth promoting *Pseudomonas* sp. strain WCS417r induce resistance in carnation to *Fusarium* wilt. Eur J Plant Pathol 98:129–139

Van Wees SCM, Pieterse CMJ, Trijssenaar A, Van ‘t Westende YAM, Hartog F, Van Loon LC (1997) Differential induction of systemic resistance in *Arabidopsis* by biocontrol bacteria. Mol Plant Microbe Interact 10:716–724

Vanholme B, De Meutter J, Tytgat T, Van Montagu M, Coomans A, Gheysen G (2004) Secretions of plant-parasitic nematodes: a molecular update. Gene 332:13–27

Veremis JC, Van Heusden AW, Roberts PA (1999) Mapping a novel heat-stable resistance to *Meloidogyne* in *Lycopersicon peruvianum*. Theor Appl Genet 98:274–280

Vos P, Simons G, Jesse T, Wijbrandi J, Heinen L, Hogers R, Frijters A, Groenendijk J, Diergaarde P, Reijans M, Fierens-Onstenk J, Both de M, Peleman J, Liharska T, Hontelez J, Zabeau M (1998) The tomato *Mi-1* gene confers resistance to both root-knot nematodes and potato aphids. Nature Biotechnol 16:1365–1369

Waetzig GH, Sobczak M, Grundler FMW (1999) Localization of hydrogen peroxide during the defence response of *Arabidopsis thaliana* against the plant-parasitic nematode *Heterodera glycines*. Nematology 1:681–686

Wei G, Kloepper JW, Tuzun S (1991) Induction of systemic resistance of cucumber to *Colletotichum orbiculare* by select strains of plant growth-promoting rhizobacteria. Phytopathology 81:1508–1512

White RF (1979) Acetylsalicylic acid (aspirin) induces resistance to tobacco mosaic virus in tobacco. Virology 99:410–412

Williamson VM (1998) Root-knot nematode resistance genes in tomato and their potential for future use. Annu Rev Phytopathol 36:277–293

Williamson VM (1999) Plant nematode resistance genes. Curr Opin Plant Biol 2:327–331

Williamson VM, Gleason CA (2003) Plant–nematode interactions. Curr Opin Plant Biol 6:327–333

Williamson VM, Hussey RS (1996) Nematode pathogenesis and resistance in plants. Plant Cell 8:1735–1745

Williamson VM, Hwang CF, Truesdell G, Bhakta AV, Fort KP (2000) The nematode resistance gene *Mi*. In: De Wit PJG, Bisseling T, Stiekema W (eds) Biology of plant–microbe interactions. St. Paul Int Soc Mol Plant Microbe Interact 2:454

Williamson VM, Kumar A (2006) Nematode resistance in plants: the battle underground. Trends Genet 22:396–403

Wolters P, Vinke H, Bontjer I, Rouppe van der Voort J, Colon L, Hoogendoorn C (1998) Presence of major genes for resistance to *Globodera pallida* in wild tuber-bearing *Solanum* species and their location on the potato genome. In: 5th Intl. Symp. Mol. Biol. Pot, August 2–6, Bogensee, Germany

Xing T, Higgins VJ, Blumwald E (1997) Race-specific elicitors of Cladosporium fulvum promote translocation of cytosolic components of NADPH oxidase to the plasma membrane of tomato cells. Plant Cell 9:249–259

Yaghoobi J, Kaloshian I, Wen Y, Williamson VM (1995) Mapping a new nematode resistance locus in *Lycopersicon peruvianum*. Theor Appl Genet 91:457–464

Yaghoobi J, Yates JL, Williamson VM (2005) Fine mapping of the nematode resistance gene *Mi-3* in *Solanum peruvianum* and construction of a *S. lycopersicum* DNA contig spanning the locus. Mol Genet Genomics 274:60–69

Zhang S, Klessig DF (2001) MAPK cascades in plant defense signaling. Trends Plant Sci 6:520–527

Zipfel C, Felix G (2005) Plants and animals: a different taste for microbes? Curr Opin Plant Biol 8:353–360

Development of the Root-Knot Nematode Feeding Cell

R. Howard Berg(✉), Thomas Fester, and Christopher G. Taylor

Abstract The root-knot nematode feeding cell is a remarkable example of the reprogramming of plant cells by biotrophic pathogens. With the aid of molecules secreted into plant cells from three esophageal gland cells, the root-knot nematode *Meloidogyne* sp. orchestrates a fundamental change in those cells surrounding its head in the plant root. These cells expand in volume over tenfold and become a virtual factory for the production of cytoplasm that is a rich source of nutrients for the nematode. Because of the large size of the cells, Treub (1887) coined the term "giant-cell" to describe them. Giant-cells have attracted the attention of biologists for over 100 years (reviewed in Christie 1936) and they continue to challenge investigators that are using modern techniques in molecular biology, biochemistry, and microscopy to answer long-held questions. This chapter examines giant-cells from a cell biologist's point of view, i.e., with an eye on identifying notable features of development at a cellular and subcellular level.

Abbreviations C: cajal body; ER: endoplasmic reticulum; FT: feeding tube; G: Golgi stack; GC: giant-cell; M: mitochondrion; MT: microtubule; N: nucleolus; Ne: nematode; Nu: nucleus; P: plastid; Pl: phloem; SER: smooth endoplasmic reticulum; TGN: trans Golgi network; V: vacuole; Ve: vesicle; WL: wall labyrinth; Wt: wall thickening; X: xylem

Unless otherwise indicated, all micrographs are thin section transmission electron micrographs of high-pressure frozen, freeze-substituted tissue (preparation described in Sect. 2.1). Sections are poststained in uranyl acetate and lead citrate. The tissue is root-knot galls of *Arabidopsis thaliana* infected with *Meloidogyne incognita*, grown in plate culture, unless otherwise noted.

R.H. Berg
Donald Danforth Plant Science Center, 975 North Warson Road, St. Louis, MO 63132, USA
e-mail: rhberg@danforthcenter.org

Plant Cell Monogr, doi:10.1007/7089_2008_30

1 Introduction

Root-knot nematode-induced giant-cells are focused on the acquisition and concentration of nutrients for their final transfer to the parasite. Nutrient acquisition by giant-cells is supported by a network of newly formed vascular tissue surrounding these feeder cells and by the cell wall ingrowths of these cells. Within the giant-cells the nutrients obtained are transformed into a dense cytoplasm with a high proportion of proteins and lipids. Critical features of giant-cells include: large number of nuclei (formed by repeated nuclear division without cytokinesis), numerous secondary cell wall ingrowths (increasing the surface area of the feeder cells) and numerous organelles including Golgi stacks, small vacuoles, plastids, endoplasmic reticulum, and mitochondria. Perhaps the most unusual cytological feature, however, is the formation of the nematode feeding tube, which is also formed in syncytia induced by the cyst nematode (Sobczak and Golonowski 2008). The feeding tube extends from the cell surface into the giant-cell for dozens of micrometers, has a crystalline wall structure, and is surrounded by plant cell endomembrane components. Its formation is elicited by secretions produced in the nematode esophageal gland cells and injected into the parasitized cell via the nematode stylet. The feeding tube serves as a conduit for cell sap uptake by the nematode. We will examine these intriguing features of giant-cells from the perspective offered by cellular imaging techniques. A historical perspective on the more controversial aspects of giant-cell development is provided for those readers new to the study of these cells.

2 Giant-Cell Initiation

Invasion of the root is effectively imaged using video-enhanced contrast light microscopy, as shown by Wyss et al. (1992). They analyzed the infection of *Arabidopsis thaliana* roots by second-stage juveniles (J2) of *Meloidogyne incognita*. The J2s enter roots in the elongation zone near root tips by penetrating and destroying epidermal cells. Within the root, the J2s intercellularly penetrate the tissue, avoiding plant wound responses and cell death, migrating to the meristem and then back into the vascular tissue, coming to rest with their head near the elongation zone of vascular tissue. This contrasts with infection of roots by cyst nematodes, the other large group of sedentary nematode parasites. Infection by these pathogens involves repeated wounding of plant cells during nematode intracellular migration within the root (Wyss et al. 1992; Sobczak and Golonowski 2008). Within 24 h of infection plant cells adjacent to the *M. incognita* J2s are in various stages of mitosis, with some cells exhibiting pre-mitotic phragmosomes and others post-mitotic structures including phragmoplasts and early stages of cell plate formation (Jones and Payne 1978). As feeding sites develop they are manifested as swollen regions on the root, referred to as the root knot or gall.

Giant-cells are most commonly formed from parenchymatic cells within the stele that surround the anterior end of the nematode, suggesting that esophageal

gland secretions are the primary molecules stimulating giant-cell formation. Indeed, removing or inactivating the nematode prevents further giant-cell development (Bird 1962). These secretions are released from the nematode stylet, a protrusible hollow mouth spear that is used to pierce cell walls and come into contact with the plant cell membrane, where a minute hole is formed in the membrane to provide access to the cytoplasm of the parasitized cell (see Sect. 6). During early development, pericycle and cortical cells of the root proliferate around the nematode (Dropkin and Nelson 1960; Jones and Payne 1978; Paulson and Webster 1970) increasing the root's girth and forming a gall. This proliferation is correlated with a high level of expression of transcriptional regulators (*PHAN*, *KNOX*) and a nodule mitogen (*ENOD40*) in this tissue (Koltai et al. 2001; Gheysen and Mitchum 2008).

A root-knot nematode secreted parasitism protein encoded by a parasitism gene designated as *16D10*, which is expressed in the two subventral pharyngeal gland cells, appears to mediate an early signaling event in the *Meloidogyne*–host interaction (Huang et al. 2003, 2006; Davis et al. 2008). This parasitism gene encodes a small, novel secreted peptide of 13 amino acids, including a 30-amino acid N-terminal hydrophobic signal peptide, and is conserved in *Meloidogyne* species. When the 16D10 peptide is over-expressed in *Arabidopsis*, root growth is significantly accelerated, giving rise to a much-enlarged root system without affecting shoot growth (Huang et al. 2006). The 16D10 peptide was shown to directly bind to the SAW domain of two *Arabidopsis* SCARECROW-like (SCL) transcription factors, AtSCL6 and AtSCL21, in a yeast two-hybrid screen for 16D10-interacting proteins (Huang et al. 2006). SCL transcription factors are members of the GRAS protein family, which play important roles in plant development and signaling (Bolle 2004). These data suggest that the 16D10 peptide functions as a signaling peptide that specifically induces root growth by directly interacting with a host intracellular SCL transcription regulator. Furthermore, since the conserved *Meloidogyne*-secreted signaling peptide is strongly expressed in the subventral pharyngeal gland cells of second-stage juveniles at the time when the giant-cells are being developed, this peptide is speculated to have a role in the reprogramming of gene expression required for giant-cell formation (Davis et al. 2008; Gheysen and Mitchum 2008).

This reprogramming of root cells includes stimulation of the production of high cytoplasmic density in giant-cells (and in syncytia, Sobczak and Golinowski 2008). Bird (1961) measured protein content with a microspectrometer in sections of giant-cells stained with mercuric-bromophenol blue, finding that protein content increases to a maximum at the egg-laying stage when nematode demands on the host are highest. The high cytoplasmic density of giant-cells is evident in the mature giant-cell (with fully developed cell walls containing transfer cell labyrinths) shown in Fig. 1a. Unlike parenchyma cells, there is no large vacuole. The cell volume is predominantly filled by cytoplasm, rich in ribosomes, plastids, mitochondria, endoplasmic reticulum (ER), Golgi stacks, and small vacuoles (Fig. 1b, c). These are not uniformly distributed (Fig. 1c), suggesting cellular processes are regionally sequestered within the cell. The large population of small vacuoles in mature giant-cells usually is not distributed near the cell periphery (Fig. 1c; Jones and Payne 1978), being excluded perhaps by cytoplasmic components functioning at the cell surface.

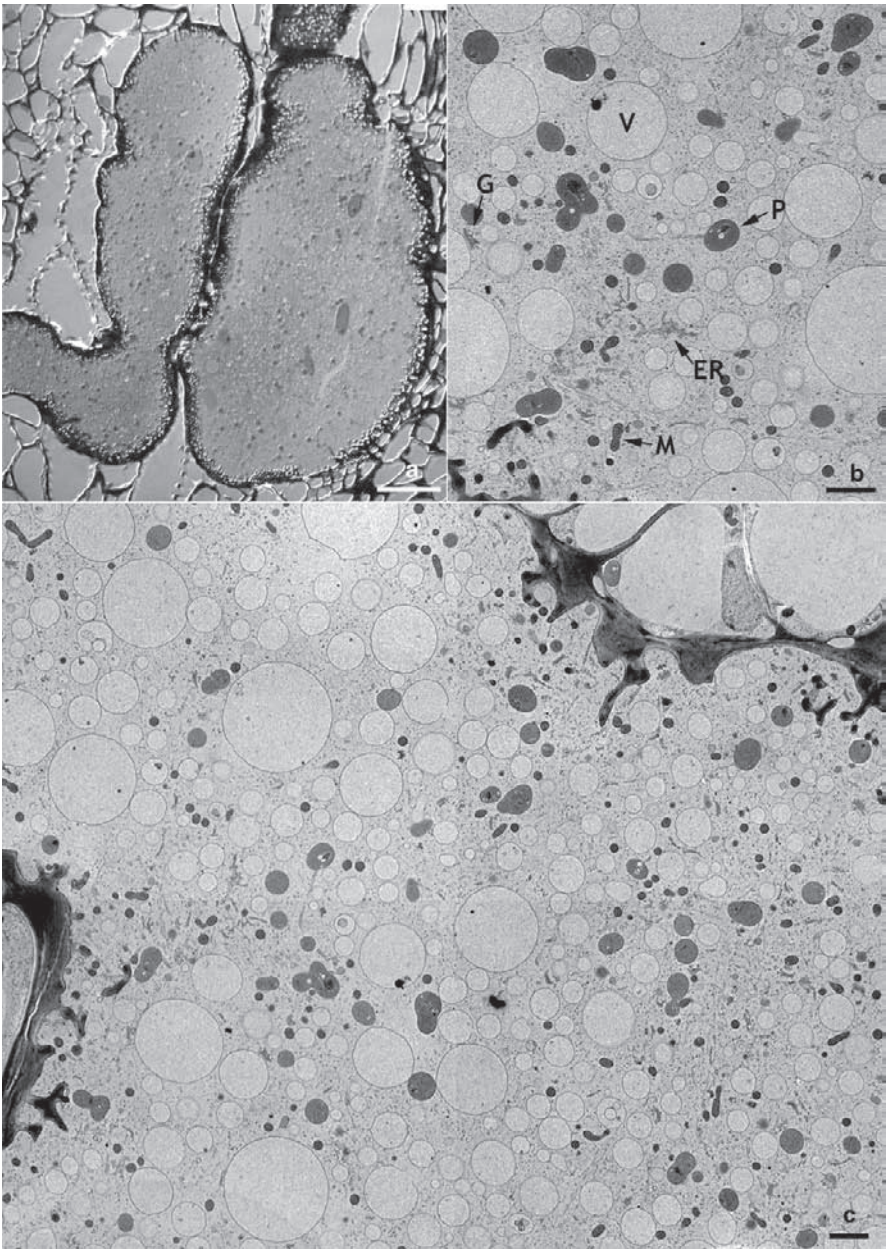


Fig. 1 Cytoplasmic density in mature giant-cells. (**a**) Light micrograph of resin section, mature giant-cell with fully developed transfer cell wall labyrinth. Note dense cytoplasm lacking a large vacuole. (**b**) Cytoplasm is rich in organelles including; Golgi stacks, small vacuoles, plastids, endoplasmic reticulum, and mitochondria. (**c**) Organelle distribution is non-uniform, forming islands of organelle-rich regions, and the small vacuoles are less abundant at the cell periphery. *Scale bars*: (**a**) 20 μm; (**b**) and (**c**) 2 μm

2.1 Electron Microscopy of Giant-Cells

The large, dense volume of giant-cells presents a challenge for preparing them adequately for thin section transmission electron microscopy (TEM). Chemical fixation (CF, typically performed with glutaraldehyde and osmium tetroxide) is an oxygen-consuming process (Hayat 1981) dependent on diffusion of fixative into the cells. This process is particularly slow in the case of large, dense giant-cells, subjecting them to a long period of chemical stress that likely compromises cellular structure. Furthermore, the subsequent dehydration steps required for embedding the tissue in resin subjects the cells to abrupt changes in water content, altering cell volume which leads to shrinkage and swelling artifacts (Hayat 1981). To circumvent these problems, we use ultra-rapid freezing (high-pressure freezing, HPF) to physically fix the tissue in a few milliseconds. Dehydration artifacts are avoided by freeze-substituting (FS) the specimen while it is frozen, at (–) 80°C, in acetone containing 2% osmium tetroxide and 0.1% uranyl acetate. The cells are then slowly thawed and embedded in conventional resin (Epon/Araldite) for thin sectioning. This approach is considered the best method for electron microscopy analysis of plant cell structure (Hess 2007). That this is the case for giant-cells is shown in Fig. 2, comparing CF (a) with HPF/FS material (b) in a region near the cell wall labyrinth of the giant-cell (see Sect. 4.3). The wall labyrinth (WL) in CF cells appears uniform in density, whereas HPF/FS displays local regions of higher electron density in the WL (arrow) which are apparently extracted using CF. The cell membrane becomes distorted by shrinkage during dehydration after CF, leading

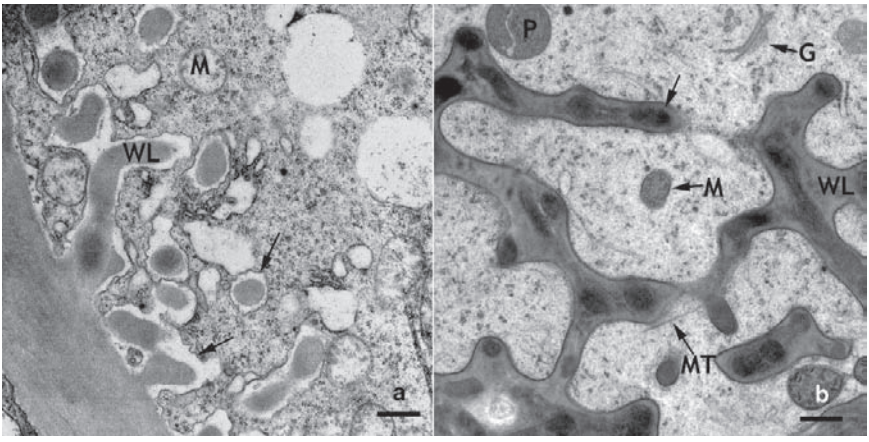


Fig. 2 Comparison of chemically fixed (**a**) and high-pressure frozen, freeze-substituted (**b**) giant-cells (see text Sect. 2.1). The wall labyrinth in freeze-substituted tissue contains electron-dense pockets (*arrow*) not preserved in chemical fixation. Chemical fixation leads to plasma membrane shrinkage artifacts at the labyrinth (*arrows*) and extraction of mitochondria and the cytoplasm. *Scale bars*: 500 nm

to gap artifacts between the membrane and wall labyrinth (arrows); HPF/FS does not produce this artifact, preserving a smooth membrane profile closely pressed to the wall labyrinth. Organelles are extracted and dilated in structure using CF. For example, mitochondria (M) in the CF image lack electron density due to leaching of matrix material in contrast to mitochondria from HPF/FS images. Plastids (P), Golgi stacks (G), and microtubules (MT) are well-preserved by HPF/FS. Ground cytoplasm is uniform in electron density in the HPF/FS specimen while it is extracted in the CF specimen, leading to non-uniformity and higher contrast. All further micrographs presented in this chapter have been produced from HPF/FS specimens, offering a more accurate evaluation of cellular structure. Though older microscopic techniques were critical and extremely useful for the early analysis of giant cells, newer techniques such as HPF/FS are improving our observational analyses of nematode feeding sites.

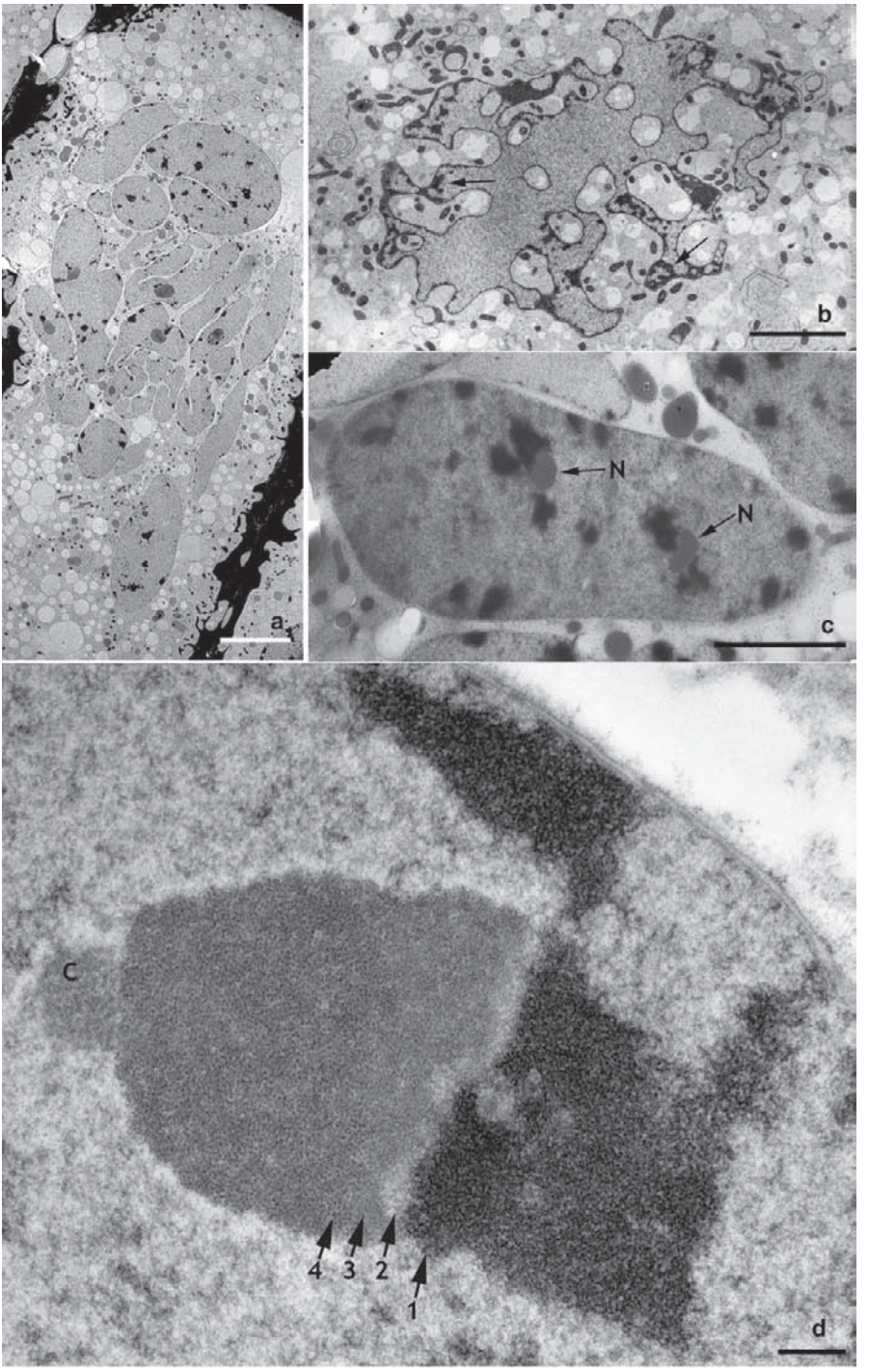
3 Giant-Cell Formation

Historically, the mode of giant-cell formation has been the subject of controversy, with one school maintaining that a giant-cell forms by the expansion of a single cell, and the other school attributing giant-cell formation to rupture or degradation of cell walls and merging of protoplasts. The latter explanation was more widely accepted in the early literature, reviewed by Christie (1936) and Huang (1985). Beille (1898) first proposed that cell walls disintegrate during giant-cell formation, with the result that protoplasts exposed by this process fuse to form a large giant-cell. He maintained that giant-cell expansion was limited by the developing vascular tissue (xylem elements) that formed around giant-cells during their development. The fusion of protoplasts was given as the explanation for the large numbers of nuclei in giant-cells. However, an alternate view of how giant-cells form was proposed soon thereafter. Nemec (1910) was the first scientist to propose the currently held view that giant-cells develop by expansion and not by cell wall degradation. In addition, he also pointed out that this expansion is accompanied by repeated rounds of mitosis without cell wall formation – providing an explanation for the high numbers of nuclei in these large cells. The occurrence of repeated cell cycles within giant-cells was shown by de Almeida Engler et al. (1999) through in situ hybridization using cell-cycle specific probes as well as the application of cell-cycle inhibitors. These experiments showed that giant-cell development is dependent on progression through G2 of the cell cycle. Further details of cell cycle activation in nematode feeding sites are reviewed in Goverse et al. (2000) and Gheysen and Mitchum 2008. Gene expression in feeding sites is reviewed by Gheysen and Fenoll (2002) and Gheysen and Mitchum (2008); Li et al. (2008). Debate between the two viewpoints of the mode of giant-cell formation revolved around whether mitosis was sufficient to explain total nuclear numbers and whether cell wall breakdown occurs during giant-cell development.

3.1 Mitosis

From the early work of Tischler (1901) and Nemec (1910), mitosis was known to occur in giant-cells. It was first quantitatively analyzed by Huang and Maggenti (1969). They counted chromosome numbers in giant-cells of *Vicia faba* infected with *M. javanica*, finding that the numbers per cell were in a geometric progression, suggesting a synchronous rate of mitosis in giant-cells. This observation is consistent with giant-cell nuclei being derived from the expansion of a single cell combined with synchronous mitotic cycles, i.e., synchronous duplication of nuclear counts. On the other hand, if a giant-cell was to result from fusion of multiple cells, then chromosome counts would vary arithmetically. The results from Huang and Maggenti were contested by Bird (1972, 1973). Using microspectrometric measurement of Feulgen-stained nuclei, he found highly variable DNA content per nucleus (Bird 1972). Like Huang and Maggenti, he observed mitotic synchrony that in older giant-cells was out of phase (Bird 1973). In contrast to Huang and Maggenti, he found the chromosome number in nuclei of a single giant-cell was arithmetically (rather than geometrically) variable, concluding that this was consistent with acquisition of nuclei via cell fusion. The source of discrepancy in chromosome counts from the two labs is not apparent, other than the possibility that giant-cell size and numbers of nuclei (up to 150 have been reported, Dropkin and Nelson 1960) make accurate measurements difficult.

The large number of giant-cell nuclei (and the endoreduplication within them, Wiggers et al. 1990; Gheysen and Mitchum 2008) correlates with the need to produce copious amounts of protein comprising the giant-cell cytoplasm. As shown in the group of approximately 20 nuclei in Fig. 3a, these organelles tend to group together in the center of giant-cells (Dropkin and Nelson 1960). The obliquely sectioned nucleus in Fig. 3b clearly shows the lobed morphology that is typical for giant-cell nuclei (and for syncytial nuclei, Sobczak and Golinowski 2008). Electron dense patches of heterochromatin are most often bound to the nuclear envelope (Fig. 3b, arrows; Fig. 3c, d). The thick section (0.5 μm) in Fig. 3c gives depth information on nuclear structure, confirming that most heterochromatin is bound to the envelope. The non-bound internal heterochromatin in this nucleus is associated with two nucleoli, which in general are prominent features of giant-cell nuclei. Salivary secretions from the nematode contain protein, some of which have nuclear localization signals (Davis et al. 2008). Whether these impact endoreduplication and nucleolar functions (as detailed below) or elicit surveillance responses from the micro-RNA (miRNA) and small-interfering RNA (siRNA) pathways associated with Cajal bodies (see below) is unknown. Giant-cells have long been known to contain prominent, deeply staining nucleoli (Christie 1936; Dropkin and Nelson 1960). Nucleoli are the sites for a number of molecular processes besides ribosome assembly. Ribosomal proteins comprise only ~27% of the nucleolus proteome (of 217 proteins) in *Arabidopsis* (Pendle et al. 2005), leaving much to understand about nucleolar cell biology. Currently the list of nucleolus-associated functions includes: ribosomal RNA synthesis/processing/assembly, transfer RNA (tRNA) transcription/


a
b
N
N
c
C
4
3
2
1
d

processing, messenger RNA (mRNA) maturation, and biogenesis or storage of signal recognition particles and telomerase ribonucleoprotein complexes (Pontes and Pikaard 2008; Shaw and Brown 2004). Cajal bodies (Fig. 3d) associated with the nucleolus and also distributed freely in the nucleoplasm are the sites of miRNA and siRNA processing in plants (Pontes and Pikaard 2008), with products impacting plant development, chromatin structure, gene silencing, and other processes. Bird (1973) quantified nucleolar volume per giant-cell nucleus, showing up to a ~tenfold increase compared to nearby parenchyma cells. The presence of this large number of nucleoli in giant-cells may be a reflection of the cell's need for protein synthesis and processes affecting gene function. The ultrastructure of the giant-cell nucleolus is comprised of four components that correlate well with steps in ribosome biogenesis as indicated in Fig. 3d: (1) condensed heterochromatin adjacent to the nucleolus (the granules are nucleosomes that participate in DNA condensation); (2) region of DNA decondensation/transcription; (3) fine fibrillar region of rRNA processing; (4) granular region of ribosome assembly.

3.2 Cell Wall Stubs

Whether giant-cell development results from cell wall breakdown/protoplast fusion or from single cell expansion was debated for decades in many papers using light microscopy evidence (e.g., Christie 1936; Dropkin and Nelson 1960). Jones and Dropkin (1975) used light microscopy to compare cell wall changes in feeding cell development in the same host (soybean) infected by three different nematodes (the root-knot nematode, *M. incognita*, and the cyst nematodes *Heterodera glycines* and *Rotylenchulus reniformis*). For the latter two species they observed a series of incomplete cell walls within the feeding cell with a gap in their middle, indicating the digestion of cell walls during the development of these cells (see Fig. 4a). However, in feeding cells elicited by *M. incognita*, no cell wall gaps were observed. Instead, there was irregular cell wall fragments anchored to one side of the cell with no corresponding wall fragment on the other side (see Fig. 4b). They concluded that cell wall breakdown was the mode for feeding cell formation in *H. glycines* and

Fig. 3 The giant-cell nucleus. (**a**) Nuclei clump in the cell center. (**b**) Obliquely sectioned nucleus showing the highly lobed character of a giant-cell nucleus. Note, heterochromatin binds the nuclear envelope (*arrows*). Chemically fixed tomato giant-cell (unpublished micrograph courtesy of Richard S. Hussey, University of Georgia). (**c**) Thick section (0.5 μm) showing most heterochromatin bound to the nuclear envelope. Two nucleoli also bind heterochromatin. (**d**) Nucleolus structure correlates with its function in ribosome synthesis/processing/assembly (see Sect. 3.1). In (**d**), (1) shows attached heterochromatin with nucleosomal beads that regulate condensation; (2) shows region of DNA decondensation/transcription of rrna genes; (3) shows fibrillar component, RNA processing; and (4) shows granular region and ribosome assembly. Additionally, a putative Cajal body is labeled (**c**). *Scale bars*: (**a**) 10 μm, (**b**) 3 μm, (**c**) 5 μm, (**d**) 250 nm

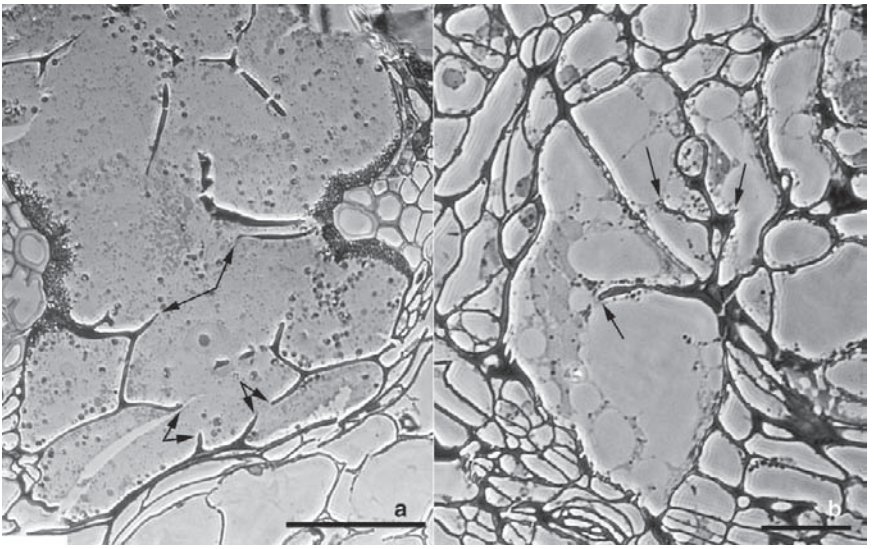


Fig. 4 Comparison of cell wall stubs in syncytia and giant-cells, phase micrographs of resin sections. (**a**) Soybean root syncytium in which cell wall stubs result from broken walls (*joined arrows*). (**b**) Developing giant-cells in *A. thaliana* in which cell wall stubs (*arrows*) do not have matching stubs in opposing walls, suggesting de novo synthesis. *Scale bars*: (**a**) 50 μm, (**b**) 25 μm

R. reniformis, and that the lack of wall gaps in feeding cells induced by *M. incognita* indicated that cell wall breakdown does not occur in this interaction. Figure 4 compares the light micrographs of the two cell types, showing the presence of matching walls on opposite sides (joined arrows) for the feeding cells from cyst nematodes and the lack of these walls in the case of root-knot nematodes. Accordingly, the multinucleate feeding cells induced by cyst nematodes (*Heterodera* spp. and others) derived from the merging of cells by cell wall breakdown are named "syncytia" while the feeding cells elicited by root-knot nematodes (*Meloidogyne* spp.) formed via expansion of a single cell are named "giant-cells."

Initial studies using electron microscopy include Bird (1961) who noted the presence of cell wall fragments within giant-cells anchored to the giant-cell wall and attributed them to cell wall breakdown during giant-cell formation. Huang and Maggenti (1969) did not report cell wall abnormalities in their electron micrographs. Paulson and Webster's paper (1970) was the first analysis of giant-cell ultrastructure aided by improved electron microscopy preparation methods developed subsequent to the early papers. They found some evidence for cell wall breakdown by light microscopy, especially in adjacent cells crushed by the expanding giant-cell. On the basis of the higher resolution images from electron microscopy, they concluded that there was no unequivocal evidence that cell wall breakdown played a role in giant-cell formation.

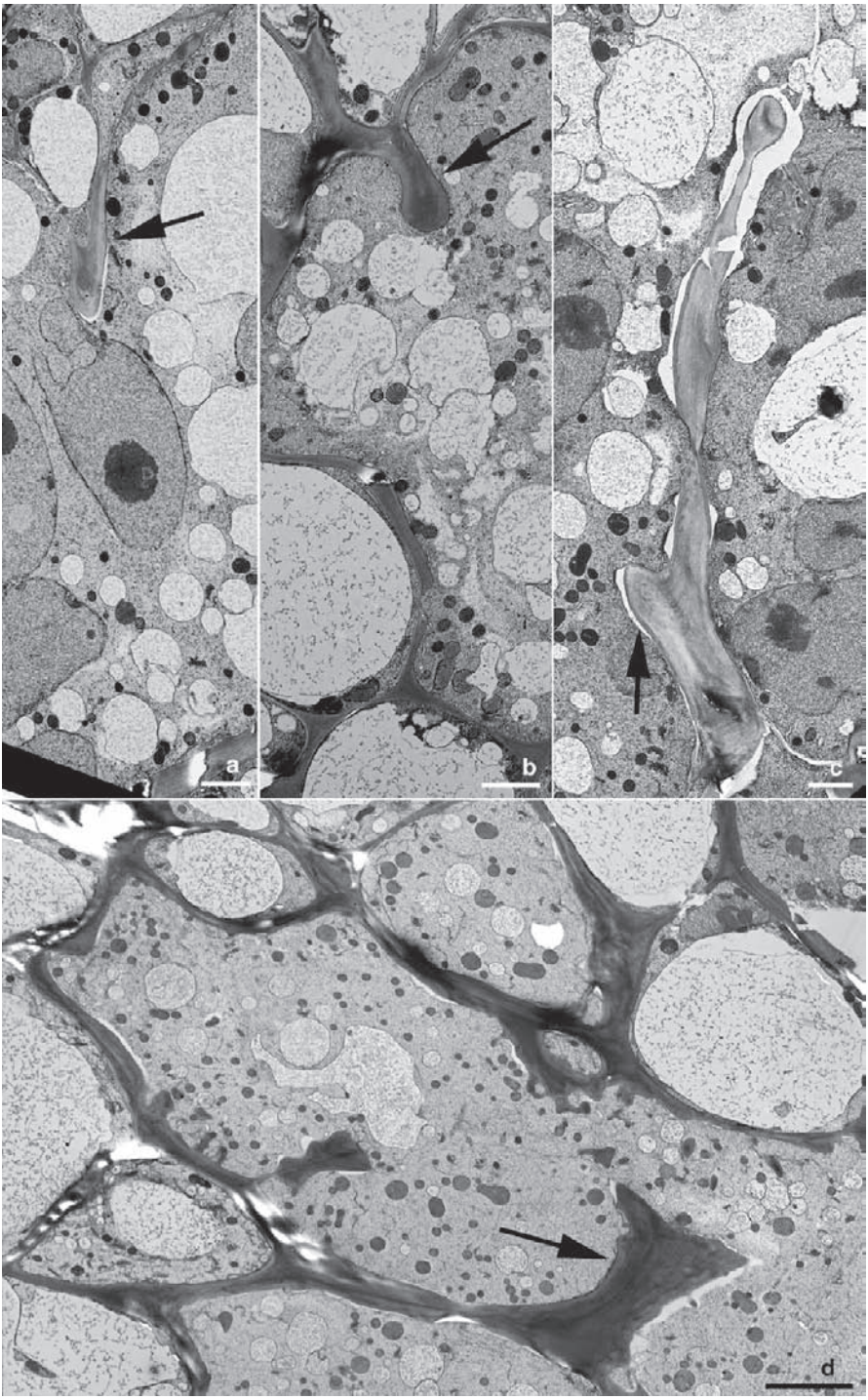
Jones and Payne (1977, 1978) offer a compelling argument that cell wall stubs/fragments in giant-cells could be a result of errant cell wall formation. They treated

dividing cells in *Impatiens* root tips with caffeine, which interferes with cell plate formation. TEM shows that this results in the formation of cell wall stubs representing incomplete cell plate formation, these being attached to opposing mother cell walls at the site where the plate normally would attach. This observation implied that for giant-cells (Jones and Payne 1978), which go through the cell cycle and mitosis repeatedly during development (Goverse et al. 2000) without cytokinesis, there is *nascent* but incomplete cell wall formation, with the new walls attached to the pre-existing cell wall as found in caffeine-treated cells. Early in development (two nuclei stage) giant-cells indeed form a nascent cell plate, comprised of aligned vesicles, which soon aborts (Jones and Payne 1978). There are further examples for the formation of multinucleate cells due to errant cell plate formation. For instance, in nitrogen fixing symbiotic cells of the actinorhizal plants *Datisca* and *Coriaria*, the symbiotic bacterium *Frankia* induces cell cycling in its host as part of the infection process. Mitosis without cytokinesis causes invaded cells to be multinucleate which has been suggested to be due to mis-targeted callose deposition which is deposited on the invading bacterium (Berg et al. 1999).

Examples of cell wall stubs/fragments are presented in Fig. 5. In all cases these stubs do not have a matching partner in the opposite wall, supporting the studies cited above that suggest they are a result of mal-formed walls rather than remnants of broken walls. The stub in Fig. 5a (arrow) appears to have been formed by folding of the wall, as has been observed by Jones and Payne (1978). The stub in Fig. 5b (arrow) may represent a later stage in development where the infolded walls have fused, forming a thick stub with a rounded tip. Stubs undergo various modifications, including uneven deposition of wall material, leading to variation in stub thickness along its length and to stub branches (arrow, Fig. 5c). Continued deposition of wall material can produce remarkably thick stubs (arrow, Fig. 5d). Wall thickening, followed by development o f the transfer cell wall labyrinth (TCL) (see Sect. 4.3), progresses during giant-cell development and may be a response to cell cycling in a cell incapable of cytokinesis. Whether the thickening of stubs and the giant cell wall (discussed in the next section) is only due to material miss-targeted because of the lack of cytokinesis remains to be determined. In conclusion, the structure of cell wall stubs strongly suggests that giant-cell expansion is not due to cell wall breakdown but to the expansion of a single cell. Evidence is presented in Sect. 5 that expansion may be due to vacuolar enlargement.

4 Cell Wall Development

The wall of giant-cells goes through significant changes during cellular development. The wall thickens and develops transfer cell labyrinths that increase the surface area for transport of nutrients into the giant-cell, which is reflected by the proliferation of vascular tissue surrounding giant-cells. This is also the case for syncytia (Chap. 6 of this book).


a
b
c
d

4.1 Vascular Tissue Development

From the earliest studies (e.g., Christie 1936) it has been observed that an extensive network of xylem elements develops around the giant-cell during its growth. Figure 6 illustrates stages of xylem element development around giant-cells in *Arabidopsis*, making use of glutaraldehyde fluorescence (green) and lignin fluorescence (red) in glutaraldehyde-fixed tissue (Fester et al. 2008). Because of extensive protein cross-linking by glutaraldehyde, the green fluorescence signal represents regions of high protein concentration. At the earliest stage of development (Fig. 6a) nematode feeding has displaced the vascular xylem (arrow). Some xylem vessels end abruptly in the region of nematode feeding; this was interpreted by Christie (1936) to mean that xylem development is arrested or aberrant under the influence of the nematode. As giant-cells expand, a network of new xylem elements forms between them (Fig. 6b, giant-cells indicated by asterisks, vascular xylem at arrows). Eventually this develops into an extensive array of xylem elements that form a cage around giant-cells (Fig. 6c, giant-cells indicated by asterisks). These are presumed to serve as a rich source of nutrients for the giant-cell, with the giant-cell transfer cell wall labyrinth developing next to them (see Sect. 4.3).

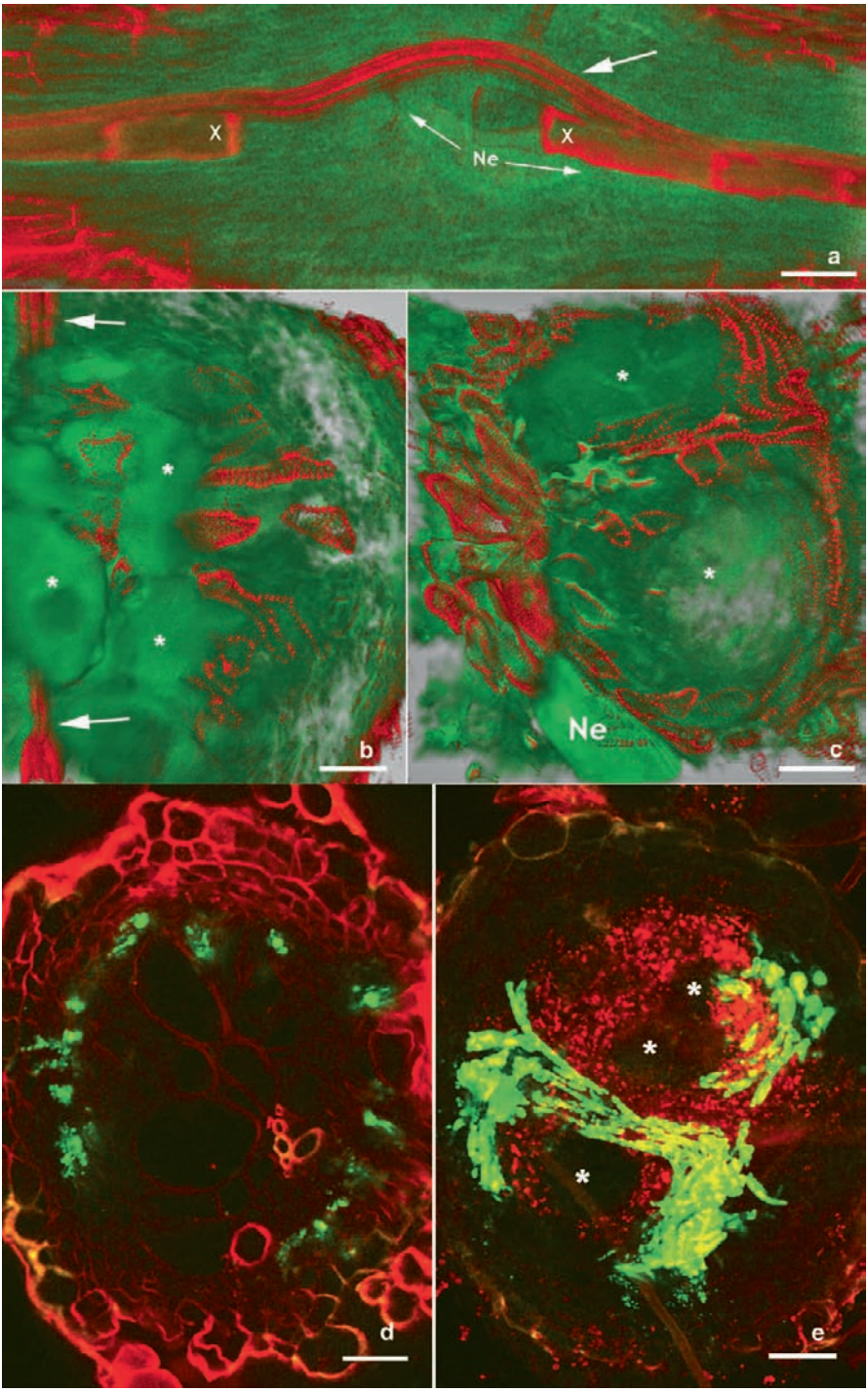
Using GFP reporter constructs of genes expressed in phloem tissue, we have shown extensive development of phloem tissue around giant-cells (Figs. 6d, e, unpublished). *AtAPLpro::GFP* (APL, Altered Phloem Development; GFP, green fluorescent protein) is a marker for phloem development (Bonke et al. 2003). Its expression pattern in root knots (Fig. 6d) shows the development of phloem around the cluster of giant-cells. This is also the case for the expression of *AtSUC2pro::GFP* (SUC2, Sucrose Transporter-2) serving as a marker for companion cells of the phloem (Fig. 6e) (Imlau et al. 1999).

That both xylem and phloem are reservoirs of nutrition for giant-cells is implied by the development of extensive transfer cell wall ingrowths in walls of giant-cells adjacent to these cells as discussed in Sect. 4.3.

4.2 Cell Wall Thickening

Wall thickenings are present early in giant-cell development and localized to small patches of the wall (Fig. 7a, b, arrows). These expand to cover larger regions (Fig. 7c), leading to extensive wall thickening (Figs. 1a, 5d and 9d). However, even in mature giant-cells (containing transfer cell wall labyrinth) the thickening is non-uniform (Figs. 8a and 9d), suggesting that the mechanism for adding cell wall material is asymmetrically distributed during giant-cell development. Golgi stacks are associated with the wall during thickening (Fig. 7d) and perhaps their cellular distribution affects this asymmetry. There are instances of unusual wall morphology

Fig. 5 Cell wall stubs in giant-cells. (**a**) Early stage of stub formation, via in-folding of a wall into the giant-cell (*arrow*). (**b**) Early stage of stub formation, the infolded wall appears to have fused together (*arrow*). (**c**) Thickened stub with a branch-like structure (arrow). (**d**) Highly thickened stub (*arrow*). *Scale bars*: (**a**), (**b**) and (**c**) 2 μm; (**d**) 5 μm


X
X
Ne
a
b
c
Ne
d
e

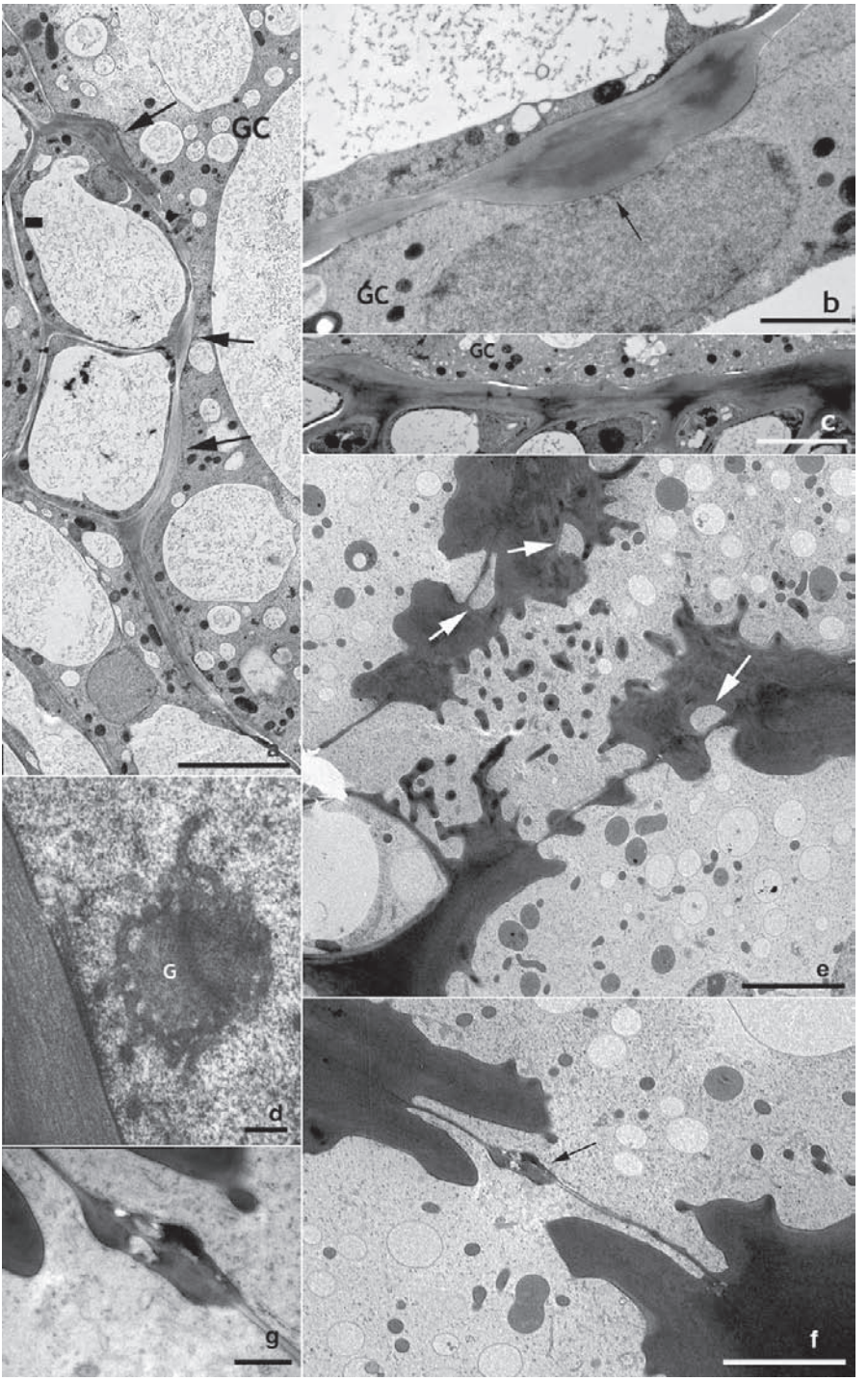
in thickenings of adjoining giant-cells. As shown in Fig. 7e, thickenings may overarch thin wall regions, even to the point of enclosing a cavity (arrows). Remarkably, in some cases, a bordered pit pair is formed (Fig. 7f). Such bordered pit pairs are a common feature of tracheids from woody gymnosperms but are rarely if ever found in *Arabidopsis*. The torus-containing wall (Fig. 7g) does not have plasmodesmata. Thickened walls are a clear example of the hyperactivity of the machinery for wall synthesis in developing giant-cells, including developmental programs for wall production that are out of character for the tissue.

4.3 Transfer Cell Wall Labyrinth

Giant-cells put a significant demand for nutrients on surrounding cells. This is manifested in their development of transfer cell wall labyrinths (TCL), which have been long known to be a hallmark of giant-cells (Jones and Gunning 1976; Jones and Northcote 1972a, b). This structure is similar to wall ingrowths of transfer cells. These significantly increase the surface area of plasma membrane, facilitating the transport of nutrients into or out of the cell, i.e., symplast–apoplast exchange (Gunning and Pate 1969, 1974; Offler et al. 2002). Transfer cells occur in a variety of tissues and are involved in the transport of nitrogenous material, minerals, and sugars. The TCL may vary in morphology according to the cell, with reticulate and flange morphologies being the predominant forms. The wall ingrowths contain cellulose, hemicellulose, pectin and likely other components (Offler et al. 2002). Transfer cell induction and regulation is reviewed in Offler et al. (2002).

Cell wall thickening in giant-cells is the first distinguishing wall modification in these cells, and most wall labyrinths develop from the thickened regions (Figs. 8a, 9a, d, e). This suggests that these two structural modifications are developmentally related, that labyrinths are rooted in these thickenings, and they develop from molecular nuclei within them. These observations fit with the model for differentiation of transfer cells in general proposed by Offler et al. (2002), which suggests that an extracellular signal leads to polarized deposition of a thickened wall (localized regions of cell wall thickening in giant-cells) from which the TCL is initialized (TCL grows from thickened regions in the wall). Two candidates for the molecular nuclei that initiate wall ingrowth formation at specific sites are arabinogalactan proteins and wall kinases (Offler et al. 2002).

Fig. 6 Vascular tissue development in the root knot. (**a**), (**b**), and (**c**) Confocal light microscopy of xylem development in feeding sites, glutaraldehyde-fixed tissue. Green is glutaraldehyde fluorescence, red is xylem fluorescence. (**a**) Early feeding stage (nematode position indicated) with vascular xylem displaced (*arrow*) and xylem development arrested at (X). (**b**) Xylem elements have formed at this intermediate stage of development, around three giant-cells (*asterisks*). Vascular xylem indicated by *arrows*. (**c**) Late in feeding site development, an extensive array of xylem elements has formed around two giant-cells (*asterisks*). (**d**) and (**e**) Confocal light microscopy of phloem development in feeding sites. GFP reporter is green with cell walls stained with Congo Red. (**d**) *Ataplpro::GFP* expression indicating phloem development around the periphery of the giant-cell cluster. (**e**) *Atsuc2pro::GFP* expression showing phloem (companion cell) proliferation around giant-cells (*asterisks*). *Scale bars*: (**a**), (**b**), (**c**), and (**e**) 50 μm; (**d**) 30 μm

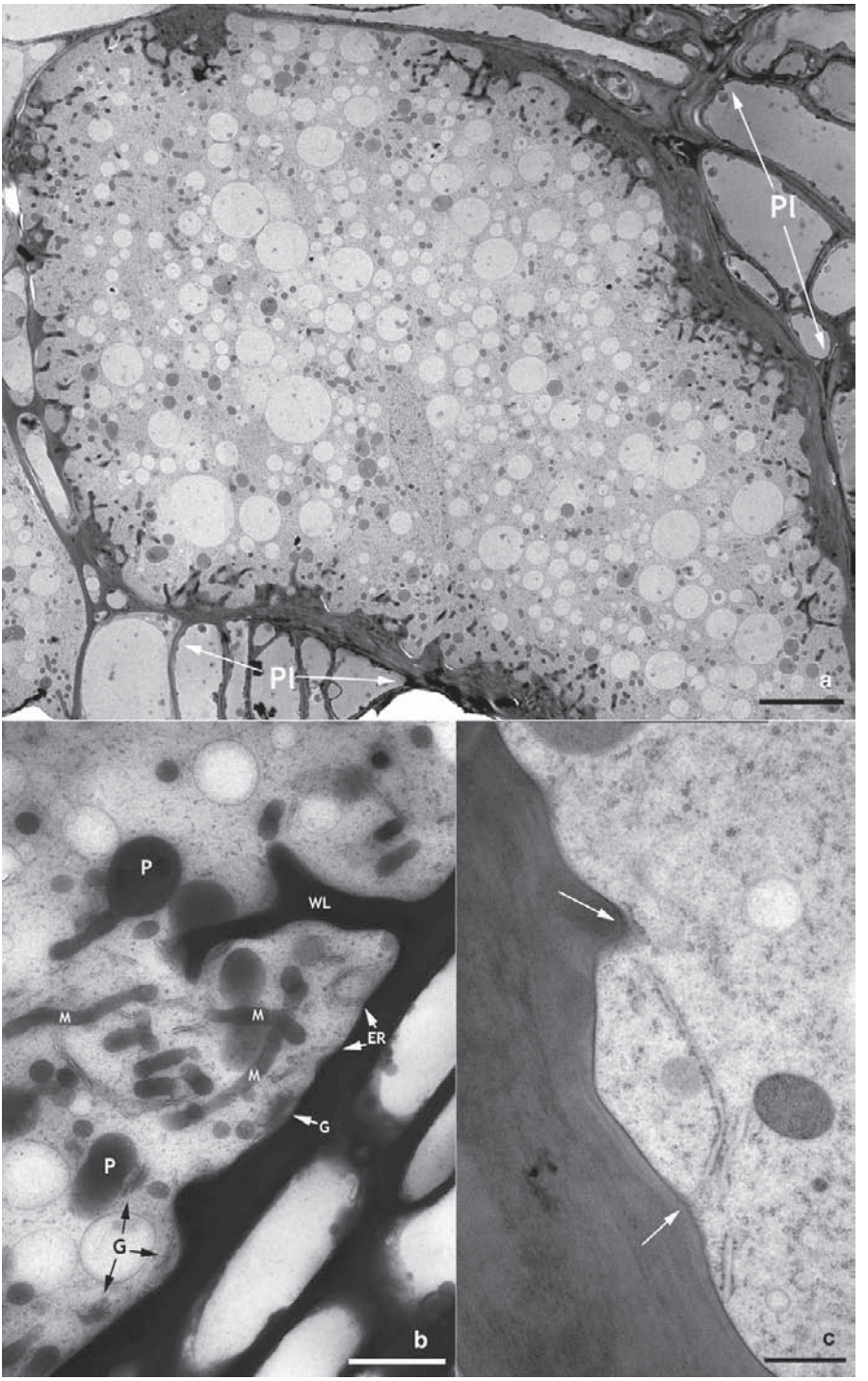

GC
GC
b
GC
c
e
G
d
g
f

Little is known about the molecular makeup of the giant-cell TCL. Histology and thin section staining provide evidence that the TCL in plant cells contains cellulose and pectins (Gunning and Pate 1974; Browning and Gunning 1977). Dropkin and Nelson (1960) note that the thickened wall of mature giant-cells (which contains the TCL) stains positively for cellulose and pectins. Immunogold analysis of pea nodule TCL shows the presence of callose in mature labyrinths in small domains, suggesting the existence of channels within the labyrinth (Dahiya and Brewin 2000), while xyloglucan (hemicellulose) and pectin are uniformly distributed in the mature labyrinth. These two major polysaccharide components of plant cell walls are secreted by Golgi stacks (Staehelin and Moore 1995) via vesicles that fuse with the cell membrane. The third major polysaccharide component, cellulose, is synthesized by cellulose synthase enzyme complexes within the cell membrane (Delmer 1999). The large number of Golgi stacks in wall regions synthesizing the labyrinth (Fig. 8b) could be responsible for secretion of pectins and hemicellulose polysaccharides in this wall-growing region.

Figure 8b is a thick (0.5 μm) section showing the distribution of organelles in a TCL-forming region. The three-dimensional morphology of the many mitochondria associated with the labyrinth can be seen. The added depth in this view shows that these mitochondria are highly elongated. The ER in labyrinth-growing wall regions comes into close association with the wall (Fig. 8b, c, arrows). Often the margins of cisternae associate specifically with the tips of growing labyrinths (Fig. 8c, arrows), which has been observed in the case of other transfer cells as well (Gunning and Pate 1969). The concentration of endomembrane secretory organelles (Golgi stacks, ER, vesicles) at the site of wall synthesis in transfer cells implicates their participation in this biosynthetic process (Offler et al. 2002). The ER in particular is a prominent feature of all transfer cells (Gunning and Pate 1969), and it has been suggested to be a compartment for concentrating solutes that have been transported into the cell, providing an avenue for transport of these solutes to other parts of the cell.

The electron-dense pockets of the TCL seen in freeze-substituted specimens (Figs. 2b and 9a), but absent from chemically fixed specimens (Fig. 2a), are likely compounds not cross-linked by glutaraldehyde and therefore leached out during dehydration. In their pioneering study of TCL ultrastructure in freeze-substituted specimens from a variety of plants, Browning and Gunning (1977) also observed electron-dense pockets in wall ingrowths of some plant species. Thin sections of

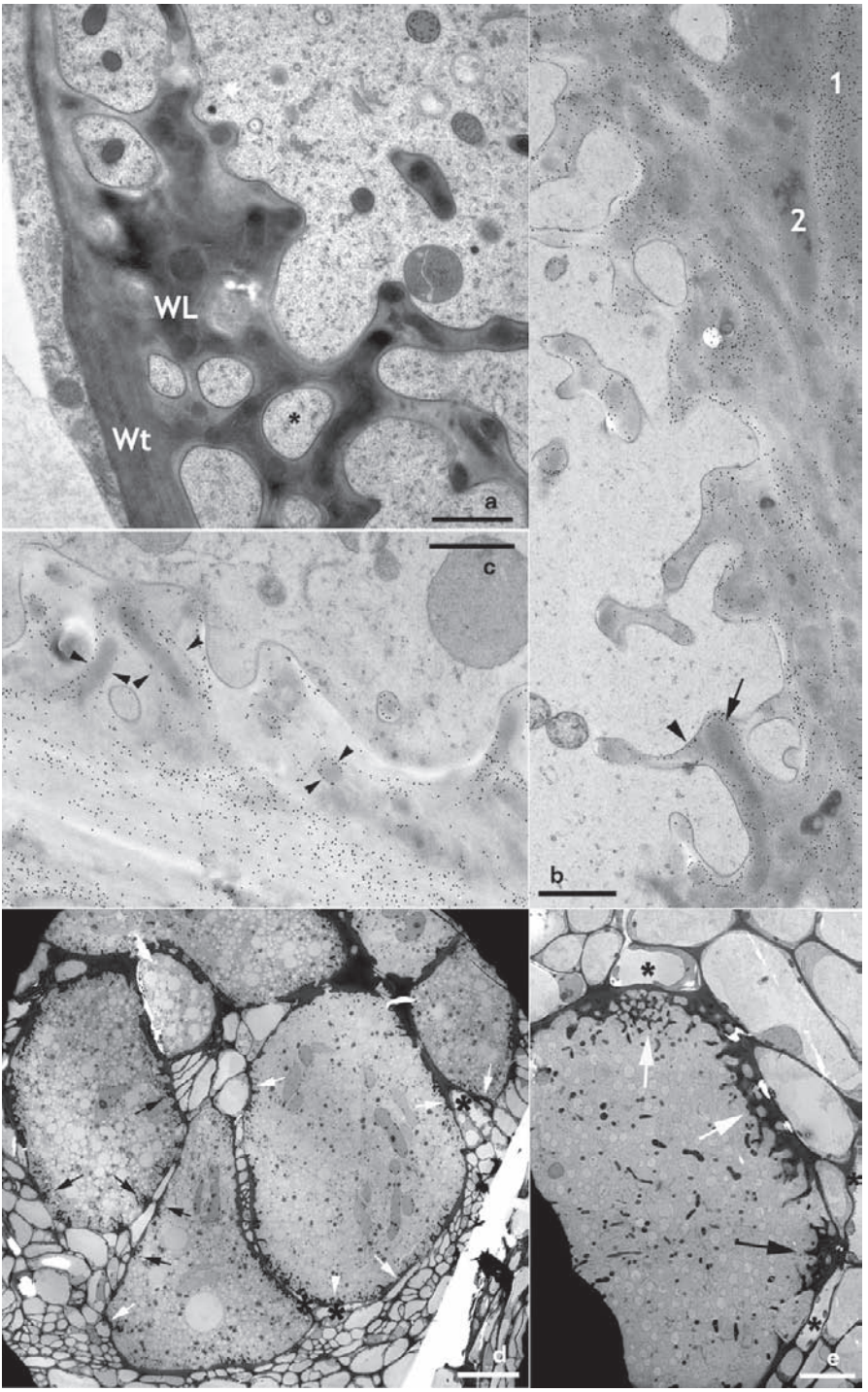
Fig. 7 Cell wall thickening during giant-cell development. (**a**) Earliest stage of slight wall thickening, in local wall regions (*arrows*). (**b**) More substantial thickening (*arrow*) in a later stage of giant-cell development. (**c**) Even later in development, thickening is more uniform across the wall. (**d**) *En face* view of a Golgi stack cisternum, with peripheral secretory vesicles apparently trafficking to the wall. (**e**) Three giant-cells with thick walls in regions joining them. Thin walls with wall thickenings on adjacent regions that are so thick that they have fused with others, overarching cavities (*arrows*). (**f**) A bordered pit pair formed between two giant-cells with torus (*arrow*). (**g**) Higher magnification of torus in (**f**). Note, there are no plasmodesmata in the torus or wall. *Scale bars*: (**a**), (**c**), (**e**) and (**f**) 5 μm; (**b**) 2 μm; (**d**) 200 nm; (**g**) 1 μm


Pl
Pl
a
P
WL
M
M
M
ER
G
P
G
b
c

these electron-dense patch regions stained lightly with alkaline Ruthenium Red and densely with the Thiery polysaccharide stain. This was interpreted to represent pectin for the former and total polysaccharide for the latter. Electron-dense pockets filling the width of labyrinthine projections (Figs. 2b and 9a) might represent water-soluble polysaccharide. In the case of cellulose, sections labeled with a cellulase-gold affinity label for this polysaccharide (Berg et al. 1988) show a non-uniform distribution of gold particles (Fig. 9b, c, unpublished). In most cases the electron-dense pockets are not labeled but rather surrounded by the label. Combining these two markers (electron-dense pockets surrounded by cellulose), it is possible to morphologically identify labyrinthine components buried in the thickened wall (opposing arrowheads, Fig. 9c), suggesting that the thickened wall contains some wall ingrowths buried in additional, less electron-dense wall material that was not labeled with the cellulose-specific probe. In contrast, the cellulose-specific label is dense and uniformly distributed in primary cell walls. Dropkin and Nelson (1960) additionally noted that the thick wall (from which the labyrinth grows) is birefringent, but the inner wall (corresponding to the labyrinth) is not, indicating more ordered cellulose microfibrils in the former and randomly oriented cellulose in the latter. Such loosely packed cellulose microfibrils have been described in reticulate TCLs of other plant cells (Browning and Gunning 1977). The cellulase-gold labeling pattern is consistent with this being the case for giant-cell TCLs as well.

Unlike conventional transfer cells, the large surface area of giant-cell walls can provide hints of the effect of differing adjacent cells on labyrinth development. Labyrinth density, a measure of the increased surface area, might be expected to reflect the importance of adjacent cells as a nutrient source. The TCL in nematode feeding cells has been reported to be most extensive when opposite vascular elements, with those adjacent to xylem elements more dense than those adjacent to phloem (Jones and Gunning 1976; Jones and Northcote 1972a, b). The giant-cells in Fig. 9d, e show patches of labyrinth that match up somewhat distinctly with specific adjacent cells (e.g., white arrows). There are other cases where the labyrinth covers only parts of the adjacent cell (e.g., white arrowhead), but this is uncommon. Xylem elements are only one example of the many different cell types eliciting labyrinth formation. The entire circumference of the three giant-cells seen in Fig. 9d has only three xylem element cells (asterisks) as adjacent cells. Similarly, in Fig. 9e, only three xylem elements (asterisks) are visible. Most of these elicit labyrinths in the giant-cell, but those in this example are a relatively small fraction of the total labyrinth. Because of this occurrence of TCLs adjacent to considerable numbers of

Fig. 8 Transfer cell wall labyrinth development in giant-cells. (**a**) Labyrinth development only occurs in regions of the wall that have thickened cell walls. Phloem cells span the regions indicated. (**b**) Thick section (0.5 μm) of a giant-cell wall region synthesizing the wall labyrinth. Thick sections permit imaging the elongated mitochondria morphology. Endoplasmic reticulum and Golgi stacks are associated with labyrinth development. (**c**) Cisterna of the endoplasmic reticulum associate with the growing tips of new wall in-growths (*arrows*). *Scale bars*: (**a**) 10 μm, (**b**) 2 μm, (**c**) 1 μm


WL
Wt
a
1
2
b
c
d
e

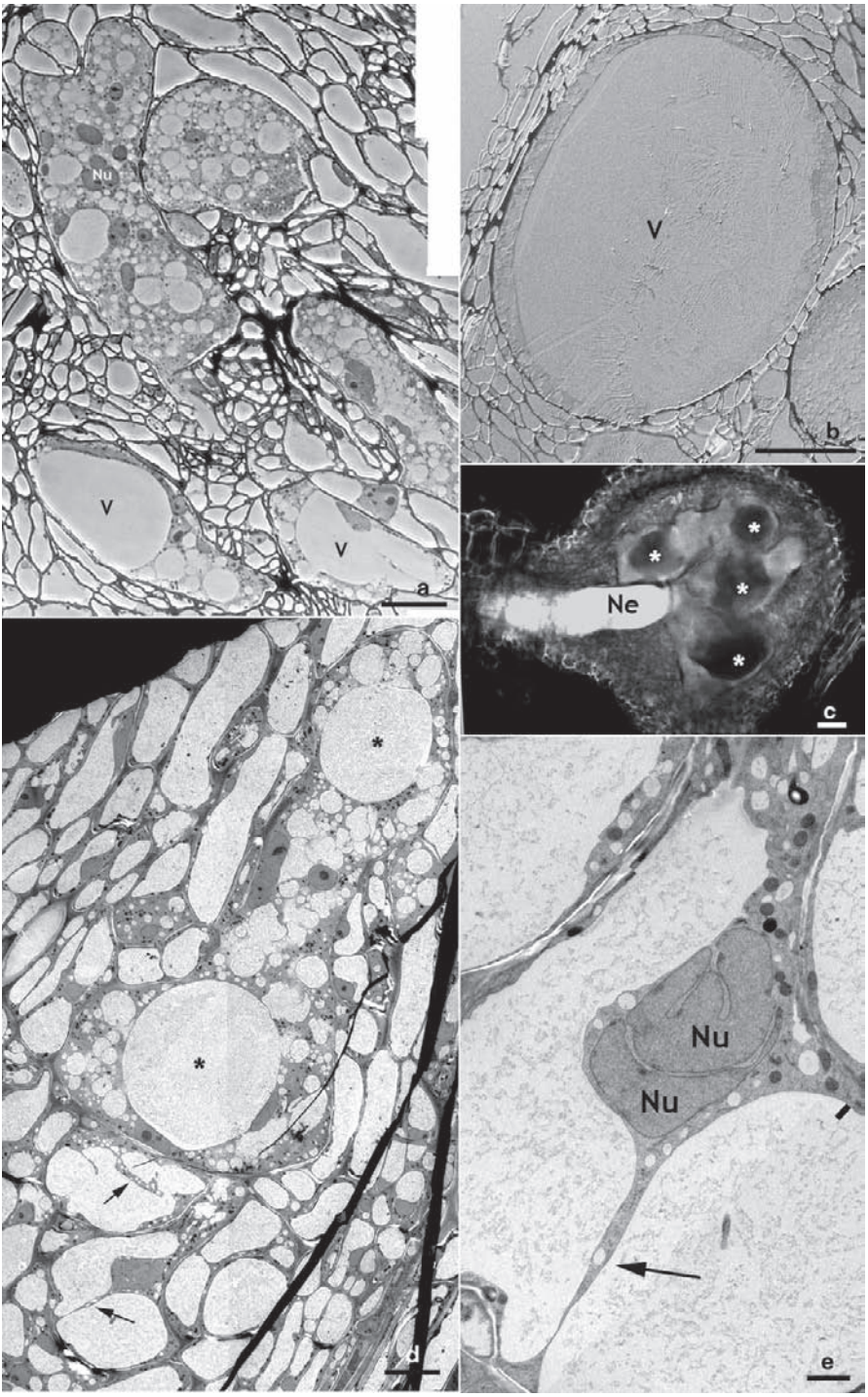
parenchyma cells, it can be concluded that these cells serve as potential nutrient sources in addition to those furnished by xylem elements. Furthermore, TCLs can be observed on cell walls terminating at the giant-cell (black arrows in Fig. 9d, e), indicating that nutrient transport in the apoplast, pooled from cells further away, may be a significant source of giant-cell nutrients. As shown in Fig. 9d, walls between giant-cells are commonly thick and labyrinth-rich, suggesting that nutrients pass between giant-cells (Jones and Gunning 1976; Jones and Northcote 1972b).

5 Cell Expansion

Giant-cells expand rapidly, reaching their full size within 14–21 days after commencement of nematode feeding (Bird 1972). On the basis of diameter measurements, giant-cells are at least ten times bigger than adjacent parenchyma cells. Most papers analyzing giant-cell development have noted that young giant-cells contain large vacuoles making up a large volume of the cell while mature giant-cells contain small vacuoles and the majority of the cell's volume is cytoplasm (Christie 1936; Dropkin and Nelson 1960; Huang 1985; Jones and Dropkin 1975; Paulson and Webster 1970). A similar change in vacuoles is found in syncytia (Sobczak and Golinowski 2008). It has been implied that the formation of the large vacuole in young giant-cells is a cause of giant-cell expansion (Hammes et al. 2005) based on a similar mechanism occurring in expanding plant tissues. Typically, rapid expansion of plant cells, e.g., in the elongation region of shoots and roots, is facilitated by the expansion of the vacuole as this largely contains water and therefore does not require extensive protein synthesis to fill the expanding volume. Nematode parasitism genes that could be involved in modification of cell walls that would facilitate expansion are identified in Davis et al. (2008).

The giant-cells in the light micrograph in Fig. 10a illustrate the vacuolar changes seen in different stages of development. The lowermost cells, with fewer nuclei, are filled with large vacuoles (V) that may aid in the expansion of these young giant-cells. Later in development, as in the multinucleate cell in the upper left (Fig. 10a),

Fig. 9 Giant-cell wall labyrinth composition and distribution. (**a**) Labyrinth grows from a thickened wall and contains electron-dense pockets; cavities (*asterisk*) are formed by labyrinthine growth. (**b**) Cellulase-gold affinity labeling of cellulose in the labyrinth; the wall thickening contains regions with uniform, dense label (1), similar to that found in primary walls, and unlabeled regions (2). The electron-dense labyrinth regions generally are unlabeled but are surrounded with label (*arrow*). Label is often aligned along the plasma membrane (*arrowhead*). (**c**) Cellulase-gold-delimited labyrinths (*opposing arrowheads*) are sometimes buried in the thickened wall. (**d**), (**e**) The giant-cell wall labyrinth is distributed completely across walls of opposing cells (*white arrows*), partly across opposing walls (*white arrowhead*) and at junctions where opposing walls terminate at the giant-cell wall (*black arrows*). Xylem elements are indicated by *asterisks*. Cell wall labyrinths can form opposite walls that are neither xylem nor phloem elements. *Scale bars*: (**a**), (**b**) and (**c**) 1 μm; (**d**) 20 μm; (**e**) 10 μm


Nu
V
V
a
V
b
Ne
c
*
*
*
*
*
d
Nu
Nu
e

there are many vacuoles of intermediate and smaller sizes, and a corresponding increase in cytoplasm content. This suggests that early giant-cell expansion is driven by vacuole expansion, and this is supplanted with synthesis of cytoplasm as nuclei increase in number and serve as a source for the extensive amount of ensuing protein synthesis (Bird 1972). As an example of the remarkable vacuole size attainable, the young giant-cell in Fig. 10b is occupied primarily by a vacuole ~200 μm in diameter. These vacuoles are much larger than vacuoles of parenchyma cells in uninfected roots. Because giant-cells derive from parenchyma cells, their vacuoles must have experienced a remarkable amount of expansion during giant-cell development. In the developing feeding site shown in Fig. 10c all of the giant-cells surrounding the nematode head contain large vacuoles (asterisks), illustrating the importance of vacuoles in expansion of the giant-cell (Hammes et al. 2005). The large amount of water uptake likely to occur during this expansion can be expected to be reflected in gene expression of aquaporin water transporters such as tonoplast intrinsic proteins (TIPs), which are required for vacuole biogenesis and rapid influx of water into expanding vacuoles (Chaumont et al. 1998). This is indeed the case for developing root knots. Opperman et al. (1994) showed high expression of *TobRB7* in root knots in tobacco, a gene that encodes a δ-TIP aquaporin (Sarda et al. 1999) and is expressed in giant-cells (Gheysen and Mitchum 2008).

The causal dynamics for vacuolar changes during later giant-cell development, from a large vacuole to many smaller vacuoles, are subject to debate and could be elucidated with time lapse studies of living cells. Hints of the mechanism can be interpreted from electron micrographs. The uppermost of the three giant-cells in Fig. 10d is the most mature. Instead of a large vacuole, there are two smaller vacuoles at the poles of the cell (asterisks) and many smaller vacuoles. The two younger giant-cells below contain large vacuoles that are penetrated by cytoplasmic strands (arrows, the lowermost at higher magnification in Fig. 10e).

Further clues regarding the change in vacuole populations during giant-cell development come from the study of cell division in highly vacuolated cells such as in the vascular cambium. Cell division in cambial cells is preceded by formation of a phragmosome, a cytoplasmic sheet transversing the vacuole that predicts the future plane of cell plate formation (Evert 2006; Sinnott and Bloch 1940). The phragmosome bisects the vacuole into smaller vacuoles that occupy the two daughter cells and is associated with extensive penetration of the

Fig. 10 Vacuole biogenesis in giant-cell expansion. (**a**) Phase micrograph of a resin section with large vacuoles in young giant-cells (lower cells) and more numerous smaller vacuoles in more developed giant-cells (upper cells). (**b**) DIC micrograph of a resin section of a young giant-cell in which a large vacuole (~200 μm in diameter) occupies 90% of the cell volume. (**c**) Confocal light micrograph of glutaraldehyde-fixed whole mount feeding site showing glutaraldehyde-fluorescence. All of the developing giant-cells surrounding the nematode head have large vacuoles (*asterisks*). (**d**) Vacuolar changes in three developing giant-cells; the uppermost has two vacuoles (*asterisks*) at poles of the cell and many smaller vacuoles. The lower two cells are early in development and cytoplasmic bridges are bisecting the vacuole (*arrows*). (**e**) Higher magnification of the lowermost cell in (**d**), showing the cell is binuclear and the structure of the cytoplasmic bridge (*arrow*). *Scale bars*: (**a**) 20 μm; (**b**) and (**c**) 50 μm; (**d**) 10 μm; (**e**) 2 μm

vacuole by cytoplasmic strands, mediated by microtubules and actin (Evert 2006; Lloyd 1991).

Fragmentation of the large central vacuole in giant-cells may be accomplished by a related mechanism. Cytoplasmic bridges in vacuolated feeding cells were observed in early cytological studies (Christie 1936). Jones and Payne (1978) describe phragmosome induction in cells near the nematode head in developing feeding sites, attributing them to cell activation by the nematode. These phragmosomes can be seen bisecting vacuoles of developing giant-cells in other studies (Payne and Webster 1970). The vacuolated cells in Fig. 10e have already initiated nuclear divisions (showing two nuclei in the ultrathin section) and the vacuolar fragmentation caused by the cytoplasmic strand (arrow) may result from phragmosomal activity. The large number of small vacuoles in later stage giant-cells (e.g., Fig. 10a, upper left cell; Fig. 10d, upper cell) could therefore result from continued vacuolar fragmentation as the cell is engaged in mitotic cycles without going through cytokinesis. Cyst and root-knot nematodes activate the cell cycle by triggering expression of cyclin-dependent kinases and mitotic cyclins (Goverse et al. 2000; Gheysen and Mitchum 2008), events that would conceivably allow production of phragmosomes.

A number of studies have emphasized similarities in plant cell signal transduction pathways induced by rhizobia and parasitic nematodes (Davis and Mitchum 2005; Koltai et al. 2001; Mathesius 2003; Weerasinghe et al. 2005; Bird et al. 2008; Gheysen and Mitchum 2008). Of particular relevance here is that root-knot nematodes produce a compound that mimics Nod factor in its effect on legume root hairs, including cytoskeletal rearrangements (Weerasinghe et al. 2005). De Almeida Engler et al. (2004) analyzed the actin and microtubule cytoskeleton in developing syncytia and giant-cells, concluding that nematode-induced perturbations of the cytoskeleton are required for successful establishment of feeding sites. Expression of actin and microtubule genes was high in developing feeding sites in giant-cells and phragmoplasts were abundant. However, they were arrested in development and no cell plates were formed.

In legume root nodule development, invading rhizobia induce phragmosome-like cytoplasmic bridges by cell cycle induction via Nod factors, and these cytoplasmic bridges provide an avenue for infection thread growth that supports rhizobial infection (van Brussel et al. 1992; Yang et al. 1994). Fragmentation of vacuoles and ensuing infection thread formation in developing symbiotic cells of actinorhizal plants has been proposed to result from a similar mechanism (Berg 1999). Like Nod factors in rhizobia, similar molecules secreted by parasitic nematodes might have a role in eliciting cell cycle-dependent changes in developing feeding cells that are critical for their development.

6 Feeding Tubes

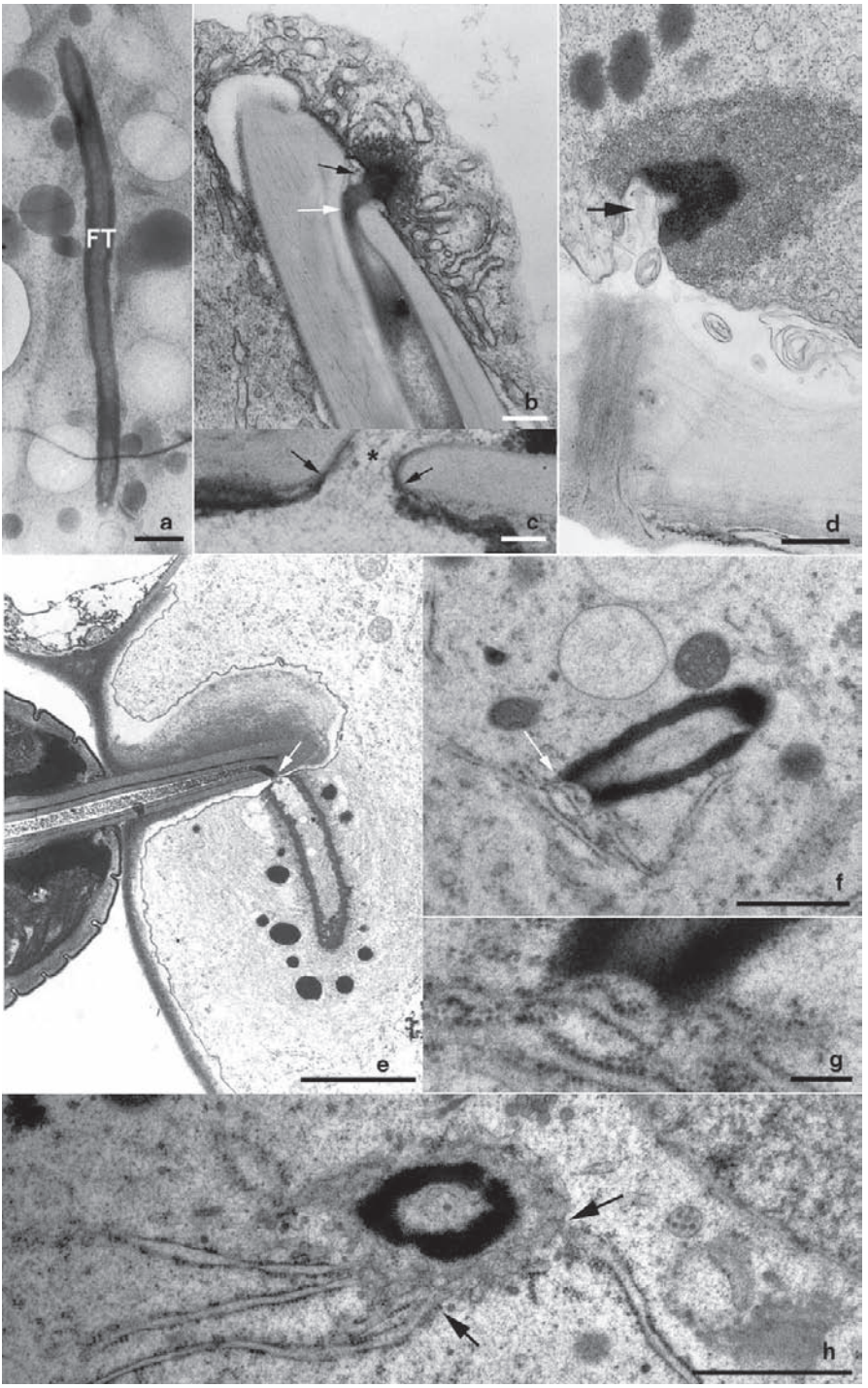
Feeding tubes are remarkable structures not only because they are central to parasitism but also because of their unique cell biology. Their formation is elicited by nematode secretions that may require participation by plant cell components to form the tube.

Feeding tubes within giant-cells have a crystalline wall structure that likely is composed of protein.

6.1 Feeding Tube Formation and Structure

Nemec (1911) was the first to identify feeding tubes formed in reaction to nematodes in giant-cells, describing them as proteinaceous threads (Nemec 1932). Using light microscopy of root knots in soybean roots, Dropkin and Nelson (1960) observed long rod-like inclusions that were up to 70-μm long, numbering up to 25 in some cells, and occurring most frequently near the clumps of nuclei in giant-cells. They suggested that these were coagulated secretions from the nematode. Paulson and Webster (1970) were the first to present electron micrographs of these structures (in tomato roots infected with *Meloidogyne*), which they identified as crystalline protein storage inclusions, as did Jones and Northcote (1972b) for protein crystal bodies observed in *Coleus* roots infected with *Meloidogyne*. The narrowness of feeding tubes (~1 μm) makes it difficult to observe their three-dimensional morphology in thin sections. Thick sections of 0.5 μm, however, clearly show that the feeding tubes in giant-cells are uniform in diameter and straight (Fig. 11a). It is easy to see why Nemec mistook them for mitochondria (Nemec 1910) as they are similar in thickness and overall shape (Fig. 8b). Feeding tubes are clearly evident in electron micrographs of soybean roots infected with *R. reniformis* published by Rebois et al. (1975), but they were interpreted as nematode secretions rather than as functional feeding tubes. Razak and Evans (1976) were the first to identify the tubular nature of these structures, coining the term "feeding tube." In their investigation of cowpea roots infected with *R. reniformis*, they used camera lucida drawings to show that the feeding tube associated with this nematode has a spiral structure. They were hesitant to interpret whether the tube was of plant or nematode origin. As for feeding tube function, their interpretation that they serve as a sieve with a large surface area that filters organelles and larger cellular colloids, preventing them from entering the tube and occluding the nematode stylet, is still plausible. Feeding tube lumen contents seen in thin sections (Figs. 11–13) are consistent with this interpretation. The posterior end of the tube is sealed, maintaining the lumen as a compartment separated from plant cytoplasm (Hussey and Mims 1991, Sobczak et al. 1999). A survey of feeding tubes in other plant parasitic nematode feeding sites (Rumpenhorst 1984) shows their occurrence in response to several nematode genera: *Globodera*, *Heterodera*, and *Meloidogyne* (Sobczak and Golinowski 2008).

There have been several investigations characterizing the size limitation of molecules taken up through the feeding tube based on fluorescence microscopy. Bockenhoff and Grundler (1994) used microinjection to introduce lucifer yellow and fluorescent dextrans of various molecular weights into syncytia in roots of *A. thaliana* infected by *H. schachtii*. They found a size exclusion of less than


FT
a
b
c
*
d
e
f
g
h

40 kDa, implying a maximum Stokes radius of 3.2–4.4 nm for molecules taken up by the nematode. Urwin et al. (1997) expressed free GFP in giant-cells of *A. thaliana* which was taken up by *M. incognita* and this was also found by Goverse et al. (1998) for the cyst nematode *G. rostochiensis* in syncytia in potato roots. The latter interpreted that proteins of ~32 kDa can be taken up by nematodes feeding on cells that form feeding tubes, implying that the feeding tube size exclusion limit is somewhere between 32 and 40 kDa.

Cell wall penetration by the stylet causes a buildup of wall material around the stylet, termed the "feeding peg" by Razak and Evans (1976). Using light microscopy of *R. reniformis* in cowpea, they observed that the feeding tube is opposite this structure, which consists of modified host cell wall material according to staining for polysaccharides (periodic acid-Schiff) and lignin (Toluidine Blue-metachromatic). Rebois (1980) used electron microscopy of *R. reniformis* in cotton roots to show that peg and feeding tube lumen are continuous, that the former is similar in structure to the cell wall, and that the feeding peg likely stabilizes the nematode stylet in its juncture with the feeding tube. Sobczak et al. (1999) show that the feeding peg (also known as feeding plug) is formed in syncytia induced by *H. schachtii* and that neither plug nor stylet penetrate the plasma membrane. Hussey and Mims (1991) found no evidence for feeding plug formation in giant-cells induced by *M. incognita*. This might result in a less stable lodging of the stylet in the giant-cell wall, evidenced by the rarity of sections with stylet in the wall in their images.

Feeding tube formation is a rapid response to the injection of nematode esophageal gland secretions into feeding cells. Using video-enhanced contrast light microscopy, Wyss and Zunke (1986) observed feeding tube formation within 15 minutes of insertion of stylet into the host cell in *Brassica* roots infected with *H. schachtii*.

Fig. 11 Feeding tube formation. (**a**) Thick section (0.5 μm) showing the uniform diameter and straight morphology of the feeding tube in giant-cells. (**b**) Juncture of the ring nematode stylet with plant cytoplasm (see Sect. 6.1). Dome-shaped secretion dome (*black arrow*) is formed where secretions contact the plant cytoplasm, surrounded by endoplasmic reticulum. Secretions within the stylet lumen are amorphous (*white arrow*). Plasma membrane surrounds the stylet, which is inserted several μm into the cytoplasm (chemically fixed, reproduced from Hussey et al. (1992) with kind permission from Springer Science + Business Media). (**c**) Junction of stylet aperture of ring nematode (*asterisk*) with pore in plant cell plasma membrane (see Sect. 6.1), formed by appression of this membrane with the stylet aperture wall (*arrows*) (chemically fixed, reproduced from Hussey et al. (1992) with kind permission from Springer Science + Business Media). (**d**) Feeding tube juncture in a giant-cell with site of *Meloidogyneincognita* stylet insertion in the cell wall (*arrow*, stylet withdrawn). Note intact plasma membrane (chemically fixed, reproduced from Hussey and Mims (1991) with kind permission from Springer Science + Business Media). (**e**) Juncture of stylet of *Rotylenchulus reniformis* with feeding tube in a cotton root cell, showing the relationship between stylet aperture (*arrow*) and feeding tube. Lumen of the stylet is apparently contiguous with the lumen of the feeding tube (chemically fixed, reproduced from Rebois (1980) with kind permission from Leiden-Brill). (**f**) and (**g**) Feeding tube-like structure formed from the surface of the endoplasmic reticulum [*arrow*, higher magnification in (**g**)] in a giant-cell in *A. thaliana*. (**h**) A membrane sheath of smooth endoplasmic reticulum surrounds the feeding tube, in some places contiguous with rough ER (*arrows*). *Scale bars*: (**a**), (**f**) and (**h**) 1 μm; (**b**) and (**g**) 200 nm; (**c**) 100 nm; (**d**) 500 nm; (**e**) 2 μm

Their images show rapid growth of the feeding tubes at the basal end from the stylet tip. Electron micrographs of the feeding tube–stylet juncture are rare due to the difficulty of serial sectioning large cells in search of this small structure. Hussey et al. (1992) sectioned the stylet tip of the ring nematode (*Criconemella xenoplax*) inserted into a cortical cell of a clover root (Fig. 11b, reproduced from Hussey et al. 1992). The plant cell membrane is not penetrated but is invaginated 5.6 µm around the stylet. The dome of granular material (black arrow) appears to be in the process of being directly discharged into the cytoplasm by the nematode. This could be interpreted to show that secretions are converted from amorphous material in the stylet lumen (white arrow) into granular material upon exposure to the cytoplasm. Endoplasmic reticulum is aggregated around this site. From the same study, a section of the ring nematode stylet inserted into a tomato feeding cell (Fig. 11c) shows that a pore in the plasma membrane is formed due to this membrane becoming tightly appressed (arrows) to the wall of the stylet aperture (asterisk). These images are evidence that the stylet does not deeply penetrate into the feeding cell; instead it comes to a rest a few µm within the cell without damaging the plasma membrane. Furthermore, when the stylet comes into physical contact with the plasma membrane it forms a seal and then releases secretions into the cytoplasm through an open pore in the plasma membrane. Our interpretation is that these secretions become granular when in contact with the cytoplasm. Although a feeding tube per se is not induced by this nematode, these observations suggest that secretions react with plant cell components to form structures separating stylet from plant cytoplasm.

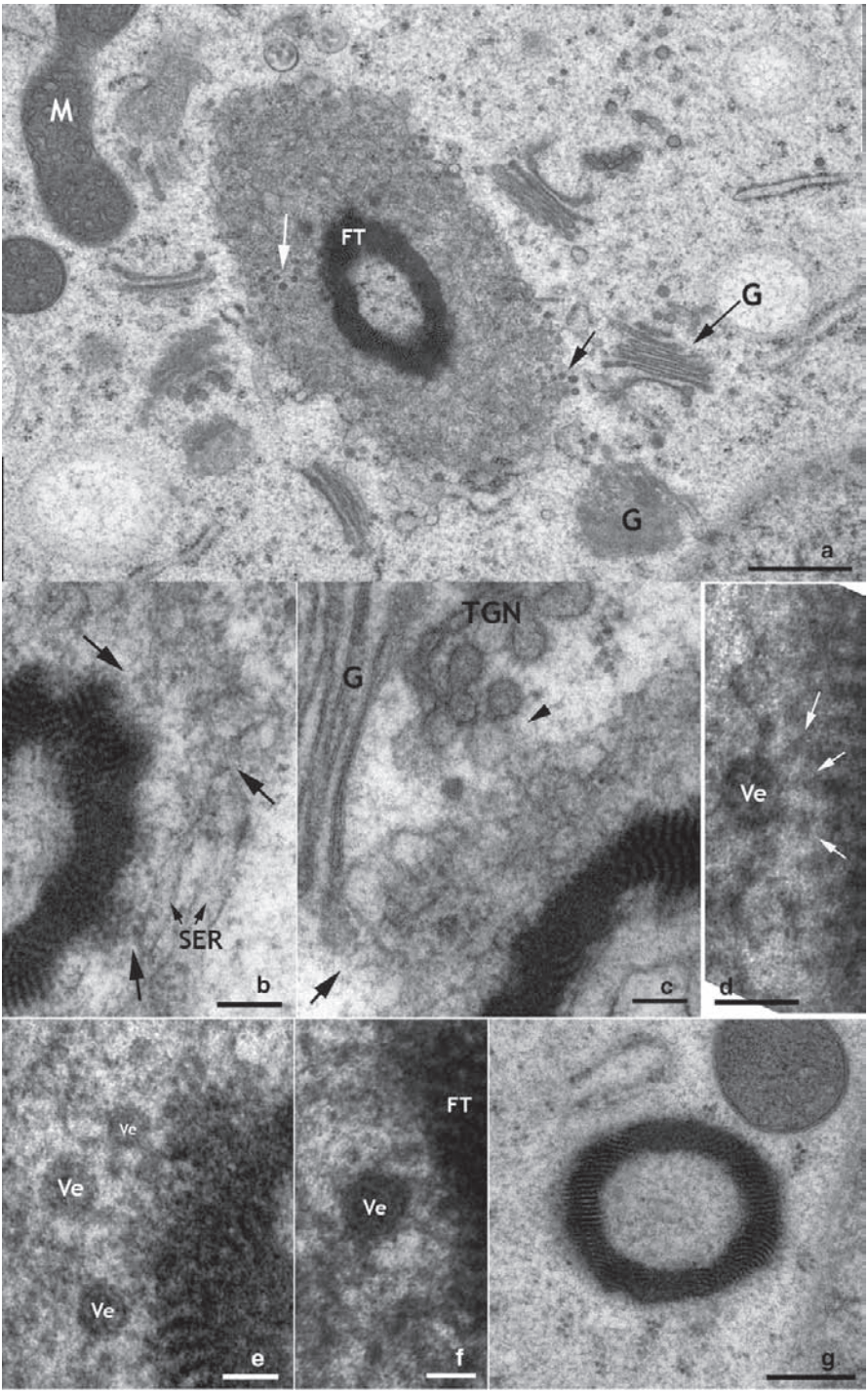
True feeding tubes are made in giant-cells for which there is scant evidence to settle the question as to whether the stylet lumen is contiguous with the feeding tube lumen. In their study of giant-cells, Hussey and Mims (1991) published two micrographs showing an intact plasma membrane at the juncture of feeding tube and cell wall, one of these is presented in Fig. 11d. In their micrographs, the stylet has been withdrawn from the plasma membrane (apparently during fixation), preventing determination of the continuity between stylet aperture and feeding tube. In the study by Rebois (1980) of feeding tubes in cotton infected with *R. reniformis*, there is apparent continuity of stylet and feeding tube lumen in one micrograph (reproduced here as Fig. 11e, arrow). A definite answer to the question of continuity, however, will require more substantial evidence.

Nevertheless, Fig. 11e provides persuasive evidence that injection of secretions into feeding cells by sedentary nematodes causes feeding tube formation, apparently due to a reaction between saliva and plant cytoplasm (Endo 1987; Hussey and Mims 1991; Rebois 1980; Sobczak et al. 1999). Giant-cell feeding tubes grow from the stylet tip of the root-knot nematode *Meloidogyne incognita* (Hussey and Mims 1991). Figure 11d (reproduced from the Hussey and Mims publication) shows an infected tomato cell where the stylet had been inserted and a new feeding tube formed from the region previously occupied by the stylet tip (arrow). The micrographs presented so far provide compelling evidence that feeding tubes are formed after injection of secretions. This does not, however, establish that feeding tubes are actually *made* of nematode secretory molecules. While its precise

composition is unknown, there is evidence that the feeding tube is proteinaceous (see Sect. 6.2). In giant-cells the feeding tube wall has a crystalline structure (Hussey and Mims 1991; Figs. 11–13); in other sedentary nematode associations it is not crystalline (Rebois 1980; Sobczak et al. 1999). In addition, there is light microscopy evidence that feeding tubes are formed in response to secretions from the nematode dorsal esophageal gland (Wyss and Zunke 1986). Secretory granules within the end sac of the dorsal gland valve of feeding *M. incognita* have a crystalline structure reminiscent of the wall of feeding tubes; but in the dorsal gland extension (which is further down the secretory path), there are no crystalline structures (Hussey and Mims 1990). In isolated juveniles (J2) of *M. incognita* induced to secrete, the secreted product does not have a crystalline or tubular ultrastructure (McClure and von Mende 1987) presumably due to the lack of plant cytoplasmic components that participate in its crystallization. Furthermore, if the tubes were composed of molecules from secretions, nematodes might choke when ingesting plant cytoplasm as this would react with secretory molecules in the esophagus to form an insoluble mass (if rapid pumping of the metacarpus during injection does not sufficiently clear plant cytoplasm from the lumen). Indeed, trapping of nematode secretions within the giant-cell forms an insoluble mass of feeding tube wall material that is associated with a large configuration of plant ER (Hussey and Mims 1991). Of the currently known components of nematode secretions (Davis et al. 2008), none have been identified as candidates for feeding tube composition.

A complex of host cell membranes surrounds growing feeding tubes, in pervasive contact with the tube wall (Figs. 11d, e, from Hussey and Mims 1991; and Rebois 1980; respectively). Figure 11h shows a feeding tube formed in *A. thaliana* infected by *M. incognita* and demonstrates that this membrane complex consists of smooth ER with regions contiguous with cisternal rough ER (arrows). That this structural relationship of ER with active feeding tubes is found for all feeding tube-eliciting sedentary nematodes emphasizes its importance in successful function of the tube. Condensation of a feeding tube as secretions meet plant cytoplasm requires a mechanism that prevents this from happening inside the nematode (for reasons described above). This requirement could be met if the condensation occurs on the outer surface of the tube.

Consistent with this, the membranes sheathing the feeding tube might have a role in tube formation. For example, the feeding tube from a giant-cell in *Arabidopsis thaliana* shown in Fig. 11f (and at higher magnification in Fig. 11g) is an unusual example of a tube growing from the surface of the endoplasmic reticulum. The fidelity of freeze-substituted specimen ultrastructure allows close examination of plant structures involved in feeding tube formation and maintenance (Fig. 12). The surface of active feeding tubes is a focus of high endomembrane system activity. In addition to the sheath of smooth ER, Golgi stacks and vesicles (arrows) also assemble around the tube (Fig. 12a) as noted by others (Hussey and Mims 1991; Paulson and Webster 1970). Details of the relationship of the smooth ER with the feeding tube surface can be seen at higher magnification (Fig. 12b). The crystalline feeding tube wall material is contiguous with the surface of the smooth ER, at the single


M
FT
G
G
a
SER
b
TGN
G
c
Ve
d
Ve
Ve
Ve
e
FT
Ve
f
g

black arrow and, in a more elaborate complex, between the opposing black arrows. The tube wall may be growing here, suggested by the domed "caps" along its surface, which are not present in older feeding tubes (Fig. 12g). Golgi stacks have not been observed within the membrane sheath; instead they interact with its surface (Fig. 12a, c). Coats on the surface of the *trans* cisterna (Fig. 12c, arrow) and the *trans* Golgi network (TGN) (arrowhead) are contiguous with coats on the smooth ER of the membrane sheath. Similarly, coats on vesicles bind to the surface of the feeding tube [Figs. 12d (arrows), e, f]. Presuming these are protein coats on the surface of endomembrane system components, it is possible that the tube is derived from coat proteins of the plant endomembrane system. This could be tested by immunogold labeling with coat protein antibodies. Work has been initiated using electron tomography (Otegui and Austin 2007) for analyzing the relationship of the feeding tube to the sheathing complex at high resolution in three dimensions. The endomembrane system is not associated with dormant, unused feeding tubes (Fig. 12g), suggesting that this association functions under the stimulus of nematode secretions or during nematode feeding.

Our hypothesis that feeding tubes contain plant material is in conflict with the more established hypothesis that they are comprised strictly of nematode material. The salient points of evidence for this (Hussey and Grundler 1998) are: (1) feeding tube ultrastructure is similar in different species of plants infected by *Meloidogyne*, (2) they are formed rapidly, (3) healthy plant cells do not form any structure similar to feeding tubes and (4) the ultrastructure of feeding tubes in a given plant species differs according to the nematode genus. The first and last points could be interpreted to show that tube ultrastructure depends on the nature of secretory molecules and that the plant components involved are of fundamental importance and found in most plant cells, as is the case for endomembrane coat proteins. There is no debate that plant cytoplasm is needed for feeding tube formation, even when they are formed rapidly. The question is: which component? That healthy plant cells do not form such structures supports the observation that nematode secretions are required for their formation. Clearly, whether the feeding tube is of plant or nematode origin (or a hybrid of both) is unresolved.

An alternate hypothesis to explain the close association of endomembranes with the tube wall is that the crystalline wall itself is of nematode origin and is composed of proteins that specifically bind coat proteins of the plant endomembrane system,

Fig. 12 Endomembrane associations with the feeding tube. (**a**) In addition to the smooth ER sheath, the feeding tube is surrounded by other components of the endomembrane system, including Golgi stacks and vesicles (*arrows*). (**b**) Smooth ER association with the feeding tube. Surface structures of the smooth ER membrane are linked to the feeding tube (*single arrow*). Between the *opposing arrows* these components are sectioned obliquely to the smooth ER surface, showing they form an extensive array with the feeding tube. (**c**) Surface structures of a Golgi stack cisternum link to the surface of the smooth ER feeding tube sheath (*arrow*), as do surface structures of the *trans* Golgi network (*arrowhead*). (**d**), (**e**) and (**f**) Coats of endomembrane system vesicles link to the surface of feeding tubes. (**g**) Transverse section of a dormant feeding tube. There are no endomembrane system associations with dormant feeding tubes

bringing them in close proximity for transport of material contained in the bound membrane complex. This would support the proposal by Sobczak et al. (1999) that the ER in contact with feeding tubes in syncytia could deliver sterols synthesized on the ER to the nematode, which is incapable of sterol synthesis. The lack of endomembranes around dormant feeding tubes supports this hypothesis. In this case disassembly of the sheath complex might involve loss of signals contained in nematode secretions that otherwise maintain coupling of coat proteins to the crystalline wall. To an extent, both ideas could be combined, i.e., endomembranes could be involved in feeding tube synthesis and also in delivering nutrients to the tube. It is notable that smooth, rather than rough, ER comprises the sheath. This suggests that potential food products made in the region of the feeding tube by this organelle are lipids rather than proteins.

6.2 Feeding Tube Composition

Feeding tube composition is largely unknown. Nemec (1932) was the first to suggest that it is composed of protein. Paulson and Webster (1970) and Jones and Northcote (1972b) both considered it to be a protein storage body, presumably due to its crystalline structure. The feeding tube is osmiophilic – osmium not only reacts with lipids but also proteins to a varying degree and nucleic acids to a limited extent (Hayat 1981). Osmium shows little reaction with polysaccharides. Further evidence in support of the tube being composed of proteins is provided by electron energy loss spectroscopy (EELS), a thin-section elemental analysis technique based on analyzing electron energies resulting from inelastic scattering of beam electrons by specimen elements (Leapman and Aronova 2007). High-resolution elemental maps of the feeding tube are presented in Fig. 13 with red dots showing elemental distributions for nitrogen (N), phosphorus (P), and sulfur (S) overlaid on 250-eV (reverse contrast) images. The feeding tube wall has a high signal for nitrogen and sulfur, indicating a composition of sulfur-rich proteins. The phosphorus map shows this element in the vacuole and broadly distributed in other cellular components, including ribosomes (e.g., those located on the ER), presumably due to the high phosphorus content of ribosomal RNA. The relatively low phosphorus signal on the tube suggests that it does not contain large amounts of nucleic acids. It also is not likely composed of cell wall material because cell walls do not exhibit high nitrogen and sulfur signals. The crystalline structure suggests high protein concentrations which should facilitate proteomic analysis given an appropriate technique for the isolation of feeding tubes.

In summary, the feeding tube is a significant cell biological component of parasitism, a conduit for delivery of plant products to the nematode and therefore a key element of plant disease caused by nematodes. It is central to a parasite that causes a billion dollar annual loss in soybean productivity – a billion dollar straw. To reduce the impact of nematode parasitism on plants, it is imperative to understand

the factors involved in feeding tube development and maintenance. Furthermore, the feeding tube represents a novel cell structure, and there is compelling evidence that this structure is derived from components present in all plant cells and assembled as a result of molecules contained in nematode secretions. Understanding how this is done will shed light on fundamental biological aspects of plant cells.

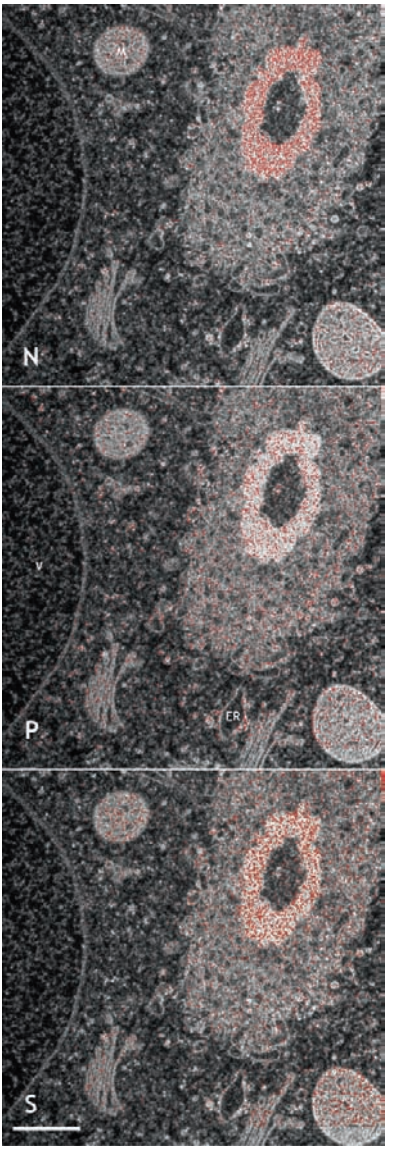


Fig. 13 Analysis of feeding tube composition using electron energy loss spectroscopy. Elemental maps of nitrogen (N), phosphorus (P), and sulfur (S) indicate the tube is rich in nitrogen and sulfur, suggesting it is composed of sulfur-rich proteins

7 Conclusion

Cellular structure in giant-cells is reviewed, with emphasis on outstanding questions in development of the giant-cell. These cells derive from root parenchyma cells, acquiring meristematic characters of proliferation without cytokinesis, and high cytoplasmic density, under the influence of a number of genes associated with these phenomena in meristematic cells. The challenge is to elucidate the cellular processes leading to this transformation and to understand how this includes prevention of cytokinesis. The prominence of nucleoli in giant-cells suggests that they have a significant function, ripe for analysis with newly emerging molecular tools. Development of the extensive vascular network surrounding giant-cells, and the concomitant development of thickened walls and transfer cell labyrinths of the giant-cell, is central to acquisition of nutrients by the giant-cell and successful nematode parasitism. Understanding the molecular and cellular biology involved might provide ideas of how to compromise these processes and the ensuing parasitism. It is now apparent that vacuolar dynamics have a role in giant-cell expansion and subsequent replacement of the vacuole by cytoplasm. As the role of cell cycle genes and the cytoskeleton in this process is explored, coupled with a successful search for feeding site-specific promoters and the application of RNA interference technology (Bakhetia et al. 2005), it is conceivable that formation of the root-knot gall could be significantly inhibited. The most unusual and remarkable structure in the giant-cell is the feeding tube, yet its molecular composition still eludes us. Its crystalline structure suggests that it has a homogenous, proteinaceous composition and, if so, proteomic analysis of even a small sample could bring forth its identification. This could shed light on whether it is composed of plant or nematode molecules and, more importantly, help in formulating a strategy to prevent its formation.

Acknowledgments There is a wide swath of literature on the cell biology of giant-cells and we apologize for those cases of omission. This review was funded in part by grants from the National Science Foundation (CGT) and the Environmental Protection Agency (CGT). We are grateful to Professor Richard S. Hussey, the University of Georgia, for his critical evaluation of this review.

References

M, Charlton WL, Urwin PE, McPherson MJ, Atkinson HJ (2005) RNA interference and plant parasitic nematodes. Trends Plant Sci 10:362–367

Beille L (1898) Sur les alterations produites par *l'Heterodera radicicola* sur les racines du *Papaya gracilis*. Compt Rend Assoc Franc Avanc Sci 27:413–416

Berg RH (1999) Cytoplasmic bridge formation in the nodule apex of actinorhizal root nodules. Can J Bot 77:1351–1357

Berg RH, Erdos GW, Gritzali M, Brown RD Jr (1988) Enzyme-gold affinity labelling of cellulose. J Electron Microsc Tech 8:371–379

Berg RH, Langenstein B, Silvester WB (1999) Development in the *Datisca-Coriaria* nodule type. Can J Bot 77:1334–1350

Bird AF (1961) The ultrastructure and histochemistry of a nematode-induced giant-cell. J Biophys Biochem Cytol 11:701–715

Bird AF (1962) The inducement of giant-cells by *Meloidogyne javanica*. Nematologica 8:1–10

Bird AF (1972) Quantitative studies on the growth of syncytia induced in plants by root-knot nematodes. Int J Parasitol 2:157–170
Bird AF (1973) Observations on chromosomes and nucleoli in syncytia induced by *Meloidogyne javanica*. Physiol Plant Pathol 3:387–391
Bird DMcK, Opperman CH, Williamson VM (2008) Plant infection by root-knot nematode. Plant Cell Monogr., doi:10.1007/7089_2008_31
Bockenhoff A, Grundler FMW (1994) Studies on the nutrient uptake by the beet cyst nematode *Heterodera schachtii* by in situ microinjection of fluorescent probes into the feeding structures in *Arabidopsis thaliana*. Parasitology 109:249–254
Bolle C (2004) The role of GRAS proteins in plant signal transduction and development. Planta 218:683–692
Bonke M, Thitamadee S, Mahonen AP, Hauser MT, Helariutta Y (2003) APL regulates vascular tissue identity in *Arabidopsis*. Nature 426:181–186
Browning AJ, Gunning BES (1977) An ultrastructural and cytochemical study of the wall-membrane apparatus of transfer cells using freeze-substitution. Protoplasma 93:7–26
Chaumont F, Barrieu F, Herman EM, Chrispeels MJ (1998) Characterization of a maize tonoplast aquaporin expressed in zones of cell division and elongation. Plant Physiol 117:1143–1152
Christie JR (1936) The development of root-knot nematode galls. Phytopathology 26:1–22
Dahiya P, Brewin NJ (2000) Immunogold localization of callose and other cell wall components in pea nodule transfer cells. Protoplasma 214:210–218
Davis EL, Mitchum MG (2005) Nematodes. Sophisticated parasites of legumes. Plant Physiol 137:1182–1188
Davis EL, Hussey RS, Baum TJ (2008) Parasitism genes: what they reveal about parasitism. Plant Cell Monogr., doi:10.1007/7089_2008_29
de Almeida Engler J, De Vleesschauwer V, Burssens S, Celenza JL Jr , Inze D, Van Montagu M, Engler G, Gheysen G (1999) Molecular markers and cell cycle inhibitors show the importance of cell cycle progression in nematode-induced galls and syncytia. Plant Cell 11:793–808
de Almeida Engler J, Van Poucke K, Karimi M, De Groodt R, Gheysen G, Engler G, Gheysen G (2004) Dynamic cytoskeleton rearrangements in giant-cells and syncytia of nematode-infected roots. Plant J 38:12–26
Delmer DP (1999) Cellulose biosynthesis: exciting times for a difficult field of study. Annu Rev Plant Physiol Plant Mol Biol 50:245–276
Dropkin VH, Nelson PE (1960) The histopathology of root-knot nematode infections in soybeans. Phytopathology 50:442–447
Endo BY (1987) Histopathology and ultrastructure of crops invaded by certain sedentary endoparasitic nematodes. In: Veech JA, Dickson DW (eds) Vistas on nematology: a commemoration of the twenty-fifth anniversary of the society of nematologists. Society of Nematologists, Hyattsville, Maryland, pp 196–210
Evert RF (2006) Esau's plant anatomy: meristems, cells, and tissues of the plant body: their structure, function and development. Wiley, Hoboken
Fester T, Berg RH, Taylor CG (2008) An easy method using glutaraldehyde-introduced fluorescence for the microscopic analysis of plant biotrophic interactions. J Microscopy. 231:342–348
Gheysen G, Fenoll C (2002) Gene expression in nematode feeding sites. Annu Rev Phytopathol 40:191–219
Gheysen G, Mitchum MG (2008) Molecular insights in the susceptible plant response to nematode infection. Plant Cell Monogr., doi:10.1007/7089_2008_35
Goverse A, Biesheuvel J, Wijers G-J, Gommers FJ, Bakker J, Schots A, Helder J (1998) In planta monitoring of the activity of two constitutive promoters, CaMV 35S and TR2, in developing feeding cells induced by *Globodera rostochiensis* using green fluorescent protein in combination with confocal laser scanning microscopy. Physiol Mol Plant Pathol 52:275–284
Goverse A, de Engler JA, Verhees J, van der Krol S, Helder JH, Gheysen G (2000) Cell cycle activation by plant-parasitic nematodes. Plant Mol Biol 43:747–761
Gunning BES, Pate JS (1969) Transfer cells: plant cells with wall ingrowths, specialized in relation to short distance transport of solutes - their occurrence, structure, and development. Protoplasma 68:107–133

Gunning BES, Pate JS (1974) Transfer cells. In: Robards AW (ed) Dynamics aspects of plant ultrastructure. McGraw-Hill Book Company, Maidenhead Berkshire England, pp 441–480
Hammes UZ, Schachtman DP, Berg RH, Nielsen E, Koch W, McIntyre LM, Taylor CG (2005) Nematode-induced changes of transporter gene expression in *Arabidopsis* roots. Mol Plant Microbe Interact 18:1247–1257
Hayat MA (1981) Principles and techniques of electron microscopy. vol. 1 University Park Press, Baltimore
Hess MW (2007) Cryopreparation methodology for plant cell biology. In: McIntosh JR (ed) Methods in cell biology, vol. 79, Cellular Electron Microscopy. Academic Press, San Diego, pp 57–100
Huang CS (1985) Formation, anatomy and physiology of giant-cells induced by root-knot nematodes. In: Sasser JN, Carter CC (eds) An advanced treatise on Meloidogyne, vol. 1, Biology & Control. North Carolina State University Graphics, Raleigh, pp 155–164
Huang CS, Maggenti AR (1969) Mitotic aberrations and nuclear changes of developing giant-cells in *Vicia faba* caused by root-knot nematode, *Meloidogyne javanica*. Phytopathology 59:447–455
Huang G, Gao B, Maier T, Allen R, Davis EL, Baum TJ, Hussey RS (2003) A profile of putative parasitism genes expressed in the esophageal gland cells of the root-knot nematode *Meloidogyne incognita*. Mol Plant Microbe Interact 16:376–381
Huang G, Dong R, Allen R, Davis EL, Baum TJ, Hussey RS (2006) A root-knot nematode secretory peptide functions as a ligand for a plant transcription factor. Mole Plant Microbe Interact 19:463–470
Hussey RS, Grundler FMW (1998) Nematode parasitism of plants. In: Perry RN, Wright DJ (eds) The physiology and biochemistry of free-living and plant-parasitic nematodes. CAN International, pp 213–243
Hussey RS, Mims CW (1990) Ultrastructure of esophageal glands and their secretory granules in the root-knot nematode *Meloidogyne incognita*. Protoplasma 156:9–18
Hussey RS, Mims CW (1991) Ultrastructure of feeding tubes formed in giant-cells induced in plants by the root-knot nematode *Meloidogyne incognita*. Protoplasma 162:99–107
Hussey RS, Mims CW, Westcott SW (1992) Ultrastructure of root cortical cells parasitized by the ring nematode *Criconemella xenoplax*. Protoplasma 167:55–65
Imlau A, Truernit E, Sauer N (1999) Cell-to-cell and long-distance trafficking of the green fluorescent protein in the phloem and symplastic unloading of the protein into sink tissues. Plant Cell 11:309–322
Jones MGK, Dropkin VH (1975) Cellular alterations induced in soybean roots by three endoparasitic nematodes. Physiol Plant Pathol 5:119–124
Jones MGK, Gunning BES (1976) Transfer cells and nematode induced giant-cells in *Helianthemum*. Protoplasma 87:273–279
Jones MGK, Northcote DH (1972a) Nematode-induced syncytium – a multinucleate transfer cell. J Cell Sci 10:789–809
Jones MGK, Northcote DH (1972b) Multinucleate transfer cells induced in coleus roots by the root-knot nematode, *Meloidgyne arenaria*. Protoplasma 75:381–395
Jones MGK, Payne HL (1977) Cytokinesis in *Impatiens balsamina* and the effect of caffeine. Cytobios 20:79–91
Jones MGK, Payne HL (1978) Early stages of nematode-induced giant-cell formation in roots of *Impatiens balsamina*. J Nematol 10:70–84
Koltai H, Dhandaydham M, Opperman C, Thomas J, Bird D (2001) Overlapping plant signal transduction pathways induced by a parasitic nematode and a rhizobial endosymbiont. Mol Plant Microbe Interact 14:1168–1177
Leapman RD, Aronova MA (2007) Localizing specific elements bound to macromolecules by EFTEM. In: McIntosh JR (ed) Methods in Cell Biology, vol 79, Cellular electron microscopy. Academic Press, San Diego, pp 593–613
Li L, Fester T, Tylor CG (2008) Transcriptomic analysis of nematode infestation. Plant Cell Monogr., doi:10.1007/7089_2008_36

Lloyd CW (1991) Cytoskeletal elements of the phragmosome establish the division plane in vacuolated higher plant cells. In: Lloyd CW (ed) The cytoskeletal basis of plant growth and form. Academic Press, San Diego, pp 245–257

Mathesius U (2003) Conservation and divergence of signalling pathways between roots and soil microbes – the *Rhizobium*-legume symbiosis compared to the development of lateral roots, mycorrhizal interactions and nematode-induced galls. Plant Soil 255:105–119

McClure MA, von Mende N (1987) Induced salivation in plant-parasitic nematodes. Physiol Biochem 77:1463–1469

Nemec B (1910) Das Problem der Befruchtungsvorgange und andere zytologische Fragen. In: Vielkernige Riesenzellen in Heterodera-Gallen, Part 4. Gebruder Borntraeger, Berlin, pp 151–173

Nemec B (1911) Uber die Nematodenkrankheiten der Zuckerrube. Zeitschrift fur Pflanzenkrankheiten 21:1–10

Nemec B (1932) Uber die Gallen von *Heterodera schachtii* auf der Zuckerrube. Studies from the Plant Physiological Laboratory of Charles University, Prague 4:1–14

Offler CE, McCurdy DW, Patrick JW, Talbot MJ (2002) Transfer cells: cells specialized for a special purpose. Annu Rev Plant Biol 54:431–454

Opperman CH, Taylor CG, Conkling MA (1994) Root-knot nematode-directed expression of a plant root-specific gene. Science 263:221–223

Otegui MS, Austin JR (2007) Visualization of membrane-cytoskeletal interactions during plant cytokinesis. In: McIntosh JR (ed) Methods in cell biology, vol 79, Cellular electron microscopy. Academic Press, San Diego, pp 221–240

Paulson RE, Webster JM (1970) Giant-cell formation in tomato roots caused by *Meloidgyne incognita* and *Meloidogyne hapla* (Nematoda) infection. A light and electron microscope study. Can J Bot 48:271–276

Pendle AF, Clark GP, Boon R, Lewandowska D, Lam YW, Andersen J, Mann M, Lamond AI, Brown JW, Shaw PJ (2005) Proteomic analysis of the *Arabidopsis* nucleolus suggests novel nucleolar functions. Mol Biol Cell 16:260–269

Pontes O, Pikaard CS (2008) siRNA and miRNA processing: new functions for Cajal bodies. Curr Opin Genet Dev 18:1–7

Razak RA, Evans AAF (1976) An intracellular tube associated with feeding by *Rotylenchulus reinforms* on cowpea root. Nematologica 22:182–189

Rebois RV (1980) Ultrastructure of a feeding peg and tube associated with *Rotylenchulus reniformis* in cotton. Nematologica 26:396–405

Rebois RV, Madden PA, Eldridge BJ (1975) Some ultrastructure changes induced in resistant and susceptible soybean roots following infection by *Rotylenchulus reniformis*. J Nematol 7:122–139

Rumpenhorst HJ (1984) Intracellular feeding tubes associated with sedentary plant parasitic nematodes. Nematologica 30:77–85

Sarda X, Tousch D, Ferrare K, Cellier F, Alcon C, Dupuis JM, Casse F, Lamaze T (1999) Characterization of closely related d-TIP genes encoding aquaporins which are differentially expressed in sunflower roots upon water deprivation through exposure to air. Plant Mol Biol 40:179–191

Shaw PJ, Brown JW (2004) Plant nuclear bodies. Curr Opin Plant Biol 7:614–620

Sijmons PC, Grundler FMW, von Mende N, Burrows PR, Wyss U (1991) *Arabidopsis thaliana* as a new model host for plant-parasitic nematodes. Plant J 1:245–254

Sinnott EW, Bloch R (1940) Cytoplasmic behaviour during division of vacuolate plant cells. Proc Natl Acad Sci USA 26:223–227

Sobczak M, Golinowski W (2008) Structure of cyst nematode feeding sites. Plant Cell Monogr., doi:10.1007/7089_2008_38

Sobczak M, Golinowski W, Grundler FMW (1999) Ultrastructure of feeding plugs and feeding tubes formed by *Heterodera schachtii*. Nematology 1:363–374

Staehelin LA, Moore I (1995) The plant golgi apparatus: structure, functional organization and trafficking mechanisms. Annu Rev Plant Physiol 46:261–288

Tischler G (1901) Ueber Heterodera-Gallen an den Wurzeln von *Circaea luteiana* L. Ber Deut Bot Gesell 19:95–107

Treub M (1887) Quelques mots sure les effets du parasitisme de *l'Heterodera javanica* dans les racines de la canne a sucre. Ann Jard Bot Buitenzorg 6:93–96

Urwin PE, Moller SG, Lilley CJ, McPherson MJ, Atkinson HJ (1997) Continual green-fluorescent protein monitoring of cauliflower mosaic virus 35S promoter activity in nematode-induced feeding cells in *Arabidopsis thaliana*. Mol Plant Microbe Interact 10:394–400

van Brussel AA, Bakhuizen R, van Spronsen PC, Spaink HP, Tak T, Lugtenberg BJ, Kijne JW (1992) Induction of pre-infection thread structures in the leguminous host plant by mitogenic lipo-oligosaccharides of R*hizobium*. Science 257:70–72

Wang X, Allen R, Ding X, Goellner M, Maier T, de Boer JM, Baum TJ, Hussey RS, Davis EL (2001) Signal peptide-selection of cDNA cloned directly from the esophageal gland cells of the soybean cyst nematode *Heterodera glycines*. Mol Plant Microbe Interact 14:536–544

Weerasinghe RR, Bird DM, Allen NS (2005) Root-knot nematodes and bacterial Nod factors elicit common signal transduction events in *Lotus japonicus*. Proc Natl Acad Sci USA 102:3147–3152

Wiggers RJ, Starr JL, Price HJ (1990) DNA content and variation in chromosome number in plant cells affected by *Meloidogyne incognita* and *M*. arenaria. Phytopathology 80:1391–1395

Wyss U, Zunke U (1986) Observations on the behaviour of second stage juveniles of *Heterodera schachtii* inside host roots. Revue Nematol 9:153–165

Wyss U, Grundler FMW, Munch A (1992) The parasitic behaviour of second-stage juveniles of *Meloidogyne incognita* in roots of *Arabidopsis thaliana*. Nematologica 38:98–111

Yang WC, de Blank C, Meskiene I, Hirt H, Bakker J, van Kammen A, Franssen H, Bisseling T (1994) *Rhizobium* nod factors reactivate the cell cycle during infection and nodule primordium formation, but the cycle is only completed in primordium formation. Plant Cell 6:1415–1426

Structure of Cyst Nematode Feeding Sites

Miroslaw Sobczak(✉) and Wladyslaw Golinowski

Abstract In susceptible plants the syncytium is generally conserved in anatomy and ultrastructure. Syncytium development starts from a single cell selected from cortical or parenchymatic vascular cylinder cells by an infective second-stage juvenile. The syncytium is enlarged and multinuclear due to fusion of protoplasts released by local cell wall dissolution. The syncytium is the only source of nutrients for all sedentary stages of cyst nematodes. Ultrastructurally, it is characterized by proliferation of cytoplasm, reduction of the volume of vacuoles, hypertrophy of nuclei and nucleoli, and abundant endoplasmic reticulum, ribosomes, plastids, and mitochondria. Resistant plants are invaded by infective juveniles that usually induce syncytia. However, these syncytia reveal atypical features during their development that lead to their necrosis, or they are surrounded by necrotic cells that result in syncytium degradation. Depending on the timing of the resistant response in such syncytia, no development of juveniles beyond the second- or third-developmental stage occurs, or only adult males develop.

1 Introduction

Sedentary parasitic cyst nematodes from the genera *Globodera* and *Heterodera* are obligatory biotrophs that induce a specific type of plant feeding site called a "syncytium." Plant roots are invaded by vermiform second-stage juveniles (J2) hatched from eggs. After root invasion and penetration of the rhizodermis, the juvenile intracellularly migrates and selects a single cell that becomes the Initial Syncytial Cell (ISC). After ISC selection the juvenile stays motionless with

M. Sobczak
Department of Botany, Faculty of Agriculture and Biology, Warsaw University of Life Sciences (SGGW), Warsaw, Poland
e-mail: miroslaw_sobczak@sggw.pl

Plant Cell Monogr, doi:10.1007/7089_2008_38

its stylet inserted into the cell for about 8 h (Wyss 1992). During this so-called "feeding-preparation period," no pumping action of the metacorporal bulb occurs. When the feeding-preparation period is completed, the juvenile retracts its stylet and re-inserts it in the ISC. Pumping action of the metacorporal bulb begins and a juvenile starts to feed. Twenty-four hours after ISC selection, cells next to the ISC are hypertrophied and interconnected with it by dissolution of cell walls. Thus, a syncytium is formed by fusion of neighboring protoplasts.

Although cyst nematodes reproduce by obligatory amphimixis, their infective J2 juveniles are not differentiated sexually. Their sex is regulated by epigenetic factors such as the amount and quality of food withdrawn from the syncytium (Betka et al. 1991; Grundler et al. 1991; Mugniéry and Bossis 1985; Mugniéry and Fayet 1981, 1984). Females will develop if their syncytia can provide sufficient food of good quality. In the event of food shortage or poor quality only males will mature. The differences in the organization of genital primordia first become evident at the end of the J2 sedentary stage (Wyss 1992). Males take up food from the syncytium only during two developmental stages (J2 and J3). After completing the J3 stage they undergo two subsequent molts and develop into adults. They become vermiform again, emerge from the cuticle and seek females. Females actively withdraw food during four developmental stages (J2, J3, J4, and adult). During molts food is not withdrawn.

Development of juveniles is concomitant with the syncytium, the latter meeting the increasing nutritional demands of the former. It is calculated that during all stages of active food uptake a female consumes 29-times more food than a male. Similarly, females need three-times more food than males during the J2 and J3 stages (Müller et al. 1982). Syncytia associated with males become necrotic soon after juveniles molt to the adult stage and leave the root (Sobczak et al. 1997). Syncytia associated with females remain active and provide food during the entire female lifetime even when eggs are produced (Müller et al. 1982).

2 Susceptible Interactions

Although the structure of the syncytium has been known for about a century, the physiological and molecular mechanisms underlying its development from the ISC into the fully functional feeding structure has only significantly progressed over the last several decades (see Davis et al. 2008; Gheysen and Mitchum 2008; Tomczak et al. 2008; Li et al. 2008). Establishment of axenic in vitro cultures for *Arabidopsis* (Sijmons et al. 1991) and other plant species like tomato, potato, or tobacco infected with sedentary cyst nematodes allows long-time in vivo screening of behavior and development of juveniles inside the roots. *Arabidopsis* also appears to be a wonderful model for comparative structural and developmental microscopy studies of syncytia associated with male and female juveniles. Unfortunately, among the more important cyst nematodes, *Arabidopsis* is only host to *H. schachtii*, *H. trifolii*, and *H. cajani*. Other species of *Heterodera* or *Globodera* are, in fact, able to invade its roots and induce the ISC but no further development of syncytium occurs (Sijmons et al. 1991; Grundler et al. 1997).

2.1 Syncytia Associated with Females

The structure of syncytia associated with females is best described for beet, soybean, and potato cyst nematodes. In the case of *H. glycines* most reports are concerned with the syncytium development in soybean roots (Endo 1964, 1965, 1991; Gipson et al. 1971; Kim et al. 1986; Subbotin 1990). The structure of syncytia induced in a broader range of hosts is described for *H. schachtii*. Reports are available describing response of infected sugar beet (Bleve-Zacheo and Zacheo 1987, Holtmann et al. 2000), radish (Grymaszewska and Golinowski 1998; Wyss et al. 1984), white mustard (Golinowski and Magnusson 1991; Soliman et al. 2005), oil-seed rape (Magnusson and Golinowski 1991), and *Arabidopsis* (Golinowski et al. 1996; Sobczak et al. 1997). There are several reports on responses of oat and wheat infected with *H. avenae* (Bleve-Zacheo et al. 1995; Grymaszewska and Golinowski 1991; Williams and Fisher 1993) as well as reports describing reactions of potato and tomato plants to infection with *G. rostochiensis* and *G. pallida* (Castelli et al. 2006; Goverse et al. 1998, Jones and Northcote 1972; Melillo et al. 1990a; Rice et al. 1985, 1987; Sobczak et al. 2005). In spite of the diversity of *Heterodera* and *Globodera* species infecting different hosts from unrelated families, there are obvious ultrastructural and anatomical similarities in their syncytium structure (Table 1). These similarities indicate that different cyst nematodes modify the same plant developmental pathways to induce syncytia.

2.1.1 Invasion

J2 may invade main and lateral roots in the elongation or root-hair zones when roots are in the primary growth state. Rarely are roots invaded in the lateral root formation zone when they are in the secondary growth state and covered by multilayered exodermis or peridermis. In such cases J2 usually enter the roots using breakages caused by emerging lateral roots. A J2 penetrates the plant cell walls using its robust stylet. However, before the stylet penetrates, cell walls are softened by a cocktail of cell-wall modifying proteins released from the nematode's esophageal glands via the stylet orifice (e.g. Qin et al. 2004; Smant et al. 1998; Vanholme et al. 2004). J2 intracellularly migrate through rhizodermal, cortical, and endodermal cells towards the vascular cylinder (Wyss 1992; Wyss and Zunke 1986). In the case of *H. schachtii* the ISC is selected in procambial or pericyclic cells (Bleve-Zacheo and Zacheo 1987; Golinowski and Magnusson 1991; Golinowski et al. 1996; Grymaszewska and Golinowski 1998; Holtmann et al. 2000; Sobczak et al. 1997; Wyss et al. 1984).

Reports dealing with responses of plants infected with *H. glycines*, *H. avenae*, *G. rostochiensis*, or *G.pallida* indicate that cortical parenchyma or endodermal cells may be selected as ISC (Bleve-Zacheo et al. 1995; Castelli et al. 2006; Endo 1964, 1965, 1991; Gipson et al. 1971; Grymaszewska and Golinowski 1991; Jones and Northcote 1972; Kim et al. 1986; Rice et al. 1985, 1987; Sobczak et al. 2005; Williams and Fisher 1993). During root invasion and migration protoplasts of cells

Table 1 Anatomical changes observed in syncytia during susceptible and resistant responses to cyst nematodes

Combination	Initial Syncytial Cell	Incorporated Cells	Hypertrophy of Syncytial Elements	Cytoplasm	Vacuole
Susceptible Response					
Heterodera sp. x susceptible hosts	pro/cambium, vascular parenchyma	pro/cambium, vascular and stellar parenchyma	strong	electron dense, strong proliferation	central vacuole is substituted by numerous small ones
Heterodera glycines x soybean	cortical parenchyma, endodermis, pericycle	cortical parenchyma, endodermis, pericycle, phloem parenchyma, cambium	strong	electron dense, strong proliferation	central vacuole is substituted by numerous small ones
Globodera sp. x susceptible hosts	cortical parenchyma, endodermis, pericycle, pro/cambium, vascular parenchyma	cortical parenchyma, endodermis, pericycle, pro/cambium, vascular parenchyma	strong	electron dense, strong proliferation	central vacuole is substituted by numerous small ones, except of elements derived from cortical cells
Resistance Response					
H. schachtii x sugar beet (*Hs1*$^{pro\text{-}1}$)	procambium, pericycle	procambium, pericycle, vascular parenchyma	weak	electron dense, proliferating	large, less numerous vacuoles
H. schachtii x *Raphanus sativus* var. o*leiformis* (cv. Pegletta)	pericycle, procambium	pericycle, procambium, vascular parenchyma	strong in pericycle derived elements, weak in others	condensed	decrease of central vacuole, later formation of numerous vacuoles that increase in size during syncytium degradation
H. schachtii x *Sinapis alba* (cv. Maxi)	pericycle, occasionally procambium	pericycle, procambium, stellar parenchyma, but xylem parenchyma resists incorporation	moderate	condensed, becoming electron translucent during syncytium development	reduced in size

H. glycines x *Glycine max* (cv. Bedford)	pericycle	primarily pericycle, but also procambium	weak	dense, but of deteriorating appearance, plasmolysed	central vacuole reduced in size and numerous small vacuoles
H. glycines x *Glycine max* (cv. Peking)	N.D.	N.D.	limited	proliferating	reduced in size, numerous small vacuoles appear in degenerating syncytia
H. glycines x *Glycine max* (cv. Forrest)	N.D.	N.D.	weak	extremely condensed	reduced in size, small vacuoles are formed
H. goettingiana x *Pisum* sp.	pericycle, procambium, cortex (future males)	pericycle, procambium, vascular parenchyma, syncytia associated with males consist of cortex cells only	moderate	proliferating, but weakly dense	reduced and replaced by small irregular vacuoles
H. avenae x *Triticum aestivum* (AUS10894)	stellar parenchyma	stellar and xylem parenchyma	moderate	proliferating and condensed till 15 DAI then degenerating	decreased in size till 15 DAI then coalescence of vacuoles
G. rostochiensis (Ro1) x *Solanum tuberosum* ssp. *andigena* (H1)	inner cortex	cortex, endodermis, outer layers of vascular cylinder	moderate	not proliferating and located paramurally	decreased in size, but usually central vacuole is preserved and small vacuoles appear
G. rostochiensis (Ro1) x *Lycopersicon esculentum* (*Hero*)	inner cortex, pericycle, procambium	cortex, endodermis, pericycle, pro/cambium, vascular parenchyma	strong	weak proliferation, located paramurally	decreased in size, but central vacuole is preserved and small vacuoles appear
G. pallida (Pa1, Pa3) x *Solanum vernei*	inner cortex	cortex, endodermis, pericycle, vascular parenchyma	N.D.	proliferates in some parts, but generally remains paramural	decreased in size, but central vacuole is preserved and small vacuoles appear
G. pallida (Pa2/3) x *Solanum canasense*	cortex	cortex, endodermis, few pericyclic and vascular parenchyma cells	weak	proliferating, granular and retracted from cell wall	decreased in size, small vacuoles appear

(continued)

Table 1 (continued)

Combination	Nucleus	Nucleolus	Plastid	Mitochondria	Endoplasmic Reticulum
Susceptible Response					
Heterodera sp. x susceptible hosts	hypertrophied, strongly amoeboid, condensed nucleoplasm	hypertrophied with numerous "vacuoles"	enlarged, dividing, different shapes	enlarged, dividing, elongated	proliferating, rough and smooth, cisternae often arranged in parallel or concentric swirls
Heterodera glycines x soybean	ranging in form from convoluted to strongly elongated	hypertrophied	enlarged	enlarged, elongated	proliferating smooth-surfaced cisternae showing parallel or concentric arrangement
Globodera sp. x susceptible hosts	strongly amoeboid, condensed nucleoplasm	hypertrophied with numerous "vacuoles"	enlarged, dividing, different shapes	enlarged, dividing, elongated	proliferating, rough and smooth, cisternae often arranged in parallel or concentric swirls
Resistance Response					
H. schachtii x sugar beet (*Hs1*$^{pro-1}$)	enlarged, amoeboid	N.D.	numerous	numerous	almost only rough flattened cisternae, forming membrane aggregations in later stages
H. schachtii x *Raphanus sativus* var. o*leiformis* (cv. Pegletta)	weakly enlarged, convoluted	N.D.	few	few	generally, poorly developed, predomination of rough-surfaced flattened cisternae

H. schachtii x *Sinapis alba* (cv. Maxi)	enlarged, amoeboid	N.D.	small number of plastids	numerous	lack of tubular smooth ER
H. glycines x *Glycine max* (cv. Bedford)	deteriorating from 5 DAI, chromatin clumps, breakdown of nuclear envelope	N.D.	N.D.	numerous	moderate proliferation
H. glycines x *Glycine max* (cv. Peking)	enlarged	N.D.	numerous	numerous	proliferating, but aggregating during necrosis
H. glycines x *Glycine max* (cv. Forrest)	N.D.	N.D.	N.D.	N.D.	N.D.
H. goettingiana x *Pisum* sp.	hypertrophied, amoeboid	hypertrophied with numerous "vacuoles"	numerous plastids with starch grains	numerous	proliferating, forming concentric swirls
H. avenae x *Triticum aestivum* (AUS10894)	hypertrophied, amoeboid, with scattered chromatin	N.D.	numerous	numerous	N.D.
G. rostochiensis (Ro1) x *Solanum tuberosum* ssp. *andigena* (H1)	enlarged, amoeboid, undergoing karyolysis	enlarged	numerous, accumulating starch and changing shapes	numerous, not changed structurally	proliferating, forming concentric aggregates in degenerating syncytium
G. rostochiensis (Ro1) x *Lycopersicon esculentum* (*Hero*)	slightly enlarged, weakly amoeboid	N.D.	few, some with starch grains	few	weakly developed
G. pallida (Pa1, Pa3) x *Solanum vernei*	enlarged	N.D.	numerous, but starch grains appear from 4 DAI	numerous	proliferates
G. pallida (Pa2/3) x *Solanum canasense*	enlarged, lobed	N.D.	few, different shapes	few	N.D.

(continued)

Table 1 (continued)

Combination	Cell wall	Cell-Wall Openings	Cell-Wall Ingrowths	Necrosis	Nematode Development
Susceptible Response					
Heterodera sp. x susceptible hosts	generally strongly thickened	wide close to nematode head, narrower in distal parts of syncytia	present at walls facing vessels from 10 DAI	single necrotized cells next to nematode or incidentally next to syncytia	adult females and males
Heterodera glycines x soybean	thickened	numerous, increasing in size during syncytium development	present at walls facing vessels	N.D.	adult females and males
Globodera sp. x susceptible hosts	generally strongly thickened	wide close to nematode head in the cortex bridge region, narrow in distal parts of syncytia derived from vascular cylinder	present at walls facing vessels from 15 DAI	single necrotized cells next to nematode or incidentally next to syncytia	adult females and males
Resistant Response					
H. schachtii x sugar beet (*Hs1^{pro-1}*)	weakly thickened	narrow	absent	single necrotized cells, degeneration of syncytium from 4 DAI	stagnating juveniles, occasionally adult males and females
H. schachtii x *Raphanus sativus* var. o*leiformis* (cv. Pegletta)	only slightly thickened	narrow and few	small and few	syncytium degradation begins at 4-5 DAI, more advanced in terminal elements than in parts close to nematode's head	males, and few females
H. schachtii x *Sinapis alba* (cv. Maxi)	thickened, heterogeneous structure due to cell wall materials depositions	numerous, but large volumes of confluent cytoplasm are exceptional	present on walls facing xylem parenchyma	abundant necrosis during invasion, then necrosis around syncytium and necrosis of syncytia induced in procambium starting from parts being in contact with the metaxylem	stagnating juveniles, adult males and few females

H. glycines x *Glycine max* (cv. Bedford)	thickened	narrow and few	N.D.	necrosis of syncytium from 5 DAI	N.D.
H. glycines x *Glycine max* (cv. Peking)	prominent thickening	pronounced	N.D.	degeneration of syncytia within 5 DAI	dead juveniles
H. glycines x *Glycine max* (cv. Forrest)	prominent thickening and appositions next to necrosis	very small	N.D.	cells surrounding syncytium necrotize from 5 DAI	N.D.
H. goettingiana x *Pisum* sp.	thickened	wide	N.D.	syncytium degradation begins at 4 DAI	juveniles up to third stage
H. avenae x *Triticum aestivum* (AUS10894)	thickened next to nematode	present	N.D.	necrosis of endodermis follows necrosis of syncytium from 15 DAI	adult males, females stopped in fourth stage
G. rostochiensis (Ro1) x *Solanum tuberosum* ssp. *andigena* (H1)	locally thickened	present	absent	necrosis of cells surrounding syncytium follows degradation of syncytium at 7 DAI	late second- and third-stage juveniles
G. rostochiensis (Ro1) x *Lycopersicon esculentum* (*Hero*)	thickened	present	absent	syncytium surrounded by layer of necrotized cells at 4 DAI	stagnating juveniles, adult males, sometimes adult females
G. pallida (Pa1, Pa3) x *Solanum vernei*	evenly thickened	present	absent	necrosis of cells next to juvenile and necrosis of syncytium by 7 DAI	stagnating second- and few third-stage juveniles
G. pallida (Pa2/3) x *Solanum canasense*	N.D.	present	absent	none around nematode, necrotic syncytium from 7 DAI	second-stage juveniles are unable to invade roots or leave roots after invasion and migration

N.D.- No Data, Not Described

punctured by a nematode stylet collapse immediately. Thus, the nematode migration path is delineated by destroyed cells.

This destructive migratory behavior changes into a subtle and exploratory one when the J2 reaches the vascular cylinder (Wyss and Zunke 1986). Cells are probed by the J2 stylet. If the protoplast of the probed cell does not collapse and its reaction is recognized by the juvenile as functional, the J2 enters into a feeding-preparation period with the stylet inserted into the selected ISC (Golinowski et al. 1997; Wyss 1992; Wyss and Zunke 1986).

2.1.2 Anatomical Changes

During the feeding-preparation period, the stylet is inserted into the ISC and covered with a thin layer of electron translucent material (Fig. 1), presumably being callose as demonstrated by Hussey et al. (1992) for the ectoparasite *Criconemella xenoplax*. Cytoplasm of the ISC proliferates and is electron dense. Concomitantly, the volume of the central vacuole decreases (Golinowski et al. 1997; Sobczak et al. 1999). Callose-like material is also locally deposited in the ISC and in some cells next to the ISC.

When the feeding preparation period is completed, the J2 withdraws the stylet and inserts it again into the ISC. This time secretions from the nematode esophageal glands are injected into the cytoplasm of the ISC (Fig. 2). The secretions are not surrounded by any membrane and mix with the ISC cytoplasm. If the ISC has been selected in procambial cells, the syncytium spreads to conductive elements of xylem and phloem via incorporation of procambial cells (Fig. 3). Thus, the syncytium assures direct contact with both conductive tissues.

If all primary xylem vessels are differentiated, they create a barrier that can not be overcome by the expanding syncytium, and the syncytium only develops on one side of the xylem bundles. However, if some xylem vessels are not differentiated, their precursors are primarily incorporated into the expanding syncytium which extends into the center of the vascular cylinder (Fig. 3). As stated by Magnusson and Golinowski (1991), successful incorporation of procambial cells located next to the vessels as well as next to sieve elements is the most crucial event to establish and develop a fully functional syncytium that will allow adult females to mature.

Cells incorporated into the syncytium during the 3 days after ISC selection are slightly enlarged but reveal ultrastructural features typical of fully efficient syncytia (Fig. 4). The cytoplasm is condensed and rich in ribosomes and Endoplasmic

Fig. 3 Anatomy of a syncytium (S) induced in *A. thaliana* associated with molting J2 male of *H. schachtii* (6 DAI). *Arrows* point to dividing pericycle forming peridermis-like tissue. *Bar* 20 μm

Fig. 4 Ultrastructure of a syncytium (S) induced by *H. schachtii* in *A. thaliana* (72 h after ISC selection). (CW, cell wall; ER, endoplasmic reticulum; M, mitochondrion; Nu, nucleus; Pl, plastid; V, vacuole). *Bar* 2 μm

Fig. 5 Ultrastructure of a syncytium (S) induced by *H. schachtii* in *A. thaliana* under conditions favoring development of males (24 h after ISC selection). (FT, feeding tube; M, mitochondrion; Ne, necrosis; Pl, plastid; V, vacuole; *arrow*-cell-wall opening). *Bar* 2 μm

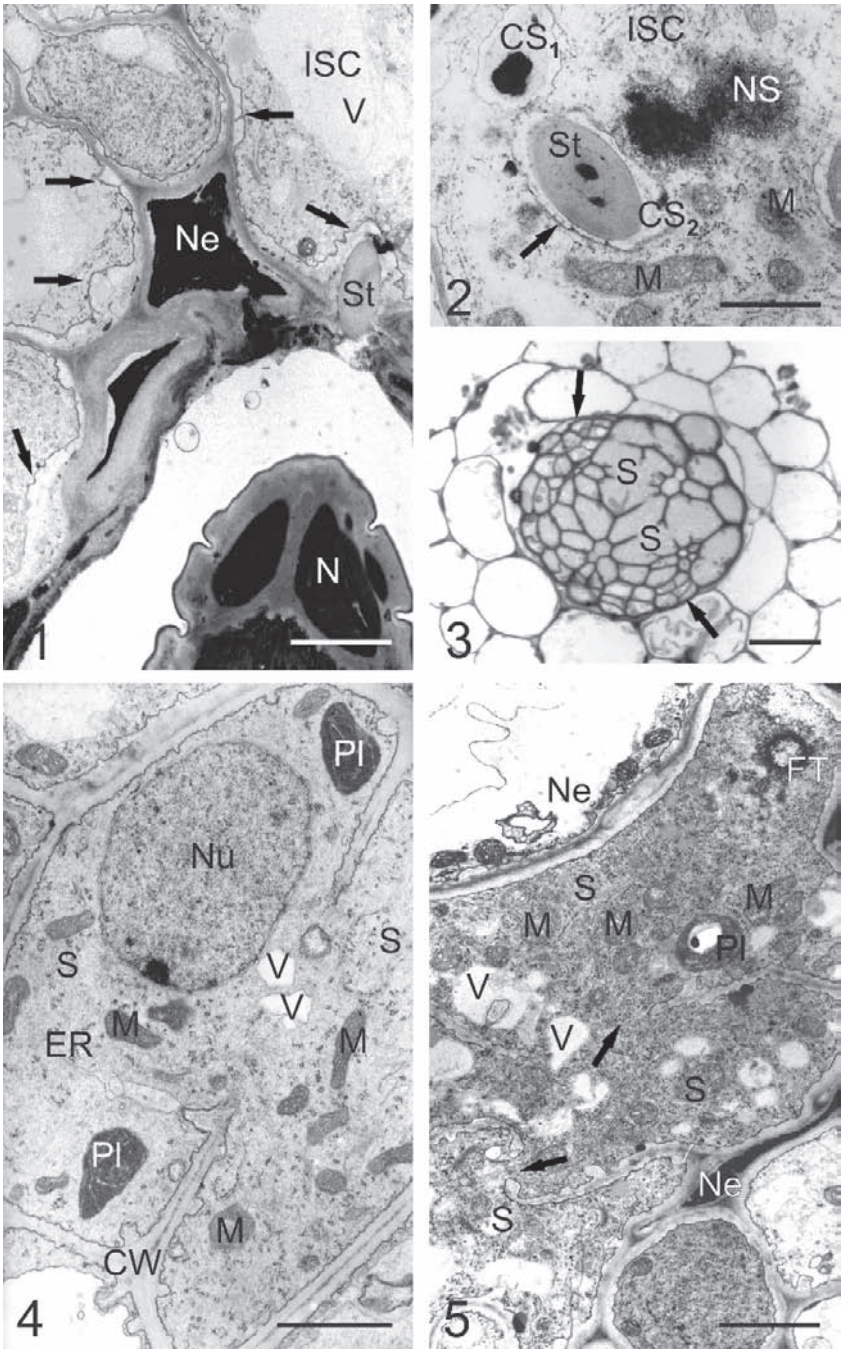


Fig. 1 Juvenile (N) of *H. schachtii* selecting the initial syncytial cell (ISC) in *A. thaliana* (6 h after ISC induction). (Ne, necrosis; St, stylet; V, vacuole; *arrow*-callose-like material). *Bar* 5 µm
Fig. 2 Nematode stylet (St) inserted into ISC selected by *H. schachtii* in *A. thaliana* (6 h after ISC induction). Collapsed callose-like sheath (CS_1) formed during feeding-preparation period and newly formed callose-like sheath around introduced stylet (CS_2). Nematode secretions (NS) injected from the stylet (M, mitochondrion; *arrow*-plasmalemma). *Bar* 1 µm

Reticulum (ER) as well as mitochondria and plastids. The volume of the central vacuole is decreased and small vacuoles appear.

G. rostochiensis and *G. pallida* juveniles usually do not enter the vascular cylinder. They select an ISC in the cortex or endodermis (see Figs. 12 and 13). From the ISC the syncytium spreads to the vascular cylinder by forming cell-wall openings between cortical and endodermal cells. This part of the syncytium is called a "cortex bridge" (Sembdner 1963) and is present only in the region next to the nematode head (Castelli et al. 2006; Jones and Northcote 1972; Rice et al. 1985, 1987; Sobczak et al. 2005). When the growing syncytium reaches the vascular cylinder, procambial and pericyclic cells are incorporated into it. Inside the vascular cylinder, the syncytium spreads axially, preferentially incorporating parenchymatic vascular cylinder cells located next to xylem or phloem elements (see Fig. 13). Rarely do J2 enter the vascular cylinder and induce syncytia in procambium. In such cases the syncytium does not contain the cortex bridge.

In *Arabidopsis* roots, a single syncytium associated with an adult female is composed of about two hundred cells (Hussey and Grundler 1998) and reaches its final maximal size at 10 days after infection (Urwin et al. 1997). For sugar beet roots infected with *H. schachtii*, it is calculated that J2 associated feeding sites occupy about 0.04 mm^3 of the root volume on average (Caswell-Chen and Thomason 1993). In comparison, the volume occupied by a J3 feeding site is about 0.27 mm^3 while feeding sites of J4 females and adults occupy about 0.5 mm^3.

Irrespective of susceptible plant species, syncytia associated with females have very similar organization. Close to the juvenile's head, the syncytium has the largest diameter and is composed of the most hypertrophied elements connected by the broadest cell-wall openings (Figs. 6 and 13). Sometimes this region of the syncytium is free of cell walls except short cell wall stubs, thus syncytial cytoplasm becomes confluent (Golinowski et al. 1996; Wyss et al. 1984). Distal parts of the syncytium are composed of smaller elements with narrower and fewer cell-wall openings than those next to the nematode head. Necroses around syncytia are rare, restricted only to single cells next to developing syncytia, sometimes even in the regions remote from the nematode head. Secondary vascular elements often differentiate around syncytia (Golinowski et al. 1996). Divisions of the pericyclic cells not incorporated into the syncytium lead to the formation of peridermis-like tissue around the vascular cylinder and syncytium (Golinowski et al. 1996, 1997).

Fig. 7 Ultrastructure of a syncytium (S) induced in *A. thaliana* associated with J3 female of *H. schachtii* (10 DAI). (CW, cell wall; ER, cisternal ER; M, mitochondrion; Pl, plastid; *arrow* tubular ER). *Bar* 2 μm

Fig. 8 Ultrastructure of nucleus (Nu) and nucleolus (No) in a syncytium induced in *A. thaliana* associated with J4 female of *H. schachtii* (14 DAI). (M, mitochondrion; *arrow*-nucleolar vacuole). *Bar* 2 μm

Fig. 9 Cell-wall ingrowths (*arrows*) formed at interface with vessels (X) in syncytium (S) induced in *A. thaliana* associated with J4 female of *H. schachtii* (14 DAI). (ER, endoplasmic reticulum; LB, lipid body; M, mitochondrion; Pl, plastid). *Bar* 2 μm

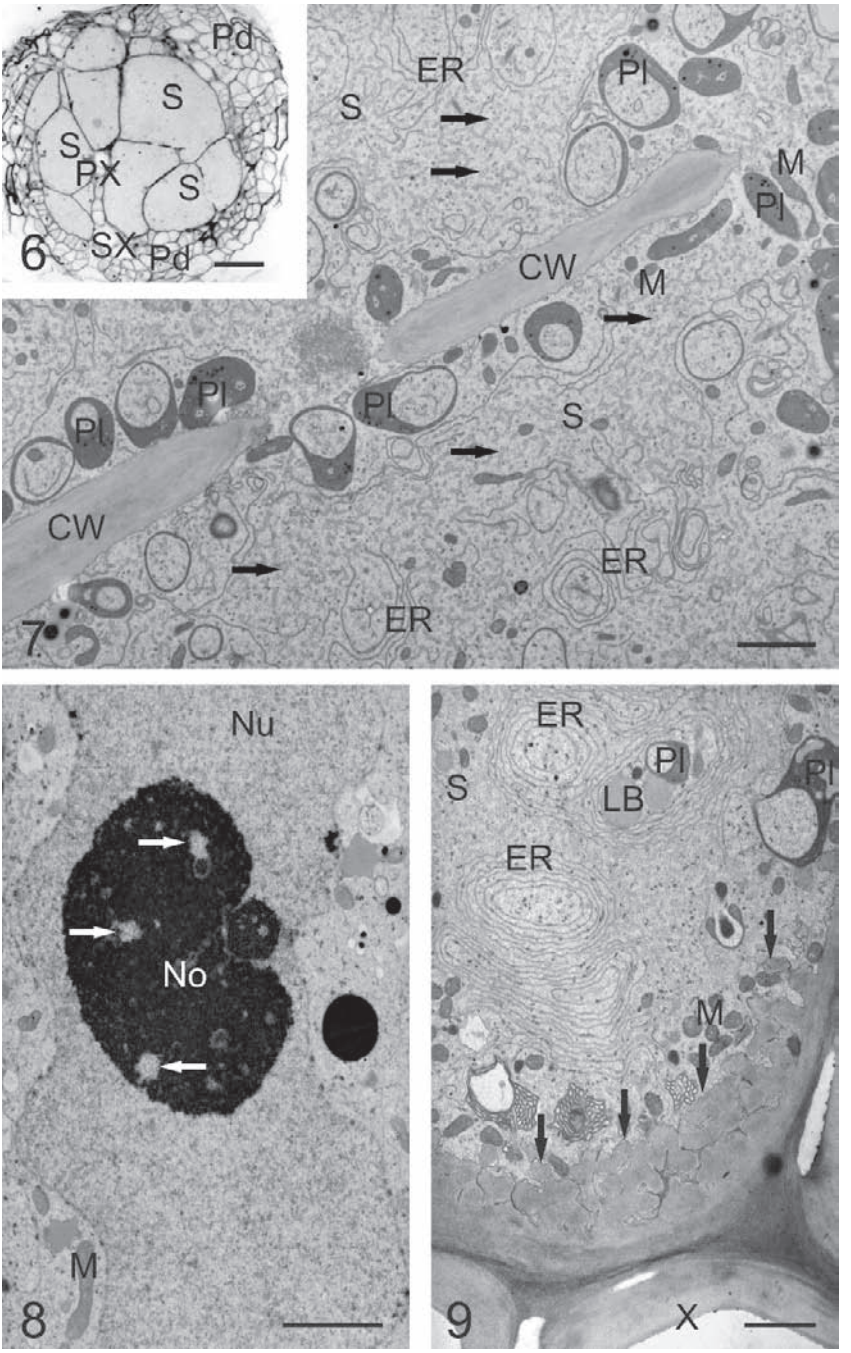


Fig. 6 Anatomy of a syncytium (S) induced in *A. thaliana* associated with J3 female of *H. schachtii* (10 DAI). Proliferating pericyclic cells surround syncytium and form peridermis-like tissue (Pd). (PX, primary xylem; SX, secondary xylem). Bar 20 µm

2.1.2.1 Cytological Changes

Like their anatomy, the ultrastructure of syncytia associated with feeding nematodes is also conserved. The syncytial cytoplasm strongly proliferates and is electron dense (Figs. 4, 5, 7, 9–12 and 14). Its streaming increases in comparison to nonsyncytial cells, indicating increased metabolic activity (Wyss 1992; Wyss and Zunke 1986).

2.1.2.2 Vacuolar Changes

In functional syncytia associated with juveniles of *Heterodera* sp., the vacuolar system consists of many small vacuoles (Bleve-Zacheo and Zacheo 1987; Bleve-Zacheo et al. 1995; Gipson et al. 1971; Golinowski et al. 1996; Grymaszewska and Golinowski 1998; Holtmann et al. 2000; Kim et al. 1986; Soliman et al. 2005). However, in syncytia associated with *Globodera* sp., large vacuoles are preserved in the cortex bridge during the entire time of syncytium development (Fig. 12). In these elements cytoplasm is paramurally located (Sobczak et al. 2005). Similarly, in syncytia of *Heterodera* and *Globodera*, elements derived from the vascular cylinder cells contain no central vacuoles that are de-differentiated during the preconditioning phase before cell incorporation into syncytium. Small vacuoles are apparently formed de novo by widening the ER cisternae (Fig. 14; Golinowski et al. 1996; Magnusson and Golinowski 1991). From electron micrographs, Subbotin (1990) calculated that the respective surface of the vacuole decreases from 85% in noninfected parenchymatic vascular cylinder cells to 37% in syncytia associated with J2 and up to 0.7% in syncytia associated with young females in syncytia induced by *H. glycines* in soybean roots. The presence of the remnants of the central vacuoles in some syncytial regions at the leading edges of the syncytium indicates that these were from recently incorporated cells and that the syncytium grows apically.

2.1.2.3 Nuclear Changes

Syncytial nuclei enlarge and are often irregular in outline with reduced number and size of heterochromatin (Bleve-Zacheo and Zacheo 1987; Bleve-Zacheo et al. 1990a, 1995; Gipson et al. 1971; Golinowski et al. 1996, 1997; Grymaszewska and

Fig. 11 Feeding tube (FT) in a syncytium (S) induced in *A. thaliana* associated with J4 female of *H. schachtii* (14 DAI). Feeding tube consists of electron opaque wall (*arrows*) and electron translucent lumen (*asterisks*). Connections with endoplasmic reticulum (ER) are indicated by *arrowheads*. (CW, cell wall; FP, feeding plug). *Bar* 2 μm

Fig. 12 A syncytium (S) induced in susceptible tomato cv. "Money Maker" by *G. rostochiensis* (3 DAI). (CB, cortex bridge; FP, feeding plug; ISC, initial syncytial cell; N, nematode; Ne, necrosis; V, vacuole). *Bar* 5 μm

Fig. 13 Anatomy of a syncytium (S) induced in susceptible potato cv. "Desiree" by *G. pallida* (6 DAI). Section was taken just above juvenile head. (CB, cortex bridge; ISC, initial syncytial cell; Ph, phloem; X, xylem). *Bar* 20 μm. (*courtesy of S Janakowski*)

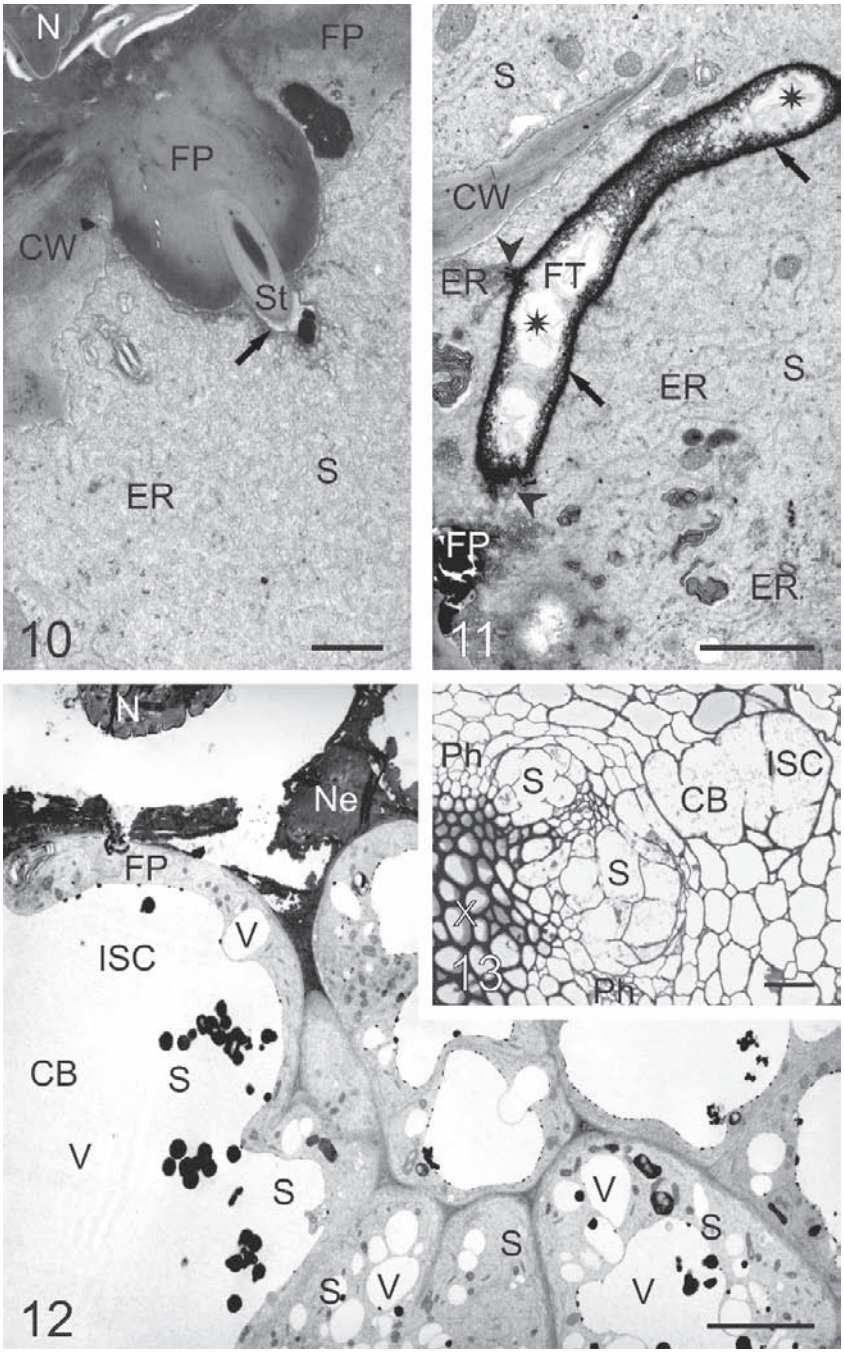


Fig. 10 Feeding plug (FP) with nematode stylet (St) covered with layer of callose-like material (*arrow*) in syncytium (S) induced in *A. thaliana* associated with J3 female of *H. schachtii* (N) (10 DAI). (CW, cell wall; ER, endoplasmic reticulum). *Bar* 1 µm

Golinowski 1991, 1998; Williams and Fisher 1993). The nucleoplasm is electron dense (Figs. 8 and 14). Nucleoli are enlarged and numerous nucleolar vacuoles are formed (Fig. 8). Structural changes of nuclei and nucleoli indicate that changes in gene expression in syncytium occur very early after its induction (Gheysen and Fenoll 2002; Opperman et al. 2008). From Endo's work (1971), it is known that DNA synthesis occurs in syncytia induced by cyst nematodes, but no mitosis is observed in syncytial elements although dividing cells are abundant around syncytia (Figs. 3, 6 and 13). Recently, it has been shown that syncytial nuclei undergo endoreduplication without karyokinesis (de Almeida Engler et al. 1999).

2.1.2.4 Changes in Other Organelles

Plastids and mitochondria rapidly proliferate in syncytial cytoplasm (Figs. 5, 7, 9 and 14). They acquire different shapes that signal that some are dividing. The mitochondria (except those that are dividing) are not morphologically or structurally changed. They are round or rod-shaped on sections and have a well-developed system of cristae (Figs. 5, 7, and 9).

Plastids reveal a mixture of ultrastructural features typical of proplastids and amyloplasts. They may morphologically and structurally change during syncytium development. In syncytia associated with J2, they are usually spherical or slightly rod-shaped (Figs. 4, 5, and 14). They are often constricted during division and usually devoid of starch grains that are subjected to intensive turnover (Hofmann et al. 2008). In syncytia associated with J3, plastids are larger and many appear cup- or ring-shaped on sections (Fig. 7). In syncytia associated with J4 or adult females, their size increases and they often have irregular outlines (Fig. 9). The majority of plastids contain no starch grains unless the syncytium is necrotic (Hofmann et al. 2008), but in many plastids, thylakoid membranes are well-developed and they easily synthesize chlorophyll when exposed to the light (Urwin et al. 1997).

In soybean, the respective surface occupied by mitochondria and plastids increases from 0.7 to 0.5%, respectively, in noninfected vascular parenchyma cells, up to 2.7 and 2.5%, respectively, in syncytia associated with young females of *H. glycines*. The mean length of the mitochondria in both axes only increases by

Fig. 15 Ultrastructure of *A. thaliana* infected with *H. glycines* (3 h after ISC selection). Juvenile (N) remains with the stylet (St) inserted into the selected initial syncytial cell (ISC). The stylet is embedded in a thick layer of callose-like material (*arrow*). Most of the vascular cylinder cells are necrotic (Ne). *Bar* 2 μm

Fig. 16 Ultrastructure of a syncytium (S) induced in resistant radish cv. "Pegletta" associated with J3 male of *H. schachtii* (10 DAI). Section taken close to the nematode head. *Arrows* point to stubby cell-wall ingrowths, and *arrowhead* indicates narrow cell-wall opening. (Nu, nucleus; X, xylem). *Bar* 2 μm. (*courtesy of G Grymaszewska*)

Fig. 17 Ultrastructure of a syncytium (S) induced in resistant white mustard cv. "Maxi" associated with J3 male of *H. schachtii* (10 DAI). Syncytium is composed of pericyclic cells and surrounded by necrotic cells (Ne). *Arrows* point to narrow cell-wall openings. Nuclei (Nu) are weakly enlarged and amoeboid. Central vacuoles (V) or their remnants are still present in some syncytial elements. *Bar* 5 μm. (*courtesy of AH Soliman*)

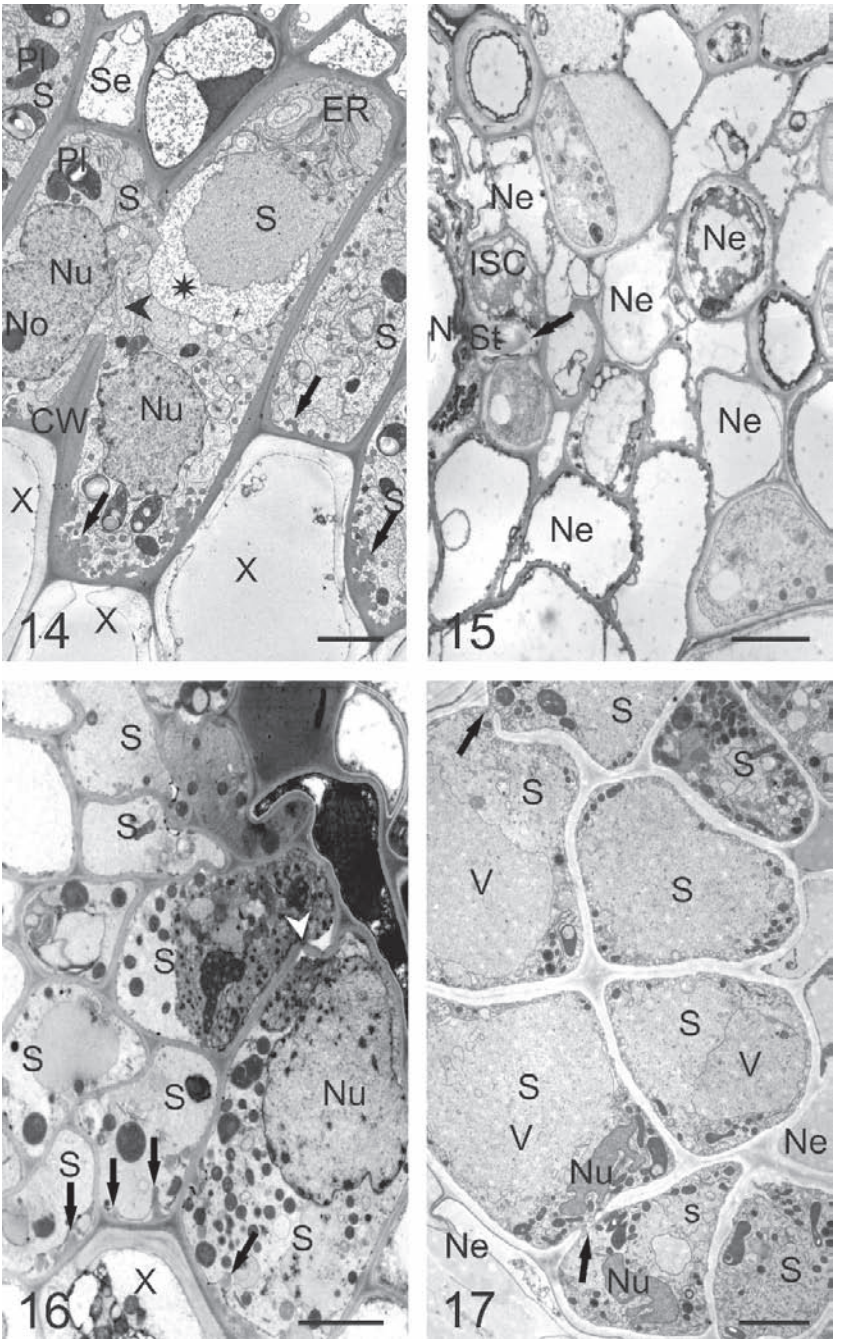


Fig. 14 Ultrastructure of vascular cylinder derived part of syncytium (S) induced in susceptible potato cv. "Desiree" by *G. pallida* (15 DAI). Strongly dilated cisterna of endoplasmic reticulum (ER) is marked with *asterisk*; *arrows* point to cell-wall ingrowths. (CW, cell wall; No, nucleolus; Nu, nucleus; Pl, plastid; Se, sieve tube; X, xylem). *Bar* 2 μm. (*courtesy of S Janakowski*)

one-third during syncytium development while the mean length of plastids is more than doubled (Subbotin 1990).

The amount of syncytial ER is high during the entire time of syncytium development (Bleve-Zacheo and Zacheo 1987; Gipson et al. 1971; Golinowski and Magnusson 1991; Golinowski et al. 1996; Grymaszewska and Golinowski 1998; Holtmann et al. 2000; Melillo et al. 1990a; Soliman et al. 2005; Wyss et al. 1984). The ER forms an extensive system of large cisternae, often arranged in parallel or in circular swirls, and numerous small tubular structures (Figs. 7 and 9–11). The former are usually located in paramural parts of syncytial cytoplasm and are sparsely covered with ribosomes. The latter predominate in central parts of syncytial elements and are free of ribosomes. Ribosomes present in the syncytial cytoplasm are arranged into polysomes.

Dictyosomes are numerous in syncytial cytoplasm and are not morphologically or structurally changed. In syncytia associated with J4 and adult females, numerous lipid bodies appear (Fig. 9; Golinowski et al. 1996; Subbotin 1990).

In spite of being abundant in syncytia, the cytoskeleton elements, microtubules and microfilaments, are randomly arranged. No cortical microtubules, mitotic spindles, or phragmoplasts are formed in functional syncytia (de Almeida Engler et al. 2004).

2.1.2.5 Cell Wall Changes

Syncytial cell walls fulfill opposing requirements. On one hand, the cell wall counteracts increasing osmotic pressure in syncytia that can increase up to 10,000 hPa (Böckenhoff and Grundler 1994). In comparison, the osmotic pressure of parenchymatic vascular cylinder cells in noninfected *Arabidopsis* roots is about 4,000 hPa. On the other hand, the syncytial cell wall has to remain flexible to allow expansion of the feeding site and incorporation of new cells as well as be permeable to facilitate uptake of nutrients necessary for syncytium maintenance and nematode feeding. In general, outer syncytial walls are thick.

Deposition of new layers of cell wall material apparently occludes plasmodesmata (Grundler et al. 1998). No functional plasmodesmata connecting syncytia with any of the neighboring cells have been reported except in syncytia induced by the false root-knot nematode *Nacobbus aberrans* (Jones and Payne 1977a, 1977b). Additionally, plasmodesmata function as pressure sensitive valves that are closed if a difference of osmotic pressures between adjacent cells exceeds 2,000 hPa (Oparka and Prior 1992). These observations together with microinjection studies (Böckenhoff and Grundler 1994) and expression analysis and localization of sucrose transporter AtSUC2 (Juergensen et al. 2003) suggest that syncytia are symplastically isolated. This idea is also supported by recent tobacco rattle virus expression studies. Juveniles of *H. schachtii* are able to withdraw from syncytia transiently expressed fluorescent protein if a tobacco rattle virus carrying the construct infects roots before syncytium induction (Valentine et al. 2007). However, translocation of symplastically unloaded fluorescent dye carboxyfluorescein (Böckenhoff et al. 1996) and phloem-expressed green fluorescent protein (GFP) (Hoth et al. 2005) into syncytial cytoplasm suggests existence

of functional plasmodesmata and symplastic loading of the syncytium. Finally, Hofmann and Grundler (2006) using transgenic scions expressing mobile GFP grafted on wild-type rootstock show that 8 days after inoculation syncytia induced by *H. schachtii* in *Arabidopsis* roots are symplastically isolated. By 8 days, GFP is transported into syncytia associated with females but not in syncytia associated with the males. This indicates de novo formation of plasmodesmata between phloem cells and well-developed syncytia. The idea of an interchangeable way of syncytium loading is additionally supported by analysis of another phloem specific sucrose transporter, AtSUC4 (Hofmann et al. 2007).

Little is known about the chemical composition of syncytial cell walls. Histological staining indicates that syncytial walls contain cellulose, hemicellulose, and pectins, but neither lignin nor suberin is detected (Sobczak 1996). Callose-like material is deposited close to the nematode head and only during early stages of syncytium development. Recently, changes have been reported in syncytia and surrounding cells in expression of wall-modifying proteins such as expansins (Fudali et al. 2008; Wieczorek et al. 2006), endoglucanases (Goellner et al. 2001; Karczmarek et al. 2008; Wieczorek et al. 2008), extensins and methyl esterases (Gheysen and Fenoll 2002; Opperman et al. 2008).

Concomitantly with the process of cell-wall thickening, cell-wall openings are formed in some parts of the syncytial wall (Figs. 5, 7, and 14). During syncytium induction and early syncytium development, the cell-wall openings are formed by widening of pre-existing plasmodesmata (Grundler et al. 1998). The widened plasmodesmata are present in the ISC at the end of the feeding-preparation period. Apparently incorporation of cells into syncytia follows plasmodesmata distribution in the root. If the ISC is selected in procambium, other procambial cells are then incorporated into it. However, if the syncytium is induced in pericycle, other pericycle cells are incorporated first. Thus, such syncytia are primarily C-shaped (Fig. 5). Later, procambial cells are incorporated into the syncytium (Golinowski et al. 1997; Sobczak et al. 1997). In the case of *Globodera*, where the ISC is selected in the cortex, cell wall dissolutions are formed in the cortex bridge straight towards the vascular cylinder (Fig. 13). In contrast, cell-wall openings in older syncytia are formed by gradual local digestion of syncytial wall lacking plasmodesmata (Grundler et al. 1998). The enzymes involved in the formation of cell-wall openings apparently are of plant origin as indicated by local over-expression of some endoglucanase isoforms inside syncytia (Goellner et al. 2001; Karczmarek et al. 2008; Wieczorek et al. 2008).

In syncytia associated with female J4 and adults that have the highest nutritional demands, a system of cell-wall ingrowths is formed on syncytial walls facing vessels (Figs. 9 and 14). The cell-wall ingrowths are structures typical of transfer cells (Jones and Northcote 1972; Offler et al. 2002). They increase the interface between syncytium and conductive xylem elements; and, thus, they increase the effectiveness of short-distance transport of water and nutrients into the syncytium (Bleve-Zacheo et al. 1995; Golinowski and Magnusson 1991; Golinowski et al. 1996; Grymaszewska and Golinowski 1998; Jones and Dropkin 1975; Jones and Northcote 1972; Kim et al. 1986; Soliman et al. 2005; Williams and Fisher 1993; Wyss et al. 1984).

2.1.3 Feeding Plug and Feeding Tube

2.1.3.1 Feeding Plug

Irrespective of cyst nematode species and host, there are two unique structures related to nematode parasitism present in the syncytia: a feeding plug and a feeding tube. The feeding plug is formed where the nematode stylet is inserted into the syncytium wall (Endo 1978; Sobczak et al. 1999). Its function is to anchor the stylet where it is inserted (Fig. 10). Cyst nematode feeding occurs in cycles. The nematode stylet is withdrawn and reinserted during the second phase of the nematode feeding cycle (Wyss 1992). The stylet does not enter the syncytial cytoplasm because it does not pierce the plasmalemma. An opening in the plasmalemma is formed only at the stylet orifice (Rebois 1980). The stylet is then isolated from the plasmalemma by callose-like material (Figs. 1, 2, and 10) except at the orifice. When the phase of food withdrawal is completed, the stylet is retracted and the callose-like sheath surrounding its tip is partially pulled inside the syncytial wall (Fig. 10). The feeding cycle repeats every 2–3 h. A striated structure embedded into syncytial wall is formed (Sobczak et al. 1999). Indications are that secretions released from nematode amphids participate in feeding plug formation. Therefore, this structure may be of both nematode and plant origin (Endo 1978; Sobczak et al. 1999).

2.1.3.2 Feeding Tube

The plasmalemma opening at the stylet orifice makes possible direct release of nematode secretions into the syncytial cytoplasm, forming feeding tubes (Figs. 2, 5 and 11). Feeding tubes are directly involved in nematode feeding (Wyss 1992; Wyss and Zunke 1986). It is generally accepted that they function as a molecular sieve allowing nematode uptake of nutrients (it is unknown what nematodes do “eat”). Feeding tubes prevent the stylet orifice and channel from occlusion caused by ingestion of plant organelles and membranes. The molecular exclusion limit for dextran uptake through feeding tubes is between 20 and 40 kDa (a calculated diameter of particles between 3.2 and 4.4 nm) (Böckenhoff and Grundler 1994). Using different forms of GFP expressed in syncytia, it appears that the molecular exclusion limit varies between cyst nematode species. Juveniles of *G. rostochiensis* ingest GFP of a molecular weight of 32 kDa (Goverse et al. 1998) while *H. schachtii* is unable to take up GFP of a molecular weight of 28 kDa (Urwin et al. 1997).

A new feeding tube is formed during each third phase of a nematode feeding cycle when the nematode secretions are injected via stylet into the syncytium (Wyss 1992). It is speculated that the feeding tubes are either formed from solidified nematode secretions (Hussey and Mims 1991; Rumpenhorst 1984) or as a result of interaction between nematode secretions and syncytial cytoplasm (Sobczak et al. 1999; Berg et al. 2008).

All feeding tubes attached to the stylet have an osmiophilic wall and electron translucent lumen (Fig. 11). The wall is often connected with ER cisternae. One end

of the feeding tube is anchored to the stylet while the lumen is opened to the stylet orifice (Rebois 1980). Older feeding tubes are detached from the nematode stylet and their lumen is filled with syncytial cytoplasm (Fig. 5).

2.2 Syncytia Associated with Males

Infective J2 of cyst nematodes are not sexually differentiated and differences in the organization of genital primordia first appear just before molting to J3 (Wyss 1992). Syncytia associated with male juveniles are smaller than the ones associated with respective developmental stages of females, and these degenerate when males become adults (Endo 1964, 1965). The calculated volume of a beet root occupied by a feeding site associated with male J4 is about 0.04 mm^3 and comparable to the volume of a root occupied by the feeding site of J2 (Caswell-Chen and Thomason 1993). Using *Arabidopsis* and changing plant culture conditions, it is possible to shift the sex ratio of *H. schachtii* in favor of one of the sexes. Thus, development of syncytia associated with males can be followed up to the J4 stage (Golinowski et al. 1997; Sobczak et al. 1997).

2.2.1 Invasion

Under conditions favoring development of males, when flowering *Arabidopsis* plants grown at low concentrations of sucrose in culture medium (Sobczak et al. 1997) are inoculated with J2, they migrate toward the vascular cylinder where they attempt to induce the ISC in procambial cells (Golinowski et al. 1997; Sobczak et al. 1997). However, these cells react hypersensitively or deposit thick callose-like sheaths around the inserted nematode stylet. J2 then probe other cells, looking for a pericyclic cell. These cells do not deposit thick callose-like sheaths around inserted stylets so the J2 are able to enter the feeding-preparation phase. A young syncytium is singly composed of pericyclic cells and is C-shaped (Fig. 5).

The more cells are probed before selection of the ISC, the greater the extent of necrosis in the nematode head region compared to the syncytia associated with females. Additionally, many cells not directly affected by migrating juveniles become necrotic around syncytia associated with males (Sobczak et al. 1997). The necrotic cells are usually xylem parenchyma cells. Necrosis of these cells separates syncytium from conductive elements. The ISC and cells preconditioned for incorporation into syncytium undergo the same changes as syncytia associated with females.

After this initial stage of syncytium development, procambial cells are incorporated into the feeding site (Fig. 3). Close to the nematode head, syncytia associated with J2 and J3 males have enlarged pericyclic cells interconnected by wide cell-wall openings and fewer enlarged procambial cells. Distal parts of syncytia are composed of slightly enlarged procambial cells interconnected by a few narrow cell-wall openings (Sobczak et al. 1997).

The anatomy of syncytia associated with males of *H. schachtii* induced in white mustard (Soliman et al. 2005) and radish (Grymaszewska and Golinowski 1998) is similar. In contrast, syncytia associated with males of *H. goettingiana* are restricted to the cortical and endodermal cells of pea roots and do not incorporate vascular cylinder cells (Melillo et al. 1990b).

2.2.2 Cytological Changes

At the cytological level male-associated syncytia are similar to syncytia associated with females at the same developmental stage. Fewer cell-wall ingrowths are formed in syncytia associated with males. They are short, unbranched, and stubby and present at different locations of the syncytial cell walls (Sobczak et al. 1997). The feeding plugs and feeding tubes formed by males have the same structure as female ones (Sobczak et al. 1999).

Male feeding is complete at the end of the J3 stage and the syncytium degenerates. Syncytial cytoplasm is electron opaque and fibrillar; nucleoplasm is electron translucent. Large vacuoles appear, membranous structures are delineated, and lipid bodies are formed (Sobczak et al. 1997). After cytoplasm degeneration, the syncytium is usually crushed and compressed by enlarging and dividing neighboring cells that are not incorporated into the syncytium (Endo 1964, 1965; Sobczak et al. 1997).

3 Resistant Interactions

Cyst nematode resistant plants are usually infected with juveniles to the same extent and in the same way as susceptible hosts (Acedo et al. 1984; Phillips et al. 1982; Turner and Stone 1984). Furthermore, root exudates obtained from resistant plants stimulate hatching as well as those collected from susceptible plants (Turner and Stone 1984). Plants that are nonhosts for a particular cyst nematode may sometimes be invaded under appropriate conditions (Grundler et al. 1997). In such cases, induction of the ISC occurs but no further syncytium development takes place (Fig. 15). Nematode stylets inserted into the ISC are embedded in a very thick layer of callose-like material occluding their orifices. The cytoplasm of the ISC is electron dense and forms many small vacuoles. Procambial cells surrounding the ISC are plasmolyzed and finally become necrotic, forming a layer of destroyed cells isolating the ISC from other root tissue.

Resistance against cyst nematodes provided by different resistance genes may be exhibited in various ways. Rarely, is it based on a hypersensitive reaction, which does not allow juveniles to induce development of the syncytium (Soliman et al. 2005). Cyst nematode resistance genes do not protect roots from invasion, or selection of the ISC, and initial syncytium development. However, syncytia developed in resistant plants reveal structural modifications (Table 1).

In some cases, syncytia become necrotic after several days of successful development (Grymaszewska and Golinowski 1998; Holtmann et al. 2000; Rice et al.

1985, 1987; Soliman et al. 2005; Wyss et al. 1984). In other cases, the necrosis of a syncytium is followed by necrosis of surrounding cells. A sheath composed of degraded cells is formed around syncytia that are then isolated from xylem and phloem elements (Grymaszewska and Golinowski 1998; Kim et al. 1987; Rice et al. 1985, 1987; Sobczak et al. 2005; Soliman et al. 2005).

Finally, syncytia are destroyed and juveniles are usually retarded in development and stagnate in one of the juvenile stages. Occasionally they develop into adult males. Resistance against cyst nematodes is highly species- or sometimes pathotype- or even race-specific. It is not absolutely effective and functional syncytia rarely allow juveniles to develop into females. This may easily lead to an emergence of new virulent nematode isolates (Müller 1998). In general, resistance genes are stable and effective for many years, but extensive use of cultivars carrying the same resistance gene may lead to the emergence of another nematode species, pathotype, or race as the main threat to a particular crop. Such a situation has occurred in the case of the *H1* gene that suppresses some *G. rostochiensis* pathotypes. Its extensive use promoted *G. pallida* as the most significant nematode pest of potato (Brodie et al. 1998). Additionally, there are only a few valuable cyst nematode resistance genes available. For these reasons, so-called "artificial resistance" against nematodes has been introduced to crops (McCarter 2008). In general, this resistance is based on specific expression of antifeedants or toxins in syncytia (Atkinson et al. 2003; Bakhetia et al. 2005), or on specific silencing of plant susceptibility factors (de Almeida Engler et al. 2005).

3.1 Resistant Responses to Heterodera sp

3.1.1 Sugar Beet

In sugar beet plants carrying the $Hs1^{pro-1}$ resistance gene, juveniles of *H. schachtii* are able to induce only small syncytia (Holtmann et al. 2000). The migration of the J2 is sometimes obstructed by coagulated cytoplasm aggregating around the nematode head. Syncytia are located in the vascular cylinder and are composed of formerly procambial and pericyclic cells. They have a well-established interface with vessels and sieve tubes. Little necrosis occurs around them. The cytoplasm becomes condensed and electron dense. The number of plastids, mitochondria, and ER structures increase. The nucleus is enlarged and the amount of heterochromatin decreases. The most obvious differences are that vacuole size increases (and their numbers decrease) and that the amount of ER increases in syncytia induced in resistant plants. ER membranes create large aggregations in older syncytia with other parts of syncytial cytoplasm being free of the ER. Such syncytia degenerate before the juveniles become adults. Few juveniles develop into adult females. Those females that do develop have syncytia that structurally resemble syncytia induced in susceptible plants (Holtmann et al. 2000). A similar mechanism of resistance based on the necrosis of the syncytium following deterioration of attached juveniles also occurs in other *Beta vulgaris* x *B. procumbens* hybrids resistant to *H. schachtii*, but its cytological background was not examined (Yu and Steele 1981).

3.1.2 Brassicaceous Plants

Among resistant responses reported for Brassicaceous plants infected with *H. schachtii* are those for white mustard (*Sinapis alba*) and radish (*Raphanus sativus*). For radish, syncytia development is the same in susceptible and resistant plants in the first 2 Days After Inoculation (DAI) (Wyss et al. 1984; Grymaszewska and Golinowski 1998). Many rough ER cisternae appear and vacuolation increases three DAI in syncytia induced in resistant plants. Seven DAI the syncytial protoplast is nearly disintegrated (Wyss et al. 1984). A few syncytia composed of slightly enlarged procambial cells that have electron dense cytoplasm can be functional long enough for adult males (Fig. 16) and some adult females to develop (Grymaszewska and Golinowski 1998). Very limited or no necrosis occurs in cells surrounding the syncytium, which maintains direct contact with vessels and sieve tubes (Grymaszewska and Golinowski 1998; Wyss et al. 1984).

A range of different defense responses are induced in cyst nematode-resistant white mustard (Golinowski and Magnusson 1991; Golinowski et al. 1997; Soliman et al. 2005). Migrating J2 induce necrosis of vascular cylinder cells along their migration paths (Golinowski et al. 1997; Soliman et al. 2005). This leads to juveniles becoming embedded in necrotic cells. The surviving cells react neoplastically and form large groups of dividing cells. Juveniles that overcome this defense reaction induce syncytia in the pericycle or procambium. Seven DAI syncytia induced in the procambium are degenerated and juveniles cannot develop after the J2 stage. Simultaneously, syncytia composed of pericycle cells survive (Fig. 17). A layer of necrotic cells separates them from the conductive elements. Syncytial cytoplasm proliferates but is relatively electron translucent. The number of organelles slightly increases. Nuclei are somewhat enlarged and acquire amoeboid shapes. The organelles are paramurally located because the central parts of syncytial elements embody remnants of the central vacuoles.

Syncytia associated with J3-stage nematodes reveal typical ultrastructural features, such as condensed cytoplasm, abundance of the ER, and a few small vacuoles (Soliman et al. 2005). However, they do not establish a direct interface with conductive elements as they are separated by necrotic cells or nonincorporated vascular parenchyma cells. Cell-wall ingrowths may be formed on syncytial walls facing xylem parenchyma cells (Golinowski and Magnusson 1991). Such syncytia are only able to support developing males (Soliman et al. 2005).

3.1.3 Soybean

The defense response to *H. glycines* is best studied in resistant soybean (*Glycine max*) plants. J2 induce syncytia in vascular cylinder cells (Endo 1991). In resistant cv. “Bedford” the syncytia are made up of a small number of slightly enlarged pericyclic cells interconnected by narrow cell-wall openings, 18 h after inoculation. The syncytial cytoplasm and nucleoplasm are electron dense and appear to be deteriorating. However, many mitochondria and dilated ER structures are recognizable.

Remnants of the central vacuole and a number of small cytoplasmic vacuoles are present (Endo 1991). At five DAI the syncytial cytoplasm appears to be decomposing, though some of the syncytial nuclei are heterochromatic while others have large patches of clumped chromatin, electron translucent nucleoplasm, and a damaged nuclear envelope (Kim et al. 1987). Syncytial walls are thickened and cell-wall openings are present. The plasmalemma is retracted from the syncytial wall. Finally, 10–15 DAI syncytial cytoplasm and nuclei are completely disintegrated, leaving only debris. No necrosis occurs in cells around the degrading syncytium (Kim et al. 1987).

A similar defense mechanism occurs in cyst nematode resistant cv. "Peking" (Endo 1965; Riggs et al. 1973). The syncytia are induced and initially develop as in susceptible plants. Four DAI in the syncytial cytoplasm numerous vacuoles, lipid bodies, and irregular aggregations of ER membranes appear prior to degradation. Cell-wall openings are formed but syncytial walls are thickened. Numerous invaginations of plasma membrane forming paramural bodies occur in close association with the thickened syncytial wall (Riggs et al. 1973). In five to seven DAI syncytia, cytoplasm is electron dense and clumped. Cell-wall thickening occurs in syncytia and in some degenerating cells next to them. Large amounts of cell wall material create a barrier that seals off the diseased region, preventing the influx of nutrients necessary for syncytium maintenance and nematode development (Riggs et al. 1973). Such degraded syncytia are associated with dead J2 (Endo 1965).

In another resistant soybean, cv. "Pickett71," syncytia are induced in the vascular cylinder, and these preferentially incorporate pericyclic cells (Endo 1991). Two DAI clusters of rough ER membranes are present in electron translucent syncytial cytoplasm that has many small vacuoles. In four DAI syncytia, enlarged syncytial elements that contain syncytial cytoplasm debris are interconnected by wide cell-wall openings.

In contrast, necrosis occurs in the cyst nematode-resistant soybean cv. "Forrest" in cells surrounding the syncytium (Kim et al. 1987). Necrosis begins about five DAI, first in the region next to the nematode head. Later this extends into the region surrounding the entire syncytium, isolating it from neighboring live cells. Additionally, syncytial walls facing the necrotized cells are thickened, preventing further nutrient influx. At 15 DAI the syncytia are completely necrotic and only have electron opaque remnants of the protoplast (Kim et al. 1987).

3.1.4 Other Fabaceae

Less is known about the structure of syncytia induced in other Fabaceous plants by different *Heterodera* sp. Juveniles of *H. ciceri* induce small syncytia in roots of cyst nematode-resistant *Cicer* plants (Di Vito and Vovlas 1990). Most of the syncytia become necrotic several DAI at which point few produce females. Unfortunately, the details of syncytium structure and a possible cytological background of this defense mechanism were not examined.

In resistant pea lines infected with J2 of *H. goettingiana*, syncytia are induced in pericycle or procambium (Bleve-Zacheo et al. 1990a; Melillo et al. 1990b). With the onset of syncytium development, the cytoplasm is less electron dense compared to syncytia induced in susceptible hosts (Bleve-Zacheo et al. 1990a). However, these syncytia reveal other typical features: proliferation of mitochondria and plastids that contain starch grains, abundant ER, hypertrophied nuclei and nucleoli, and replacement of the central vacuole by numerous small vacuoles. The syncytial elements are enlarged and interconnected by wide cell-wall openings. At four DAI the degeneration process begins in the syncytial elements furthest from the nematode head. These elements contain condensed cytoplasm with large vacuoles and ER structures accumulating around them. Ten DAI syncytial cytoplasm and nuclei are completely disintegrated and electron opaque (Bleve-Zacheo et al. 1990a; Melillo et al. 1990b). The juveniles associated with such syncytia fail to develop after the J3 stage (Melillo et al. 1990b).

3.1.5 Monocots

For cyst nematodes infecting monocot plants the best known are syncytia induced by *H. avenae* in wheat and oat (Bleve-Zacheo et al. 1995; Grymaszewska and Golinowski 1991; Williams and Fisher 1993). In resistant wheat, juveniles invade roots and induce syncytia inside the vascular cylinder. However, these syncytia may not incorporate xylem parenchyma cells but are restricted to the cells located at some distance from the xylem tracheary elements (stellar parenchyma) (Grymaszewska and Golinowski 1991). Initial development of syncytia in resistant plants is the same as in susceptible plants. Syncytia in resistant plants become necrotic about 15 DAI. Degrading syncytia contain large vacuoles and less cytoplasm (Williams and Fisher 1993). In addition, they are surrounded by a layer of necrotic cells (Grymaszewska and Golinowski 1991). These syncytia only support developing males while females are arrested in the J4 stage (Williams and Fisher 1993). Different timing of defense reactions occurs in other resistant wheat genotypes, where necrotic reactions begin at five DAI and are followed by deposition of callose-like material and accumulation of osmiophilic granules at syncytial walls (Bleve-Zacheo et al. 1995).

In cyst nematode resistant oat infected with two different isolates of *H. avenae*, different defense reactions are induced depending on the nematode genotype (Bleve-Zacheo et al. 1995). One nematode isolate could invade roots and induce syncytia in vascular cylinder cells, however, xylem parenchyma was not incorporated into the syncytium. Three DAI syncytia are composed of a few enlarged cells interconnected by narrow cell-wall openings. The cytoplasm proliferates and is electron dense. The syncytial nuclei are enlarged and amoeboid, and chromatin is condensed. The most remarkable feature is the lack of cristae in syncytial mitochondria. The syncytium is surrounded by necrotic endodermal, pericyclic, and nonmodified procambial cells. Another isolate of *H. avenae* induces syncytia in cortical cells of resistant oat roots (Bleve-Zacheo et al. 1995). The syncytial cytoplasm is electron dense and contains many tubular ER structures and numerous

mitochondria. Syncytial walls are thickened and contain callose-like deposits. Cell-wall openings are formed and cytoplasm is confluent.

3.2 Resistant Responses to Globodera sp

Interaction between *G. rostochiensis* and potato carrying the *H1* resistance gene is probably the best known defense reaction against *Globodera* sp. (Bleve-Zacheo et al. 1990b; Rice et al. 1985, 1987; Turner and Stone 1984). As in susceptible interactions, juveniles invade host roots and induce syncytia in cortical cells. By forming cell-wall openings, syncytia develop centripetally and incorporate cortical, endodermal, pericyclic, and procambial cells. The syncytium spreads into the vascular cylinder and is in direct contact with conductive elements (Bleve-Zacheo et al. 1990b; Rice et al. 1985). However, the incorporation of vascular cylinder cells usually fails and the syncytium is located alongside the stele. Proliferation of the syncytial cytoplasm does not occur or is weak. Cytoplasm is electron dense and in a paramural location.

Central vacuoles are present in syncytial elements, and many small vacuoles appear within the syncytial cytoplasm. Depending on the report, syncytial nuclei and nucleoli enlarge (Rice et al. 1985) or are amoeboid and degenerated with advanced karyolysis (Bleve-Zacheo et al. 1990b). Plastids contain starch grains and crystalline inclusions (Bleve-Zacheo et al. 1990b; Rice et al. 1985). The protoplast is extremely vacuolated and the cytoplasm and mitochondria are degenerated in seven DAI syncytia. Syncytial cell walls are locally thickened and no cell-wall ingrowths are formed. Necrosis appears first around the juvenile and then along syncytia that surround and isolate them from direct contact with conductive elements and other non-necrotic cells (Bleve-Zacheo et al. 1990b; Rice et al. 1985).

A similar response occurs in a resistant *Solanum andigena* clone infected with *G. pallida* (Blok, Janakowski, Sobczak, unpubl.). In this case the syncytium is small and composed of cortical, endodermal, and pericyclic cells (Fig. 18) that may have failed to incorporate procambial cells and thus lack a direct prominent interface with the conductive elements. Proliferation of the syncytial cytoplasm is weak and central vacuoles are present. Syncytial cytoplasm appears to be deteriorating. The necroses around syncytium are abundant four DAI and lead to syncytium isolation and total degeneration seven DAI.

In resistant tomato carrying the *Hero* gene cluster infected with the Ro1 pathotype of *G. rostochiensis*, a syncytium also develops (Sobczak et al. 2005). By two DAI necrotic cells appear around the syncytium. At four DAI the syncytium is surrounded by a continuous layer of necrotic cells and its cytoplasm is granular and electron translucent (Fig. 19). Large vacuoles and numerous small vacuoles are present. Later, the syncytium is empty or contains debris of the degraded cytoplasm. However, in the case of the *Hero* gene-containing plants, some syncytia resist this initial defense response and develop further. Most of the associated juveniles develop into males, but a few are able to develop into females (Sobczak et al. 2005). Syncytia associated with J3 males are smaller and composed of fewer cells

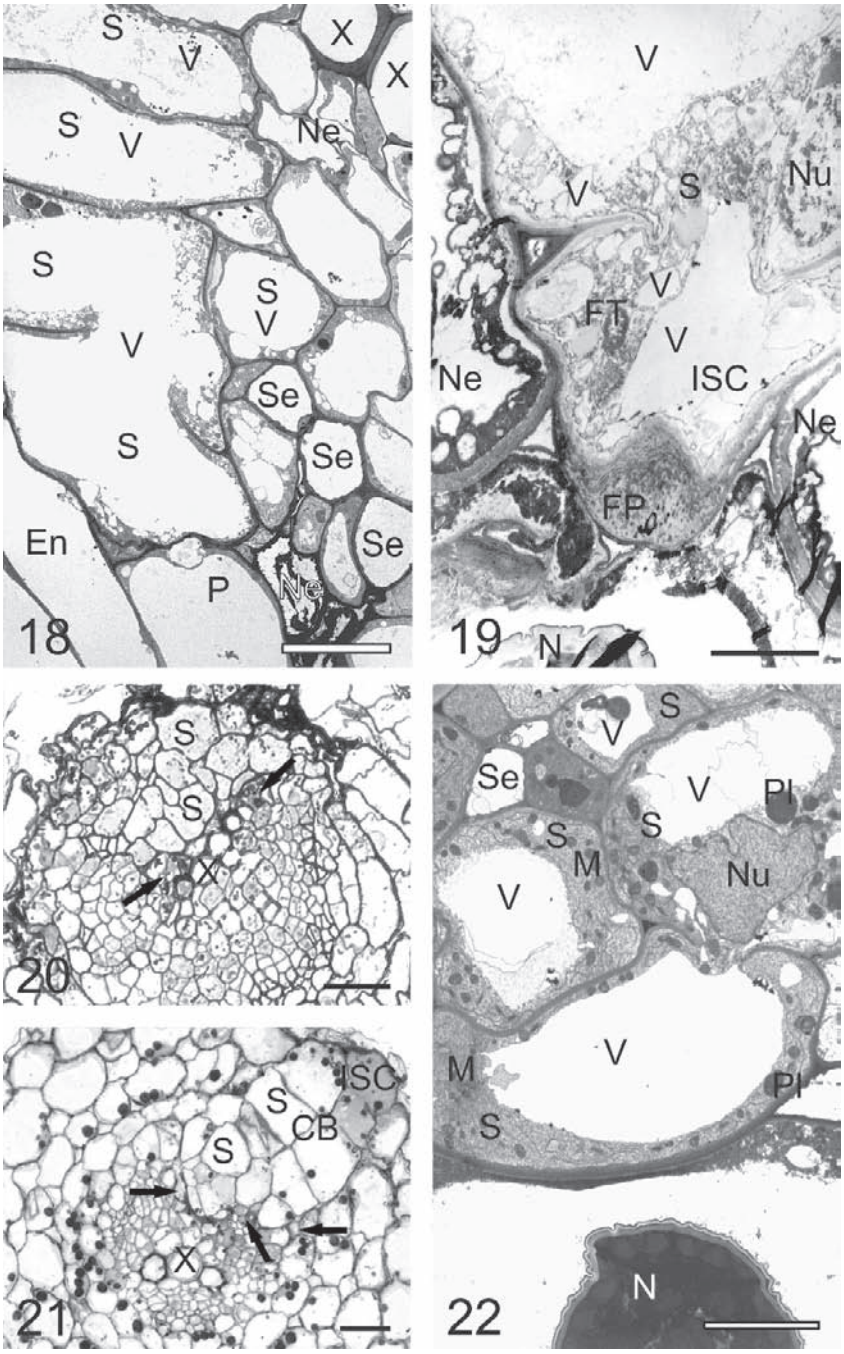


Fig. 18 Ultrastructure of a syncytium (S) induced in resistant *S. andigena* clone by *G. pallida* (4 DAI). Cytoplasm is located paramurally and central vacuoles (V) are still present. (En, endodermis; Ne, necrosis; P, pericycle; Se, sieve tube; X, xylem). *Bar* 2 μm. (*courtesy of S Janakowski*)

compared to syncytia associated with J3 females. They invade the vascular cylinder and incorporate procambial cells. In contrast to syncytia associated with females, they are separated from vessels by a layer of necrotic cells (Fig. 20). Compared to J3 females, there are minor ultrastructural differences in syncytia associated with J3 males (Sobczak, Kumar, unpubl.): fewer and smaller cell-wall openings, weaker cytoplasm proliferation, less hypertrophied nuclei, and more small vacuoles.

When the *Hero* gene was cloned (Ernst et al. 2002) and transgenic plants became available, its mode of action was examined. In general, the features of the defense reaction are the same as in plants containing the *Hero* gene cluster (Sobczak et al. 2005). Syncytia are induced in the cortex and spread toward the vascular cylinder, but therein they only incorporate pericyclic cells (Fig. 21). By four DAI they are surrounded by necrotic cells. However, in the case of the transgenic plants, the defense response seems to be more rapid and abrupt. The cytoplasm is granular and osmiophilic, and organelles are unrecognizable. The syncytial protoplast is plasmolyzed and there is callose-like material deposition. In the examined transgenic line, only about 6% of juveniles are able to reach J3 stage in contrast to 39% of invading juveniles that develop into J3 and adults in plants with the *Hero* gene cluster (Sobczak et al. 2005).

Resistance against *G. pallida* occurs in some clones of *S. vernei* (Rice et al. 1987) and *S. canasense* (Castelli et al. 2006). In the former case, syncytia are induced in the cortex and centripetally spread to the vascular cylinder. Therein they only incorporate pericyclic cells (Rice et al. 1987). The proliferation of syncytial cytoplasm is very weak. Syncytia continuously enlarge until seven DAI by hypertrophy of incorporated cells. The cytoplasm is electron dense in syncytia four DAI and many small vacuoles are formed. The syncytial nuclei are enlarged and often plastids accumulate in their vicinity. The degree of syncytial abnormality is variable, with some syncytia showing no signs of degradation and resembling syncytia induced in susceptible plants. Seven DAI syncytia are usually vacuolated and only contain degraded cytoplasm. But there still are fewer syncytia developed that show no structural abnormality. During the entire time of syncytium development, there is no necrotic layer formed around them. Only a few J3 are able to develop by about 10 DAI (Rice et al. 1987).

Fig. 19 Ultrastructure of a syncytium (S) induced by juvenile (N) of *G. rostochiensis* in resistant tomato line LA1792 carrying *Hero* gene cluster (4 DAI). Syncytium is surrounded by necrotic cells (Ne) and contains degenerated cytoplasm with numerous small and large vacuoles (V). (FP, feeding plug; FT, feeding tube; ISC, initial syncytial cell; Nu, nucleus). *Bar* 3 μm

Fig. 20 Anatomy of a syncytium (S) induced in resistant tomato line LA1792 carrying the *Hero* gene cluster associated with J3 male of *G. rostochiensis* (10 DAI). Syncytium is separated from xylem (X) by a layer of necrotic cells (*arrows*). *Bar* 20 μm

Fig. 21 Anatomy of a syncytium (S) induced by *G. rostochiensis* in resistant transgenic tomato line carrying the *HeroA* gene alone (2 DAI). Syncytium is separated from the vascular cylinder by a layer of necrotic cells (*arrows*). (CB, cortex bridge; ISC, initial syncytial cell; X, xylem). *Bar* 20 μm

Fig. 22 Ultrastructure of a syncytium (S) induced by *G. rostochiensis* in transgenic potato line having the silenced *cel9C1* gene (7 DAI). Syncytial elements are weakly hypertrophied and still contain central vacuoles (V). (N, nematode; Nu, nucleus; M, mitochondrion; Pl, plastid; Se, sieve tube). *Bar* 5 μm. (*courtesy of W Kurek*)

In the case of *S. canasense*, *G. pallida* J2 infect roots with low efficiency and yet fewer numbers successfully induce ISC (Castelli et al. 2006). The whole process of root invasion, migration, and ISC selection is delayed compared to the susceptible interaction. Additionally, many juveniles apparently leave the roots after invasion. If successful, J2 induce syncytia in the cortex. The syncytia consist of cortex bridge elements and a few pericyclic and parenchymatic vascular cylinder cells. The syncytial cytoplasm is granular and retracts from walls in seven DAI syncytia. Numerous small vacuoles are formed. Plastids and mitochondria are not structurally changed, and some plastids appear to divide. The nuclei are hypertrophied and amoeboid. No necrotic cell layer is formed around syncytia that appear to degenerate before seven DAI (Castelli et al. 2006).

3.3 Artificial Resistance of Transgenic Plants

Because of the shortage of valuable cyst nematode resistance genes and the considerable limitations in their transfer to crops as well as their narrow specificity, the idea of an "artificial" resistance against plant nematodes has been raised (Sijmons et al. 1994). This idea concerns detailed examination of the susceptible interaction and selection of the most important plant susceptibility traits that thereafter could be specifically inhibited or silenced in syncytia (de Almeida Engler et al. 2005). Artificial resistance should lead to syncytium degradation or at least to its disturbed development, and promote development of males or decrease the number or fecundity of females. Such resistance seems promising as most cyst nematode resistance genes act this way.

In the case of *G. rostochiensis*, these susceptibility traits appear to be tomato endoglucanases *Cel7* and *Cel9C1* (Karczmarek et al. 2008). Transgenic potato plants having silenced one of these genes via RNAi appear to reduce reproduction of *G. rostochiensis*. At the anatomical level, syncytia induced in transgenic plants are usually induced in the cortex. Through formation of the cortex bridge, they spread into the vascular cylinder (Kurek, Janakowski, Fudali, Sobczak, Karczmarek, Helder, Goverse, unpubl.). Inside the vascular cylinder all parenchymatic cells are incorporated into the syncytium. Thus, there are no cells remaining that could develop into secondary xylem or phloem elements or form a peridermis-like tissue surrounding the syncytium. The extent of hypertrophy of syncytial elements is relatively low and only a few cell-wall openings are formed (Fig. 22). Ultrastructurally, syncytial protoplasts reveal most of the typical syncytia features such as condensed cytoplasm, abundance of plastids, mitochondria and ER, and hypertrophy of nuclei and nucleoli. However, large vacuoles are present long after syncytium induction. In general, it seems that silencing of *Cel7* and *Cel9C1* endoglucanases decreases the number of cell-wall openings, but more importantly it hampers the hypertrophy of syncytial elements, making syncytia less efficient and unable to support development of females and oviposition.

Acknowledgments The authors are grateful to Vivian Blok, Sylwia Fudali, Aska Goverse, Florian Grundler, Grazyna Grymaszewska, Hans Helder, Slawomir Janakowski, Aneta Karczmarek, Wojciech Kurek, Didier Mugniéry, and Ahmend Soliman for providing unpublished figures, data, and research material that were used to prepare this publication. Some of the author's research presented above was supported by the "NONEMA" (QLRT-1999-1501) and "DREAM" (QLRT-1999-1462) projects of the 5th EU Framework Programme and research grants of the Polish State Committee for Scientific Research.

References

Acedo JR, Dropkin VH, Luedders VD (1984) Nematode population attrition and histopathology of *Heterodera glycines*-soybean associations. J Nematol 16:48–57

Atkinson HJ, Urwin PE, McPherson MJ (2003) Engineering plants for nematode resistance. *Annu* Rev Phytopathol 41:615–639

Bakhetia M, Charlton WL, Urwin PE, McPherson MJ, Atkinson HJ (2005) RNA interference and plant parasitic nematodes. Trends Plant Sci 10:362–367

Berg RH, Fester T, Taylor CG (2008) Development of the root-knot nematode feeding cell. Plant Cell Monogr, doi:10.1007/7089_2008_30

Betka M, Grundler F, Wyss U (1991) Influence of changes in the nurse cell system (syncytium) on the development of the cyst nematode *Heterodera schachtii*: single amino acids. Phytopathology 81:75–79

Bleve-Zacheo T, Zacheo G (1987) Cytological studies of the susceptible reaction of sugarbeet roots to *Heterodera schachtii*. Physiol Mol Plant Pathol 30:13–25

Bleve-Zacheo T, Melillo MT, Zacheo G (1990a) Syncytia development in germplasm pea accessions infected with *Heterodera goettingiana*. Nematol Mediter 18:93–102

Bleve-Zacheo T, Melillo MT, Zacheo G (1990b) Ultrastructural response of potato roots resistant to cyst nematode *Globodera rostochiensis* pathotype Ro1. Rev Nématol 13:29–36

Bleve-Zacheo T, Melillo MT, Andres M, Zacheo G, Romero MD (1995) Ultrastructure of initial response of graminaceous roots to infection by *Heterodera avenae*. Nematologica 41:80–97

Böckenhoff A, Grundler FMW (1994) Studies on the nutrient uptake by the beet cyst nematode *Heterodera schachtii* by in situ microinjection of fluorescent probes into the feeding structures in *Arabidopsis thaliana*. Parasitology 109:249–254

Böckenhoff A, Prior DAM, Grundler FMW, Oparka KJ (1996) Induction of phloem unloading in *Arabidopsis thaliana* roots by parasitic nematode *Heterodera schachtii*. Plant Physiol 112:1421–1427

Brodie B, Evans K, Franco J (1998) Nematode parasites of potato. In: Evans K, Trudgill DL, Webster JM (eds) Plant parasitic nematodes in temperate agriculture. CAB International, Wallingford, pp 87–132

Castelli L, Bryan G, Blok VG, Ramsay G, Sobczak M, Gillespie T, Phillips MS (2006) Investigations of *Globodera pallida* invasion and syncytia formation within roots of the susceptible potato cultivar Désirée and resistant species S*olanum canasense*. Nematology 8:103–110

Caswell-Chen EP, Thomason IJ (1993) Root volumes occupied by different stages of *Heterodera schachtii* in sugarbeet, *Beta vulgaris*. Fundam Appl Nematol 16:39–42

Davis EL, Hussey RS, Baum TJ (2008) Parasitism genes: what they reveal about parasitism. Plant Cell Monogr, doi: 10.1007/7089_2008_29

de Almeida Engler J, De Vleesschauwer V, Burssens S, Celenza JL, Inzé D, Van Montagu M, Engler G, Gheysen G (1999) Molecular markers and cell cycle inhibitors show the importance of cell cycle progression in nematode-induced galls and syncytia. Plant Cell 11:793–807

de Almeida Engler J, Van Poucke K, Karimi M, De Groodt R, Gheysen G, Engler G, Gheysen G (2004) Dynamic cytoskeleton rearrangements in giant cells and syncytia of nematode-infected roots. Plant J 38:12–26

de Almeida Engler J, Favery B, Engler G, Abad P (2005) Loss of susceptibility as an alternative for nematode resistance. Curr Opin Biotechnol 16:112–117

Di Vito M, Vovlas N (1990) Differential histological response of selected *Cicer bijugum* and *C. arietinum* lines to *Heterodera ciceri*. Nematol Mediter 18:165–167

Endo BY (1964) Penetration and development of *Heterodera glycines* in soybean roots and related anatomical changes. Phytopathology 54:79–88

Endo BY (1965) Histological responses of resistant and susceptible soybean varieties, and back-cross progeny to entry and development of *Heterodera glycines*. Phytopathology 55:375–381

Endo BY (1971) Synthesis of nucleic acids at infection sites of soybean roots parasitized by *Heterodera glycines*. Phytopathology 61:395–399

Endo BY (1978) Feeding plug formation in soybean roots infected with the soybean cyst nematode. Phytopathology 68:1022–1031

Endo BY (1991) Ultrastructure of initial responses of susceptible and resistant soybean roots to infection by *Heterodera glycines*. Rev Nématol 14:73–94

Ernst K, Kumar A, Kriseleit D, Kloos D-U, Phillips MS, Ganal MW (2002) The broad-spectrum potato cyst nematode resistance gene (*Hero*) from tomato is the only member of a large gene family of NBS-LRR genes with an unusual amino acid repeat in the LRR region. Plant J 31:127–136

Fudali S, Janakowski S, Sobczak M, Griesser M, Grundler FMW, Golinowski W (2008) Two tomato α-expansins show distinct spatial and temporal expression patterns during development of nematode-induced syncytia. Physiol Plant 132:370–383

Gheysen G, Fenoll C (2002) Gene expression in nematode feeding sites. Annu Rev Phytopathol 40:191–210

Gheysen G, Mitchum MG (2008) Molecular insights in the susceptible plant response to nematode infection. Plant Cell Monogr, doi:10.1007/7089_2008_35

Gipson I, Kim KS, Riggs RD (1971) An ultrastuctural study of syncytium development in soybean roots infected with *Heterodera glycines*. Phytopathology 61:347–353

Goellner M, Wang X, Davis EL (2001) Endo-ß-1,4-glucanase expression in compatible plant–nematode interactions. Plant Cell 13:2241–2255

Golinowski W, Magnusson C (1991) Tissue response induced by *Heterodera schachtii* (Nematoda) in susceptible and resistant white mustard cultivars. Can J Bot 69:53–62

Golinowski W, Grundler FMW, Sobczak M (1996) Changes in the structure of *Arabidopsis thaliana* during female development of the plant-parasitic nematode *Heterodera schachtii*. Protoplasma 194:103–116

Golinowski W, Sobczak M, Kurek W, Grymaszewska G (1997) The structure of syncytia. In: Fenoll C, Grundler FMW, Ohl S (eds) Cellular and molecular aspects of plant–nematode interactions. Kluwer, Dordrecht, pp 80–97

Goverse A, Biesheuvel J, Wijers G-J, Gommers FJ, Bakker J, Schots A, Helder J (1998) *In planta* monitoring of the activity of two constitutive promoters, CaMV 35S and TR2', in developing feeding cells induced by *Globodera rostochiensis* using green fluorescent protein in combination with confocal laser scanning microscopy. Physiol Mol Plant Pathol 52:275–284

Grundler F, Betka M, Wyss U (1991) Influence of changes in the nurse cell system (syncytium) on sex determination and development of the cyst nematode *Heterodera schachtii*: total amounts of proteins and amino acids. Phytopathology 81:70–74

Grundler FMW, Sobczak M, Lange S (1997) Defence responses of *Arabidopsis thaliana* during invasion and feeding site induction by the plant-parasitic nematode *Heterodera glycines*. Physiol Mol Plant Pathol 50:419–429

Grundler FMW, Sobczak M, Golinowski W (1998) Formation of wall openings in root cells of *Arabidopsis thaliana* following infection by the plant-parasitic nematode *Heterodera schachtii*. Europ J Plant Pathol 104:545–551

Grymaszewska G, Golinowski W (1991) Structure of syncytia induced by *Heterodera avenae* Woll. in roots of susceptible and resistant wheat (*Triticum aestivum* L.). J Phytopathol 133:307–319

Grymaszewska G, Golinowski W (1998) Structure of syncytia induced by *Heterodera schachtii* Schmidt in roots of susceptible and resistant radish (*Raphanus sativus* L. *var. oleiformis*). Acta Soc Bot Pol 67:207–216

Hofmann J, Grundler FMW (2006) Females and males of root-parasitic cyst nematodes induce different symplasmic connections between their syncytial feeding cells and the phloem in *Arabidopsis thaliana*. Plant Physiol Biochem 44:430–433

Hofmann J, Wieczorek K, Blöchl A, Grundler FMW (2007) Sucrose supply to nematode-induced syncytia depends on the apoplasmic and symplasmic pathways. J Exp Bot 58:1591–1601

Hofmann J, Szakasits D, Sobczak M, Daxböck-Horvath S, Golinowski W, Bohlmann H, Grundler FMW (2008) Starch serves as carbohydrate storage in nematode-induced syncytia. Plant Physiol 146:228–235

Holtmann B, Kleine M, Grundler FMW (2000) Ultrastructure and anatomy of nematode-induced syncytia in roots of susceptible and resistant sugar beet. Protoplasma 211:39–50

Hoth S, Schneidereit A, Lauterbach C, Scholz-Starke J, Sauer N (2005) Nematode infection triggers the de novo formation of unloading phloem that allows macromolecular trafficking of green fluorescent protein into syncytia. Plant Physiol 138:383–392

Hussey RS, Grundler FMW (1998) Nematode parasitism of plants. In*:* Perry RN, Wright DJ (eds) The physiology and biochemistry of free-living and plant-parasitic nematodes. CAB International, Wallingford, pp 213–243

Hussey RS, Mims CW (1991) Ultrastructure of feeding tubes formed in giant-cells induced in plants by the root-knot nematode *Meloidogyne incognita*. Protoplasma 162:99–107

Hussey RS, Mims CW, Westcott III SW (1992) Immunocytochemical localization of callose in root cortical cells parasitized by the ring nematode *Criconemella xenoplax*. Protoplasma 171:1–6

Jones MGK, Dropkin VH (1975) Cellular alterations induced in soybean roots by three endoparasitic nematodes. Physiol Plant Pathol 5:119–124

Jones MGK, Northcote DH (1972) Nematode-induced syncytium – a multinucleate transfer cell. J Cell Sci 10:789–809

Jones MGK, Payne HL (1977a) Scanning electron microscopy of syncytia induced by *Nacobbus aberrans* in tomato roots. Nematologica 23:172–176

Jones MGK, Payne HL (1977b) The structure of syncytia induced by the phytoparasitic nematode *Nacobbus aberrans* in tomato roots, and the possible role of plasmodesmata in their nutrition. J Cell Sci 23:299–313

Juergensen K, Scholz-Starke J, Sauer N, Hess P, van Bel AJE, Grundler FMW (2003) The companion cell-specific Arabidopsis disaccharide carrier AtSUC2 is expressed in nematode-induced syncytia. Plant Physiol 131:61–69

Karczmarek A, Fudali S, Lichocka M, Sobczak M, Kurek W, Janakowski S, Roosien J, Golinowski W, Bakker J, Goverse A, Helder J (2008) Expression of two functionally distinct plant endo-β-1, 4-glucanases is essential for the compatible interaction between potato cyst nematode and its hosts. Mol Plant Microbe Interact 21:791–798

Kim YH, Kim KS, Riggs RD (1986) Morphological characteristics of syncytia in susceptible hosts infected by the soybean cyst nematode. Phytopathology 76:913–917

Kim YH, Riggs RD, Kim KS (1987) Structural changes associated with resistance of soybean to *Heterodera glycines*. J Nematol 19:177–187

Li L, Fester T, Taylor CG (2008) Transcriptomic analysis of nematode infestation. Plant Cell Monogr., doi: 10.1007/7089_2008_36

Magnusson C, Golinowski W (1991) Ultrastructural relationships of the developing syncytium induced by *Heterodera schachtii* (Nematoda) in root tissue of rape. Can J Bot 69:44–52

McCarter JP (2008) Molecular approaches toward resistance to plant-parasitic nematodes. Plant Cell Monogr., doi:10.1007/7089_2008_32

Melillo MT, Bleve-Zacheo T, Zacheo G (1990a) Ultrastructural response of potato roots susceptible to cyst nematode *Globodera pallida* pathotype Pa3. Rev Nématol 13:17–28

Melillo MT, Bleve-Zacheo T, Zacheo G, Perrino P (1990b) Morphology and enzyme histochemistry in germplasm pea roots attacked by *Heterodera goettingiana*. Nematol Mediter 18:83–91

Mugniéry D, Bossis M (1985) Influence de l'hôte, de la compétition et de l'état physiologique des juvéniles sur la pénétration, le développement et la sexe d'*Heterodera carotae* Jones. Nematologica 31:335–343

Mugniéry D, Fayet G (1981) Détermination du sexe chez *Globodera pallida* Stone. Rev Nématol 4:41–45

Mugniéry D, Fayet G (1984) Détermination du sexe de *Globodera rostochiensis* Woll. et influence des niveaux d'infestation sur la pénétration, le développement et le sexe de ce nématode. Rev Nématol 7:233–238

Müller J (1998) New pathotypes of the beet cyst nematode (*Heterodera schachtii*) differentiated on alien genes for resistance in beet (*Beta vulgaris*). Fundam Appl Nematol 21:519–526

Müller J, Rehbock K, Wyss U (1982) Growth of *Heterodera schachtii* with remarks on amounts of food consumed. Rev Nématol 4:227–234

Offler CE, McCurdy DW, Patrick JW, Talbot MJ (2002) Transfer cells: cells specialized for special purpose. Annu Rev Plant Biol 54:431–454

Oparka KJ, Prior DAM (1992) Direct evidence for pressure-generated closure of plasmodesmata. Plant J 2:741–750

Opperman CH, Bird DMcK, Schaff JE (2008) Genomic analysis of the root-knot nematode genome. Plant Cell Monogr, doi:10.1007/7089_2008_37

Phillips MS, Forrest JMS, Farrer LA (1982) Invasion and development of juveniles of *Globodera pallida* in hybrids of *Solanum vernei* x *S. tuberosum*. Ann Appl Biol 100:337–344

Qin L, Kudla U, Roze EHA, Goverse A, Popeijus H, Nieuwland J, Overmars H, Jones JT, Schots A, Smant G, Bakker J, Helder J (2004) A nematode expansin acting on plants. *Nature* 427:30

Rebois RV (1980) Ultrastructure of a feeding peg and tube associated with *Rotylenchulus reniformis* in cotton. Nematologica 26:396–405

Rice SL, Leadbeater BSC, Stone AR (1985) Changes in cell structure in roots of resistant potatoes parasitized by potato cyst nematodes. I. Potatoes with resistance gene H1 derived from *Solanum tuberosum* ssp. *andigena*. Physiol Plant Pathol 27:219–234

Rice SL, Stone AR, Leadbeater BSC (1987) Changes in cell structure in roots of resistant potatoes parasitized by potato cyst nematodes. 2. Potatoes with resistance derived from *Solanum vernei*. Physiol Mol Plant Pathol 31:1–14

Riggs RD, Kim KS, Gipson I (1973) Ultrastructural changes in Peking soybeans infected with *Heterodera glycines*. Phytopathology 63:76–84

Rumpenhorst HJ (1984) Intracellular feeding tubes associated with sedentary plant parasitic nematodes. Nematologica 30:77–85

Sembdner G (1963) Anatomie der *Heterodera-rostochiensis*-Gallen an Tomatenwurzeln. Nematologica 9:55–64

Sijmons PC, Grundler FMW, von Mende N, Burrows PR, Wyss U (1991) *Arabidopsis thaliana* as a new model host for plant-parasitic nematodes. Plant J 1:245–254

Sijmons PC, Atkinson HJ, Wyss U (1994) Parasitic strategies of root nematodes and associated host cell responses. Annu Rev Phytopathol 32:235–259

Smant G, Stokkermans JPWG, Yan Y, de Boer JM, Baum TJ, Wang X, Hussey RS, Gommers FJ, Henrissat B, Davis EL, Helder J, Schots A, Bakker J (1998) Endogenous cellulases in animals: Isolation of ß-1,4-endoglucanase genes from two species of plant-parasitic cyst nematodes. Proc Natl Acad Sci USA 95:4906–4911

Sobczak M (1996) Investigations on the structure of syncytia in roots of *Arabidopsis thaliana* induced by the beet cyst nematode *Heterodera schachtii* and its relevance to the sex of the nematode. PhD Thesis, University of Kiel

Sobczak M, Golinowski W, Grundler FMW (1997) Changes in the structure of *Arabidopsis thaliana* roots induced during development of males of the plant parasitic nematode *Heterodera schachtii*. Europ J Plant Pathol 103:113–124

Sobczak M, Golinowski W, Grundler FMW (1999) Ultrastructure of feeding plugs and feeding tubes formed by *Heterodera schachtii*. Nematology 1:363–374

Sobczak M, Avrova A, Jupowicz J, Phillips MS, Ernst K, Kumar A (2005) Characterization of susceptibility and resistance responses to potato cyst nematode (*Globodera* spp.) infection of tomato lines in the absence and presence of the broad-spectrum nematode resistance *Hero* gene. Mol Plant Microbe Interact 18:158–168

Soliman AH, Sobczak M, Golinowski W (2005) Defence responses of white mustard, *Sinapis alba*, to infection with the cyst nematode *Heterodera schachtii*. Nematology 7:881–889

Subbotin SA (1990) Ultrastructural changes in cells of roots of *Glycine max* (Fabaceae) infected with cyst and root-knot nematodes. Botan Z 75:315–325

Tomczak A, Koropacka K, Smant G, Goverse A, Bakker E (2008) Resistant plant responses. Plant Cell Monogr, doi:10.1007/7089_2008_39

Turner SJ, Stone AR (1984) Development of potato cyst-nematodes in roots of resistant *Solanum tuberosum* ssp. *andigena* and *S. vernei* hybrids. Nematologica 30:324–332

Urwin PE, Møller SG, Lilley CJ, McPherson MJ, Atkinson HJ (1997) Continual green-fluorescent protein monitoring of cauliflower mosaic virus 35S promoter activity in nematode-induced feeding cells in *Arabidopsis thaliana*. Mol Plant Microbe Interact 10:394–400

Vanholme B, De Meutter J, Tytgat T, Van Montagu M, Coomans A, Gheysen G (2004) Secretions of plant-parasitic nematodes: a molecular update. Gene 332:13–27

Valentine TA, Randall E, Wypijewski K, Chapman S, Jones J, Oparka KJ (2007) Delivery of macromolecules to plant parasitic nematodes using a tobacco rattle virus vector. Plant Biotech J 5:827–834

Wieczorek K, Golecki B, Gerdes L, Heinen P, Szakasits D, Durachko DM, Cosgrove DJ, Kreil DP, Puzio PS, Bohlmann H, Grundler FMW (2006) Expansins are involved in the formation of nematode-induced syncytia in roots of *Arabidopsis thaliana*. Plant J 48:98–112

Wieczorek K, Hofmann J, Blöchl A, Szakasits D, Bohlmann H, Grundler FMW (2008) Arabidopsis endo-1,4-ß-glucanases are involved in the formation of root syncytia induced by *Heterodera schachtii*. Plant J 53:336–351

Williams KJ, Fisher JM (1993) Development of *Heterodera avenae* Woll. and host cellular responses in susceptible and resistant wheat. Fundam Appl Nematol 16:417–423

Wyss U (1992) Observations on the feeding behaviour of *Heterodera schachtii* throughout development, including events during moulting. Fundam Appl Nematol 15:75–89

Wyss U, Zunke U (1986) Observations on the behaviour of second stage juveniles of *Heterodera schachtii* inside host roots. Rev Nématol 9:153–165

Wyss U, Stender C, Lehmann H (1984) Ultrastructure of feeding sites of the cyst nematode *Heterodera schachtii* Schmidt in roots of susceptible and resistant *Raphanus sativus* L. var. *oleiformis* Pers. cultivars. Physiol Plant Pathol 25:21–37

Yu MH, Steele AE (1981) Host–parasite interaction of resistant sugarbeet and *Heterodera schachtii*. J Nematol 13:206–212

Transcriptomic Analysis of Nematode Infestation

Yuhong Li, Thomas Fester, and Christopher G. Taylor(✉)

Abstract The establishment and maintenance of nematode feeding sites and subsequent nematode development are associated with significant changes in expression level of both plant and nematode genes. Analysis of the changes in transcript abundance during feeding site establishment and function provides insight into the complexity of plant–nematode interactions and may lead to the discovery of novel strategies for managing nematodes. Early techniques of gene discovery had identified a small cadre of genes induced in the plant host or invading nematode. More recently, the adoption of high-throughput gene expression tools such as microarrays has allowed the identification of hundreds of both plant and nematode genes that are regulated over the course of nematode infestation. Initially, microarray studies were used to examine gene expression in whole infested roots or nematode feeding site-enriched tissues. More recently, the combination of laser-assisted microdissection of nematode feeding sites and subsequent transcriptomic analyses using microarrays has provided an unprecedented view of gene expression within the feeding site itself. This chapter is intended to present a broad overview of the knowledge gained to date about techniques that have been used to identify differentially regulated genes of both plant and nematode during the plant–nematode interaction and recent methodological improvements crucial for future studies.

1 Introduction

1.1 Temporal and Spatial Analysis of Tissues for Gene Expression Studies

During the infestation process, nematodes induce changes in gene expression ranging from local effects to those that affect whole plants. Thus, the choice of root materials in analysis becomes an important factor in approaching the interaction

C.G. Taylor
Donald Danforth Plant Science Center,
St. Louis, MO 63132, USA
e-mail: ctaylor@danforthcenter.org

Plant Cell Monogr, doi:10.1007/7089_2008_36

between plants and parasitic nematodes. Experiments using whole infested root tissues will likely yield different results as compared to those with a focus on cell-specific (for example, nematode feeding structures) gene-expression analyses. Notably, a variety of tissues including whole roots, isolated galls, and microdissected feeding sites have been examined for changes in gene expression upon nematode infections (Fig. 1). Transcriptomic analyses of whole infested root tissues identify genes that are indirectly regulated by the infection of nematodes. Feeding sites induced by nematodes may contribute only a small percentage (less than 5%) of whole infested roots, thus identification of genes directly responsive to invading nematodes may be problematic due to dilution of target RNA, specifically RNA from feeding structures. However, studies using whole infested roots provide a global view of gene expression in host plants and offer a broader perspective on plant health and ultimate yield potentials in the context of plant–nematode interactions. On the other hand, a localized approach including laser-assisted microdissection or microaspiration may be required to identify genes that are directly regulated by nematodes. Analyses of specific tissues or cell-types in nematode feeding sites produce important clues on localized responses of plants to invading or developing nematodes and generate potential targets for disrupting the intimate interaction between host and nematode. Alternatively, localized responses to nematode infection may not have any significant effects on overall plant health and will likely have little impact on practical applications in the field.

For researchers who are interested in studying the expression of nematode genes, the same spatial considerations of gene expression given to host plants apply.

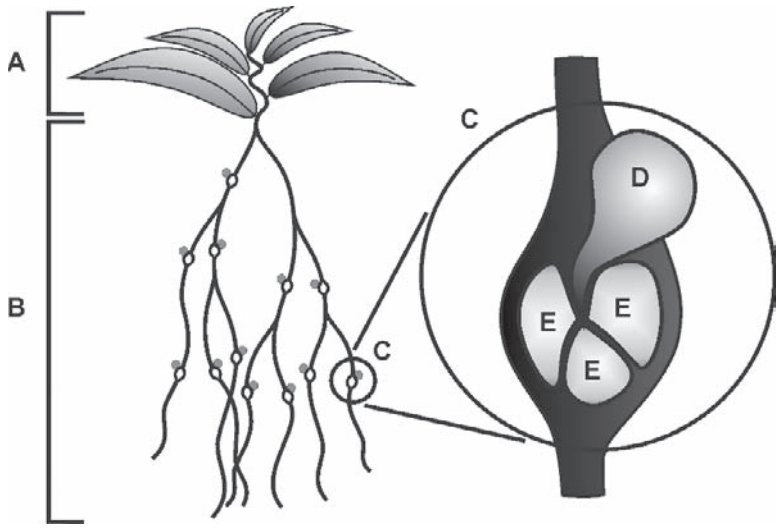


Fig. 1 Pictorial diagram of tissues used for gene expression analyses of plant–nematode interactions. **a** Whole plant (leaf and infested roots). **b** Whole infested roots. **c** Tissues enriched with nematode feeding sites. **d** The nematode. **e** Nematode-induced feeding sites (i.e. giant-cells or syncytia)

For instance, esophageal glands of nematodes are thought to be one of the organs within the nematode that produce parasitism molecules that are secreted and injected into host cells for the initiation and development of nematode feeding sites (Davis et al. 2008). With only three gland cells among over 950 cells in an intact nematode, the targeted isolation of glands using microaspiration techniques is required to find potential genes essential for nematode parasitism. Several studies using the microaspiration approach identified over 50 different putative secreted parasitism genes from the esophageal glands of root-knot and cyst nematodes (Davis et al. 2008). In contrast, Roze et al. (2008) isolated only eight secreted gland expressed genes after screening more than 12,000 expressed sequence tags (ESTs) generated from whole nematodes (representing three developmental stages). Clearly, a targeted approach can uncover more putative genes involved in parasitism; however, it can be time consuming and require specialized equipment and expertise.

Temporal changes in gene expression over the process of host and parasite interactions have been examined to identify genes that play important roles in initiating or maintaining feeding sites. Nematode-induced changes in gene expression can occur even before the entry of juvenile nematodes into host plants (Bird et al. 2008). Upon entering, infective juveniles migrate to the vascular cylinder within the root to begin setting up a feeding site (Berg et al. 2008; Sobczak and Golinowski 2008). Nematode feeding sites undergo numerous transformations from a single cell to a multi-cellular structure, all of which are controlled by activation or suppression of plant developmental, hormonal, and stress pathways (Gheysen and Mitchum 2008). Following the induction of feeding sites, nematodes resume development and undergo multiple morphological changes from a motile infective juvenile to a mature male or egg-laying adult female. The enormous developmental changes over the life cycle of nematodes are likely reflected in dramatic changes in gene expression (Davis et al. 2008).

A third consideration for gene expression studies involves specific interactions between nematodes and their corresponding host plants. The majority of research on plant and nematode interactions has focused on four host plants including *Arabidopsis thaliana* as a model plant, and the crop plants *Glycine max*, *Nicotiana tabacum*, or *Lycopersicon esculentum*, where nematode infestation is of particular economic and practical importance. Many of these plant systems have cultivars or mutants that are susceptible, tolerant, or resistant to parasitic nematodes. Careful use of susceptible and resistant recombinant inbred lines has been proven to be a powerful tool in identifying plant genes that regulate host responses to invading nematodes (Tomczak et al. 2008; Berg et al. 2008). Likewise, cyst nematodes including *Heterodera schachtii*, *H. glycines*, *Globodera pallida*, and *G. rostochiensis* and the root-knot nematodes including *Meloidogyne incognita*, *M. javanica*, *M. arenaria*, and *M. hapla* are frequently used in the analysis of gene expression primarily due to their economic impact on agriculture. Different populations of nematodes can be virulent or avirulent on the same host, thus allowing additional comparative tools to identify genes important in the parasitism process.

2 Early Methods Analyzing Gene Expression during Nematode Parasitism

Prior to the introduction of DNA microarrays, early studies on plant–nematode interactions typically involved the generation of cDNA libraries from the RNA of whole infested roots and subsequent isolation and expression studies with individual cDNA clones (Niebel et al. 1995). As molecular biology techniques improved, PCR enrichment strategies were employed to identify transcripts directly from cDNA libraries in response to nematode infections. Frequently, whole root systems were used as a starting material for RNA isolation (Hermsmeier et al. 1998; Mahalingam et al. 1999; Vaghchhipawala et al. 2001). Furthermore, chances of identifying genes that were differentially regulated by nematode infections were improved by collecting tissues enriched for nematode feeding sites using approaches of preselecting infested tissues (for example, giant-cell or syncytial containing tissues) (Hermsmeier et al. 2000; Vercauteren et al. 2001), by manually dissecting feeding sites (Wilson et al. 1994) or by microaspiration (Wieczorek et al. 2006). RNA isolated from such tissues was either immediately transcribed into cDNA or first enriched for feeding-site specific transcripts by subtractive hybridization. A summary of experiments on the direct examination of plant gene expression during nematode infestation can be found in Table 1 of this chapter. Indirect approaches, including the generation of promoter::*GUS* transgenic plants or the examination of genes or promoters not originally cloned from nematode-infested tissues, were also conducted (reviewed in Gheysen and Fenoll 2002). For the expression of genes in nematodes, studies have been carried out to identify and characterize gene transcripts in an attempt to understand the location and timing of gene expression at the various stages of nematode development and during the parasitism process (Davis et al. 2008).

2.1 Early Gene Expression Analyses of Whole Infested Roots

The following sections will focus only on those studies that directly involved infested materials to identify differentially regulated genes in response to nematode infections. The authors of this chapter direct you to the review by Gheysen and Fenoll (2002) for more detailed descriptions about the early analysis of gene expressions in nematode feeding sites through indirect analyses.

Differential screening of a cDNA library from potato roots infested with the cyst nematode *G. pallida* identified a catalase (*Cat2St*) transcript (Niebel et al. 1995). Under normal conditions, the expression of the *Cat2St* gene was very strong in stems in comparison to other parts of the plant. However, Northern blot analysis showed that the expression of the *Cat2St* gene was induced in roots upon nematode infection and the increase in transcript abundance progressed until the nematodes became fully mature. Furthermore, the expression of the *Cat2St* gene was systemic throughout the entire infected plants. Similar patterns of expression were obtained

Table 1 Summary of early gene expression analyses of plant–nematode interactions

Experimental material	Host plant	Nematode	Type of interaction	Time point[a]	Reference
Whole infested roots	Potato	*G. pallida*	Compatible	Early and late	Niebel et al. (1995)
	Soybean	*H. glycines*	Compatible	Early and late	Hermsmeier et al. (1998)
	Soybean	*H. glycines*	Compatible and incompatible	Early	Mahalingam et al. (1999)
	Soybean	*H. glycines*	Compatible	Middle	Vaghchhipawala et al. (2001)
	Alfalfa	*M. incognita*	Compatible and incompatible	Early	Potenza et al. (2001)
Feeding site enriched samples	Tomato	*M. incognita*	Compatible	Late	Eycken et al. (1996)
	Arabidopsis	*H. schachtii*	Compatible	Early	Hermsmeier et al. (2000)
	Arabidopsis	*M. incognita*	Compatible	Early	Vercauteren et al. (2001, 2002)
Nematode feeding sites only	Tomato	*M. incognita*	Compatible	Late	Wilson et al. (1994); Bird and Wilson (1994)
	Tomato	*M. javanica*	Compatible	Early and middle	Ramsay et al. (2004)
	Soybean	*H. glycines*	Compatible	Early	Klink et al. (2005)

[a]Early, middle, and late time points refer to 1 week after inoculation, 2 week after inoculation, and 3–4 week after inoculation, respectively

when plants were attacked by the root-knot nematode, *M. incognita* and the plant-pathogenic bacteria, *Erwinia carotovora* and *Corynebacterium sepedonicum*. Nearly ubiquitous to all aerobic organisms, catalases convert H_2O_2 to water and oxygen, providing protection against damage caused by reactive oxygen species. These observations led to the speculation that plants gradually induce the expression of the *Cat2St* gene for protection against local damage caused by parasitic nematodes and bacteria.

A differential display analysis comparing transcript abundance in *H. glycines* infested and noninfested soybean roots identified 15 gene transcripts that were differentially regulated one day after inoculation (Hermsmeier et al. 1998). To identify nematode-regulated transcripts, Hermsmeier and colleagues used primers that were specially designed to amplify random cDNAs from infested and control libraries. PCR amplified bands that showed differences in intensity during nematode infestation were cloned and sequenced. Among 15 identified transcripts, ten were down-regulated while five were up-regulated in infested roots. All 15 genes were

confirmed to be of plant origin by Southern blot analysis. Northern blot analysis was used to confirm changes in transcript abundance during nematode infestation. Sequence analysis revealed the identity of several genes including a transcription factor *TFIIA*, an auxin down-regulated gene, a small GTP-binding protein gene and several other genes with unknown function. At the early time point chosen for this analysis, nematodes are in the process of penetration and intracellular migration towards the vascular bundle (Endo 1964). Accordingly, changes in gene expression observed in this study might pertain to wounding responses in addition to the initiation of nematode feeding sites.

Using a nearly identical differential display method, Mahalingam et al. (1999) identified two polygalacturonase (*PG*) transcripts whose expression was altered in a compatible interaction. By comparing gene expression in roots of susceptible (Essex) and resistant cultivars (PI 437654) challenged with *H. glycines*, Mahalingam and colleagues eliminated the expression of genes associated with wounding response to nematode intracellular migration since this should be consistent between the two cultivars. In roots of the susceptible cultivar, expression of the *PG* genes was induced during early infestation. In contrast, the expression of the *PG* genes was barely detectable in infested roots of the resistant cultivar. Polygalacturonases are enzymes that catalyze the degradation of pectins, major plant cell wall components, and are involved in processes like fruit ripening, when cell wall softening is necessary (Brummell and Harpster 2001). Mahalingam and colleagues speculated that soybean cyst nematodes induce expression of plant polygalacturonases possibly to help in the formation of syncytia through loosening of cell wall.

Vaghchhipawala et al. (2001) took the cDNA subtractive hybridization and differential display work a step further by mapping nematode-regulated genes to the host genome in an effort to identify any genes that might be linked to soybean resistance to *H. glycines*. RNA was isolated from infested (1–2 weeks after inoculation) and noninfested roots of a susceptible soybean line (PI 89008) and used in a subtractive approach to identify nematode-regulated genes. Several differentially regulated genes were successfully mapped to the soybean genome and one transcript, coding for formylglycinamidine ribonucleotide synthase, was found to be tightly linked to a major soybean cyst nematode resistance quantitative trait locus (QTL) on linkage group G. However, molecular markers that were recently developed to further refine this QTL region likely exclude this transcript from being "the resistance gene" responsible for providing resistance to *H. glycines* in soybean (Ruben et al. 2006).

Subtractive hybridization approaches were also used to identify genes regulated during the invasion of roots by the root-knot nematode, *M. incognita* in alfalfa (Potenza et al. 2001). RNA was collected from infested and mock-inoculated alfalfa plants (cv. Lahontan; susceptible to *M. incognita*) at 72 h after inoculation and used to make a cDNA library. Thirteen cDNA clones were shown with an increase in transcript abundance in infested roots as compared to control tissues. Four of them were sequenced and identified as glycine-rich RNA binding protein, a phosphoenolpyruvate carboxykinase, an isoflavone reductase-like protein, and a metallothionein-like protein. Furthermore, Northern blot analysis revealed that these four genes were induced in response to nematode infections in both susceptible and resistant cultivars (cv. Moapa 69) of alfalfa as compared

to their corresponding controls. These analyses coupled with spatial and temporal differences in the expression level of these genes provided initial evidence that the early response of plants to nematode infection is likely associated with the induction of metabolic or stress pathways.

2.2 Early Gene Expression Analyses of Tissues Enriched for Nematode Feeding Sites

Beginning with RNA from galls induced by *M. incognita*, Eycken et al. (1996) used a differential display approach to identify genes that were up-regulated in infection sites. Clones were subjected to three rounds of differential hybridization with cDNA probes from gall mRNA as compared with probes from control roots. Two gall-induced transcripts (*Lemmi8* and *Lemmi11*) encoded extensin proteins were identified. Extensins are important structural components of plant cell walls (Kieliszewski and Lamport 1994). A recent report showed that one crucial function of extensins may be to provide a structural scaffold for the self-assembly of newly formed plant cell walls during cell division (Cannon et al. 2008) or during nematode parasitism, as structural components of the cell wall of developing feeding sites (Eycken et al. 1996).

Another gene identified from this study was *Lemmi9* that shared sequence homology to the *Lea* genes that are normally expressed during late embryogenesis. Strong expression of *Lea* genes predominantly in late embryogenesis of both monocot and dicot plants led to the speculation that *Lea* genes may be involved in protecting the embryo during seed desiccation. Since giant-cells or syncytia are characterized by an increase in cytoplasmic density (Jones 1981; Golinowski et al. 1996), the enhanced expression of the *Lemmi9* gene in giant-cells was assumed to protect the cytoplasm of giant-cells from osmotic stress (Eycken et al. 1996). However, unlike *Lea* genes, drought stress or ABA application did not induce the expression of the *Lemmi9* gene in tomato.

Hermsmeier et al. (2000) examined early changes in transcript abundance in developing synctia induced by *H. schachtii* in Arabidopsis. Root segments with protruding juveniles were dissected 4 days after inoculation and compared to adjacent nematode-free tissues. This sampling approach was designed to minimize systemic and wounding responses. Differential display analysis identified a total of 36 cDNA clones whose transcript abundance changed over the course of nematode infestation; 24 clones were of plant and 12 of nematode origins. A plant-specific clone with a decrease in transcript abundance was confirmed to be down-regulated specifically within feeding sites by in-situ hybridization and belongs to the ethylene-responsive element-binding protein (EREBP) family of transcription factors. Members of this family have been observed to regulate the expression of pathogenesis-related proteins in plant defense responses (Zhou et al. 1997) by binding to the promoter regions of their target genes (Stepanova and Ecker 2000). Local suppression of such genes specifically within young syncytia has been suggested to be important for the induction and establishment of feeding sites.

In a similar differential display study, Vercauteren et al. (2001, 2002) examined changes in transcript abundance of genes in Arabidopsis roots during infestation by the root-knot nematode, *M. incognita*. They dissected infested gall tissues and compared gene expression in gall tissues at five early time points (2 through 7 days after inoculation) to corresponding controls. Seven cDNA clones with an increase in transcript abundance during nematode infestation were identified as; a serine proteinase inhibitor, a peroxidase, a mitochondrial uncoupling protein, a putative endomembrane protein, a 20S proteasome α-subunit, diaminopimelate decarboxylase, and pectin acetylesterase. All seven genes were confirmed to be of plant origin and were further characterized by in-situ hybridization over a 3-week period of nematode infestation. Annotations of these genes and temporal changes in their expression level suggested that these genes are likely involved in cell wall modification of giant-cells, an increase of organelle and amino acid content in giant-cells, and plant defense responses.

2.3 Early Gene Expression Analyses of Nematode Feeding Sites

In tomato, 58 cDNA clones were identified from a library that was produced from giant-cells induced by *M. incognita* after subtractive hybridization with cDNA from uninfected roots (Bird and Wilson 1994; Wilson et al. 1994). Several transcripts were confirmed to be of plant origin and changes in transcript abundance were further validated by RNA dot-blot analysis. One transcript was similar to the tobacco gene *TobRB7* that was previously shown to be up-regulated specifically in giant-cells of tobacco roots infected by *M. incognita* (Opperman et al. 1994). Although the identity of the majority of genes could not be assigned, many of the giant-cell expressed genes were found to be normally expressed in the aerial parts of plants. This was one of the first reports to show that genes expressed in giant-cells are likely expressed in other tissues of host plants and that they are triggered or recruited during the formation of nematode feeding sites.

Laser-capture microdissection (LCM) is a powerful alternative method for the isolation of purified populations of cells from tissues (reviewed by Schnable et al. 2004). This state-of-the-art technology allows identification of specific cell types and their precise removal from the tissue of origin (Fig. 2). LCM offers several advantages over other techniques for cell purification: it is simple, involves no manual or enzymatic microdissection, allows microscopic verification of the specificity of captured materials, and freezes the metabolic state of the cell. These captured cells can then be used in a wide range of downstream assays, such as EST library generation, RT-PCR amplification, microarray analysis, single and two-dimensional gel electrophoresis analyses of protein products, Western blotting, and MALDI-TOF mass spectrometry (Burgess and McParland 2002; Craven and Banks 2002; Lindeman et al. 2002; Neira and Azen 2002; Todd et al. 2002; Xu et al. 2002). The LCM process does not alter the chemistry or morphology of sampled cells or tissues. For this reason, LCM is a powerful tool that enables the researcher to perform DNA, RNA and/or protein analyses on collected cells. A variety of fixation methods have

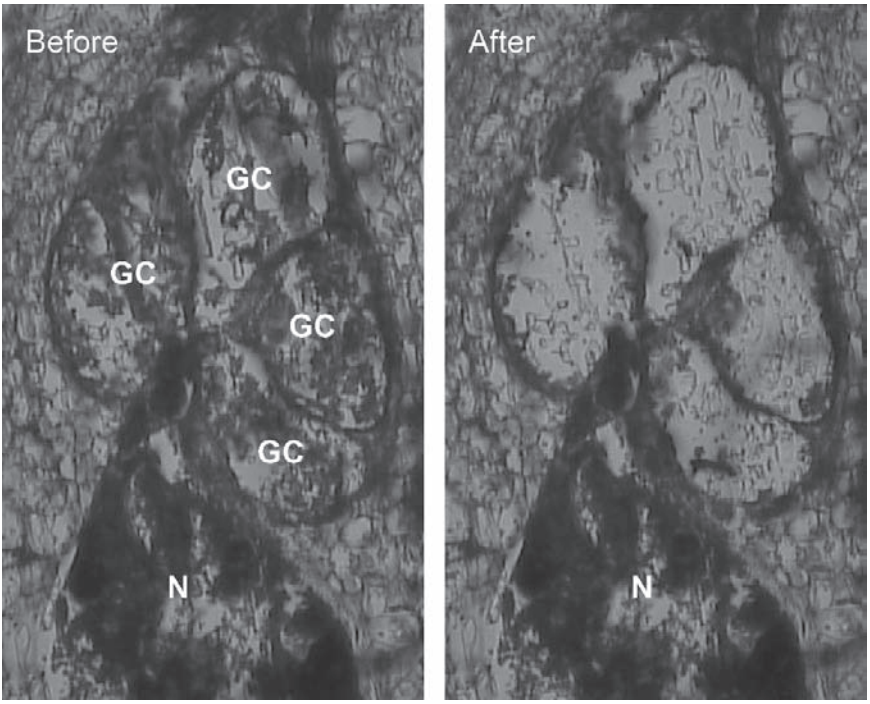


Fig. 2 Laser-capture microdissection of a root-knot nematode (N; *M. incognita*) induced giant-cells (GC) in *Z. mays* roots before and after capture

been developed for the preparation of tissues prior to sectioning and the isolation of target molecules. In fact, the ever increasing use of LCM has warranted the publishing of an entire "Methods of Enzymology" book devoted to LCM and the various protocols used for DNA, RNA, and protein analysis (Conn 2002).

LCM has been extensively used in the medical field to study cell-specific gene expression and pathology (reviewed in Conn 2002). The first documented use of LCM on plants was by Asano et al. (2002), using LCM to isolate rice phloem in constructing a rice phloem-specific cDNA library. Since then, several reports of using LCM to investigate gene expression in nematode-induced feeding sites have been published (Ithal et al. 2007b; Klink et al. 2005, 2007a; Ramsay et al. 2004). To investigate the expression of cyclin genes in giant-cells, Ramsay and associates (2004) captured RNA from giant-cells and demonstrated that two D-type cyclin genes were expressed at higher levels in giant-cells as compared to other cyclin genes. Later, Klink and colleagues (2005) isolated RNA from LCM-captured syncytia induced by *H. glycines* in soybean and used it to create a syncytial-specific cDNA library. Real-time PCR was performed to examine gene expression of several ESTs from the cDNA library in LCM-captured syncytia and total root RNA. Transcripts of an aquaporin and several tubulin (α and β) genes were enriched in syncytia and in situ hybridizations showed that they were highly induced in nematode feeding sites as well as in adjacent parenchyma cells.

2.4 Early Gene Expression Analyses using Promoter::GUS Transgenic Plants

Another approach to identifying host genes that are differentially regulated during nematode infestation is through the use of a scorable marker. Promoter-driven scorable markers have been routinely used by plant molecular biologists to identify the location and timing of gene expression in transgenic plants. Two scorable markers, the β-glucuronidase gene (*uidA*) and the green fluorescent protein (*GFP*), have been the most widely adopted. In studies of plant–nematode interactions, the expression of GUS or GFP has been used to demonstrate expression of specific genes in nematode feeding sites. Many studies using promoter::*GUS* or *GFP* constructs show differences in expression based on the selection of different nematodes, time points, and tissue types. Corresponding to the multi-nucleated nature of nematode feeding sites, Niebel et al. (1996) demonstrated that transgenic Arabidopsis lines containing the cell-cycle constructs *cdc2a::GUS* or *cyc1At::GUS* showed an increase in the level of GUS expression in giant-cells or synctia induced by *M. incognita* or *H. schachtii*, respectively. In addition, Huang et al. (2003) found that transgenic *Arabidopsis* plants carrying a *PROLIFERA* (a gene associated with maintenance of minichromosomes) promoter::*GUS* construct showed GUS expression that was induced in giant-cells and syncytia by *M. javanica* and *H. schachtii*, respectively. To monitor the expression of several monosaccharide and disaccharide transporter genes in *H. schachtii*-induced syncytia, Arabidopsis plants were transformed with constructs containing *GUS* or *GFP* reporter genes driven by promoters of these transporter genes (Juergensen et al. 2003). Among the various transgenic lines, the expression of the GUS reporter inside syncytia was only detected when the reporter gene was under the control of the promoter of the disaccharide transporter gene, *AtSUC2*. When the respective transgenic lines were challenged with *M. incognita*, no GUS activity was found in giant-cells. Temporal monitoring of *AtSUC2*::*GFP* activity showed a maximal expression in the syncytia at 20-day post infestation. This observation provided a strong correlation between the activity of AtSUC2 and the general development of nematode feeding sites, which positively reflected the growth of the cyst nematode towards maturity.

Promoter trapping studies have also been employed to identify genes that are differentially regulated in nematode feeding structures. Favery et al. (1998) used a promoterless::*GUS* construct to identify possible genes regulated during nematode infestation. Among 3,000 T-DNA-tagged Arabidopsis lines, 25 were detected with an increase and three with a decline in GUS expression during infestation by *M. incognita*. The T-DNA fragment of one nematode-induced tagged line was inserted in a gene encoding a protein similar to d-ribulose-5-phosphate 3-epimerase (RPE), a key enzyme in the Calvin cycle and the oxidative pentose phosphate pathway. Expression of GUS in this transgenic line was exclusively restricted to nematode-induced galls and the expression was observed throughout the course of infestation by the root-knot nematodes including *M. javanica* and *M. hapla*. However, GUS expression was not detected until later stages of infestation by the cyst nematodes,

H. schachtii or G. *rostochiensis*. Fully devoted to providing nutrients for sedentary endoparasites (root-knot and cyst nematodes), giant-cells and syncytia are characterized as sink tissues with active metabolism (Jones 1981). The high expression of the *RPE* gene in both feeding structures at later stages of nematode development was anticipated as nematodes mature into adults and they require large amounts of nutrients for reproduction (Favery et al. 1998). In noninfested roots, GUS expression was localized to root fragments undergoing rapid cell division, including the root meristem, the elongation zone, and sites of lateral root initiation.

2.5 Early Gene Expression Analyses of Plant-Parasitic Nematodes

Little is known about the expression of genes in plant-parasitic nematodes over the course of parasitism in plants. Current genome sequencing projects (Opperman et al. 2008) indicate that a majority of plant-parasitic nematodes have more than 14,000 genes. Likely, many of these genes are differentially and spatially regulated over the developmental cycle of nematodes. Numerous projects have generated cDNA libraries for several yield damaging plant-parasitic nematodes. These libraries have been partially sequenced producing a treasure trove of valuable ESTs (Dautova et al. 2001; McCarter et al. 2000; McCarter et al. 2003; Popeijus et al. 2000; Roze et al. 2008). Sequence information about ESTs from plant-parasitic nematodes is accessible through websites dedicated to nematode research (for example www.nematode.net). However, the majority of EST collections are focused on libraries from egg or second-stage infective juveniles as these stages are the easiest to isolate and process. Furthermore, little is known about where and when these genes are expressed in the nematode during the parasitism process. A significant amount of evidence on changes in gene expression within plant-parasitic nematodes comes from the studies of esophageal glands and the genes that are expressed within the three gland cells. Davis et al. (2008) provide an in depth analysis of gene expression in the esophageal glands of root-knot or cyst nematodes. Little is known about the spatial and temporal expression of genes outside the esophageal glands that could be important for the parasitism process. New tools such as microarrays and RNA interference will provide a more detailed analysis of gene expression and the importance of expressed genes to the parasitism process.

Aside from transcriptomic analysis of nematode genes identified in esophageal glands, one report employed a subtractive approach to identify genes whose expression was altered during the parasitism process. Two cDNA libraries were separately constructed for preparasitic second-stage juveniles (J2) and parasitic third-stage juveniles (J3) of *M. incognita* by suppression subtractive hybridization (Dubreuil et al. 2007). Comparison between the two libraries yielded four clones in common, suggesting that each library was enriched with stage specific transcripts. Among 187 unique gene fragments in the J3 library, 57 of them were up-regulated. For sedentary endoparasites including *M. incognita*, the initiation of nematode feeding

sites corresponds to the transition of nematode development from mobile to sedentary. Some genes including several protease genes and the gene encoding a putative glutathione-*S*-transferase (*GST*) gene were dramatically up-regulated in J3 nematodes in comparison to J2. Furthermore, the introduction of RNAi by soaking J2 in dsRNA of the *GST* gene followed by immediate inoculation of J2 on tomato roots resulted in a significant reduction in the number of egg masses per plant in the case of dsRNA-treated nematodes compared with control J2. In the model nematode *Caenorhabditis elegans*, over-expression of a single *GST* gene was clearly correlated to an increase in nematode resistance to oxidative stress (Leiers et al. 2003). Plants are known to produce an array of reactive oxygen species in response to abiotic and biotic stresses. Accordingly, the dramatic induction of the *GST* gene in J3 nematodes likely plays an essential role in protecting sedentary nematodes or enhancing nematode resistance to plant defense responses.

3 Microarray Analyses of Plant–Nematode Interactions

Microarrays have now become the platform of choice for identifying genes that are regulated during plant–pathogen interactions. With the release of complete genome sequences and/or accumulation of vast numbers of EST libraries, numerous platforms (either cDNA- or oligo-based) have been generated for a variety of plant species. Microarrays allow the interrogation of the transcriptome during specific interactions or under defined treatments. The power of microarrays comes from the simultaneous analysis of virtually every gene within an organism and the use of standardized platforms and databases, thus the meta-analysis of different experiments become possible. These meta-analyses allow researchers to identify commonalities of transcription among specific gene sets or pathways across different conditions and gain deeper insight into the co-regulation of genes.

The design and production of DNA microarrays faces three significant challenges: the number of elements that can be reproducibly applied to a solid and two-dimensional support, the uniformity of hybridization across various elements on an array, and the selection of suitable sequences representing all target genes for a given study. While the latter two issues are still problematic, modern technologies have successfully addressed the problem of spot density. The first application of microarrays in plant science used spotted PCR-products corresponding to selected cDNAs from the model plant *A. thaliana* (Schena et al. 1995). Improvements in spotting technology now routinely allow up to 30,000 cDNA fragments to be spotted on a single chip. These cDNA chips are routinely created in research labs and used for specific studies (Alkharouf et al. 2006; Khan et al. 2004; Schaff et al. 2007). However, the use of cDNA microarrays has been somewhat problematic due to potential errors caused by cross-hybridization and/or nonuniformity of hybridization as the result of the presence of palindromes or homopolymeric runs. The increased availability of complete genome sequences and development of large EST libraries has improved DNA selection by allowing for the careful selection of probe sequences (Allemeersch et al.

2005; Hilson et al. 2004). Nevertheless microarrays relying on PCR for element production are being gradually replaced by those containing elements generated by oligonucleotide synthesis that offers an even higher density of spot elements per chip. Commercial suppliers (i.e. Affymetrix, NimbleGen, and Agilent) have pushed spot density to 6.5 million elements per array (Galbraith 2006). Commercially available microarrays are composed of oligonucleotides in 25 nt (Affymetrix), 60 nt (Agilent), and 85 nt (NimbleGen) and are available for Arabidopsis, maize, rice, tomato, and *Medicago truncatula* (Rensink and Buell 2005). Such arrays are easily produced for organisms with completely sequenced genomes, but can be derived from EST sequence collections with somewhat less confidence in producing gene-specific elements with minimal cross-hybridization problems.

Working with microarrays, researchers must become familiar with potential limitations of the various platforms that have been developed. How to measure, normalize, and statistically analyze microarray results has been subjected to discussions and whole reviews and chapters have been devoted to microarray analysis (Clarke and Zhu 2006; Meyers et al. 2004; Nettleton 2006; Rensink and Buell 2005). In this section we will focus rather on the microarray experiments to evaluate plant–nematode interactions and discuss results and refer readers to aforementioned references for critical evaluation of microarray platforms, experimental design and statistical evaluations.

Nematologists have come to recognize the importance of microarrays and have applied them in a variety of experiments, monitoring global changes in gene expression within the host and/or parasite at different stages of infestation. A summary of studies of microarray analyses on plant–nematode interactions can be found in Table 2 of this chapter. Over the past several years, a number of microarray analyses have been conducted to investigate differential expression of genes in whole roots infested by root-knot or cyst nematodes (Alkharouf et al. 2006; Hammes et al. 2005; Ithal et al. 2007a; Khan et al. 2004; Klink et al. 2007b; Puthoff et al. 2003). Microarray analyses using materials enriched with nematode feeding structures have also been conducted (Bar-Or et al. 2005; Fuller et al. 2007; Jammes et al. 2005; Schaff et al. 2007). Recently, the incorporation of laser-capture microdissection (LCM) of nematode feeding structures (i.e. giant-cells or syncytia) now offers researchers a close-up view of gene expression within a single cell-type essential to the parasitic interaction (Ithal et al. 2007b; Klink et al. 2005, 2007a; Ramsay et al. 2004).

Direct comparisons among the various nematode microarray data sets have been difficult due to differences in hosts and nematodes used, methods of inoculation, inoculum densities, sample materials and sampling methods, time points, conditions for a particular study, microarray platforms and statistical methods for data analysis. However, as experimental conditions are refined, platforms standardized and more consistent data sets developed [i.e. following specified guidelines (i.e. MIAME; Minimum Information About a Microarray Experiment; http://mged.org/Workgroups/MIAME/miame.html)], comparative analysis of transcriptional data sets will allow researchers to investigate the role of specific pathways or groups of genes during different nematode interactions and/or other plant biotic interactions or treatments. These analyses will in turn promote the development of

Table 2 Summary of microarray analyses of plant–nematode interactions

Experimental material	Host plant	Nematode	Type of interaction	Time point[a]	Reference
Whole infested roots	*Arabidopsis*	*H. glycines and H. schachtii*	Compatible and incompatible	Early	Puthoff et al. (2003)
	Soybean	*H. glycines*	Compatible	Early	Khan et al. (2004)
	Soybean	*H. glycines*	Compatible	Early	Alkharouf et al. (2006)
	Soybean	*H. glycines*	Compatible	Early and middle	Ithal et al. (2007a)
	Soybean	*H. glycines*	Compatible and incompatible	early and middle	Klink et al. (2007b)
	Arabidopsis	*M. incognita*	Compatible	Early, middle and late	Hammes et al. (2005)
	Tomato	*M. incognita and M. hapla*	Compatible and incompatible	Early and late	Schaff et al. (2007)
Feeding site enriched samples	*Arabidopsis*	*M. incognita*	Compatible	Early, middle and late	Jammes et al. (2005)
	Tomato	*M. javanica*	Compatible	Early and middle	Bar-Or et al. (2005)
	Arabidopsis	*M. incognita*	Compatible	Late	Fuller et al. (2007)
	Soybean	*H. glycines*	Compatible	Middle and late	Puthoff et al. (2007)
Nematode feeding sites only	Soybean	*H. glycines*	Compatible	Early and middle	Ithal et al. (2007b)
	Soybean	*H. glycines*	Compatible and incompatible	Early and middle	Klink et al. (2007a)
	Arabidopsis	*H. schachtii*	Compatible	Early, middle and late	Wieczorek et al. (2006); Hofmann et al. (2008)

[a]Early, middle, and late time points refer to 1 week after inoculation, 2 week after inoculation, and 3–4 week after inoculation, respectively

testable hypotheses about the importance of specific genes or pathways during the nematode parasitism process (Hofmann et al. 2008; Wieczorek et al. 2006).

3.1 Microarray Analyses of Whole Infested Roots

One of the first published microarray studies on plant–nematode interactions was described by Puthoff et al. (2003). Changes in transcript abundance were examined during the early infection by infective juveniles of *H. schachtii* or *H. glycines*. The cyst nematodes, *H. schachtii* and *H. glycines*, can both enter the roots of Arabidopsis,

but only *H. schachtii* is able to develop, reproduce and complete its life cycle. Though capable of infecting Arabidopsis roots, juveniles of *H. glycines* fail to induce a viable feeding site, thus Arabidopsis is considered a nonhost for *H. glycines* (Grundler et al. 1997). Arabidopsis plants were challenged with similar numbers of freshly hatched juveniles of *H. schachtii* or *H. glycines.* Roots were harvested at 3 days after inoculation. RNA was extracted and used to hybridize the Arabidopsis GeneChip from Affymetrix. Of the >8,200 genes represented on the chip, 128 genes showed significant changes in expression level in infested roots as compared to mock-inoculated control roots. A total of 82 genes were detected with a significant increase in transcript abundance in *H. schachtii*-infested roots and 11 of them were also up-regulated in roots infested by *H. glycines*. In contrast, 46 genes were repressed in *H. schachtii*-infested roots and only one in *H. glycines*-infested roots. The majority of differentially expressed genes were involved in stress and defense responses, cell wall alteration, metabolism and nutrient allocation, signal transduction, or phytohormone action. Changes in transcript abundance of several genes in response to nematode infection were further confirmed by quantitative RT-PCR analysis. Interestingly, no genes were found to be unique to the incompatible interaction between *A. thaliana* and *H. glycines* under the conditions tested in this study. These findings led to the consideration that an active defense response might not be involved in the incompatible interaction between *A. thaliana* and *H. glycines* (Puthoff et al. 2003).

In 2004, Khan and colleagues described the use of microarrays to study early gene expression in a compatible interaction between soybean and *H. glycines* (Khan et al. 2004). A total of 1,305 unique soybean ESTs were identified in three cDNA libraries that were constructed from soybean cyst nematode susceptible and resistant roots, 2 days after inoculation. The ESTs were used to create a custom mini-array through PCR amplification of EST target sequences. RNA was isolated from the whole roots of the susceptible cultivar Kent at 2-day post-inoculation with freshly hatched *H. glycines* juveniles and mock-inoculated control roots. Analysis of the mini-array showed limited differences (less than twofold) in fold-change between infested and noninfested roots. Over 7% of the genes on the mini-array showed an increase in transcript abundance in infested roots in comparison to control roots and many genes with the highest fold-change values were associated with cell wall proteins such as proline-rich proteins and hydroxyproline-rich glycoproteins (Khan et al. 2004). Since syncytia begin to form as early as two days after inoculation in susceptible plants (Endo 1991), genes involved in cell wall modification are expected to be up-regulated. Genes involved in general plant defenses were also induced, including a stress-induced gene and a peroxidase gene. The induction of defense-related genes in a susceptible cultivar might be explained as the result of physical damage caused by intracellular migration of infective juveniles. Possibly due to the small size of the array, no genes were reported to show a decrease in transcript abundance in response to nematode infection in Kent (Khan et al. 2004). This study also highlights a pitfall of using whole roots for analysis; the masking or dilution of rare RNAs that are regulated at sites of nematode infestation by transcripts of noninfested root tissues.

A larger soybean cDNA microarray was generated to examine the compatible interaction between the susceptible cultivar Kent and *H. glycines* (Alkharouf et al. 2006). An array representing 6,543 different transcripts from four different nematode-infested root cDNA libraries was printed onto slides. A time course series of RNA isolated from nematode infested roots (6, 12, and 24 h and 2, 4, 6, and 8 days after inoculation) was isolated and compared to RNA from mock-inoculated roots of the same time points. The time-course series covered the entry and migration of infective juveniles (6 and 12 h after inoculation), the initiation and formation of syncytia (1, 2, and 4 days after inoculation), and the development of syncytia (6 and 8 days after inoculation). Similar to previous microarray studies, this study was challenged by the fact that a large amount of root tissue used for RNA extraction was not infected by nematodes. This compromised the sensitivity of the microarray to detect nematode-regulated transcripts. As a result, only few transcripts with low-fold change values were identified, especially at the early time points.

Among 6,543 genes on the array, the number of genes with an increase greater than 1.5-fold in transcript abundance rose from 61 at 1-day post-inoculation to 287 at 6-day post-inoculation. Similarly, the number of genes with a reduction greater than 1.5-fold in expression level increased from 128 at 1-day post-inoculation to 243 at 6-day post-inoculation. Genes encoding proteins with unknown or unclassified functions constituted 45–55% of the differentially expressed genes on the array. Clustering analysis identified four distinct expression profiles for differentially regulated genes. The first group consisted of genes that were only induced at 6 and 12 h after inoculation. Genes encoding Kunitz trypsin inhibitor, stress-related proteins, germin-like proteins and others were included in this group. The genes in the first profile are likely induced due to wound response caused by nematode entry and intracellular migration in the root. Genes in the second group or cluster were induced at 6 and 8 days after inoculation. These genes encoded proteins involved in protein synthesis, metabolism (i.e. sugar) and transport (i.e. sugar and phosphate) and are likely important in the development of syncytia. The third group of genes was those induced in most or at all time points. This group included a WRKY transcription factor, a calmodulin, a polyubiquitin, and a lipoxygenase. Many genes in this group function in stress and defense responses. The fourth group of genes was repressed across all time points. A large number of genes with unknown or putative functions were found in this group. Seven genes were selected for quantitative real-time RT-PCR analysis and the changes in expression levels generally agreed with the microarray results (Alkharouf et al. 2006).

The production of the Affymetrix soybean GeneChip made it possible to screen a large portion of soybean transcripts publicly available in soybean literature and databases. Over 35,000 soybean transcripts and nearly 7,500 soybean cyst nematode transcripts are represented on this chip providing an unprecedented view of gene expression in this important agricultural crop and pest. Ithal and colleagues used this chip to examine gene expression in the compatible interaction between soybean and *H. glycines* (Ithal et al. 2007a). Synchronized inoculation was con-

ducted to only allow infective juveniles of *H. glycines* to enter roots for 24 h at approximately 1 cm above root tips. Infested and mock treated roots were harvested at 2, 5, and 10 days after inoculation representing early (2 days), middle (5 days), and advanced stages (10 days) of syncytial development. Labeled cRNA was hybridized to the Affymetrix chips with three biological replicates for each time point. Transcript abundance for a total of 429 genes was significantly altered with greater than 1.5-fold change in infested roots as compared to noninfested roots at the three time points. Quantitative real-time RT-PCR analysis was used to confirm the change in expression level of 19 differentially regulated genes.

With little functional information available for soybean transcripts on the array, a similarity search using the TAIR Arabidopsis sequence database identified putative functions for over 7% of the genes (Ithal et al. 2007a). According to this analysis, genes involved in plant defense responses were up-regulated in roots upon infection by *H. glycines* across all time points (Ithal et al. 2007a). These induced genes included genes encoding PR proteins, glutathione-S-transferase, stress-related transcription factors, and harpin-induced gene-1. The up-regulation of defense-related genes in the compatible interaction was in agreement with a previous study (Khan et al. 2004). This demonstrated that defense-related genes were induced in response to physical damage caused by intracellular migration of infective juveniles and by stress from expanding syncytia and developing nematodes (Ithal et al. 2007a). In addition, significant up-regulation of a large number of soybean genes involved in cell wall modification was detected in infested roots across all time points, further supporting the role of cell-wall modifying proteins in the initiation and development of syncytia (Ithal et al. 2007a; Klink et al. 2007b). Moreover, an increase in transcript abundance in infested roots across all time points was observed for a number of genes involved in plant secondary metabolism, specifically genes participating in the phenylpropanoid and flavonoid pathways (Ithal et al. 2007a). The up-regulation of these genes resulting in the accumulation of flavonoid or isoflavonoid phytoalexins or in the deposition of lignin is often observed in hypersensitive responses in incompatible interactions. However, the function of secondary metabolites in compatible interactions remains largely unknown (Ithal et al. 2007a). Changes in expression level of nematode genes in this study are described later in this chapter.

Using the same soybean microarray chips from Affymetrix, Klink and colleagues examined the compatible and incompatible interactions between the soybean line Peking and *H. glcyines* (Klink et al. 2007b). To minimize changes in expression levels that may be caused by different genetic background of host plants, two different nematode populations (NL1-RHg and TN8) were selected to infect the same host plant Peking. This selection represented the compatible interaction between TN8 and Peking and the incompatible interaction between NL1-RHg and Peking. Similar to the previous report (Ithal et al. 2007a), a synchronized inoculation method was also employed to ensure that juveniles of *H. glycines* had only a limited period (12 h) to infect roots. This inoculation approach allowed a relatively uniform infection process so that the ability to detect changes in transcript abun-

dance would not be compromised by continuous entry of juveniles. Throughout the course of infestation, the compatible and incompatible interactions were histologically examined to ensure adequate infestation of root tissues by nematodes. Using two biological replicates for each time point, RNA was isolated from infested lateral roots at 0.5, 3, and 8 days after inoculation, hybridized to the soybean microarray chips and analyzed for changes in transcript abundance. The cutoff for all transcripts on the array was changes equivalent to or greater than 1.5-fold in transcript abundance and with a *P*-value lower than 0.05 in infested tissues as compared to noninfested roots. Across all time points, a total of 339 transcripts were induced while 740 transcripts were repressed in the incompatible interaction. In the compatible interaction, 181 transcripts were induced and 491 genes were repressed across all time points.

Klink and colleagues also conducted a comparative analysis of compatible and incompatible interactions at individual time points. Because of the amount of data generated from this study and the limited space within this chapter, the authors direct you to Klink et al. (2007b) for data analysis and annotation of genes. These analyses revealed a large number of transcripts that were differentially regulated at individual time points over the infestation process. Changes in transcript abundance for some genes were unique to either interaction while similar changes in expression level were detected in both interactions (Klink et al. 2007b). One interesting finding of this study was that the host plant could distinguish virulent and avirulent nematodes as early as 12 h after inoculation and continued to respond differently to the two populations throughout the infestation process (3 and 8 days post-inoculation). Also interesting was that the number of repressed genes unique to a single time point declined with the development of nematodes in the incompatible interaction while the number of repressed genes unique to a single time point in the compatible interaction increased over time. By 8 days after inoculation, the number of repressed genes unique to the compatible interaction was nearly five fold greater than that of the incompatible interaction. Furthermore, the number of induced genes that were common to both interactions was similar across all time points, suggesting the presence of basic and fundamental responses in soybean to *H. glycines*. In addition, these observations suggest that successful development of nematodes in the compatible interaction may require continuous suppression of host genes (Klink et al. 2007b).

Another way researchers use microarrays is to address specific hypotheses. Our laboratory was interested in the role of transporters during the nematode parasitism process. Previous studies have shown that water transporters and sucrose transporters likely play roles in the nematode parasitism process (Juergensen et al. 2003; Opperman et al. 1994). In our study we used the ATH1 chip (Affymetrix) carrying nearly 24,000 individual genes from Arabidopsis to examine gene expression during the later stages of nematode infestation. This chip is widely used by the Arabidopsis community, with over 3,000 different arrays available for meta-analysis (Zimmermann et al. 2005). Our initial interest in using these arrays was sparked by the 805 transcripts on the ATH1 chip that were represented in the Arabidopsis

Membrane Protein Library Database and were annotated as transport proteins. Given the symplastic isolation of nematode feeding sites and associated transfer cell-like characteristics, we hypothesized that transport plays an important role in the parasitism process (Offler et al. 2002; Berg et al. 2008; Sobczak and Golinowski 2008).

RNA was isolated from whole infested roots of Arabidopsis grown under sterile conditions at 1, 2, and 4 weeks after inoculation. RNA from three separate biological replicates for each time point was converted to cRNA and hybridized to the ATH1 chip. Combined analysis of three time points yielded 533 induced genes and 665 repressed genes (Fig. 3, unpublished data). Categorization of differentially regulated genes into function classes revealed that the genes associated with metabolism could be activated or repressed. Interestingly, 17% of the induced genes belonged to the category of cell wall modification with only 4% of down-regulated genes in the same category. This result is not surprising given the development of giant-cells and subsequent gall formation. Conversely, 18% of the down-regulated genes fell into of the transcription category while only 5% of the up-regulated genes were involved in transcription. The repression associated with transcription during the nematode parasitism process appears to be a common theme among cyst and root-knot nematodes (Klink et al. 2007b; Schaff et al. 2007). However, this reduction could be the result of the possible global effect on limited root growth due to resource consumption by nematodes and its indirect impact on gene expression (i.e. down-regulation of genes).

Among the 805 transporter genes represented on the ATH1 array, 50 genes were found to be differentially regulated during nematode infestation. Twenty of these

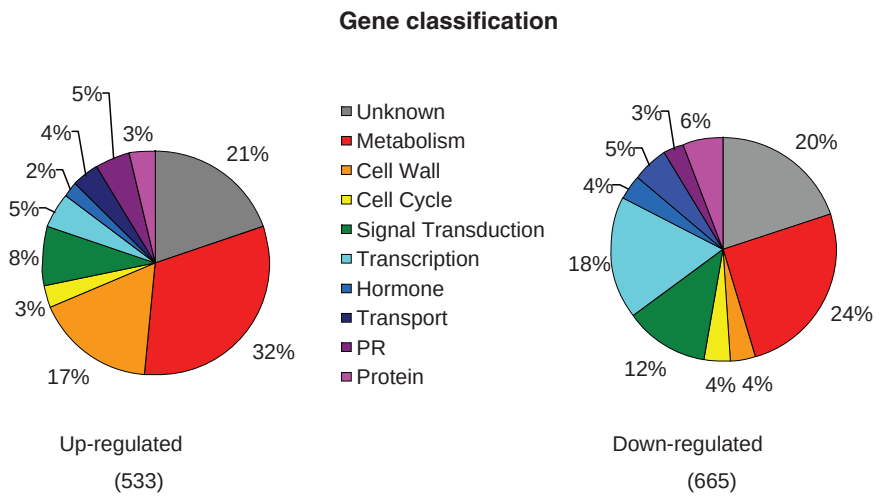


Fig. 3 Gene classification of root-knot nematode (*M. incognita*) regulated genes in whole infested roots

were down-regulated across all three time points. Interestingly, six members of the peptide transporter/proton-coupled oligopeptide transporter family were down-regulated at all time points. Of the remaining 30 differentially regulated genes, 26 were up-regulated at 1 and 2 weeks after inoculation and 16 were up-regulated throughout all three time points. Three members of the auxin/amino acid permease family were induced. Quantitative real-time RT-PCR was used to confirm changes in expression levels during nematode infestation and transcript abundance for many transporter genes was greater in the gall as compared to noninfected root tissues (Hammes et al. 2005). Notably, several transporters including the aquaporin gene, *AtPIP2.5* and an amino acid transporter, *AtCAT6* were significantly induced in galls (Hammes et al. 2005, 2006). Furthermore, recent analysis of T-DNA knockouts in *Arabidopsis* for several amino acid transporter genes has led to alterations in plant susceptibility to the root-knot nematode, *M. incognita* (CG Taylor, unpublished results).

Schaff and colleagues investigated gene expression in susceptible and resistant tomato plants challenged by root-knot nematodes (Schaff et al. 2007). The resistant cultivar "Motelle" (carrying the resistance gene *Mi*) and the susceptible cultivar "Moneymaker" were examined at early (12, 36, and 72 h) and late (4 weeks) time points after inoculation with *M. incognita* or *M. hapla* with a synchronized inoculation approach. The *Mi* gene in the resistant cultivar provides resistance to *M. incognita*, *M. javanica*, and *M. arenaria* but fails to provide protection against *M. hapla* and *M. chitwoodii* while the susceptible cultivar is equally susceptible to all root-knot nematodes. A cDNA microarray was constructed from ESTs from root cDNA and giant-cell cDNA libraries (Bird and Wilson 1994; Wilson et al. 1994). In total, 1,547 root-expressed and 186 giant-cell-expressed transcripts were spotted onto a glass slide. Differences in genetic background between the two cultivars were investigated first by comparing gene expression in mature roots to the two uninfested cultivars. RNA was isolated from infested and noninfested plants, labeled and used to hybridize to the miniarrays. Data from an experimental loop design with four replicates per sample were collected and analyzed.

Upon infestation by infective juveniles of *M. incognita*, changes in transcript abundance between inoculated and mock-inoculated control roots were observed for 14% (217 genes across all time points) of total transcripts on the array for the compatible interaction. In pooled susceptible interactions, one-third of differentially regulated genes were induced and two-thirds were repressed. In the incompatible interactions, 58 transcripts were differentially regulated and 31 of them were consistently induced in all resistance responses across the three early time points. This suggested that resistance responses were activated and maintained in the first several days following the infection of root-knot nematodes (Schaff et al. 2007). Moreover, genes induced in incompatible interactions were not differentially regulated in compatible interactions between "Moneymaker" and *M. incognita* (Schaff et al. 2007). For a more detailed list of genes identified in this study, the authors refer you to the published report, Schaff et al. (2007). Comparison of gene expression between uninfested resistant and susceptible cultivars identified only one gene encoding a glycosyltransferase with a greater level of expression in the resistant cultivar. In addition, the expression of the glycosyltransferase gene was nearly six

fold greater in infested roots of the resistant cultivar "Motelle" as compared with noninfested roots. Interestingly, silencing of this gene using virus-induced gene silencing in *Mi*-containing plants resulted in plant susceptibility to root-knot nematodes. This suggests that high expression of the glycosyltransferase gene may be necessary in the resistance response to root-knot nematode in tomato; however, the actual role of this gene remains to be elucidated.

3.2 Microarray Analyses of Tissues Enriched for Nematode Feeding Sites

To increase the chances of finding genes that are differentially regulated in plant–nematode interactions, some investigators have limited their analysis to tissues enriched for nematode feeding sites. Jammes et al. (2005) used this approach to examine gene expression in Arabidopsis over a time course series of 7, 14, and 21 days after inoculation with infective juveniles of *M. incognita*. These time points were selected to represent the induction of giant-cells, the development of giant-cells, and mature giant-cells, respectively. For each time point, RNA was isolated from 1,500 root-knots individually sectioned from infected or control roots, excluding root tips and lateral root meristems. RNA from control and infested roots was labeled and used to hybridize to an array (CATMA chip) carrying 24,576 specific gene-sequence tags (GST) for Arabidopsis. After microarray analysis, 50 genes were further examined by quantitative real-time RT-PCR to verify microarray results.

A total of 3,373 genes represented on the CATMA chip displayed a significant change in transcript abundance in response to the infection by the root-knot nematode. This included 1,606 induced and 1,742 repressed genes in galls. Genes identified by this study were described in Jammes et al. (2005) and the publication has more detailed information. Changes in expression level reached as high as tenfold, highlighting the power of enrichment of infection sites in identifying differentially expressed genes. Changes in gene expression were further analyzed and grouped according to gene function to gain a better understanding of the formation and development of giant-cells (Jammes et al. 2005). The group with the highest number of differentially regulated genes in galls consisted of genes involved in metabolism. This was expected due to the nature of giant-cells as nutrient sinks (Jammes et al. 2005). Interestingly, the number of up-regulated and down-regulated genes was similar in this group. In contrast, genes involved in cell wall modification appeared to be induced, including genes encoding pectate lyases, β-xylosidases, β-1,4-endoglucanases, xyloglucan endotransglycosylases, polygalacturonases and many cell-wall loosening expansins. The expression of many *WRKY* genes decreased in galls, suggesting that sedentary endoparasites like root-knot nematodes may have evolved strategies to actively suppress plant defense signaling (Jammes et al. 2005). Results were further validated using promoter::*GUS* constructs to examine the temporal and spatial expression of a down-regulated gene encoding a trypsin protease inhibitor (TPI) and an up-regulated formin gene (Jammes et al. 2005).

To investigate gene expression in an agriculturally important crop, Bar-Or and colleagues examined the compatible interaction between tomato and *M. javanica* using a tomato microarray containing 12,500 clones randomly selected from cDNA libraries for various tomato tissues (Bar-Or et al. 2005). Tomato roots were inoculated with infective juveniles of *M. javanica* and individual galls were dissected from infested roots at 5 and 10 days after inoculation for RNA isolation. Control RNA was isolated from mock-inoculated roots at the same time points. Comparative hybridizations were conducted between labeled RNA isolated from infested and mock-inoculated roots.

At each time point, a total of 516 genes were found with significant changes in transcript abundance in galls as compared with control roots. For the identity of these genes the authors of this chapter direct you to the paper, Bar-Or et al. (2005). Cluster analysis of expression ratios (galls vs. control roots) revealed that changes in transcript abundance were more pronounced at the later time point (10 days after inoculation) as compared to the early time point. Among differentially regulated genes in galls, 118 were common to both time points, suggesting that a large number of plant genes could be specifically regulated in response to different developmental stages of *M. javanica* (Bar-Or et al. 2005). Even in compatible interactions, plants seem to recognize parasitic nematodes as a pathogen. This was demonstrated by a significant increase in transcript abundance of several genes encoding pathogenesis-related (PR) proteins in tomato (Bar-Or et al. 2005). The induction of some *PR* genes in compatible interactions could be the result of general stress due to nematode infection. In addition, several hormone-related genes, such as genes encoding a gibberellin 2-oxidase-like protein and two ethylene responsive element binding proteins, were up-regulated in galls. Interestingly, similar increases in transcript abundance of these genes were also reported in plants infested by cyst nematodes (Klink et al. 2007b; Mazareri et al. 2002; Puthoff et al. 2003; Wubben et al. 2001). Infections by root-knot and cyst nematodes possibly result in similar fluctuations in the levels of gibberellin or ethylene in plants causing similar expression patterns of genes regulated by hormone signaling (Bar-Or et al. 2005). The expression of several development-associated genes was down-regulated in galls at both time points (Bar-Or et al. 2005).

In 2007, Fuller and associates used the same CATMA microarray chips as Jammes et al. (2005) to examine changes in expression levels of genes induced by the root-knot nematode, *M. incognita* in Arabidopsis (Fuller et al. 2007). Root knots were individually collected from infested roots at 21 days after inoculation (Fuller et al. 2007). In response to nematode infection, general changes in transcript abundance of genes in this study were in agreement with the result in Jammes et al. (2005). Nearly 4% of the genes on the array showed significant changes in transcript abundance in root knots and this included 258 induced and 701 repressed genes. For a more detailed analysis about the identified genes, the authors of this chapter refer you to the publication, Fuller et al. (2007). Genes with a decrease in transcript abundance were classified into groups for signal transduction, responses to biotic or abiotic stress, electron transport and energy metabolism or other biological processes. In contrast, genes with an increase in transcript abundance could

be categorized into groups for cell organization and biogenesis, DNA or RNA metabolism, as well as other biological processes. These findings further support the hypothesis that gene repression is essential in maintaining mature giant-cells (Fuller et al. 2007). The changes in expression levels of 17 genes were verified by quantitative real-time RT-PCR. Promoter::*GUS* constructs were also generated for several genes and tested in transgenic Arabidopsis for detailed expression profiles of these genes in giant-cells and syncytia induced by *H. schachtii*.

Similar to the microarray analysis using whole infested root by Ithal et al. (2007a), Puthoff and colleagues used the same set of soybean microarrays to interrogate gene expression in syncytial enriched tissues (Puthoff et al. 2007). The Affymetrix Soybean GeneChip array representing 35,593 soybean transcripts was used to analyze gene expression in infested roots of the compatible interaction between Williams and *H. glycines* (NL1-RHg, Hg-type 7, race 3) over the course of 8, 12, and 16 days after inoculation. Infested tissues containing syncytia and developing nematodes were dissected under a stereomicroscope to minimize non-infested tissues. For controls, root pieces were collected from roots at similar positions on noninfested roots. Isolated RNA was hybridized to chips and examined for changes in transcript abundance.

A total of 1,404 induced and 739 repressed genes were identified with greater than two fold change in transcript abundance. The authors of this chapter direct you to the publication, Puthoff et al. (2007), for more detailed information on the identity of genes and level of transcript abundance. Over the course of nematode infestation, differentially regulated genes include those associated with defense, dehydration, the phenylpropanoid pathway, cell wall modification, DNA replication, auxin and ethylene regulation.

3.3 Microarray Analyses of Nematode Feeding Sites

For sedentary endoparasitic nematodes such as root-knot and cyst nematodes, feeding sites are the sole nutrient source to support nematode development and reproduction. These feeding structures consist of three to six giant-cells in the case of root-knot nematodes or a syncytia formed by the coalescence of up to 200 root cells in the case of cyst nematodes. The development of nematodes and the growth of feeding structures are intimately associated. Disruption or degradation of nematode feeding sites can lead to changes or cessation of nematode development. Conversely, removal or death of nematodes results in the deterioration of feeding sites. Virtually nothing was known about the expression of genes within feeding sites, partially due to the fact that feeding structures are located in the vascular cylinder and buried underneath layers of cells. A number of technologies have been adopted to isolate these cells for analysis. Wilson et al. (1994) used hand-microdissected root knots to investigate gene expression in feeding sites. Recently, laser-capture microdissection has been integrated in the isolation of feeding structures from root sections (Ramsay et al. 2004). The combination of laser-capture microdissection with

microarrays has proven to be a very powerful technique to investigate gene expression in nematode feeding sites.

One of the first published reports on using microarrays to evaluate RNA isolated by laser-capture microdissection from feeding sites was described by Ithal et al. (2007b). Infected and control roots of susceptible soybean cultivar Williams 82 at 2, 5, and 10 days after inoculation with *H. glycines* were fixed and embedded in paraffin, sectioned and syncytia were captured using LCM. Only a very small amount of RNA was isolated from captured syncytia, thus a linear amplification procedure was used to produce sufficient amounts of RNA for microarray analysis. Three independent biological replicates for each treatment were collected and analyzed on the Affymetrix Soybean GeneChip array. A total of 1,765 genes including 1,116 induced genes and 649 repressed genes were significantly regulated in syncytia of a compatible interaction at 2 days after inoculation. Over 27% of the differentially regulated genes shared no significant sequence homology to genes in public databases. Quantitative real-time RT-PCR was used to validate the microarray results for 25 genes. Clustering analysis of expression patterns over the sampling points led to the discovery of some key biological processes in initiation and formation of syncytia (Ithal et al. 2007b). A number of genes involved in biotic and abiotic stress responses including genes encoding pathogen-responsive receptor-like kinases were down-regulated in syncytia, suggesting a suppression of plant defense mechanisms in compatible interactions (Ithal et al. 2007b). In contrast, a general increase in transcript abundance was detected among genes involved in primary and secondary metabolism and in cell wall modification. A corresponding increase in transcript abundance was detected for peroxidase genes in syncytia (Ithal et al. 2007b), suggesting that peroxidases may be involved in cell wall loosening and cross-linking of cell wall components in the formation and development of syncytia (Passardi et al. 2004). In addition to cell-wall modifying enzymes, a number of differentially regulated genes in the analysis were found to be part of phytohormone signaling including auxin and ethylene (Ithal et al. 2007b). The importance of auxin and ethylene in the formation and development of syncytia has been established in previous targeted studies (Goverse et al. 2000; Mazareri et al. 2002; Wubben et al. 2001). These analyses were further supported by a recent report on expression profiling of cell wall genes in the compatible interaction between soybean and *H. glycines* demonstrating that many induced genes in nematode infested roots were also up-regulated during ethylene-induced petiole abscission (Tucker et al. 2007). Furthermore, changes in transcript abundance for a number of genes involved in gibberellin (GA) and cytokinin biosynthesis and respective signaling pathways were detected in syncytia (Ithal et al. 2007b). For example, a gene encoding a GA-induced cysteine-rich cell wall protein was induced while genes encoding proteins functioning as cytokinin receptors were repressed in syncytia. Interestingly, the expression of the majority of genes regulated by jasmonic acid (JA) was repressed in syncytia (Ithal et al. 2007b). Known to inhibit plant growth and to promote various processes such as pollen formation and fruit ripening, JA, or jasmonates in general, are induced by a variety of biotic and abiotic stresses (Ellis et al. 2002). Cell-wall degrading enzymes are among the

elicitors of JA responses. Root-knot and cyst nematodes are known to release an array of cell-wall degrading enzymes to aid their migration towards the vascular cylinder. For both nematodes, the formation of syncytia or giant-cells involves changes in expression of a number of plant cell wall proteins. Thus, the level or status of phytohormones and downstream signaling pathways are expected to change in response to the infection by parasitic nematodes. A systemic defense response against *M. incognita* was induced in susceptible tomato by foliar application of JA (Cooper et al. 2005). The suppression of many genes involved in JA biosynthesis and signaling pathways in syncytia is likely to inhibit plant defense response to *H. glycines*. However, the connection of phytohormone signaling and the initiation and formation of syncytia or giant-cells remains elusive.

In a related study, Klink and colleagues examined changes in gene expression in compatible and incompatible interactions between soybean and *H. glycines* by comparing whole infested roots with laser-captured syncytia at 3 and 8 days after inoculation (Klink et al. 2007a). Additional comparisons included changes in transcript abundance in laser-captured syncytia among compatible and incompatible reactions and in syncytia across the two time points. Experimental conditions were virtually the same as described in Klink et al. (2007b) and previously covered in this chapter. Labeled cRNA was used to hybridize to Affymetrix Soybean GeneChip microarrays with two replicates per treatment. Syncytia were identified in both compatible and incompatible interactions at 3 days after inoculation. At 8 days after inoculation, continuous development of syncytia was only detected in the compatible interaction, however, syncytia deterioration was observed in the incompatible interaction. Overall, a greater number of differentially regulated genes was found in syncytia or whole infested roots from the incompatible interaction as compared to the compatible interaction at 3 days after inoculation. For a more detailed analysis and report, the authors direct you to Klink et al. (2007a).

Analysis of gene expression in incompatible interactions identified 425 genes that were only differentially regulated in syncytia at 3 days after inoculation. The number of repressed genes (278) was nearly two fold greater than that of induced genes (147) at this sampling point. A total of 287 differentially regulated genes including 77 induced genes and 210 repressed genes were found to be unique to syncytia in compatible interactions at 3 days after inoculation. At this early time point, the comparison of up-regulated genes in syncytia from compatible and incompatible interactions showed that 30 genes were shared by both interactions, but 136 genes were specific to the incompatible interaction and 49 were unique to the compatible interaction. Similarly, analysis of down-regulated genes identified that 154 genes were common to both interactions, but 163 genes were unique to the incompatible interaction and 118 genes to the compatible interaction. At 8 days after inoculation, a total of 269 differentially regulated genes were unique to the syncytia from the compatible interaction and over 76% of these genes were induced, suggesting a fundamental change in expression of genes in relation to the development of syncytia. Analysis of differentially regulated genes in syncytia from the compatible interaction across the sampling points revealed that 22 induced and 49 repressed genes were common to both time points. However, among differentially regulated genes unique to syncytia

from compatible interactions, the number of induced genes increased but the number of repressed genes declined over time. From these results, Klink and colleagues (2007a) concluded that changes in gene expression in syncytia were detected not only between compatible and incompatible interactions but also over the development of syncytia in the compatible interaction.

An alternative approach of using arrays to examine expression level of all genes is to focus on genes in specific pathways or functional groups. Syncytia induced by cyst nematodes are characterized by the thickening of cell walls and increase in subcellular organelles including plastids and mitochondria. These morphological changes led researchers in two separate microarray studies to evaluate a group of cell wall genes (i.e. expansins; Wieczorek et al. 2006) and metabolic pathways (i.e. starch accumulation and degradation; Hofmann et al. 2008). Expansin proteins are involved in cell wall breakdown or softening during cell expansion and are hypothesized to play important roles in the development of nematode feeding sites (Li et al. 2003; Cosgrove 2000; Gheysen and Mitchum et al. 2008). Syncytial RNA was microaspirated from *H. schachtii*-induced syncytia in infested Arabidopsis roots and used to construct a cDNA library. Using gene-specific primers to 26 α- and three β-expansin genes, ten expansin genes were amplified from the syncytial library (Wieczorek et al. 2006). Analysis of ATH1 microarrays of RNA from control root tissues (nonsyncytial containing roots) and microaspirated syncytia indicated that five (*AtEXPA3*, *AtEXPA6*, *AtEXPA8*, *AtEXPA10*, and *AtEXPA16*) were up-regulated specifically in the syncytia. Promoter::*GUS* and in situ RT-PCR were used to confirm expression of expansin genes in the feeding structures. Interestingly, two expansin genes *AtEXPA3* and *AtEXPA16* were found to be exclusively expressed in the shoots of noninfested *Arabidopsis* plants.

Similarly, Hofmann and colleagues (2008) used microarrays to address the role of starch accumulation and degradation in syncytia induced by *H. schachtii*. Close examination of syncytia revealed that large starch granules were present in numerous plastids and dramatic changes in the morphology of plastids were associated with the development of syncytia (Hofmann et al. 2008). In 15-day-old syncytia, a significant induction was detected for a majority of the 56 genes that are involved in starch synthesis and degradation. The accumulation of starch in syncytia serves as an intermediate in carbohydrate storage and balance changes in sugar levels throughout plant–nematode interactions. The demand for nutrients increases with the development of nematodes, especially with mature females that are known to feed on syncytia over an extended period of time. The dramatic up-regulation of starch-metabolism genes in 15-day-old syncytia corresponds to the development of adult females. Furthermore, the analysis of starch content in the *Arabidopsis* T-DNA knockout line for STARCH SYNTHASE1 (*SS1*) revealed that the mutant produced 23% less starch in leaves. In addition, this mutant line supported significantly fewer numbers of female and male nematodes as compared to wild-type plants (Hofmann et al. 2008). The use of microarrays in a selective manner clearly allows researchers to address important biological questions about gene families or pathways in plant–nematode interactions.

3.4 Microarray Analyses of Plant-Parasitic Nematodes

Little is known about global changes in gene expression in parasitic nematodes. On the Affymetrix soybean GeneChip, nearly 7,500 oligos specific to *H. glycines* genes have been arrayed in addition to 35,000 soybean gene transcripts and 7,500 *Phythopthora sojae* genes. Using whole infested roots, Ithal and colleagues (2007a) examined the expression of nematode genes in a compatible interaction. Significant changes in transcript abundance were detected for 1,859 transcripts of *H. glycines* on the array (Ithal et al. 2007a). An increase in transcript abundance for genes involved in nematode development and metabolism (including genes encoding nematode ribosomal proteins) was observed across the time points of 2, 5, and 10 days after inoculation. However, expression of genes previously identified as nematode parasitism gene candidates from esophageal gland cells declined over time, suggesting the importance of these genes in nematode migration and initiation of feeding sites. A majority of nematode genes encoding cell-wall modification proteins were represented in this group. In addition, some genes, including the gene encoding a putative cuticular collagen, expressed at very low levels in infective juveniles, but the expression increased in parasitic juveniles and reached a peak at 5 days after inoculation. Changes in the expression level of the putative cuticular collagen gene are associated with the development of *H. glycines* from motile to sedentary. In contrast, high expression of the gene encoding a putative secreted glutathione peroxidase was found in infective juveniles, but the expression declined at 2 days after inoculation, followed by an increase and peak expression at 5 days after inoculation (Ithal et al. 2007a). Notably, a large number of differentially regulated nematode genes were found to encode proteins with unknown functions. Thus, studying expression patterns of these genes would provide the first hints regarding the role of these genes over the course of nematode infection.

4 Conclusions

Induced by nematodes, root cells are transformed to become specialized feeding structures (giant-cells or syncytia) that serve as the sole nutrient sources for nematode development and reproduction. Despite a decade of study in gene expression, both plant and nematode genes associated with the formation and development of feeding structures remain poorly understood. However, recent application of microarray technology coupled with the integration of laser-capture microdissection has become a powerful tool for simultaneously monitoring changes in transcript abundance of thousands of genes within feeding structures. Microarrays are available for both model plants and economically important crops such as soybean and tomato, allowing researchers to examine gene expression in several different systems. In addition, susceptible, resistant, and nonhost plants along with virulent and avirulent strains of nematodes are excellent materials for comparative analyses providing new insights into dissecting plant susceptibility and resistance to parasitic nematodes.

Taken together, the generation of enormous data sets by microarray analyses using different plant systems opens up multiple directions in developing a better understanding of plant–nematode interactions.

Acknowledgments There is a wide swath of literature on the analyses of changes in expression levels of plant or nematode genes and we apologize for those cases of omission. This review was funded in part by grants from the National Science Foundation, the Environmental Protection Agency, and the United Soybean Board. We are grateful to Heather Marella, Joe Kamalay, Christine Ehret, and Marti Shafer for their critical evaluation and editing of this review.

References

Alkharouf NW, Klink VP, Chouikha IB, Beard HS, MacDonald MH, Meyer S, Knap HT, Khan R, Matthews BF (2006) Timecourse microarray analyses reveal global changes in gene expression of susceptible *Glycine max* (soybean) roots during infection by *Heterodera glycines* (soybean cyst nematode). Planta 224:838–852

Allemeersch J, Durinck S, Vanderhaeghen R, Alard P, Maes R, Seeuws K, Bogaert T, Coddens K, Deschouwer K, Hummelen PV, Vuylsteke M, Moreau Y, Kwekkeboom J, Wijfjes AHM, May S, Beynon J, Hilson P, Kuiper MTR (2005) Benchmarking the CATMA microarray: a novel tool for Arabidopsis transcriptome analysis. Plant Physiol 137:588–601

Asano T, Masumura T, Kusano H, Kikuchi S, Kurita A, Shimada H, Kadowaki K (2002) Construction of a specialized cDNA library from plant cells isolated by laser capture microdissection: toward comprehensive analysis of the genes expressed in the rice phloem. Plant J 32:401–408

Bar-Or C, Kapulnik Y, Kpltai H (2005) A broad characterization of the transcriptional profile of the compatible tomato response to the plant-parasitic root-knot nematode *Meloidogyne javanica*. Eur J Plant Pathol 111:181–192

Berg RH, Fester T, Taylor CG (2008) Development of the root-knot nematode feeding cell. Plant Cell Monogr., doi:10.1007/7089_2008_30

Bird D, Wilson M (1994) DNA sequence and expression analysis of root-knot nematode-elicited giant-cell transcripts. Mol Plant Microbe Interact 7:419–424

Bird DMcK, Opperman CH, Williamson VM (2008) Plant infection by root-knot nematode. Plant Cell Monogr., doi:10.1007/7089_2008_31

Brummell DA, Harpster MH (2001) Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. Plant Mol Biol 47:311–340

Burgess JK, McParland BE (2002) Analysis of gene expression. Method Enzymol 356:259–270

Cannon MC, Terneus K, Hall Q,Tan L, Wang Y, Wegenhart BL, Chen L, Lamport DTA, Chen Y, Kieliszewski MJ (2008) Self-assembly of the plant cell wall requires an extensin scaffold. Proc Natl Acad Sci USA 105:2226–2231

Clarke JD, Zhu T (2006) Microarray analysis of the transcriptome as a stepping stone towards understanding biological systems: practical considerations and perspectives. Plant J 45:630–650

Conn MP (2002) Laser-capture microscopy. Method Enzymol 356

Cooper WR, Jia L, Goggin L (2005) Effects of jasomiate-induced defenses on root-knot nematode infection of resistant and susceptible tomato cultivars. J Chem Ecol 31:1953–1967

Cosgrove DJ (2000) Loosening plant cell walls by expansins. Nature 407:321–326

Craven RA, Banks RE (2002) Use of laser-capture microdissection to selectively obtain distinct populations of cells for proteomic analysis. Method Enzymol 356:33–49

Dautova M, Rosso MN, Abad P, Gommers FJ, Bakker J, Smant G (2001) Single pass cDNA sequencing-a powerful tool to analyse gene expression in preparasitic juveniles of the southern root-knot nematode *Meloidogyne incognita*. Nematology 3:129–139

Davis EL, Hussey RS, Baum TJ (2008) Parasitism genes: what they reveal about parasitism. Plant Cell Monogr., doi:10.1007/7089_2008_29

Dubreuil G, Magliano M, Deleury E, Abad P, Rosso MN (2007) Transcriptome analysis of root-knot nematode functions induced in the early stages of parasitism. New Phytologist 176:426–436

Ellis C, Karafyllidis I, Wasternack C, Turner JG (2002) The Arabidopsis mutant *cev1* links cell wall signaling to jasmonate and ethylene responses. The Plant Cell 14:1557–1566

Endo BY (1964) Penetration and development of *Heterodera glycines* in soybean roots and related anatomical changes. J Nematol 54:79–88

Endo BY (1991) Ultrastructure of initial responses of susceptible and resistant soybean roots to infection by *Heterodera glycines*. Rev de Nématol 14:73–94

Eycken WVD, Engler JDA, Inze D, Montagu AV, Gheysen G (1996) A molecular study of root-knot nematode-induced feeding sites. Plant J 9:45–54

Favery B, Lecomte P, Gil N, Bechtold N, Bouchez D, Dalmasso A, Abad P (1998) *RPE*, a plant gene involved in early developmental steps of nematode feeding sites. EMBO J 17:6799–6811

Fuller VL, Lilley CJ, Atkinson HJ, Urwin PE (2007) Differential gene expression in *Arabidopsis* following infection by nematodes *Meloidogyne incognita* and *Heterodera schachtii*. Mol Plant Pathol 8:595–609

Galbraith DW (2006) Microarray analyses in higher plants. OMICS 10:455–473

Gheysen G, Fenoll C (2002) Gene expression in nematode feeding sites. Ann Rev Phytopathol 40:191–219

Gheysen G, Mitchum MG (2008) Molecular insights in the susceptible plant response to nematode infection. Plant Cell Monogr., doi:10.1007/7089_2008_35

Golinowski W, Grundler FMW, Sobczak M (1996) Changes in the structure of *Arabidopsis thaliana* during female development of the plant-parasitic nematode *Heterodera schachtii*. Protoplasma 194:103–116

Goverse A, Overmars H, Engelbertink J, Schots A, Bakker J, Helder J (2000) Both induction and morphogenesis of cyst nematode feeding cells are mediated by auxin. Mol Plant Microbe Interact 13:1121–1123

Grundler FMW, Sobczak M, Lange S (1997) Defence responses of *Arabidopsis thaliana* during invasion and feeding site induction by the plant-parasitic nematode *Heterodera glycines*. Physiol Mol Plant Pathol 50:419–429

Hammes UZ, Schachtman DP, Berg RH, Nielsen E, Koch W, Mclntyre LM, Taylor CG (2005) Nematode-induced changes of transporter gene expression in *Arabidopsis* roots. Mol Plant Microbe Interact 18:1247–1257

Hammes UZ, Nielsen E, Honaas LA, Taylor CG, Schachtman DP (2006) AtCAT6, a sink-tissue-localized transporter for essential amino acids in Arabidopsis. Plant J 48:414–426

Hermsmeier D, Mazarei M, Baum TJ (1998) Differential display analysis of the early compatible interaction between soybean and the soybean cyst nematode. Mol Plant Microbe Interact 11:1258–1263

Hermsmeier D, Hart JK, Byzova M, Rodermel SR, Baum TJ (2000) Changes in mRNA abundance within *Heterodera schachtii*-infested roots of *Arabidopsis thaliana*. Mol Plant Microbe Interact 13:309–315

Hilson P, Allemeersch J, Altmann T, Aubourg S, Avon A, Beynon J, Bhalerao RP, Bitton F, Caboche M, Cannoot B, Chardakov V, Cognet-Holliger C, Colot V, Crowe M, Darimont C, Durinck S, Eickhoff H, de Longevialle AF, Farmer EE, Grant M, Kuiper MT, Lehrach H, Léon C, Leyva A, Lundeberg J, Lurin C, Moreau Y, Nietfeld W, Paz-Ares J, Reymond P, Rouzé P, Sandberg G, Segura MD, Serizet C, Tabrett A, Taconnat L, Thareau V, Van Hummelen P, Vercruysse S, Vuylsteke M, Weingartner M, Weisbeek PJ, Wirta V, Wittink FR, Zabeau M, Small I. (2004) Versatile gene-specific sequence tags for *Arabidopsis* functional genomics: transcript profiling and reverse genetics applications. Genome Res 14:2176–2189

Hofmann J, Szakasits D, Blochl A, Sobczak M, Daxbock-Hovath S, Golinowski W, Bohlmann H, Grundler FMW (2008) Starch serves as carbohydrate storage in nematode-induced syncytia. Plant Physiol 146:228–235

Huang X, Springer PS, Kaloshian I (2003). Expression of the Arabidopsis *MCM* gene *PROLIFERA* during root-knot and cyst nematode infection. Nematology 93:35–41

Ithal N, Recknor J, Nettleton D, Hearne L, Maier T, Baum TJ, Mitchum MG (2007a) Parallel genome-wide expression profiling of host and pathogen during soybean cyst nematode infection of soybean. Mol Plant Microbe Interact 20:293–305

Ithal N, Recknor J, Bettleton D, Maier T, Baum TJ, Mitchum MG (2007b) Developmental transcript profiling of cyst nematode feeding cells in soybean roots. Mol Plant Microbe Interact 20:510–525

Jammes F, Lecomte P, Almeida-Engler J, Bitton F, Martin-Magniette M, Renou JP, Abad P, Favery B (2005) Genome-wide expression profiling of the host response to root-knot nematode infection in *Arabidopsis*. Plant J 44:447–458

Jones MGK (1981) Host cell responses to endoparasitic nematode attack: structure and function of giant-cells and syncytia. Annu Appl Biol 97:353–372

Juergensen K, SchoLz-Starke J, Sauer N, Hess P, van Bel AJE, Grundler FMW (2003) The companion cell-specific Arabidopsis disaccharide carrier AtSUC2 is expressed in nematode-induced syncytia. Plant Physiol 131:61–69

Khan R, Alkharouf N, Beard H, Macdonald M, Chouikha I, Meyer S, Grefenstette J, Knap H, Matthews B (2004) Microarray analysis of gene expression in soybean roots susceptible to the soybean cyst nematode two days post invasion. J Nematol 36:241–248

Kieliszewski M, Lamport DTA (1994) Extensin:repetitive motifs, functional sites, posttranslational codes, and phylogeny. Plant J 5:157–172

Klink VP, Alkharouf N, MacDonald M, Matthews BF (2005) Laser capture microdissection (LCM) and expression analyses of *Glycine max* (soybean) syncytium containing root regions formed by the plant pathogen *Heterodera glycines* (soybean cyst nematode). Plant Mol Biol 59:965–979

Klink VP, Over CC, Alkharouf NW, MacDonald MH, Matthews BF (2007a) Laser capture microdissection (LCM) and comparative microarray expression analysis of syncytial cells isolated form incompatible and compatible soybean (*Glycine Max*) roots infested by the soybean cyst nematode (*Heterodera glycines*). Planta 226:1389–1409

Klink VP, Overall CC, Alkharouf NW, MacDonald MH, Matthews BF (2007b) A time-course comparative microarray analysis of an incompatible and compatible response by *Glycine max* (soybean) to *Heterodera glycines* (soybean cyst nematode) infection. Planta 226:1423–1447

Leiers B, Kampkotter A, Grevelding CG, Link CD, Johnson TE, Henkle-Duhrsen K (2003) A stress-responsive glutathione-*S*-transferase confers resistance to oxidative stress in *Caenorhabditis elegans*. Free Radic Biol Med 34:1405–1415

Li Y, Jones L, McQueen-Mason SJ (2003) Expansins and plant cell growth. Curr Opin Plant Biol 6:603–610

Lindeman N, Waltregny D, Signoretti S, Loda M (2002) Gene transcript quantitation by real-time RT-PCR in cells selected by immunohistochemistry-laser capture microdissection. Diag Mol Pathol 11:187–192

Mahalingam R, Wang G, Knap HT (1999) Polygalaturonase and polygalacturonase inhibitor protein: gene isolation and transcription in *Glycine max–Heterodera glycines* interactions. Mol Plant Microbe Interact 12:490–498

Mazareri M, Puthoff DP, Hart JK, Rodermel SR, Baum TJ (2002) Identification and characterization of a soybean ethylene-responsive element-binding protein gene whose mRNA expression changes during soybean cyst nematode infection. Mol Plant Microbe Interact 15:577–586

McCarter JP, Abad P, Jones JT, Bird DM (2000) Rapid gene discovery in plant parasitic nematodes via expressed sequence tags. Nematology 2:719–731

McCarter JP, Mitreva MD, Martin J, Dante M, Wylie T, Rao U, Pape D, Bowers Y, Theisin B, Murphy CV, Kloek AP, Chiapelli BJ, Clifton SW, Bird DM, Waterston RH (2003) Analysis and functional classification of transcripts from the nematode *Meloidogyne incognita*. Genome Biol 4:R26

Meyers BC, Galbraith DW, Nelsen T, Agrawal V (2004) Methods for transcriptional profiling in plants. Be fruitful and replicate. Plant Physiol 135:637–652

Neira M, Azen E (2002) Gene discovery with laser-capture microscopy. Method Enzymol 356:282–289
Nettleton D (2006) A discussion of statistical methods for design and analysis of microarray experiments for plant scientists. Plant Cell 18:2112–2121
Niebel A, Heungens K, Barthels N, Inze D, Montagu MV, Gheysen G (1995) Characterization of a pathogen-induced potato catalase and its systemic expression upon nematode and bacterial infection. Mol Plant Microbe Interact 8:371–378
Niebel A, Engler JDA, Hemerly A, Derreira P, Inze D, Montagu MV, Ghensen G (1996) Induction of *cdc2* and *cyc1At* expression in *Arabidopsis thaliana* during early phases of nematode-induced feeding cell formation. Plant J 10:1037–1043
Offler CE, McCurdy DW, Patrick JW, Talbot MJ (2002) Transfer cells: cells specialized for a special purpose. Annu Rev Plant Biol 54:431–454
Opperman CH, Taylor CG, Conkling MA (1994) Root-knot nematode directed expression of a plant root-specific gene. Science 263:221–223
Opperman CH, Bird DMcK, Schaff JE (2008) Genomic analysis of the root-knot nematode genome. Plant Cell Monogr., doi:10.1007/7089_2008_37
Passardi F, Penel C, Dunand C (2004) Performing the paradoxical: how plant peroxidases modify the cell wall. Trends Plant Sci 9:534–540
Popeijus H, Blok VC, Cardle L, Bakker E, Phillips MS, Helder J, Smant G, Jones JT (2000) Analysis of genes expressed in second stage juveniles of the potato cyst nematode *Globodera rostochiensis* and *G. pallida* using the expressed sequence tag approach. Nematology 2:567–574
Potenza C, Thomas SH, Sengupta-Gopalan C (2001) Genes induced during early response to *Meloidogyne incognita* in roots of resistant and susceptible alfalfa cultivars. Plant Sci 161:289–299
Puthoff D, Nettleton D, Rodermel SR, Baum TJ (2003) Arabidopsis gene expression changes during cyst nematode parasitism revealed by statistical analysis of microarray expression profiles. Plant J 33:911–921
Puthoff DP, Ehrenfried ML, Vinyard BT, Tucker ML (2007) Genechip profiling of transcriptional responses to soybean cyst nematode, *Heterodera glycines*, colonization of soybean roots. J Exp Bot 58:3407–3418
Ramsay K, Wang Z, Jones MGK (2004) Using Laser capture microdissection to study gene expression in early stages of giant-cells induced by root-knot nematodes. Mol Plant Pathol 5:587–592
Rensink WA, Buell CR (2005) Microarray expression profiling resources for plant genomics. Trends Plant Sci 10:603–609
Roze EHA, Hanse B, Mitreva M, Vanholme B, Bakker J, Smant G (2008) Mining the secretome of the root-knot nematode *Meloidogyne chitwoodi* for candidate parasitism genes. Mol Plant Pathol 9:1–10
Ruben E, Aziz J, Afzal J, Njiti VN, Triwitayakorn K, Iqbal MJ, Yaegashi S, Arelli P, Town C, Meksem K, Lightfoot DA (2006) Genomic analysis of the 'Peking' *rhg1* locus: candidate genes that underlie soybean resistance to the cyst nematode. Mol Gen Genomics 276:320–330
Schaff JE, Nielsen DM, Smith CP, Scholl EH, Bird DM (2007) Comprehensive transcriptome profiling in tomato reveals a role for glycosyltransferase in *Mi*-mediated nematode resistance. Plant Physiol 144:1079–1092
Schena M, Shalon D, Brown PO, Davis RW (1995) Quantitative monitoring of gene-expression patterns with a complementary cDNA microarray. Science 270:467–470
Schnable PS, Nakazono M, Hochholdinger F (2004) Global expression profiling applied to plant development. Cur Opin Plant Biol 7:50–56
Sobczak M, Golinowski W (2008) Structure of cyst nematode feeding sites. Plant Cell Monogr., doi:10.1007/7089_2008_38
Stepanova AN, Ecker JR (2000) Ethylene signaling: from mutants to molecules. Curr Opin Plant Biol 3:353–360

Todd R, Lingen MW, Kuo WP (2002) Gene expression profiling using laser capture microdissection. Expert Rev Mol Diagn 2:497–507

Tomczak A, Koropacka K, Smant G, Goverse A, Bakker E (2008) Resistant plant responses. Plant Cell Monogr., doi:10.1007/7089_2008_39

Tucker ML, Burke A, Murphy CA, Thai VK, Ehrenfried ML (2007) Gene expression profiles for cell wall modifying proteins associated with soybean cyst nematode infection, petiole abscission, root tips, flowers, apical buds and leaves. J Exp Bot 58:3395–3406

Vaghchhipawala Z, Bassüner R, Clayton K, Lewers K, Shoemaker R, Mackenzie S (2001) Modulations in gene expression and mapping of genes associated with cyst nematode infection of soybean. Mol Plant Microbe Interact 14:42–54

Vercauteren I, Van Der Schueren E, Van Montagu M, Gheysen G (2001) *Arabidopsis thaliana* genes expressed in the early compatible interaction with root-knot nematodes. Mol Plant Microbe Interact 14:288–299

Vercauteren I, Engler JDA, Groodt RD, Gheysen G (2002) An *Arabidospsis thaliana* pectin acetylesterase gene in upregulated in nematode feeding sites induced by root-knot and cyst nematodes. Mol Plant Microbe Interact 15:404–407

Wieczorek K, Golecki B, Gerdes L, Heinen P, Azakasits D, Durachko DM, Cosgrove DJ, Kreil DP, Puzio PS, Bohlmann H, Grundler FMW (2006) Expansins are involved in the formation of nematode-induced syncytia in roots of *Arabidopsis thaliana*. Plant J 48:98–112

Wilson MA, Bird DM, van der Knaap E (1994) A comprehensive subtractive cDNA cloning approach to identify nematode induced transcripts in tomato. Phytopathology 84:299–303

Wubben MJE, Su H, Rodermel SR, Baum TJ (2001) Susceptibility to the sugar beet cyst nematode in modulated by ethylene signal transduction in *Arabidopsis thanliana*. Mol Plant Microbe Interact 14:1206–1212

Xu BJ, Caprioli RM, Sanders ME, Jensen RA (2002) Direct analysis of laser-capture microdissected cells by MALDI mass spectrometry. J Am Soc Mass Spectr 13:1292–1297

Zhou J, Tang X, Martin GB (1997) The Pto kinase conferring resistance to tomato bacterial speck disease interacts with proteins that bind to a *cis*-element of pathogenesis-related genes. EMBO J 16:3207–3218

Zimmermann P, Hennig L, Gruissem W (2005) Gene-expression analysis and network discovery using GENEVESTIGATOR. Trends Plant Sci 10:407–409

Genomic Analysis of the Root-Knot Nematode Genome

Charles H. Opperman(✉), David McK. Bird, and Jennifer E. Schaff

Abstract Plant-parasitic nematodes cause substantial agricultural damage throughout the world, triggering as much as $100 billion in economic losses per year. Measures to control these pests are limited and include the use of agrichemicals such as methyl bromide (now available only on a "critical use exemption" basis) or the planting of crops that have natural resistance. However, the availability of chemical pesticides is decreasing and host resistance is limited. A better understanding of the complex interaction between plant-parasitic nematodes and their hosts is needed to develop new control strategies (including new chemicals). The vast majority of the damage is caused by sedentary endoparasitic forms in the order Tylenchida, which fall into clade IV of the Nematoda (Blaxter et al. 1998). In particular, the root-knot nematodes (Meloidogyne spp.), soybean cyst nematodes (Heterodera glycines), and potato cyst (Globodera spp.) nematodes are devastating parasites of plant roots.

In this chapter, we will present a brief overview of the status of genomic research on root-knot nematodes. Root-knot nematodes, in particular *M. hapla*, are emerging as a model species for research on sedentary endoparasites. We will discuss the impact of root-knot nematodes on the host plant and focus on genomic approaches to unraveling the complex nature of the interaction from the nematode's perspective. In addition to the complete genome sequence of *M. hapla*, a complete genome sequence has simultaneously been obtained for the aneuploid species, *M. incognita* (Abad, personal communication). In the future, comparison between these two genomes will provide fundamental clues as to the evolution and biology of root-knot nematodes.

C.H. Opperman
Center for the Biology of Nematode Parasitism, North Carolina State University,
Raleigh, NC 27695, USA,
Department of Plant Pathology, North Carolina State University,
Raleigh, NC 27695, USA,
and Department of Genetics, North Carolina State University,
Raleigh, NC 27695, USA
e-mail: warthog@ncsu.edu

Plant Cell Monogr, doi:10.1007/7089_2008_37

1 Introduction

Nematodes are the most abundant and speciose metazoans, and they account for up to 80% of the animal kingdom's members (Boucher and Lambshead 1994). Most, like *Caenorhabditis elegans*, are free-living and graze on microbes or detritus and as such have no obvious influence on humans. Others, however, are adapted as parasites and have a substantial and direct impact on human health, animal production, and food and fiber crops worldwide. Although the evolutionary relationship of nematodes to other animals (especially insects) is controversial (Aguinaldo et al. 1997; Mallatt and Winchell 2002), a robust phylogeny dividing Nematoda into five major clades has been established (Blaxter et al. 1998). Interestingly, this analysis implies that plant and animal parasitism arose multiple times along independent branches over the course of nematode evolution.

The *C. elegans* genome was the first multicellular eukaryote genome completed (*C. elegans* Sequencing Consortium 1998). The power of this 100-Mb sequence is due in a large degree to the extensive functional biology generated over the past decades (Wood 1998; Riddle et al. 1997) and integrated into WormBase (http://www.wormbase.org). The *C. elegans* genome has proven to be an essential resource for annotating parasitic nematode genes identified from Expressed Sequence Tags (EST) sequencing projects (McCarter et al. 2003; Scholl et al. 2003), and it also "anchors" the phylogeny of this large phylum as the reference clade V species. In particular, *C. elegans* represents an out-group to establish specific relationships within the Nematoda.

The 104-Mb *Caenorhabditis briggsae* genome has also been completed (Stein et al. 2003), providing a platform for comparative genomics. Like *C. elegans*, this species encodes ~20,000 proteins. Approximately 12,200 *C. briggsae* genes have clear *C. elegans* orthologs, and a further 6,500 have one or more clearly detectable *C. elegans* homologs, with ~800 *C. briggsae* genes having no detectable match in *C. elegans*. Essentially all of the known noncoding RNAs are shared between the two species and operons are highly conserved. The two genomes exhibit extensive colinearity, and the rate of divergence appears to be higher in the chromosomal arms than in the centers. Although almost indistinguishable by eye and apparently sharing identical biology, *C. briggsae* and *C. elegans* split from a common ancestor ~100 million years ago, and this is reflected in divergence of noncoding regions. It is anticipated that identification of conserved elements within this divergent background will point to subtle primary and higher-order regulatory elements. Currently, many genomes of free-living *Caenorhabditids* have been completed including *C. japonicum*, *C. remaniae*, and *Caenorhabditis* sp. CB 5161. These sequences will prove invaluable to improving annotation of the *C. elegans* genome, as well as providing key information on the evolution of Nematoda. Another free-living nematode, *Pristiochus pacificus*, is also being completed and will enable further comparisons across long evolutionary distances about nematode biology. Comparisons of these genomes will help to further understanding of the evolutionary forces that mold nematode genomes.

The Filarial Genome Project has now finished a draft genome sequence *of Brugia malayi* which is responsible for human filariasis and elephantiasis (Ghedin et al. 2007). Like *C. elegans*, *B. malayi* has six chromosomes which comprise the predicted 90–95-Mb genome. Although diverged by over 350 million years, comparative analysis with *C. elegans* revealed that genes largely remain in linkage on chromosomal units though local synteny is not observed. In addition, more than 100 conserved operons were identified. Although preliminary, there appears to be evidence for adaptations of *B. malayi* to niches in its human and vector hosts as well as to the molecular basis of a mutualistic relationship with its *Wolbachia* endosymbiont. In addition to contributing to enhanced understanding of filarial parasite biology and suggesting new vaccine candidates and drug targets, *Brugia* defines the reference clade III nematode genome (Blaxter et al. 1998) and, like *C. elegans* and *C. briggsae*, will be invaluable for annotating new nematode genomes (Bird et al. 1999).

At this writing, there are two complete plant parasitic nematode genomes, *M. hapla* (54 Mb) and *M. incognita* (80 Mb), a partial sequence from *Heterodera glycines* (92.5 Mb) (Monsanto), and the planned sequence of *Globodera pallida*, the white potato cyst nematode. There are numerous genome-sequencing projects underway for animal-parasitic nematode species including the animal parasites: *Ascaris suum*, *Haemonchus contortus*, *Heterorhabditis bacteriophora* (entomopathic), *Necator americanum*, *Telatodorsagia circumcincta*, *Trichinella spiralis*, and *Trichuris muris*. These completed sequences will be a platform for analysis of traits related to parasitic abilities. Obviously, obtaining more nematode genomes provides additional platforms for comparative genomics and, as such, will enhance the annotation of the *Caenorhabditis* and *Brugia* genomes, and vice versa. Furthermore, genomes from clades other than III (*Brugia*) or V (*Caenorhabditis*) will provide a wealth of evolutionary information for the phylum as a whole. Importantly, comparing nematodes from different niches (free-living vs. animal-parasite vs. plant-parasite) will permit niche-specific genes to be discerned, and even with just small EST data-sets, this has proven powerful (McCarter et al. 2003) and led to possible new control strategies.

2 Root-Knot Nematodes as Model Parasites

The Northern root-knot nematode, *Meloidogyne hapla*, was an ideal choice for a full genome sequence project. Most economically important root-knot nematode species reproduce by mitotic parthenogenesis, making genetic crossing impossible as well as the development of well-characterized recombinant inbred lines. In contrast, *M. hapla* has a meiotic reproduction lifestyle and genetic crosses are possible. This biology was exploited to create robust inbred lines and to produce a genetic map based on controlled crosses. Combined with the small genome size (54 MB) and the deep EST database, this made *M. hapla* an ideal candidate for genome sequencing. Additionally, *M. hapla* represents the smallest multicellular eukaryote yet sequenced. The extreme importance of the root-knot nematodes in production agriculture, along with the biological features of *M. hapla*, suggested that the information gleaned from the full genome sequence

would provide clues to not only management of these parasites, but also to the evolution of parasitic abilities and deep phylogenetic relationships. The concurrent project at INRA to sequence *M. incognita* provides an exceptionally powerful platform for nematologists to study the relationships of these parasites.

Root-knot nematodes have a very broad host range, encompassing more than 2,000 plant species, and most cultivated crops are attacked by at least one species of *Meloidogyne* (Sasser 1980). Three species, *M. incognita*, *M. javanica*, and *M. arenaria*, have short life cycles (typically 4 weeks at 25°C), high fecundity (typically 1,000 eggs/female) and reproduce by mitotic parthenogenesis. In cooler, more temperate climates, other species of root-knot nematode are more prevalent. These include *M. hapla*, *M. naasi*, and *M. chitwoodi* which reproduce either by meiotic parthenogenesis or amphimixis. The problem in the sub-tropics and tropics is particularly severe, and many developing nations are seriously impacted in both food security and economics by root-knot nematodes.

Mature female root-knot nematodes release hundreds of eggs into a proteinaceous matrix on the surface of the root. Following a first molt in the egg, motile second-stage juveniles (J2) hatch in the soil and typically re-infect the same plant. It has been proposed that the infective stages of the sedentary endo-parasitic forms, including the *Meloidogyne* J2, function as dauers (Bird and Opperman 1998; Bird et al. 2008). Dauer larvae were first described (Fuchs 1915) as a parasitism adaptation to overcome adverse environmental conditions and facilitate dispersal; they have been best studied in *C. elegans*. There is no strict definition of a "dauer," but these larvae share the properties of being developmentally arrested, motile, nonfeeding, nonageing and hence long-lived (Cassada and Russell 1975; Klass and Hirsh 1976; Riddle and Albert 1997). Dauer entry and exit is controlled by the environmental cues of "food signal" and nematode population density which is established based on a secreted pheromone; the latter process is often termed "quorum sensing." It is clear that although the various arrested parasites functionally resemble *C. elegans* dauers, they must respond to a widely different range of cues for entry and exit.

Root-knot nematode J2 "dauers" destructively penetrate the root, preferentially in the zone of elongation or at the site of a lateral root emergence, and migrate intercellularly into the vascular cylinder, causing little or no injury. Once in the vascular cylinder, the nematode makes a commitment to establish a feeding site. Although the basis for this decision is unknown, the events that immediately ensue are central to the host–parasite interaction and involve dramatic changes both in plant and nematode, leading to giant-cell induction and gall formation (Gheysen and Mitchum 2008; Berg et al. 2008).

2.1 Root-Knot Nematode's Impact on Host Gene Expression

The formation of giant-cells, a highly specialized feeding site, is complex, and it is clear that plant gene expression is altered in host tissue during induction and maintenance. Microarrays and now, second-generation sequencing platforms are powerful tools in determining gene expression patterns in both root-knot nematodes (Schaff,

unpublished data) and host plants. Bird and Wilson (1994) used a subtractive cDNA approach to provide the first comprehensive analysis of host gene expression when they revealed a set of nearly 150 genes up-regulated in giant-cells (as compared to uninfected root tissue). This gene set includes the gene *Le-phan* (Thiery et al. 1999), a canonical member of the myb-family of transcription regulators. Using in situ hybridizations, transcripts from *Le-phan* have been localized to root-knot nematode feeding cells as well as to meristematic tissue (Koltai and Bird 2000). Other gene discovery and gene testing strategies, such as promoter trapping (Sijmons et al. 1994; Barthels et al. 1997; Favery et al. 2002), have yielded other candidate genes associated with feeding sites and collectively, these experiments have revealed that genes regulating the cell cycle (Niebel et al. 1996; de Almeida-Engler et al. 1999) and cell wall synthesis (Niebel et al. 1993; Goellner et al. 2001; Vercauteren et al. 2002) are very important in the development of nematode feeding sites. Gheyson and Fenoll (2002), Gheysen and Mitchum (2008) and Li et al. (2008) provide a detailed review of many of these genes and the techniques used to discover and/or verify them.

The advent of microarrays enabled researches to simultaneously examine the gene expression of thousands of genes, propelling many nematologists into the genomics era (Li et al. 2008). The first microarrays with plant-parasitic nematodes exploited the already substantial body of knowledge accumulated on the model plant *Arabidopsis*. Puthoff et al. (2003) examined the changes in *Arabidopsis* gene expression upon infection with both beet cyst nematode and soybean cyst nematode. Hammes et al. (2005) examined expression of families of transporter genes in root-knot nematode-infected and healthy root tissue and demonstrated that multiple transport processes are regulated in the feeding site as well as in noninfected tissue. In another study, Jammes et al. (2005) interrogated giant-cell-enriched root tissue, establishing that as many genes are repressed as up-regulated upon nematode infection. They further substantiated that successful root-knot nematode infection is associated with suppression of a number of plant defenses. Soon after, microarrays were used to probe gene expression of specific host plant/plant-parasitic nematode reactions such as tomato and root-knot nematode (Bar-Or et al. 2004; Schaff et al. 2007), and soybean and SCN (Khan et al. 2004; Ithal et al. 2007). Several studies address specific types of interactions, such as those involved in resistance (Schaff et al. 2007), or (like those mentioned above) specific gene/gene categories, such as identification of stress-induced genes and genes involved in carbohydrate metabolism (Khan et al. 2004); genes encoding enzymes involved in primary metabolism, biosynthesis of phenolic compounds, lignin, and flavonoids; and genes related to stress and defense responses, cell wall modification, cellular signaling, and transcriptional regulation (Ithal et al. 2007).

2.2 Parasite Gene Expression

The migration phase within the root is accompanied by extensive secretion of proteins in the J2. Nematodes have a number of secretory systems, and secretions play numerous and central roles in host–parasite interactions (Blaxter and Bird

1997; Davis et al. 2000; Davis et al. 2008). Various enzymatic functions for the secretions have been proposed and initial cloning and sequencing of genes encoding gland proteins have allowed the nature of the secretion products to be discerned with confidence. Importantly, annotation of the completed genome sequence will permit a complete catalogue of secretion products to be obtained.

Using monoclonal antibodies directed to the subventral gland, antigens were truly demonstrated to be secreted (de Boer et al. 1996). Genes defining a small family of endoglucanases have been isolated from cyst (Smant et al. 1998; Yan et al. 1998) and also root-knot nematodes (Rosso et al. 1999). Transcripts for these and other enzymes, including pectinases, polygalacturonase, and phenol oxidase, have subsequently been identified in EST sequencing projects (Sect. 2.3 of this chapter; Popeijus et al. 2000; McCarter et al. 2002; McCarter et al. 2003). Expression of the *eng* genes, which encode cellulases used during migration and perhaps also host penetration, recapitulates subventral pharyngeal gland activity; their expression ceases and expression of host cellulases begins at some point during feeding-site induction (Goellner et al. 2001). Significantly, these enzymes appear to have been acquired via horizontal gene transfer from prokaryotes (Lambert et al. 1999; Smant et al. 1998; Yan et al. 1998). A recent computational approach confirmed their prokaryotic origin and identified additional horizontal gene transfer candidates (Scholl et al. 2003). As discussed later in this chapter, it appears that both the root-knot nematodes and the cysts nematodes have acquired bacterial genes via horizontal gene transfer.

2.3 EST Sequencing Sets the Stage

Expressed sequence tags represent a rapid and accurate avenue to gene discovery and set the stage for complete genome sequencing. Because they represent transcripts, each sequence represents an expressed gene. As a first step, this approach begins to pay off immediately and as such has been employed to great effectiveness on parasitic nematodes. However, EST sequencing only does part of the job. ESTs do not generally represent full length sequences and provide no information on promoters or gene structure. In addition, since ESTs are biased towards abundantly expressed genes, they will generally only identify approximately 50% of genes in a given organism. That being said, they are absolutely essential for annotation of a full genomic sequence and have made tremendous contributions to our knowledge and understanding of parasitic nematodes.

The ability to explore the transcriptome of specific tissue (such as feeding sites) first by EST sequencing and then microarray analysis added tremendously to the body of knowledge of the nematode–host interaction in just a few short years. For some time, examination of plant-parasitic EST libraries from *M. incognita* (McCarter et al. 2003) and a comparison of ESTs between root-knot nematode and free-living nematodes (Mitreva et al. 2005) were the only analyses that attempted to use nematode transcripts to distinguish which nematode genes are involved in

parasitism. McCarter et al. (2003) analyzed 5,700 ESTs from the root-knot nematode infective stage (J2) by clustering and classified them by function. They found a diverse array of ligand-binding proteins and abundant cytoskeletal proteins. Also present were transcripts encoding glyoxylate pathway enzymes which supports the belief that root-knot nematode J2s metabolize lipid stores while searching for a host. More recently, Mitreva et al. (2005) used comparative genomics to analyze over 500,000 nematode ESTs and genome survey sequences from over 38 species of nematodes. The data were used to classify nematodes and provide additional evidence for horizontal gene transfer.

Many nematode genes have been identified from expressed sequence tag projects (McCarter et al. 2000). As recently as 2000, there were only 24,000 ESTs sequenced from nematodes other than *C. elegans*, but that number is now greater than 375,000, with a large emphasis on human parasites (e.g., Daub et al. 2000; Blaxter et al. 2002), animals (e.g., Tetteh et al. 1999), and plant parasites (e.g., Bird et al. 2002; McCarter et al. 2003; Mitreva et al. 2004). An analysis was completed using >250,000 ESTs originating from 30 species, clustered into 93,000 genes and grouped into 60,000 gene families (Parkinson et al. 2004). Using these data, it has been calculated that the diversity of genes within the phylum is great. In nematodes, despite the availability of multiple *Caenorhabditis* species genomes, the addition of new species to the analysis has yielded a rapid increase in discovery of new genes, and it appears that nematodes are more diverse at the molecular level than was previously recognized. The set of ~20,000 genes and 12,000 gene families represented by *C. elegans* provides a baseline for nematodes and many of the conserved gene families are shared with other eukaryotes, but these findings represent only a portion of the expanding total nematode molecular diversity. It seems very likely that the parasitic species are evolving genes specialized for their niche. The genes that may be critical in the development and evolution of parasitism are the ones that are different from the free-living nematodes and other multicellular eukaryotes.

3 Genome Sequencing

Most genome sequences have been obtained using the classic Sanger dideoxy chain-terminator protocol (Sanger and Coulson 1974) on automated platforms. Typically, the read length of these sequences is 500–800 bp. There are several approaches to genome sequencing using this technology; they include both whole genome shotgun and minimum tile approaches. As the name implies, whole genome shotgun is an approach that does not require a priori physical mapping and relies instead upon computational technologies to reconstruct the genome after sequencing. In this approach, both small and large insert libraries are constructed and clones selected at random for sequencing. The bulk of the sequencing is performed on the smaller insert libraries, with paired end sequences obtained from a number of the large insert (fosmid, BAC, etc.) libraries to help with assembly. In the minimum tile approach, physical maps are constructed from large insert libraries,

typically BACs. From these maps, a minimum tile, or set of overlapping clones, are selected for sequencing. For smaller genomes like nematodes (typically 50–100 Mb), whole genome shotgun has proved most efficient.

The advent of second-generation technologies, which perform shorter reads (30–250 bp, depending on platform), enables the elimination of library construction. The second-generation technologies allow many more reads per run and are less expensive than the conventional approaches. However, shorter reads make assembly of whole genomes more difficult unless there is a reference genome available. In the case of nematodes, it should be possible in the future to obtain survey sequences from nematodes related to fully sequenced genomes, thus enhancing the value of both. For example, with two root-knot nematodes fully sequenced and assembled, obtaining lower coverage sequences using the short read platforms will provide an attractive economic alternative to a whole genome project, and will likely provide key information on relationships between species.

3.1 The M. hapla Genome

The *M. hapla* genome has features that provide insight not only into parasitism but also basic nematode biology. Several of the broad findings from this study are listed below:

- *M. hapla* has a very compact genome with high A + T content, relatively few repetitive elements, very densely packaged genes, and greatly reduced gene number compared to other nematodes.
- Developmental pathways for basic nematode processes, including dauer formation, cuticle formation, and RNA interference, are conserved with *C. elegans*.
- The *M. hapla* genome encodes suites of genes apparently acquired by horizontal transfer and thought to be involved in parasitic abilities.
- Expansion of some families of horizontal gene transfer candidates and reduction of a major family of sensory receptors were found.

Unlike many root-knot nematode species, lines of *M. hapla* exist which are diploid, amphimictic and diocious. These same reasons point to *M. hapla* as a platform for genetic studies and a system has been established (Liu et al. 2007). Our current estimate of 54.5 Mbp makes the *M. hapla* genome the smallest multicellular animal sequenced to date. This compares, for example, with SCN at 92.5 Mbp (Opperman and Bird 1998), and *C. elegans* at 100 Mb. Further, the *M. hapla* genome is largely composed of unique or moderately repeated sequences (such as the rDNA cluster and other gene family members). Importantly for genome assembly, less than 5% of the genome represents highly repetitive sequence, and this is largely accounted for by tandem repeats of a 169-bp satellite sequence (Castagnone-Sereno et al. 1995) which is believed to be associated with the telomeres (P. Castagnone-Sereno, pers. comm.). We have completed a 10.4X draft sequence covering approximately 54 Mb, or approximately >98% of the genome, in approximately 1,523 scaffolds.

3.1.1 General Characterization

The *M. hapla* strain VW9 (16 chromosomes) was chosen for the primary sequence due to the availability of a genetic system, including an AFLP linkage map developed by the Williamson Lab at the University of California-Davis. The genome possesses a very low amount of moderately repetitive DNA (~12%), comprised mostly of low complexity sequence. We examined the repetitive sequence and found that the vast majority is simple repeats. Approximately 1% of the repetitive sequence encodes characterized repeats, including DNA transposons. We found 323 copies of the *M. hapla* equivalent of the Tc1 transposon, a number almost identical to that found in *C. elegans*. Similarly, in both the number of genes and structure, the SL-1 trans-splice leader in *M. hapla* is equivalent to *C. elegans*, but the SL-1 loci appear dispersed throughout the *M. hapla* genome. Additionally, small groups of satellites were found, as were the rRNA sequences (5S, 16S-5.8S-28S) in clusters. As noted above, only 5% is highly repetitive sequence, resulting in 83% unique sequence. The genome exhibits an unusually low GC content (27%) compared to the free-living nematode *Caenorhabditis elegans* at 36%. Despite this anomaly, *M. hapla* VW9 is an extremely tractable system for genomic analysis.

3.1.2 Estimation of Gene Number

Using a combination of EST sequence and Glimmer/fGENESh predictions from genomic sequence, we have identified/predicted 14,454 genes in *M. hapla*. This is in stark contrast to *C. elegans* which has 20,000+ genes. This difference may reflect a diminished need for the parasite to carry as full a gene complement as free-living species due to dependence on the host to provide essential resources. A protein dataset (*HapPep1*) was constructed from the *M. hapla* Freeze 1 of the predicted genes dataset, resulting in 13,336 deduced protein sequences. These protein sequences were used as queries in an HMM search against the Pfam22 database and the top ten matches with an e-value of less than e^{-05} were recovered. A similar analysis was done using the wormpep185 build (peptides verified/predicted from the *C. elegans* genome) (www.wormbase.org(for comparison between *M. hapla* and *C. elegans*. There were 4,952 matches in *M. hapla* and 18,062 matches in *C. elegans*. The number of times the top 20 protein domains represented in the *M. hapla* dataset were found (top match only) was compared to the number of times each of these domains appeared in the same analysis for *C. elegans*. Nine of the top 20 pfam domains found in *M. hapla* are also in the top 20 for *C. elegans*.

3.1.3 Gene Families

The *M. hapla* genome contains many of the same gene families as *C. elegans*, and fully half of the most abundant families in *M. hapla* are also among the most abundant in *C. elegans*. In some cases, however, gene family size is drastically

different between the two species. The most obvious example of this is the G-protein coupled receptor (GPCR) family, which is the largest gene family in *C. elegans* (>1,000 genes). Analysis of the *M. hapla* sequence indicates that it possesses only 18% of the *C. elegans* complement. Other large gene families in *C. elegans* are somewhat reduced in *M. hapla*, but the GPCRs are the most extreme example. This difference may represent gene loss that occurs during niche specialization to become an internal parasite of plants, with the life stages outside the plant being restricted to egg and J2 dauer (stages both with restricted neuronal access to the environment). Alternatively, this disparity may reflect gene expansion in *C. elegans* due to its soil environment. In contrast, although other gene families in *M. hapla* are reduced compared to *C. elegans*, the disparity is much smaller. For example, *M. hapla* encodes 81 collagen genes, compared to 165 in *C. elegans*. Collagens likely play key roles in basic nematode biology (Page and Johnstone 2007).

Whether this reflects the parasitic lifestyle or some other facet of biology remains to be seen, but it is clear that *M. hapla* carries fewer genes than its free-living counterpart. We have found numerous genes that we predicted to have been acquired by horizontal gene transfer, including all of the previously reported horizontal gene transfer candidates as well as a number of new and previously unknown genes. Because these genes are potentially very significant in the evolution of parasitism, they are the subject of intense scrutiny. In addition to horizontal gene transfer candidates, previously identified parasitism genes secreted by the nematode during pathogenesis have been observed. Genome annotation has provided positional information, demonstrating in some cases that cellulase genes may be clustered in the *M. hapla* genome.

3.1.4 General Similarities

Approximately 45% of the genes found in *M. hapla* show the highest similarities to *C. elegans* genes where similarity can be detected. The next largest group is similarity to animal parasitic nematodes probably because there is little genomic sequence data on any plant-parasitic nematode in the public database. Of the genes we can recognize approximately 55% have highest similarities to nematode genes. We expect that when the genome sequence of *M. incognita*, a related root-knot nematode species, is published by INRA in France, it will show substantial similarities to *M. hapla*.

3.1.5 Genome Organization

Approximately 15% of the *C. elegans* genome is organized into operons. We identified orthologs of the 4,685 *C. elegans* proteins encoded by genes found in operons and found 3,693 matches in the *M. hapla* assembly. We examined the positions of the matches for the individual members of each operon and identified

101 operons from *C. elegans* that are at least partially conserved in *M. hapla*, having at least two genes from an operon within the same genomic local. The largest operon fully conserved consists of three genes, though a larger cassette of four genes from the 5-gene operon CEOP3272 also has been conserved. This analysis does not preclude the possibility that other *M. hapla* genes may be organized into operons but suggests that the genes co-regulated by virtue of such organization in *C. elegans* are not regulated in a similar manner in *M. hapla*. Because of its small genome size, *M. hapla* intergenic regions also tend to be quite short, making ab initio prediction of operon organization difficult. However, comparison of conserved operons has shed light on microsynteny between *M. hapla* and *C. elegans*.

In most cases, lack of operon conservation with *C. elegans* simply results from orthologous genes not being present in *M. hapla*. The corresponding genes in *C. elegans* generally do not have an RNAi phenotype (www.wormbase.org) which suggests either redundant or dispensable function. We also observed some areas of microsynteny occurring in the *M. hapla* genome, but synteny is either broken or nonexistent over most of the genome. Taken as a whole, these data may point to *M. hapla* having a minimal genome due to its obligate biotrophic lifestyle.

3.1.6 Parasitism Genes

Probing the public databases revealed a total of 70 putative orthologs to previously identified plant-parasitic nematode secreted proteins. To identify new putative *M. hapla* secreted proteins, we searched all 13,336 predicted peptides from *HapPep1* for signal sequences using the SignalP algorithm (Bendtsen et al. 2004). A total of 1,583 proteins that had predicted signal sequences using both available SignalP search algorithms were identified. Because gene prediction tools do not always accurately predict the most 5 exons, this number is likely an underestimate of the total number of secreted proteins, but it probably represents an unbiased sampling. This set of proteins was then subjected to a search for putative trans-membrane spanning regions using TM-HMM. This analysis identified a total of 847 proteins that have a predicted secretion signal but which lack membrane spanning helices. These proteins were compared to the total set of *C. elegans* proteins in WormPep release 185 (www.wormbase.org), revealing 116 to have a significant match to a *C. elegans* protein. The remaining 731 proteins were then compared to the National Center for Biotechnology Information (NCBI) NR database of peptide sequences using BlastX, revealing a total of 21 sequences with a significant match to known proteins. Many of these matches are to plant-parasitic nematode proteins suggested to play a role in parasitism as well as to proteins from the animal pathogenic nematode *Brugia malayi*. The remainder are candidates for proteins that may play a role in defining the host–parasite interface; their discovery helps validate the power of whole genome analysis.

3.1.7 Pathway Conservation with Free-Living Nematodes

Many important developmental pathways in *C. elegans* are partially conserved in the *M. hapla* genome. Sex determination is a key developmental event in all nematodes, yet *M. hapla* carries a very limited number of proteins with significant similarity to those involved in sex determination in *C. elegans*. Of the major sex-determining genes in *C. elegans*, *tra-1* and *tra-2* alone are highly conserved. Although several dosage compensation genes are also conserved, this pathway as a whole remains obscure in *M. hapla*. Genes that are the earliest in the pathway such as *xol-1*, *sdc-1*, *sdc-3*, and *her-1* were not found, suggesting that the signals that trigger these pathways are substantially diverged. In contrast, many of the genes in other *C. elegans* pathways have clear orthologs in *M. hapla*. For example, genes involved in function of small RNAs including *drsh-1*, *pash-1*, *dcr-1 drh-2*, *drh-3*, *agl-1*, *agl-2*, *rrf-3*, *eri-1*, and *pir*-1 can unequivocally be found in *M. hapla*, the sole exception being *rde-4* which is not well conserved across phylogenetic distances. Not surprisingly, the RNAi phenomenon can be experimentally induced in *M. hapla*, where it is persistent over several generations. Similarly, many genes involved in basic nematode development are well conserved, reflecting their primary roles in generalized nematode developmental biology as opposed to response to the environment.

The ability to form dauer larvae is broadly conserved across the Nematoda. Genetic analysis in *C. elegans* identified 32 genes as dauer affecting (*daf*) and the molecular nature of 20 of these has been characterized. *C. briggsae* encodes 19 of these 20 but lacks *daf-28*, the beta-insulin molecule involved in signal transduction. *M. hapla* carries strong orthologs of 14 *C. elegans daf* genes and weak orthologs of three more. Like *C. briggsae*, *M. hapla* lacks an ortholog of *daf-28*. The molecular identities of those genes not found in *M. hapla* appear related to specific developmental cues related to life style and demonstrate that although the basic mechanical aspects of development are conserved, response to environment in parasite versus free-living nematode is substantial.

3.1.8 Preliminary Comparison Between *M. hapla* and *Heterodera glycines*

Recently, a low-coverage draft sequence of the soybean cyst nematode (SCN), *Heterodera glycines*, was released to the public databases (Monsanto). Although a 3X coverage, it is still very useful for comparative purposes in other plant nematode genomes. The number of gaps in the SCN sequence precludes examination of conserved synteny, and gene prediction is also difficult. However, comparison between SCN and *M. hapla* has revealed several fascinating clues to the relationship between these two sedentary endoparasites. Comparison with *C. elegans* indicates that *H. glycines* has greater similarities than *M. hapla* using a translated BLAST algorithm. This may reflect a gene number discrepancy among *M. hapla* and *H. glycines* or may also suggest that they diverged very early in the evolution of nematodes. It does appear, however, that many of the key facets of nematode parasitism, including horizontal gene transfer, have been conserved. In addition, the key developmental

pathways for nematode biology are conserved. Further comparison will likely yield important information regarding evolution of the sedentary lifestyle in plant parasitic nematodes.

Obtaining the complete genome sequence from *M. hapla* has immediate ramifications for both plant nematology and broader biology. The ability to perform comparative genomics using *C. elegans*, *C. briggsae*, and *B. malayi* will provide insight into evolution of both parasitic ability and general nematode development. These studies will reveal critical junctures in the life cycle of the obligate parasites that may be unique and specific targets for anti-nematode therapies. In particular, events such as sex determination, arrested development, and response to host and environmental cues may be examined in a detail heretofore impossible. These events represent key points in the parasites' life cycle, and we expect that the mechanisms will be conserved between species although the precise machinery may differ. Interestingly, many pathogenic bacteria are evolving to lose genes from pathways in which they rely on their host and therefore have smaller genomes than their free-living counterparts. The smaller size of the *M. hapla* genome as compared to the free-living *C. elegans* could point to a similar strategy, and as noted above, current evidence is pointing to *M. hapla* possessing fewer genes than its free-living counterpart. If indeed the parasite has a reduced gene repertoire, those lost or disabled pathways may also provide clues to the complex interaction between host and parasite. Needless to say, areas such as the identification of pathogenicity islands, virulence operons, and horizontally transferred genes will also be amenable to detailed study.

The acquisition of the sequences of diploid *M. hapla* and aneuploid *M. incognita* (Abad, personal communication) represent a huge step forward for the discipline, and the fact that these projects were completed simultaneously is doubly fortuitous. Both projects' commitment to collaborative efforts and public access to the data demonstrate an unprecedented resource for the entire community. On a broader scale, the complete genomes from *M. hapl*a, *M. incognita*, and *H. glycines* provide a platform for studies that take advantage of whole genome transcriptome analysis to ask any multitude of pathogenicity and developmental questions. Further, the sequences provide a springboard for both survey sequencing of other species and genera in the Tylenchida and the Nematoda.

Acknowledgments We thank CSREES for funding the acquisition of the *M. hapla* genome sequence, all members of the CBNP both past and present, and our colleagues at the Joint Genome Institute, Walnut Creek, CA for invaluable collaboration and support of this project.

References

Aguinaldo AM, Turbeville JM, Linford LS, Rivera MC, Garey JR, Raff RA, Lake JA (1997) Evidence for a clade of nematodes, arthropods and other moulting animals. Nature 387:489–493

Barthels N, van der Lee FM, Klap J, Goddijn OJ, Karimi M, Puzio P, Grundler FM, Ohl SA, Lindsey K, Roberson L, Robertson WM, Van Montagu M, Gheysen G, Sijmons PC (1997)

Regulatory sequences of Arabidopsis drive reporter gene expression in nematode feeding structures. Plant Cell 9:2119–2134

Bar-Or C, Kapulnik Y, and Koltai H (2005) A broad characterization of the transcriptional profile of the tomato response to the plant parasitic root knot nematode. *European Journal of Plant Pathology* 111:181–192

Bendtsen JD, Nielsen H, von Heinje G, Brunak S (2004) Improved prediction of signal peptides: SignalP 3.0. J Mol Bio 340:783–795

Berg RH, Fester T, Taylor CG (2008) Development of the root-knot nematode feeding cell. Plant Cell Monogr., doi:10.1007/7089_2008_30

Bird DM, Opperman CH (1998) *Caenorhabditis elegans*: a genetic guide to parasitic nematode biology. J Nematol 30:299–308

Bird DM, Wilson MA (1994) DNA sequence and expression analysis of root-knot nematode-elicited giant-cell transcripts. Mol Plant Microbe Interact 7:419–424

Bird DM, Opperman CH, Jones SJM, Baillie DL (1999) The *Caenorhabditis elegans* genome: a guide in the post genomics age. Annu Rev Phytopathol 37:247–265

Bird DM, Clifton SW, Kepler T, Kieber JJ, Thorne J, Opperman CH (2002) Genomic dissection of a nematode-plant interaction: a tool to study plant biology. Plant Physiol 129:394–395

Bird DMcK, Opperman CH, Williamson VM (2008) Plant infection by root-knot nematode. Plant Cell Monogr., doi:10.1007/7089_2008_31

Blaxter M, Bird DM (1997) Parasitic nematodes. In: Riddle DL, Blumenthal T, Meyer BJ Priess JR (eds) *C. elegans* II. Parasitic nematodes. Cold Spring Harbor Laboratory, Cold Spring Harbor, pp 851–878

Blaxter ML, DeLey P, Garey J, Liu LX, Scheldeman P, Vierstraete A, Vanfleteren JR, Mackey LY, Dorris M, Frisse LM, Vida JT, Thomas WK (1998) A molecular evolutionary framework for the phylum Nematoda. Nature 392:71–75

Blaxter M, Daub J, Guiliano D, Parkinson J, Whitton C, The Filarial Genome Project (2002) The *Brugia malayi* genome project: expressed sequence tags and gene discovery. Trans R Soc Trop Med Hyg ISO 96:7–17

Boucher G, Lambshead PJD (1994) Ecological biodiversity of marine nematodes in samples from temperate, tropical, and deep-sea regions. Conserv Biol 9:1594–1604

C. elegans Sequencing Consortium (1998) Genome sequence of the nematode *C. elegans*: a platform for investigating biology. Science 282:2012–2018

Cassada RC, Russell RL (1975) The dauer larva, a post-embryonic developmental variant of the nematode *Caenorhabditis elegans*. Dev Biol 46:326–342

Castagnone-Sereno P, Esparrago G, Abad P, Leroy F, Bongiovanni M (1995) Satellite DNA as a target for PCR-specific detection of the plant-parasitic nematode *Meloidogyne hapla*. Curr Genet 28:566–570

Daub J, Loukas A, Pritchard DI, Blaxter M (2000) A survey of genes expressed in adults of the human hookworm, *Necator americanus*. Parasitology 120:171–184

Davis EL, Hussey RS, Baum TJ, Bakker J, Schots A, Rosso MN, Abad P (2000) Nematode parasitism genes. Annu Rev Phytopath 38:341–372

Davis EL, Hussey RS, Baum TJ (2008) Parasitism genes: what they reveal parasitism. Plant Cell monogr., doi:10.1007/7089_2008_29

de Almeida-Engler J, de Vleesschauwer V, Burssens S, Celenza JL Jr, Inze D, Van Montagu M, Engler G, Gheyson G (1999) Molecular markers and cell cycle inhibitors show the importance of cell cycle progression in nematode-induced galls and syncytia. Plant Cell 11:793–807

de Boer JM, Smant G, Goverse A, Davis EL, Overmars HA, Pomp H, van Gent-Pelzer M, Zilverentant JF, Stokkermans JP, Hussey RS, Gommers FJ, Bakker J, Schots A (1996) Secretory granule proteins from the subventral esophageal glands of the potato cyst nematode identified by monoclonal antibodies to a protein fraction from second-stage juveniles. Mol Plant Microbe Interact 9:39–46

Favery B, Complainville A, Vinardell JM, Lecomte P, Vaubert D, Mergaert P, Kondorosi A, Crespi M, Abad P (2002) The endosymbiosis-induced genes *ENOD40* and *CCS52a* are involved in endoparasitic nematode interactions in *Medicago truncatula*. Mol Plant Microbe Interact 15:1008–1013

Fuchs AG (1915) Die Naturgeschichte der Nematoden und einiger anderer Parasiten. I. Des *Ips typographus* L. 2. Des *Hylobius abietis* L. Zoologische Jahrbücher Abteilung für Systematik ökologie und Geographie der Tiere, Jena 38:109–122

Ghedin E, Wang S, Spiro D, Caler E, Zhao Q, Crabtree J, Allen JE, Delcher AL, Guiliano DB, Miranda-Saavedra D, Angiuoli SV, Creasy T, Amedeo P, Haas B, El-Sayed NM, Wortman JR, Feldblyum T, Tallon L, Schatz M, Shumway M, Koo H, Salzberg SL, Schobel S, Pertea M, Pop M, White O, Barton GJ, Carlow CK, Crawford MJ, Daub J, Dimmic MW, Estes CF, Foster JM, Ganatra M, Gregory WF, Johnson NM, Jin J, Komuniecki R, Korf I, Kumar S, Laney S, Li BW, Li W, Lindblom TH, Lustigman S, Ma D, Maina CV, Martin DM, McCarter JP, McReynolds L, Mitreva M, Nutman TB, Parkinson J, Peregrin-Alvarez JM, Poole C, Ren Q, Saunders L, Sluder AE, Smith K, Stanke M, Unnasch TR, Ware J, Wei AD, Weil G, Williams DJ, Zhang Y, Williams SA, Fraser-Liggett C, Slatko B, Blaxter ML, Scott AL (2007) Draft genome of the filarial nematode parasite *Brugia malayi*. Science 317:1756–1760

Gheysen G, Mitchum MG (2008) Molecular insights in the susceptible plant response to nematode infection. Plant Cell Monogr., doi:10.1007/7089_2008_35

Gheyson G, Fenoll C (2002) Gene expression in nematode feeding sites. Annu Rev Phytopath 40:191–219

Goellner M, Wang X, Davis EL (2001) Endo-beta-1,4-glucanase expression in compatible plant-nematode interactions. Plant Cell 13:2241–55

Hammes UZ, Schachtman DP, Berg RH, Nielson E, Koch W, McIntyre LM, Taylor CG (2005) Nematode-induced changes of transporter gene expression in *Arabidopsis* roots. Mol Plant Microbe Interact 18(12):1247–1257

Ithal N, Recknor J, Nettleton D, Hearne L, Maier T, Baum TJ, Mitchum MG (2007) Parallel genome-wide expression profiling of host and pathogen during soybean cyst nematode infection of soybean. Mol Plant Microbe Interact 20:293–305

Jammes F, Lecomte P, de Almeida-Engler J, Bitton F, Martin-Magniette M, Renou JP, Abad P, Favery B (2005) Genome-wide expression profiling of the host response to root-knot nematode infection in *Arabidopsis*. Plant J 44:447–458

Khan R, Alkharouf N, Beard H, MacDonald M, Chouikha I, Meyer S, Grefenstette J, Knap H, Matthews B (2004) Microarray analysis of gene expression in soybean roots susceptible to the soybean cyst nematode two days post invasion. J Nematol 36:241–248

Klass M, Hirsh D (1976) Non-ageing developmental variant of *Caenorhabditis elegans*. Nature 260:523–525

Koltai H, Bird DM (2000) Epistatic repression of *PHANTASTICA* and class I *KNOTTED* genes is uncoupled in tomato. Plant J 22:455–459

Lambert KK, Allen KD, Sussex IM (1999) Cloning and characterization of an esophageal gland specific chorismate mutase from the phytoparasitic nematode *Meloidogyne javanica*. Mol Plant Microbe Interact 12:328–336

Li L, Fester T, Taylor CG (2008) Transcriptomic analysis of nematode infestation. Plant Cell Monogr., doi:10.1007/7089_2008_36

Liu QL, Thomas VP, Williamson VM (2007) Meiotic parthenogenesis in a root-knot nematode results in rapid genomic homozygosity. Genetics 176:1483–1490

Mallatt J, Winchell CJ (2002) Testing the new animal phylogeny: first use of combined large-subunit and small-subunit rRNA gene sequences to classify the protostomes. Mol Biol Evol 19:289–301

McCarter JP, Abad P, Jones J, Bird DM (2000) Rapid gene discovery in plant-parasitic nematodes *via* expressed sequence tags. Nematology 2:719–731

McCarter JP, Clifton SW, Bird DM, Waterston R (2002) Nematode gene sequences; update for march 2002. J Nematol 34:71–74

McCarter JP, Mitreva MD, Martin J, Dante M, Wylie T, Rao U, Pape D, Bowers Y, Theising B, Murphy C, Kloek AP, Chiapelli B, Clifton SW, Bird DM, Waterston R (2003) Analysis and functional classification of transcripts from the root-knot nematode *Meloidogyne incognita*. Genome Biol 4:R26.1–R26.19

Mitreva M, McCarter JP, Martin J, Dante M, Wylie T, Chiapelli B, Pape D, Clifton SW, Nutman TB, Waterston RH (2004) Comparative genomics of gene expression in the parasitic and free-living nematodes *Strongyloides stercoralis* and *Caenorhabditis elegans*. Genome Res 14:209–220

Mitreva M, Blaxter ML, Bird DM, McCarter JP (2005) Comparative genomics in nematodes. Trends Genet 21:573–581

Niebel A, de Almeida-Englar J, Tire C, Engler G, Van Montagu M, Gheysen G (1993) Induction patterns of an extension gene in tobacco upon nematode infection. Plant Cell 5:1697–1710

Niebel A, de Almeida-Engler J, Hemerly A, Ferriera P, Inze D, Van Montagu M, Gheyson G (1996) Induction of *cdc2A* and *cyc1At* expression in *Arabidopsis thaliana* during early phases of nematode-induced feeding cell formation. Plant J 10:1037–1043

Opperman CH, Bird DM (1998) The soybean cyst nematode, *Heterodera glycines*: a genetic model system for the study of plant-parasitic nematodes. Cur Opin Plant Biol 1:342–346

Page AP, Johnstone IL (2007) The cuticle. In: *WormBook* (ed) The *C. elegans* research community. WormBook, doi/10.1895/wormbook.1.138.1, http://www.wormbook.org

Parkinson J, Mitreva M, Whitton C, Thomson M, Daub J, Martin J, Schmid R, Hall N, Barrell B, Waterston RH, McCarter JP, Blaxter ML (2004) A transcriptomic analysis of the phylum Nematoda. Nature Genet 36:1259–1267

Popeijus M, Blok VC, Bakker E, Phillips MS, Smant G, Jones JT (2000) Analysis of genes expressed in second-stage juveniles of the potato cyst nematodes *Globodera rostochiensis* and *G. pallida* using the expressed sequence tag approach. Nematology 2:567–574

Puthoff DP, Nettleton D, Rodermel SR, Baum TJ (2003) Arabidopsis gene expression changes during cyst nematode parasitism revealed by statistical analyses of microarray expression profiles. Plant J 33:911–921

Riddle DL, Albert PS (1997) Genetic and environmental regulation of dauer larva development. In: Riddle DL, Blumenthal T, Meyer BJ, Priess JR (eds) *C. elegans* II. Cold Spring Harbor Laboratory, Cold Spring Harbor, pp 739–768

Riddle DL, Blumenthal T, Meyer BJ, Priess JR, eds. (1997). *C. elegans* II. Cold Spring Harbor Laboratory Press, Cold Spring Harbor. pp 1222

Rosso MN, Favery B, Piotte C, Arthaud L, De Boer JM, Hussey RS, Bakker J, Baum TJ, Abad P (1999) Isolation of a cDNA encoding a beta-1,4-endoglucanase in the root-knot nematode *Meloidogyne incognita* and expression analysis during plant parasitism. Mol Plant Microbe Interact 12:585–591

Sanger F, Coulson AR (1975) A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. Journal of Molecular Biology 94:441–446

Sasser JN 1980 Root-knot nematodes: a global menace to crop production. Plant Dis 64:36–41

Schaff JE, Scholl EB, Smith CP, Nielsen DL, Bird DM (2007) Transcriptome profiling in tomato reveals a role for glycosyltransferase in *Mi*-mediated nematode resistance. Plant Physiol 144:1079–1092

Scholl EH, Thorne JL, McCarter JP, Bird DM (2003) Horizontally transferred genes in plant-parasitic nematodes: a high-throughput genomic approach. Genome Biol 4:R39.1–R39.12

Sijmons PC, Cardol EF, Goddijn OJM (1994) Gene activities in nematode-induced feeding structures. In: Daniels MJ, Downie JA, Osbourn AE (eds) Advances in molecular genetics of plant microbe interactions. Kluwer, Dordrecht, pp 333–338

Smant G, Stokkermans JPWG, Yan YT, de Boer JM, Baum TJ, Wang XH, Hussey RS, Gommers FJ, Henrissat B, Davis EL, Helder J, Schots A, Bakker J (1998) Endogenous cellulases in animals: isolation of beta-1,4-endoglucanase genes from two species of plant-parasitic cyst nematodes. Proc Natl Acad Sci USA 95:4906–4911

Stein LD, Bao Z, Blasiar D, Blumenthal T, Brent MR, Chen N, Chinwalla A, Clarke L, Clee C, Coghlan A, Coulson A, D'Eustachio P, Fitch DH, Fulton LA, Fulton RE, Griffiths-Jones S, Harris TW, Hillier LW, Kamath R, Kuwabara PE, Mardis ER, Marra MA, Miner TL, Minx P, Mullikin JC, Plumb RW, Rogers J, Schein JE, Sohrmann M, Spieth J, Stajich JE, Wei C, Willey D, Wilson RK, Durbin R, Waterston RH (2003) The genome sequence of *Caenorhabditis briggsae*: a platform for comparative genomics. PLoS Biol 1:E45

Tetteh KK, Loukas A, Tripp C, Maizels RM (1999) Identification of abundantly expressed novel and conserved genes from the infective larval stage of *Toxocara canis* by an expressed sequence tag strategy. Infect Immun 67:4771–4779

Thiery MG, Green AE, Bird DM (1999) The *Lycopersicon esculentum* orthologue of *PHANTASTICA*/*rough sheath2* is expressed in feeding sites induced by root-knot nematodes. Plant Physiol 120:934

Vercauteren I, De Groodt R, de Almeida Engler J, Gheyson G (2002) An *Arabidopsis thaliana* pectin acetylesterase gene is up-regulated in nematode feeding sites induced by root-knot and cyst nematodes. Mol Plant Microbe Interact 15:404–407

Wood WB (1988) The nematode *Caenorhabditis elegans*. CSHL, Cold Spring Harbor, 667 pp

Yan YT, Smant G, Stokkermans J, Qin L, Helder J, Baum T, Schots A, Davis E (1998) Genomic organization of four beta-1,4-endoglucanase genes in plant-parasitic cyst nematodes and its evolutionary implications. Gene 220:61–70

Molecular Approaches Toward Resistance to Plant-Parasitic Nematodes

J.P. McCarter

Abstract Basic research in molecular plant nematology is expanding the inventory of knowledge that can be applied to provide crop resistance to parasitic nematodes in an economically and environmentally beneficial manner. Approaches to transgenic nematode control can be classified as acting (1) on nematode targets, (2) at the nematode–plant interface, and (3) in the plant response. Strategies aimed at nematode targets include disruption of nematode intestinal function through recombinant plant expression of protease inhibitors or *Bacillus thuringiensis* (BT) toxins, expression of double-stranded RNAs (dsRNAs) that cause silencing of essential nematode genes, disruption of sensory nervous system function, and generation of nematicidal metabolites. Methods directed at disruption of the nematode–plant interface include expression of proteins, or dsRNAs, that block the function of nematode parasitism gene products involved in migration through the plant vasculature or feeding site establishment, production of molecules repellent to the nematode, or conversion of the plant to a non-host. Approaches acting through the plant response include expression of a cloned plant resistance gene triggering a hypersensitive response, expression of gene(s) deleterious to the feeding site with a feeding site-specific promoter, and conversion of the plant from sensitive to tolerant. Degrees of resistance have been demonstrated through recombinant expression of protease inhibitors, dsRNAs, and cloned plant resistance genes, although none of these discoveries has yet reached commercialization. The focus of molecular plant nematology on root-knot and cyst nematodes makes it likely that transgenic technology will first be commercially applied to these sedentary endoparasites with eventual application to other species. Successful commercialization of biotechnology-derived crops with nematode resistance that result in large yield benefits for producers as well as environmental benefits will be an important milestone for the discipline of molecular plant nematology and should accelerate further progress.

J.P. McCarter
Divergence, Inc., St. Louis, MO 63141, USA and Genome Sequencing Center
Washington University School of Medicine, St. Louis, MO 63108, USA
e-mail: mccarter@divergence.com

Plant Cell Monogr, doi:10.1007/7089_2008_32

1 Introduction

1.1 The Importance of Progress in Applied Nematology

Research on nematode parasitism is shedding light on fundamental questions in plant biology and host–parasite interaction. The preceding chapters provide numerous examples of the improvement in core knowledge surrounding plant nematode pathogenesis. At the same time, plant nematology is also an applied science that receives financial support largely because of the importance of nematode parasites to plant damage and yield loss in major crops (Sasser and Freckman 1987; Whitehead 1998). The growth in plant nematology as an academic discipline in the 1950s to 1960s followed the demonstration in the 1940s to 1950s that fumigants and nematicides controlling parasitic nematodes could substantially raise crop yields (Johnson 1985a; Taylor 1978). Further successes came from breeding, including the movement on the Mi gene for *Meloidogyne* resistance into commercial tomato cultivars in the 1950s (Gilbert and McGuire 1956; Watts 1947) as well as the introduction of soybeans resistant to *Heterodera glycines* (soybean cyst nematode) in the 1970s that had an estimated economic benefit of $400 million (Brady and Duffy 1982; Starr et al. 2002a). Applied nematology, with its core mission of diagnosis and control of plant diseases caused by parasitic nematodes using methods such as cultural practices, chemical application, and host plant genetics, therefore has a history with some noteworthy successes.

Early technology for nematode control, however, has left much to be desired. Nematicides such as organophosphates and carbamates are non-specific neurotoxins with poor environmental and worker-safety profiles and many have been restricted in use or withdrawn from the market (Haydock et al. 2006; Risher et al. 1987). 1,2-Dibromo-3-chloropropane (DBCP) was an effective nematicide that also tragically caused human sterility and was widely banned in the late 1970s (Slutsky et al. 1999). The fumigant methyl bromide has been largely phased out because of its role in ozone depletion (Schneider et al. 2003). With the exception of progress in the use of abamectin seed treatments (e.g. Rich and Kavitha 2006), no new class of effective nematicidal chemistry has been commercialized since the 1970s. Nematode control through genetic resistance is also insufficient. While some crops benefit from resistance, many lack identified resistant germplasm. Furthermore, resistance breaking through selection of virulent nematode populations (e.g. soy parasites) or selection for non-susceptible species (e.g. potato parasites) can occur, lessening the trait's value (Starr et al. 2002b). Lastly, despite the substantial progress in transgenic approaches to resistance described in this chapter, as of 2006, no biotechnology-derived crop with nematode resistance has yet reached commercialization and nematode control lags behind progress in transgenic control of insects, viruses, and fungi (Adkisson et al. 2000). This lack of new and improved technology reaching the grower has been detrimental to nematology as a discipline and has coincided with static-to-declining numbers of trained applied nematologists, particularly in the United States (US).

The tools of molecular and genomic analysis are now creating a new wave of interest in nematology from the free-living model organism *Caenorhabditis elegans* (The *C. elegans* Sequencing Consortium 1998) to the parasitic nematodes of plants and mammals, including humans (Mitreva et al. 2005). Capitalizing on this momentum and applying newly acquired knowledge to create commercial products for nematode diagnosis and control is vitally important. It is safe to say that the future of plant nematology as a discipline is dependent on the value of the commercial solutions delivered to the grower. Economically and environmentally sound new methods of nematode control with a real world impact on yield will be the drivers that validate the enterprise of molecular plant nematology and result in reinvestment in the field. Advances can and should come from a variety of methodologies including traditional and molecular breeding (Dale and De Scurrah 1998; Starr et al. 2002b; Young and Mudge 2002) as well as new classes of safer nematicides (McCarter 2004). This chapter will focus specifically on methods of nematode control that are driven by a molecular biological understanding of the nematode and host plant and implemented by plant transgenic expression. There are reasons to be optimistic about these approaches and their potential impact. For example, while the USDA database of field test release applications lists only 53 proposals to examine nematode resistance out of 14,774 documents from 1987 to 2008, 39 of these filings come from 2004 to 2008, indicating an upswing in projects reaching field trials in recent years (http://www.isb.vt.edu/CFDOCS/fieldtests1.cfm).

2 Definitions and Goals

Formally defined terms applied to nematode pathogenicity and plant response are susceptible, resistant, tolerant, intolerant, non-host, compatible interaction, incompatible interaction, virulence, and avirulence (Dropkin 1989; Roberts 2002; Zijlstra et al. 1997). Susceptible and resistant are terms that describe the capability of a parasitic nematode to reproduce on a host plant. A susceptible host plant is vulnerable to invasion and reproduction by a specific nematode species or population whereas a resistant host is one that supports either diminished or no reproduction by the nematode. Resistance can be quantified with decreases in nematode reproduction relative to a susceptible control ranging from mild effects to complete. Naturally occurring resistance frequently involves detection of the nematode by the plant, followed by a hypersensitivity response (Williamson and Kumar 2006; Tomczak et al. 2008). Like resistant hosts, non-host plants do not support reproduction by a given nematode, although the mechanism of their non-host status may or may not be related to a hypersensitivity response (Kaplan and Keen 1980). The interaction between a parasite and a susceptible host is called a compatible interaction. Conversely, the interaction between a parasite and a resistant host is termed an incompatible interaction. Tolerance and intolerance relate to plant damage caused by the nematode. Intolerant plants experience symptoms including injury and death following nematode invasion whereas tolerant plants experience proportionally less

damage with the same nematode inoculum. Resistant or susceptible plants may have varying degrees of tolerance or intolerance. An avirulent nematode population fails to reproduce on a resistant plant, while a virulent nematode population overcomes the plant's resistance, reproducing within a resistant host.

Beyond these formal definitions, when researchers discuss the goal of achieving "resistance" in the host plant, they generally mean both reducing nematode reproduction as well as decreasing symptoms of disease that include root damage, vulnerability to stressors such as drought, and especially yield loss. The key objective for commercial crop resistance can be simply stated as providing economically significant yield gain in the presence of nematode pressure without yield drag in the absence of nematode pressure. In real world settings, nematode control strategies must compete with numerous other management concerns of the grower. Bred or engineered resistance has some distinct advantages over alternative methods of nematode control. Nematicidal chemicals can be expensive, require a management decision to treat, and have associated application costs in labor, fuel, and equipment. Chemical control can be incomplete and require multiple treatments over the growing season. In addition, conventional nematicides pose dangers to the applicator and the environment and most are restricted use pesticides (Sect. 1 above). Rotation to non-host crops for one or more growing seasons, an option for the control of some nematodes, can be uneconomical. Soil amendments, cultural practices, and biocontrol strategies can offer partial control but are rarely employed as stand-alone solutions (Johnson 1985b). Host-plant resistance has the advantage of being intrinsic to the seed so that there are no costs or decisions beyond the initial choice to purchase seed containing the trait.

3 Resistance Strategies

There have been numerous excellent reviews of engineered nematode resistance over the past 10 years (Atkinson et al. 1998a, 1998b, 2003; Burrows and De Waele 1997; Jung et al. 1998; Lilley et al. 1999a, 1999b; McPherson et al. 1997; Ohl et al. 1997; Stiekema et al. 1997; Thomas and Cottage 2006; Williamson 1999; Williamson and Hussey 1996; Williamson and Kumar 2006). The major contribution of this chapter is to provide a classification system for the strategies that have been or could be undertaken to achieve biotechnology-based control of plant-parasitic nematodes. References are selected to illustrate these approaches and are extensive but not all inclusive. Parasitic nematode species, their lifecycles, and their interactions with the plant are interjected here in the context of specific control strategies and markets. The reader is referred to the other Chaps. 1–8 of this book (Dropkin 1989; Perry and Moens 2006; Wyss 1997) for more thorough descriptions of their biology. Approaches to control can be classified as acting (1) on targets within the nematode, (2) at the nematode–plant interface, and (3) in the plant response (Fig. 1). Strategies aimed at nematode targets include disruption of the intestine by protease inhibitors or BT toxins, triggering of RNA interference

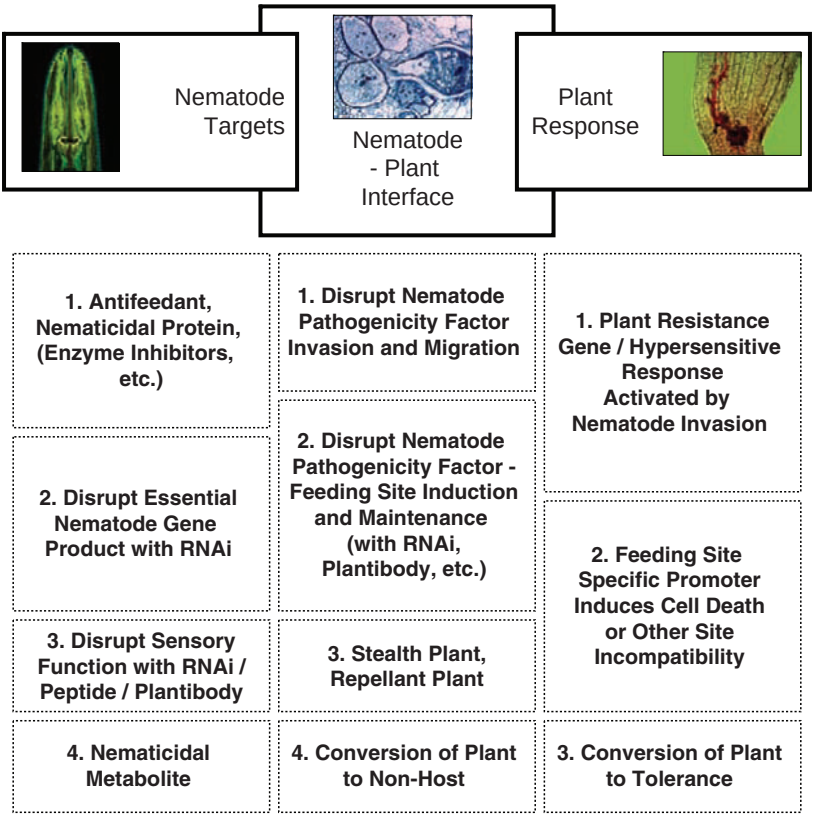


Fig. 1 Three broad classes of transgenic nematode control strategies are displayed in columns; nematode targets (*left*, Sect. 3.1), nematode–plant interface (*center*, Sect. 3.2), and plant response (*right*, Sect. 3.3). Within each broad class are three or four specific strategies of nematode control (also called methods or approaches). Images show, respectively, the head anatomy of a *Heterodera glycines* second-stage juvenile (Photo by Burton Endo, Nemapix Volume 1), a cross-section of a *Meloidogyne* species nematode at the feeding site (Photo by Roger Lopez-Chaves, Nemapix Volume 1), and a plant hypersensitive response to a nematode (Photo from Jonathan Eisenbach)

(RNAi) to cause silencing of nematode genes, disruption of sensory function, and generation of nematicidal metabolites. Methods to disrupt the nematode–plant interface include disrupting nematode parasitism gene products involved in migration or feeding site establishment, producing repellents, or converting the plant to a non-host. Approaches acting through the plant response include expression of a plant-resistance gene triggering a hypersensitive response, generation of gene products deleterious to the feeding site with specific promoters, and conversion of the plant from sensitive to tolerant. There is, of course, some overlap between these broad classifications and categories (e.g. nematicides can be repellents, repellents can act via nematode sensory function, etc.) and some strategies could be placed in

more than one class (e.g. non-host status may be due to events at the nematode–plant interface or due to the plant response). Nevertheless, the classification scheme provides a useful way to organize information about the numerous approaches to nematode resistance. The strengths and weaknesses of each approach are discussed along with commercialization potential.

3.1 Disruption of Nematode Target Genes

3.1.1 Antifeedant/Nematicidal Proteins

The most extensively studied approach to transgenic nematode control is the expression of rice oryzacystatin protein, an inhibitor of cysteine proteases. Nematode intestinal proteases are attractive targets for disruption for several reasons (Lilley et al. 1999b). The lumen of the nematode intestine is a surface that comes in contact with ingested plant materials and its function of digestion is essential to growth and reproduction. Most other essential physiological processes of the nematode are protected behind cell membranes and the cuticle, such that they do not usually come in direct contact with undigested macromolecules from the host plant. Plant-parasitic nematodes have been demonstrated to have multiple types of active intestinal proteases including cysteine proteases. Several of these genes have been cloned (Lilley et al. 1999b). Inhibition of protease activity might be expected to have broad activity across many plant-parasitic nematodes including both migratory and sedentary parasites. Lastly, naturally occurring proteins with protease inhibitor activity like cystatins have been characterized from edible plants such as rice and maize and have long been part of the human diet (McPherson et al. 1997).

Multiple studies have demonstrated that transgenic expression of a modified version of oryzacystatin, Oc-1ΔD86, can interfere with nematode replication (Urwin et al. 1995). In *Arabidopsis thaliana*, expression of Oc-1ΔD86 using the cauliflower mosaic virus (CaMV35S) promoter and infection with the beet cyst nematode *Heterodera schachtii* resulted in adult females that were greatly diminished in size relative to controls (Urwin et al. 1997a). Cryosections of *H. schachtii* recovered from the plants showed diminished cysteine protease activity. Infection of the plants with root-knot nematode *Meloidogyne incognita* resulted in fewer full-size adults (Urwin et al. 1997a). Transformation of potato plants expressing Oc-1ΔD86 from the same promoter and challenged with potato cyst nematode *Globodera pallida* in a field trial resulted in a 55–70% decrease in cyst number. However, cysts that did form were of normal size with a similar number of eggs to control, suggesting the potential for escape from digestive disruption. Transgenic banana plants expressing Oc-1ΔD86 from the maize ubiquitin gene promoter and challenged with burrowing nematode *Radopholus similis* in greenhouse trials identified eight of 115 lines that expressed the protein and showed substantial control (Atkinson et al. 2004). Two possible explanations for the partial degree of control

observed in multiple studies are that some nematodes within a population can maintain viability despite a large decrease in intestinal cysteine protease activity, perhaps due to redundancy in digestive processes, or that feeding-site expression is suboptimal. Beyond cysteine protease inhibitors, a serine protease inhibitor has been examined for control of the cereal cyst nematode *Heterodera avenae* in wheat (Vishnudasan et al. 2005). Another suggested approach is the expression of inhibitors of alpha-amylases, enzymes important for carbohydrate digestion in insects (Burrows and De Waele 1997).

One key feature of cystatins for nematode control is that they are relatively small proteins (~11 kD). Sedentary endoparasitic nematodes, including root-knot and cyst species, withdraw molecules from their plant-feeding site through a feeding tube that restricts the size of molecules entering the intestine (Bockenhoff and Grundler 1994; Berg et al. 2008). *Heterodera schachtii* excludes plant-expressed green fluorescent protein (GFP), a 28-kD protein (Urwin et al. 1997b), whereas *Globodera rostochiensis* (Goverse et al. 1998) allows entry of GFP.

Bacillus thuringiensis bacteria produce specific toxins (Cry proteins, 54–140 kDa) that can control insects in both spray-on (biocontrol) and transgenic approaches (Schnepf et al. 1998). Cry protein (Bt) expressing lines have been commercialized for control of Lepidoptera and Coleoptera in corn, cotton, and other crops and are grown on 26-million hectares annually (James 2005). Some Cry proteins have been described as nematicidal (e.g. Schnepf et al. 2003) and testing of a Bt panel against free-living nematodes demonstrated nematicidal activity of Cry5B, Cry6A, Cry14A, and Cry21A (Wei et al. 2003). Cry5B interacts with the luminal surface of the *C. elegans* intestine via an invertebrate-specific glycolipid, loss of which conveys resistance (Griffitts et al. 2001, 2005). Expression of codon-optimized Cry6A in transgenic tomato roots by the CaMV35S promoter reduced egg production by *Meloidogyne incognita* 56–76% (Li et al. 2007). Use of Cry proteins against parasites with lower molecular weight size restrictions like cyst nematodes will require truncation to allow uptake into the worm intestine. Cry6A can be truncated to 43 KDa before activity is lost (Wei et al. 2003).

An approach that has been tested as an anthelmintic treatment for control of animal nematodes could potentially be adapted for plant-parasitic nematode control. Plant proteases (as opposed to protease inhibitors) found in the latex of papaya and pineapple disrupt the integrity of the cuticle in parasitic nematodes including *Heligmosomoides polygyrus* (Stepek et al. 2004). Transgenic expression of such proteases in crop roots could be tested for plant-parasitic nematode control. Other enzymes suggested for expression in disrupting nematode reproduction include a variety of collagenases to disrupt the cuticle, chitinases to disrupt egg shells, ribosome inactivating proteins, and patatin, a non-specific lipid acyl hydrolase (Burrows and De Waele 1997; Jung et al. 1998). An alternative approach to enzymes is to develop proteins with novel-binding properties specifically tailored to nematode targets. An example of this strategy is the selection of monoclonal antibodies binding nematode targets of interest and their expression in plants (i.e. plantibodies) (Sect. 3.2 below).

3.1.2 Disruption of Essential Nematode Gene Products with RNAi

In many organisms, exposure of cells to sequence-specific dsRNA has been demonstrated to result in degradation of corresponding mRNAs, a process called RNA interference (RNAi). Gene silencing in plants occurs by this mechanism (Waterhouse et al. 1998). The critical role of dsRNA in silencing was first elucidated in *C. elegans* (Fire et al. 1998). dsRNA microinjection, soaking, and feeding protocols have been used to knock down expression of all *C. elegans* genes and phenotypic effects have been observed for several thousand (Fraser et al. 2000; Gonczy et al. 2000; Kamath et al. 2003; Maeda et al. 2001). RNAi occurs by an endogenous cellular pathway, which includes the dicer protein that processes long dsRNAs into 21mers (siRNAs) and the RISC protein complex that guides siRNA-mRNA base pairing and degradation (Mello and Conte 2004).

Recently, laboratories have reported gene silencing by dsRNA soaking for plant-parasitic nematodes including *Heterodera*, *Globodera*, and *Meloidogyne* species (Bakhetia et al. 2005a; Chen et al. 2005; Fanelli et al. 2005; Huang et al. 2006a; Lilley et al. 2005; Rosso et al. 2005; Urwin et al. 2002). RNAi is emerging as an extremely useful research tool in plant nematology to elucidate gene function by observing the phenotypic effect of transcript knock-down. For instance, in *G. rostochiensis*, reduction of the transcript-encoding amphidial secretory protein AMS-1 by dsRNA soaking of second-stage juveniles (J2) greatly reduced the ability of worms to locate host plants (Chen et al. 2005). Soaking with a dsRNA for *M. incognita* dual oxidase gene reduced reproduction by 70% (Bakhetia et al. 2005a).

It is also possible that RNAi will result in commercial nematode control through transgenic plant-delivered dsRNA. The first published demonstration of transgenic plants with RNAi-based resistance to plant-parasitic nematode infection was reported by Yadav et al. (2006) for tobacco challenged with *Meloidogyne incognita*. Expression of dsRNA for a *Meloidogyne* splicing factor protein decreased gall formation and nematode reproduction almost entirely. Subsequent demonstration of RNAi-based resistance has come from work by Huang et al. (2006a) for *Arabidopsis* challenged with *M. incognita*, *M. javanica*, *M. arenaria*, and *M. hapla*. Expression of dsRNA for a secreted *Meloidogyne* parasitism gene 16D10 (3.3–1 below) decreased eggs per gram of root by 69–93%.

Advantages of transgenic RNAi include the likelihood of excellent biosafety and the possibility of stacking resistance by targeting multiple essential genes (Bakhetia et al. 2005b). Challenges and unknowns include feeding-tube size limitations on uptake of dsRNA, siRNAs, or RISC-siRNA complexes, particularly for cyst nematodes as discussed for antifeedants above. Also unknown is how robust the RNAi response is in each plant-parasitic nematode species, how much variation there is in response across populations within a species, and how quickly resistance can be expected to develop by disruption of the RNAi pathway in the parasite. In *C. elegans*, multiple viable mutants have been identified that are RNAi resistant (Mello and Conte 2004). Details regarding the selective pressures needed to maintain an intact RNAi pathway remain largely unexplored.

Yadav et al. (2006) selected their RNAi gene candidates based on orthology to essential *C. elegans* genes, whereas Huang et al. (2006a) selected a gland secreted protein that appears to play a role in feeding-site formation. Genome projects are beginning to make available catalogs of plant-parasitic nematode genes that can be used to identify targets of interest for RNAi studies (McCarter et al. 2003, 2005; Mitreva et al. 2004; Parkinson et al. 2004; Vanholme et al. 2006; Opperman et al. 2008). Other targets have been identified by differential cDNA expression analyses (Qin et al. 2000) and proteomics approaches (Jaubert et al. 2002).

3.1.3 Disruption of Sensory Function

Nematodes have sensory processes that allow them to adjust their movements when they sense chemical and temperature gradients and physical barriers in their surroundings. An amphid, for instance, is a chemosensory sensillum made up of a neuron, sheath, and socket cell. The *C. elegans* cellular and molecular pathways for chemosensation have been extensively described (Bargmann et al. 1993; Bergamasco and Bazzicalupo 2006). Migratory ecto- and endo-parasitic nematodes are motile throughout most of their life cycle. Sedentary endo-parasitic nematodes rely on movement and migratory path-finding in the J2 stage prior to feeding-site formation as well as in the adult male for species reliant on sexual reproduction (Perry 1998). Disruption of sensory neurons may interfere with multiple aspects of movement including host-plant finding, migration within the host, plant cell-type localization for feeding-site formation, and mating.

Winter et al. (2002) demonstrated that the acetylcholinesterase-blocking nematicide aldicarb interferes with *H. glycines* chemosensation at a 1,000,000-fold lower dose (1 picomolar) than was required for inhibition of locomotion, indicating that disruption of chemosensation is likely a key feature of aldicarb's efficacy. The authors used phage display to identify peptide-mimics of aldicarb that disrupt chemosensation. These molecules are likely taken-up through cuticular openings into sensory sensilla and moved via retrograde transport to synapses. Expression of the aldicarb-like peptides as secretory products in transgenic potato resulted in root exudates with acetylcholinesterase-blocking activity, which in greenhouse trials reduced *Globodera pallida* infection with cyst number declining 36–48% relative to vector controls (Liu et al. 2005). Peptide mimics of levamisole also reduced *Globodera* infection in a potato hairy root system. In an alternative approach, Fioretti et al. (2002) characterized monoclonal antibodies directed against *Globodera pallida* amphidial secretions and found that J2 soaked in these antibodies showed diminished mobility and delayed invasion of potato roots. Transgenic plants could conceivably disrupt amphidial proteins by RNAi (Chen et al. 2005), peptides (Liu et al. 2005), plantibodies, or other factors. Lectins, which have been shown to bind to carbohydrates in amphidial exudates (McClure 1988), could also be considered, though initial tests of lectin expression have not resulted in resistance (Burrows and De Waele 1997). One challenge for any approach aimed at disrupting nematode sensory function is the timing and location of delivery of the expressed factor.

Disrupting sensory function outside the plant would be advantageous. Once a sedentary endoparasitic female has already established a feeding site, disruption of sensory function may be too late to be efficacious.

3.1.4 Nematicidal Metabolites

Dozens of naturally occurring nematicidal metabolites from plants have been characterized including certain polythienyls, alkaloids, lipids, isoflavonoids, and diterpenoids (Chitwood 2002; Kaplan and Keen 1980; Valette et al. 1998). For instance, accumulation of a terpenoid aldehyde has been associated with *Meloidogyne* resistance in cotton (Veech and McClure 1977) and Alfalfa resistant to *Pratylenchus* accumulates the isoflavonoid medicarpin (Baldridge et al. 1998). Nematicides can also have repellant properties (Sects. 3.2 and 3.3 below). Pesticidal compounds induced in the plant in response to pathogen entry are called phytoalexins (Dropkin 1989; Rich et al. 1977), and could be considered a subgroup of nematicidal metabolites. Phytoalexins that may play a role in naturally occurring resistance to plant-parasitic nematodes include coumestrol and psoralidin (Rich et al. 1977), and the flavone-C-glycoside O-methyl-apigenin-C-deoxyhexoside-O-hexoside (Soriano et al. 2004a). Soriano et al. (2004b) have also characterized the phytoecdysteroid 20-hydroxyecdysone (20E) as a phytoalexin. The molecule, which is a metabolite of spinach and mimics a molting hormone in insects, can cause abnormal molting, immobility, and impaired development in cyst, root-knot, and lesion nematodes. Treatment of spinach with methyl jasmonate increases levels of 20E and decreases plant-parasitic nematode infectivity (Soriano et al. 2004b). Organisms other than plants can also be a rich source of nematicidal molecules. For instance, nematicidal and insecticidal compounds from fungi have been characterized and broadly deployed as semi-synthetic parasite control agents (e.g. macrocyclic lactones) (Yoon et al. 2004).

By manipulation of biosynthetic pathways, some natural-product nematicides could potentially be produced in biotechnology-derived crops as secondary metabolites in sufficiently high concentrations within plants to disrupt infection. In addition to intrinsic potency, desired characteristics of such metabolites include: (1) an ability to disrupt the nematode after plant invasion; (2) accumulation of the active molecule in the plant, as opposed to accumulation in the soil during decomposition; (3) generation by a limited number of synthetic steps from a chemical precursor present in roots of commercial crops; (4) absence of pathways for rapid degradation; (5) absence of phytotoxicity at the concentration required for nematicidal activity; and (6) absence of toxicity to non-target species and a favorable food-safety profile. These requirements may disqualify molecules such as glycosinolates from *Brassica* species which kill nematodes by breaking down to active isothiocyanates during decomposition in the soil (Chitwood 2002) and macrocyclic lactones like avermectin that require a dozen or more synthetic steps (Yoon et al. 2004). Probably the major limiting factor in transgenic production of nematicidal metabolites is a lack of knowledge of the biosynthetic pathways responsible for the production of many of these molecules. A natural-product nematicide with a broad

spectrum of activity across the plant-parasitic nematodes would be particularly appealing for transgenic expression since it could provide a single solution applicable to many crops and parasites, thereby spreading the cost of research and development over many markets. Other approaches, such as RNAi, plant resistance genes, or use of feeding-site-specific promoters, are more likely to be specific to a particular parasite genus or species.

3.2 Disruption at the Nematode–Plant Interface

3.2.1 Disruption of Nematode Pathogenicity Factors

Parasitic nematodes differ from free-living (bacteriovorus) species in their ability to enter a host and use the host's resources for their own replication. Plant-parasitic nematodes use a number of anatomical specializations (e.g. stylet, specialized gland cells) and an arsenal of gene products to accomplish this complex process. The last decade has seen the identification and characterization of numerous specialized parasite gene products that are absent from free-living nematodes with a focus on secretory proteins from the subventral and dorsal esophageal glands (Davis et al. 2004, 2000, 2008; Vanholme et al. 2004). Gland genes encoding these proteins are sometimes called parasitism genes or the "parasitome" (Gao et al. 2001, 2003; Huang et al. 2003). Disrupting the function of parasitism gene products may be a way to selectively block nematode infection.

Prior to entering the root, *Meloidogyne incognita* J2s appear to produce a secretory factor, NemF, similar to a rhizobial Nod factor, which can serve as a signal to the plant at a distance (Bird et al. 2008). Mutant plants defective in Nod factor reception show diminished galling relative to controls (Weerasinghe et al. 2005). *Meloidogyne* species express a homolog of *NodL*, a gene involved in Nod factor biosynthesis (McCarter et al. 2003; Scholl et al. 2003). On entry into the root, invading root-knot and cyst J2 secrete a number of enzymes from their subventral glands likely to be involved in plant cell-wall degradation. These include β-1, 4-endoglucanases (cellulases) (Gao et al. 2004; Smant et al. 1998), a pectate lyase (Doyle and Lambert 2002; Popeijus et al. 2000), an expansin (Qin et al. 2004), and an endo-1,4-β-xylanase (Mitreva-Dautova et al. 2006).

Over 20 gland expressed root-knot and cyst nematode gene products have been identified that may play important roles in establishment and maintenance of the feeding site (Vanholme et al. 2004; Davis et al. 2008; Gheysen and Mitchum 2008). *Meloidogyne incognita* subventral gland-secreted peptide 16D10 is similar in sequence to the plant CLAVATA3 peptide involved in cell-fate determination (Huang et al. 2006b; Olsen and Skriver 2003). Two-hybrid studies suggest that the 13-amino acid 16D10 peptide binds a plant transcription factor, and RNAi experiments (Sect. 3.1 above) indicate that it is essential for root-knot nematode reproduction (Huang et al. 2006a). *Heterodera glycines* also encodes a CLAVATA3-like peptide (SYV46), which is secreted from the dorsal gland. Over-expression of SYV46 in *Arabidopsis*

thaliana results in apical meristem termination (Wang et al. 2005). Chorismate mutase, an enzyme expressed within the glands of root-knot nematode, may enter the feeding site and increase the metabolism of chorismate via the shikimate pathway at the expense of aromatic amino acid biosynthesis. Transgenic expression of *Meloidogyne javanica* chorismate mutase alters the cellular morphology of soybean hairy roots (Doyle and Lambert 2003). Other gland-secreted proteins have nuclear localization signals, suggesting that they may alter gene expression within the feeding site (Gao et al. 2003; Huang et al. 2003). Additional gene products characterized include a root-knot calreticulin that accumulates along the cell wall of giant-cells (Jaubert et al. 2005) and a cyst nematode ubiquitin extension protein that may localize to the host nucleolus (Tytgat et al. 2004).

Nematode parasitism genes are candidates for disruption by transgenic RNAi. In addition to 16D10 (Sect. 3.1; Davis et al. 2008), J2 soaking RNAi directed at a *Globodera rostochiensis* cellulase resulted in diminished cyst formation after inoculation (Chen et al. 2005). However, parasitism genes may not be superior targets compared to other essential nematode genes for RNAi, and some could be particularly difficult to disrupt by in planta delivery. Many of the proteins, particularly those from the subventral gland involved in J2 invasion and migration, have already been translated and packaged for secretion prior to the nematode entering the host plant, making mRNA degradation irrelevant. Dorsal-gland genes required on a sustained basis during feeding-site maintenance could potentially be superior targets in this regard.

Another strategy implemented for interfering with parasitism gene products is the transgenic production of antibody-like proteins that bind to a specific target, also called plantibodies (Jobling et al. 2003; Stoger et al. 2002). This approach has two potential advantages over RNAi. First, it directly targets the protein rather than the upstream mRNA, and second, delivery of a macromolecule into the nematode through a feeding tube with size restrictions is not necessary (Sect. 3.1 above) (Bockenhoff and Grundler 1994; Urwin et al. 1997b). Plantibodies capable of binding nematode proteins with high affinity can be generated by immunization and monoclonal antibody selection or by in vitro methods including phage display. Plantibodies have been successfully used to confer viral resistance in transgenic plants such as resistance to tomato-spotted wilt virus in tobacco (Prins et al. 2005). Monoclonal antibodies have been raised against root-knot and cyst nematode salivary secretions and the corresponding heavy or light chain encoding-genes expressed in planta, but successful disruption of infection by this approach has not yet been reported (Baum et al. 1996; Rosso et al. 1996; Stiekema et al. 1997). As an alternative to mammalian-derived antibodies, the expression of which may be controversial in food crops, the selection of binding molecules from peptide display libraries has also been proposed. For most parasitism genes that could be plantibody or RNAi targets, it is not known whether or not they play essential roles in the infective process. It is possible that the redundancy of targets involved in some processes (e.g. multiple cellulases secreted by cyst nematode) (Gao et al. 2003) will require multiple points of intervention to achieve efficacy.

3.2.2 Repellent Or Stealth Plants

Just as disruption of nematode sensory function (Sect. 3.1 above) may prevent successful host finding, it may be possible to alter nematode migration by changing the molecular profile of the plant, particularly the root exudate. Strategies could include creating a stealth plant by removing attractants from the rhizosphere or creating an undesirable plant by adding repellants. Plant-parasitic nematodes chemotax toward the roots of host plants, although other than carbon dioxide the plant chemicals sensed by the nematode that form the basis of the attraction are poorly defined (Dusenbery 1983; Prot 1980; Riddle and Bird 1985; Robinson 1995). In the case of the entomopathogenic (insect parasitic) nematode *Heterorhabditis megidis*, a chemical cue whereby maize infested with western corn rootworm actively recruits the nematode has been identified. Injured plants release (E)-beta-caryophyllene, which attracts the nematodes in a laboratory "olfactometer" and in the field (Rasmann et al. 2005). It is possible that plant-parasitic nematodes, particularly those with narrow host ranges, may be taking advantage of similar gradients of specific molecules to enable host finding. Selected non-essential metabolic pathways in the plant could be silenced to prevent production of metabolites sensed by the nematode, resulting in a stealth plant. However, it also seems likely that there is redundancy in the host-finding system, with the nematode taking advantage of multiple chemical cues so that elimination of one might only have a minor effect. Rather than relying on loss of an attractant, repellant plants could be generated by the addition of a selected molecule that repels plant-parasitic nematodes while being relatively innocuous to non-target organisms in the environment and to humans and animals in their food. *C. elegans* actively migrates away from a number of molecules (Hilliard et al. 2002) and, while less characterized, plant-parasitic nematodes appear to do the same. Asparagus roots may release a glycoside that is both repellant and nematicidal to *Paratrichodorus minor,* and bitter cucumber may repel *Meloidogyne incognita* with curcurbitacin triterpenoids (Kaplan and Keen 1980). Use of root-specific promoters may be necessary to limit the food exposure of repellent molecules that are bitter or otherwise unacceptable to food taste.

3.2.3 Conversion of Plant to Non-Host

Little is known about the molecular mechanisms that make a particular plant a host or non-host for a given plant-parasitic nematode species. For the majority of plant-parasitic nematode species, most plants are actually classified as non-hosts (Roberts 2002). Root-knot nematodes have an extraordinarily broad host range with the ability to infect thousands of plant species, whereas cyst nematodes generally have a narrow host range (Shurtleff and Averre 2000). Some non-host plants react to the invading nematode with an active hypersensitive response, indicating the presence of a resistance gene (Sect. 3.3 below) (Kaplan and Keen 1980; Starr et al. 2002b). Subsequently, the nematodes either die or migrate out of the roots. Preformed nematicidal metabolites such as alkaloids, phenolics, and sesquiterpenes appear to

play a role in nematode rejection as may the phytoalexins produced in response to invasion (Sect. 3.1 above) (Kaplan and Keen 1980; Rich et al. 1977). There is less evidence to support a role for anatomical attributes or physical barriers that can block penetration and nutrient deprivation (Kaplan and Keen 1980). In other instances, non-host plants may lack the plant genes required for susceptibility. A case of a gene required for susceptibility to the powdery mildew fungal pathogen has been identified in *Arabidopsis* (Vogel et al. 2002). De Almeida Engler et al. (2005) have considered the opportunities for generating nematode resistance via a loss of susceptibility genes. Developing a more fundamental understanding of what causes a plant to be a non-host could be an extremely fruitful line of inquiry with immediate applications to commercial nematode resistance.

3.3 Disruption of Plant Responses

3.3.1 Plant Resistance Gene/Hypersensitive Response Activated by Nematode Invasion

There are numerous examples of plants that have an innate immune response to invasion by specific nematode species (Starr et al. 2002b). This resistance response is characterized by two features. First, it is dependent on specific plant resistance genes (R-genes) that detect the invading nematode, and second, the plant responds by a localized hypersensitive response (HR) that can include cell death, atrophy, or abnormal development of the feeding site. Nematodes remaining at these feeding sites are either dead or greatly diminished in size and fertility. Phytoalexins can also be induced (Sect. 3.1 above). A major area of progress over the last decade has been the molecular genetic characterization of endogenous-plant-resistance responses to parasitic nematodes (Williamson and Hussey 1996; Williamson and Kumar 2006). Six R-genes have now been cloned from sugar beet (Cai et al. 1997), tomato (Ernst et al. 2002; Milligan et al. 1998; Vos et al. 1998), and potato (Paal et al. 2004; van der Vossen et al. 2000). An additional ten R-genes have been mapped, some to narrow intervals (e.g. Ruben et al. 2006). All of these R-genes are involved in resistance to sedentary endoparasites including cyst nematodes (*Heterodera* spp., *Globodera* spp.) or root-knot nematodes (*Meloidogyne* spp.).

One example is the tomato *Mi*-1.2 gene (Milligan et al. 1998; Vos et al. 1998), which encodes a leucine-rich repeat protein and confers resistance to three *Meloidogyne* species as well as aphids and white flies. *Mi*-1.2 can be transgenically expressed and provide *Meloidogyne* resistance in some tomato-related plant species (such as eggplant) but not in others (Goggin et al. 2006). Mi-1.2 is likely part of a surveillance cascade that detects a specific nematode factor and triggers localized host cell death where giant-cells would normally form near the head of the invading J2 worm. Some R-genes, such as *Hero A* (Ernst et al. 2002), can alter the sex ratio of cyst nematodes with more males being formed, a known nematode stress response. Nematode avirulence (*Avr*) genes encoding proteins detected by the

corresponding plant R-genes have yet to be cloned and characterized (Williamson and Kumar 2006) with the exception of Mi-1.2 where a *M. javanica* avirulence gene *Cg-1* was recently identified (Gleason et al. 2008).

Plant R-genes have been the underlying basis for successes in breeding efforts generating nematode-resistant tomato, soybeans, tobacco and other crops with pronounced economic benefits (Sect. 1 above) (Starr et al. 2002a). Introduction of such traits within a species or genus (when possible) by mating has the advantage of avoiding transgenic technology with its associated development and regulatory costs and market introduction issues. Transgenics can extend the introduction of R-genes to more distantly related species. One benefit of R-genes versus other possible approaches is that it takes advantage of endogenous plant pathways that merely require activation. Given that R-genes are widely deployed in many crops, issues around phytotoxicity and toxicology are limited. Potential disadvantages of transgenic R-genes include limitations on the spectrum of activity (a small subset of parasites are recognized) and the likelihood of rapid resistance breaking and selection of non-susceptible nematodes (Starr et al. 2002b).

3.3.2 Feeding Site-Specific Promoter Induces Cell Death Or Other Site Incompatibility

Most approaches to biotechnology-based nematode control will require expression of the transgene such that the nematode or the plant cell interacting with the nematode comes into contact with the needed effector molecule. Given the economic importance of root-knot, cyst, and other sedentary endoparasitic species that form feeding sites, characterizing gene expression at these nematode-induced structures within the plant has become one of the most extensively studied areas in molecular plant nematology (Gheysen and Fenoll 2002; Hammes et al. 2005; Jammes et al. 2005; Klink et al. 2005; Li et al. 2008). The introduction of methods including microarrays and laser-capture microdissection to isolate feeding sites is improving the resolution of such studies. This research has the potential to not only provide key insights into the physiological processes and cellular structure of feeding sites but also identify promoters that are useful for transgene expression.

The cauliflower mosaic virus 35S promoter, an archetypal strong promoter common in transgenic plant applications, may not be optimal for driving nematode resistance genes, particularly for cyst nematodes. Down-regulation of this promoter in nematode-induced syncytium has been observed for both *Heterodera schachtii* infecting *Arabidopsis thaliana* (Goddijn et al. 1993; Urwin et al. 1997b) and *Globodera tabacum* infecting tobacco (Bertioli et al. 1999). However, a growing collection of genes have been identified that are reproducibly expressed in feeding sites. Examples of genes up-regulated in syncytium formed by *Heterodera schachtii* infecting *Arabidopsis* include *Hs1pro-1* (Thurau et al. 2003), *AtSUC2* normally expressed in companion cells (Juergensen et al. 2003),

At17.1 expressed in vascular tissues and root tips (Mazarei et al. 2004), *FGAM* synthase (phosphoribosylformyl-glycinamidine synthase) (Vaghchhipawala et al. 2004), and *ABI3* (De Meutter et al. 2005). Examples of genes up-regulated in giant-cells formed by *Meloidogyne incognita* include *TobRB7* (Opperman et al. 1994) (see below) and the small heat-shock gene *Hahsp17.7G4* (Escobar et al. 2003) in tobacco as well as D-ribulose-5-phosphate-3-epimerase (*RPE*) (Favery et al. 1998) in *Arabidopsis*. Genes up-regulated in both syncytia and giant-cells include *AtPGM* (co-factor dependent phosphoglycerate mutase enzyme) in *Arabidopsis* (Mazarei et al. 2003) and endo-β-1,4-glucanases in tobacco (Goellner et al. 2001).

Depending on the effector molecule being generated, expression outside the feeding site may still be adequate to provide nematode control. Nematodes migrate through the root vasculature prior to feeding-site establishment and would come in contact with effector molecules produced by ubiquitous or root-specific promoters. Further, feeding sites are metabolic sinks that take up photo assimilates from the phloem. Syncytia formed in response to cyst nematodes have been described as symplastically isolated and lacking plasmodesmata to surrounding tissues (Bockenhoff and Grundler 1994; Bockenhoff et al. 1996). However, a recent study has shown that macromolecules up to 30 kD can move from phloem companion cells into the syncytium (Hoth et al. 2005). Therefore, gene expression in the phloem may be suited for delivery of effector molecules into feeding sites.

Beyond merely providing feeding-site expression, approaches that disrupt plant physiological processes to block nematode replication face the much higher hurdle of restricting expression to only the feeding site to prevent phytotoxic effects elsewhere in the plant. TobRB7 is a tobacco water channel protein expressed in roots and induced in root-knot nematode giant-cells (Opperman et al. 1994). Deletion analysis identified a promoter element (Δ0.3) that retained feeding site expression but had no expression in the remainder of the root. If such a promoter was entirely feeding-site specific, it could be used to drive expression of a cytotoxic product to kill cells within the feeding site. One suggested killing effector is the Barnase toxin (Conkling et al. 1998; Van Pouche et al. 2001). The risk of such a potent cytotoxin is that “leakage” of the promoter in any other plant cells under any conditions can result in unacceptable phytotoxicity including necrosis in cells outside the feeding site (Bertioli et al. 2001). Since it has been demonstrated that mitotic blocking agents halt giant-cell development, other suggested approaches include disruption of cell-cycle genes (de Almeida Engler et al. 1999). It may be possible to drive expression of a construct that interferes with the feeding site but also tolerates some leakage of expression elsewhere in the plant such as over-expressing a gene that must be down-regulated for proper feeding-site function or using RNAi to silence a gene that is essential for the feeding site but non-essential in most plant tissues. The approach of promoter-induced feeding-site cell death is limited to species that form feeding sites, mostly sedentary endoparasites such as *Meloidogyne* spp., *Heterodera* spp., *Globodera* spp., *Nacobbus* spp., *Rotylenchulus* spp., and *Tylenchulus* spp. (Wyss 1997).

3.3.3 Conversion of Plant to Tolerance

In general, molecular strategies for achieving protection from nematode damage have focused on achieving resistance (decreased nematode reproduction), but producing plants that are tolerant (decreased plant damage despite nematode burden) could be a valuable alternative strategy. Tolerance has been selected for in subsistence farming under continued high nematode pressure such as with small-fruited tomatoes in West Africa that display tolerance to *Meloidogyne* (Starr et al. 2002a). Tolerance traits have also been identified in germplasm screens for *Meloidogyne* and *Rotylenchus reniformis* (reniform nematode) tolerance in US cotton (Roberts 2002; Robinson 2002) and *Globodera* tolerance in United Kingdom (UK) potatoes (Trudgill et al. 1998). Little is known about the molecular mechanisms that underlie nematode tolerance in plants, and new research into understanding what makes a plant tolerant could be valuable.

4 Applications by Crop and Nematode Species

There is no doubt that parasitic nematodes cause substantial yield loss across many temperate, subtropical, and tropical crops (Luc et al. 2005b; Whitehead 1998). However, detailed knowledge of exact economic damage is often inadequate. More extensive data on yield loss experienced by growers and about which crops, pathogens, and geographies present the best near-term opportunities for putting molecular plant nematology knowledge to use could be beneficial.

Most nematology publications cite a 1987 international opinion survey of 371 nematologists to support a staggering estimated yield loss due to nematodes of $78–125 billion US dollars world-wide annually (Sasser and Freckman 1987). Estimated loss by crop in the survey, reflective of informed opinion but not necessarily field data, ranged from 3.3% (rye) to 20% or more (e.g. bananas, tomatoes). Reliability of these estimates remains difficult to assess. For the US, surveys of opinion on nematode-induced crop losses by state have also been conducted, and detailed information is available for some geographies and crops (e.g. cotton, soy) (Koenning et al. 1999; McSorley et al. 1987; Monson and Schmitt 2004). Trials of crops with and without nematicides can also be quite informative (Johnson 1985a, 1985b). As a starting point to better understand the value-capture proposition for nematode control, we applied the 1987 international loss estimates by crop (Sasser and Freckman 1987) to 2001 data for crops and value by country including yield and currency exchange rates (Divergence Inc. 2003, unpublished). The extrapolated 2001 loss for the 40 crops in the survey totaled $118 billion (11% of production). (The decline in value of the dollar relative to other currencies and the increase in commodity prices since 2001 inflate this estimate further.) Damage estimates for non-food plants including ornamentals, turf, and forest trees (e.g. pine) were not included.

More important than the total estimated damage is the representation of loss by crop (Fig. 2). Nearly half of the total (48%) derives from just two crops, rice (Bridge et al. 2005) and maize (McDonald and Nicol 2005), which dominate because of their overall predominance in world agriculture. Twenty-eight percent of the total derives from rice in China ($22.2 billion) and maize in the US ($10.3 billion) alone. Despite the impact of rice and maize on the estimated total yield loss, few molecular nematologists focus their research on these crops and the associated nematode pathogens. While 29 species of nematodes parasitize rice, studies of disease distribution and yield loss in rice have been limited (Bridge et al. 2005; Padgham et al. 2004), and international agricultural research centers have employed a minimal number of nematologists (Luc et al. 2005a). Despite cases of substantial yield loss documented in certain states, such nematode damage of corn acreage in Nebraska (Grower Survey by the National Corn Growers Association and Divergence Inc. 2003, unpublished), little persuasive information has been provided to US corn growers or breeders to make the case for a focus on nematode control as a yield-boosting strategy (Koenning et al. 1999). Growing market demand for US corn (e.g. ethanol production) may increase interest in yield boosting traits like nematode resistance that are currently overlooked by industry.

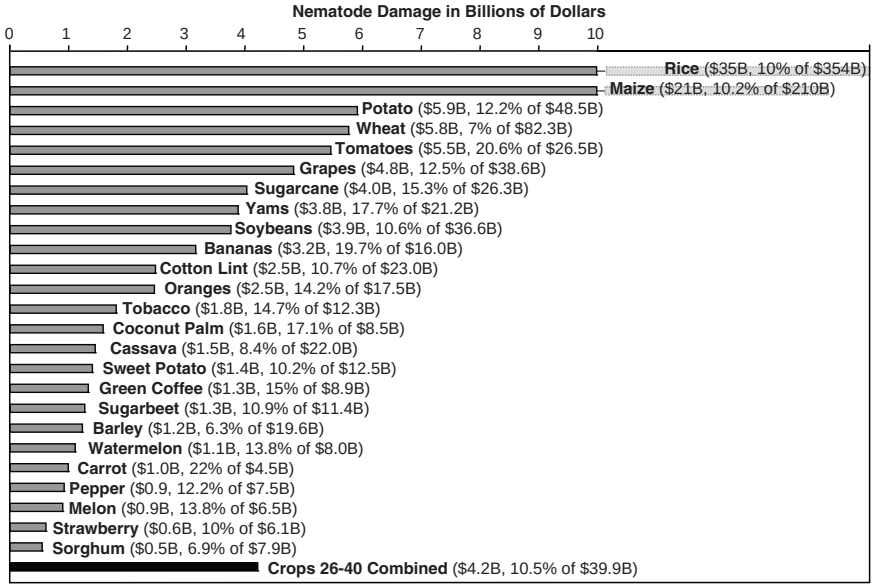


Fig. 2 An estimate of potential 2001 global nematode damage in billions of dollars based on a 1987 yield loss survey (Sasser and Freckman 1987). Of the 40 crops in the survey, 1–25 are shown in rank order. Estimates for rice (#1) and maize (#2) are not to scale. Crops ranked 26–40 are millet, cow peas, lemons/limes, chick peas, pineapple, broad beans (*green*), cocoa beans, tea leaves, eggplant, papaya, oats, pigeon pea, grapefruit, broad beans (*dry*), and rye

Beyond the scientific and technical progress detailed in this book, the likely impact of molecular nematology on agriculture over the next several decades may be determined by two interacting factors: (1) the focus of molecular nematology on certain crops and parasitic species, and (2) the development and adoption of plant biotechnology by crop and country. The major focus of molecular nematologists studying resistance, most of whom are located in Europe and North America, has been on major crops parasitized by root-knot (*Meloidogyne* spp.) and cyst nematodes (*Heterodera* and *Globodera* spp.). The near-term impact of this work could be seen with soybeans, cotton, tomatoes, tobacco, peanuts, potatoes, sugar beets, and various other solanaceous vegetables and cucurbits (peppers, eggplants, melons, etc.), an opportunity for yield improvements worth perhaps $25 billion (2001 dollars) based on yield loss estimates (Sasser and Freckman 1987). Soybeans and cotton are prime candidates for early technology introduction because of the broad adoption of biotechnology-derived traits (herbicide tolerance, insect resistance) in these crops in North and South America and Asia (Brookes and Barfoot 2005). For instance, 2004 US market penetration of transgenic traits in soybeans and cotton was 85 and 77% of total acreage, respectively (Sankula et al. 2005). Worldwide in 2005, biotechnology-based crops were grown on 90 million hectares in 21 countries by 8.5 million farmers, a 53-fold increase from 1.7 million hectares planted in 1996 (James 2005). Addition of second- or third-traits (i.e. stacking) in crops that are already transgenic is less controversial than the initial decision to deploy biotechnology in a crop. Opposition to biotechnology-derived crops by advocacy groups and delays in regulatory approvals of traits in the European Union have been detrimental to introduction of nematode resistance in potato and sugar beet, crops where European nematologists have substantial expertise.

Following soybeans and cotton, biotechnology-based resistance strategies could gradually be expected to find application in broad-acreage grains (e.g. rice, maize, wheat), citrus, vines, plantation crops (e.g. banana, sugarcane, and coffee), potatoes, sugar beets, subsistence crops in developing countries (e.g. yams, cassava, sweet potato) (Atkinson et al. 2001; Luc et al. 2005b), ornamentals and turf. Capture of all these additional opportunities will require an expansion of knowledge into a variety of nematode species including migratory endoparasites. For instance, nematode parasites of corn include *Hoplolaimus galeatus* (lance), *Longidorus breviannulatus* (needle), *Pratylenchus* spp. (lesion), *Xiphinema americanum* (dagger), *Belonolaimus* spp. (sting), *Helicotylenchus* spp. (spiral), *Tylenchorhynchus* spp. (stunt), and *Paratrichodorus* spp. (stubby-root), in addition to *Heterodera* spp. and *Meloidogyne* spp. Certain approaches to nematode control (e.g. antifeedants, nematicidal metabolites) may have broader application than technologies that are more tailored to individual species or genera (e.g. plant resistance genes, feeding site disruption). In almost any scenario, the long-term goal of greatly reducing the negative impact of plant-parasitic nematodes will require a sustained and multifaceted assault, including biotechnology-derived resistance, advances in marker-assisted breeding (Young and Mudge 2002) and introduction of safer next-generation nematicides on top of current best practices (Sikora et al. 2005).

5 Impact and Conclusions

In closing, the development of successful biotechnology-based nematode resistance should be viewed as an integral part of a more extensive endeavor to improve crop yields through pathogen and insect resistance, tolerance to drought, salinity and other stressors, superior efficiency in photosynthesis, and improved utilization of nitrogen and phosphate. Exponential technological improvements will be needed for agriculture to keep pace with the increasing global human population and increasing per capita consumption that are anticipated to double world-wide food demand by 2050. Even assuming similar yield improvements through breeding, fertilizers, pesticides, and irrigation to those achieved from 1965 to 2000, some models estimate that providing a sufficient food supply in 2050 (relative to 2000) will require a 23% increase in land devoted to crops (1.89 billion hectares), a 16% increase in pasture land (4.01 billion hectares), a 90% increase in irrigated land, a 270% increase in nitrogen fertilizer, and a 240% increase in phosphate fertilizer (Tilman et al. 2001). The ecological impact of these trends, including the conversion of a land mass nearly the size of the United States from natural ecosystems to agriculture, and the accompanying degradation of marine and freshwater ecosystems, soil and water resources, and species extinction, could be devastating and is clearly not sustainable. Failing to provide future food security and opportunities for poverty alleviation is equally unacceptable.

Forestalling future environmental effects by limiting the footprint of agriculture will require overcoming the current barriers that limit optimal plant yield, including nematode damage. Seen in this broader context, molecular plant nematology has a major role to play worldwide in both food security and environmental protection in the coming decades. Superior technology for nematode control has the opportunity to substantially raise yields for most major temperate crops in developed countries and perhaps, especially, for tropical subsistence crops in developing countries where pathogenicity is particularly high (Luc et al. 2005a). This will require both increased attention to nematode control by agricultural biotechnology companies with established pipelines for commercializing transgenic traits in major row crops, as well as strategies for successfully transferring technology and intellectual property rights to partners capable of making these traits widely available for staple food crops in developing countries (Atkinson et al. 2001; Beachy 2003; Serageldin 1999).

Insights from research in molecular plant nematology are now providing a more detailed picture of parasitic nematode gene products and physiology, interactions occurring at the nematode–plant interface, and features of the plant response to parasitism. At least 11 general strategies for biotechnology-based nematode control have been described to date and new discoveries will likely introduce further possibilities. Progress with some approaches has already been substantial, culminating in field trials for a few cases. Successful commercialization of biotechnology-derived crops with nematode resistance that result in large yield benefits for growers as well as environmental benefits will be an important milestone for the discipline of molecular plant nematology and should accelerate further progress.

Acknowledgments I thank Divergence colleagues Martha Schlicher for market analysis and Bingli Gao for improvements to Fig. 1. The manuscript was improved by critical reading and feedback from David Bird, Makedonka Mitreva, and Michael Crawford. Support of nematode control research at Divergence Inc. has been provided by collaborations with Monsanto Company and the National Corn Growers Association, and grants from the National Science Foundation (NSF), the US Department of Agriculture, the Environmental Protection Agency, and the National Institutes of Health (NIH). Support of parasitic nematode genomics at Washington University has been provided by NSF, NIH, the Helen Hay Whitney Foundation, and Merck and Company Inc.

References

Adkisson P, Abraham S, Baenzinger S, Betz F, Carrington JC, Goldburg RJ, Gould F, Hodgson E, Jones T, Levin M, Lichtenberg E, Snow A, Kuzma J, Phillips MJ (2000) Genetically modified pest-protected plants, science and regulation. National Research Council, Washington DC.

Atkinson HJ, Lilley CJ, Urwin PE, McPherson MJ (1998a) Engineering resistance in the potato to potato cyst nematodes. In: Marks RJ, Brodie BB (eds) Potato cyst nematodes, biology, distribution and control. CAB International, Wallingford, pp 209–236

Atkinson HJ, Lilley CJ, Urwin PE, McPherson MJ (1998b) Engineering resistance to plant-parasitic nematodes. In: Perry RN, Wright DJ (eds) The physiology and biochemistry of free-living and plant-parasitic nematodes. CABI Publishing, Wallingford pp 381–405

Atkinson HJ, Green J, Cowgill S, Levesley A (2001) The case for genetically modified crops with a poverty focus. Trends Biotech 19:91–96

Atkinson HJ, Urwin PE, McPherson MJ (2003) Engineering plants for nematode resistance. Annu Rev Phytopathol 41:615–639

Atkinson HJ, Grimwood S, Johnston K, Green J (2004) Prototype demonstration of transgenic resistance to the nematode *Radopholus similis* conferred on banana by a cystatin. Transgenic Res 13:135–142

Bakhetia M, Charlton W, Atkinson HJ, McPherson MJ (2005a) RNA Interference of dual oxidase in the plant nematode *Meloidogyne incognita*. Mol Plant Microbe Interact 18:1099–1106

Bakhetia M, Charlton WL, Urwin PE, McPherson MJ, Atkinson HJ (2005b) RNA interference and plant-parasitic nematodes. Trends Plant Sci 10:362–367

Baldridge GD, O'Neill NR, Samac DA (1998) Alfalfa (*Medicago sativa* L) resistance to the root-lesion nematode, *Pratylenchus penetrans*: defense-response gene mRNA and isoflavonoid phytoalexin levels in roots. Plant Mol Biol 38:999–1010

Bargmann CI, Hartwieg E, Horvitz HR (1993) Odorant-selective genes and neurons mediate olfaction in *C elegans*. Cell 74:515–27

Baum TJ, Hiatt A, Parrot WA, Pratt H, Hussey RS (1996) Expression in tobacco of a functional monoclonal antibody specific to stylet secretions of the root-knot nematode. Mol Plant Microbe Interact 9:382–387

Beachy RN (2003) IP policies and serving the public. Science 299:473

Berg RH, Fester T, Taylor CG (2008) Development of the root-knot nematode feeding cell. Plant Cell Monogr., doi:10.1007/7089_2008_30

Bergamasco C, Bazzicalupo P (2006) Chemical sensitivity in *Caenorhabditis elegans*. Cell Mol Life Sci 63:1510–1522

Bertioli DJ, Smoker M, Burrows PR (1999) Nematode-responsive activity of the cauliflower mosaic virus 35S promoter and its subdomains. Mol Plant Microbe Interact 12:189–196

Bertioli DJ, Guimaraes PM, Jones JDG, Thomas CM, Burrows PR, Monte DC, Leal-Bertioli SCDeM (2001) Expression of tomato *Cf* genes and their corresponding avirulence genes in transgenic tobacco plants using nematode responsive promoters. Ann Appl Biol 138:333–342

Bird DMcK, Opperman CH, Williamson VM (2008) Plant infection by roo-knot nematode. Plant Cell Monogr., doi:10.1007/7089_2008_31

Bockenhoff A, Grundler FM (1994) Studies on the nutrient uptake by the beet cyst nematode *Heterodera schachtii* by *in situ* microinjection of fluorescent probes into the feeding structure in *Arabidopsis thaliana*. Parasitol 109:249–254

Bockenhoff A, Prior DA, Grundler FM, Oparka KJ (1996) Induction of phloem unloading in *Arabidopsis thaliana* roots by the parasitic nematode *Heterodera schachtii*. Plant Physiol 112:1421–1427

Brady EB, Duffy M (1982) The value of plant resistance to soybean cyst nematodes: a case study of Forrest soybean. US Department of Agriculture, Natural Resources Economics Division, Washington DC

Bridge J, Plowright RA, Peng D (2005) Nematode parasites of rice. In: Luc M, Sikora RA, Bridge J (eds) Plant-parasitic nematodes in subtropical and tropical agriculture, pp 87–130. CAB International, Wallingford

Brookes G, Barfoot P (2005) GM crops: the global economic and environmental impact – the first nine years 1996–2004. Ag Bio Forum 8:187–196

Burrows PR, De Waele D (1997) Engineering resistance against plant-parasitic nematodes using anti-nematode genes. In: Fenoll C, Grundler FMW, Ohl SA (eds) Cellular and molecular aspects of plant–nematode interactions, pp 217–236. Kluwer, Dordrecht

Cai D, Kleine M, Kifle S, Harloff H-J, Sandal NN, Marcker KA, Klein-Lankhorst RM, Salentijn EMJ, Lange W, Stiekema WJ, Wyss U, Grundler FMW, Jung C (1997) Positional cloning of a gene for nematode resistance in sugar beet. Science 275:832–834

Chen Q, Rehman S, Smant G, Jones JT (2005) Functional analysis of pathogenicity proteins of the potato cyst nematode *Globodera rostochiensis* using RNAi. Mol Plant Microbe Interact 18:621–625

Chitwood DJ (2002) Phytochemical based strategies for nematode control. Annu Rev Phytopathol 40:221–249

Conkling MA, Opperman CH, Taylor CG Pathogen-resistant transgenic plants. United States Patent #5,750,386, May 12, 1998

Dale MFB, De Scurrah MM (1998) Breeding for resistance to the potato cyst nematodes *Globodera rostochiensis* and *Globodera pallida*: strategies, mechanisms, and genetic resources. In: Marks RJ, Brodie BB (eds) Potato cyst nematodes, biology, distribution, and control. CAB International, Wallingford, pp 167–195

Davis EL, Hussey RS, Baum TJ, Bakker J, Schots A, Rosso MN, Abad P (2000) Nematode parasitism genes. Annu Rev Phytopathol 38:365–396

Davis EL, Hussey RS, Baum T (2004) Getting to the root of parasitism by nematodes. Trends Parasitol 20:134–141

Davis EL, Hussey RS, Baum TJ (2008) Parasitism genes: what they reveal about parasitism. Plant Cell Monogr., doi:10.1007/7089_2008_29

de Almeida Engler J, De Vleesschauwer V, Burssens S, Celenza JL Jr, Inze D, Van Montagu M, Engler G, Gheysen G (1999) Molecular markers and cell cycle inhibitors show the importance of cell cycle progression in nematode-induced galls and syncytia. Plant Cell 11:793–808

de Almeida Engler J, Favery B, Engler G, Abad P (2005) Loss of susceptibility as an alternative for nematode resistance. Curr Opin Biotechnol 16:112–117

De Meutter J, Robertson L, Parcy F, Mena M, Fenoll C, Gheysen G (2005) Differential activation of *ABI3* and *LEA* genes upon plant-parasitic nematode infection. *Mol Plant Path* 6:321–325

Doyle EA, Lambert KN (2002) Cloning and characterization of an esophageal-gland-specific pectate lyase from the root-knot nematode *Meloidogyne javanica*. Molec Plant-Microbe Interacts 15:549–56

Doyle EA, Lambert KN (2003) *Meloidogyne javanica* chorismate mutase 1 alters plant cell development. Mol Plant Microbe Interact 16:123–131

Dropkin VH (1989) Introduction to plant nematology. Wiley, New York

Dusenbery DB (1983) Chemotactic behavior of nematodes. J Nematol 15:168–173

Ernst K, Kumar A, Kriseleit D, Kloos DU, Phillips MS, Ganal MW (2002) The broad-spectrum potato cyst nematode resistance gene (*Hero*) from tomato is the only member of a large gene family of NBS-LRR genes with an unusual amino acid repeat in the LRR region. Plant J 31:127–136

Escobar C, Barcala M, Portillo M, Almoguera C, Jordano J, Fenoll C (2003) Induction of the *Hahsp177G4* promoter by root-knot nematodes: involvement of heat-shock elements in promoter activity in giant-cells. Mol Plant Microbe Interact 12:1062–1068

Fanelli E, Di Vito M, Jones JT, De Giorgi C (2005) Analysis of chitin synthase function in a plant-parasitic nematode, *Meloidogyne artellia*. Gene 349:87–95

Favery B, Lecomte P, Gil N, Bechtold N, Bouchez D, Dalmasso A, Abad P (1998) *RPE*, a plant gene involved in early developmental steps of nematode feeding cells. Embo J 17:6799–6811

Fioretti L, Porter A, Haydock PJ, Curtis R (2002) Monoclonal antibodies reactive with secreted-excreted products from the amphids and the cuticle surface of *Globodera pallida* affect nematode movement and delay invasion of potato roots. Int J Parasitol 32:1709–1718

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

Fraser AG, Kamath RS, Zipperlen P, Martinez-Campos M, Sohrmann M, Ahringer J (2000) Functional genomic analysis of *C elegans* chromosome I by systematic RNA interference. Nature 408:325–330

Gao B, Allen R, Maier T, Davis EL, Baum TJ, Hussey RS (2001) Identification of putative parasitism genes expressed in the esophageal gland cells of the soybean cyst nematode *Heterodera glycines*. Mol Plant Microbe Interact 14:1247–1254

Gao B, Allen R, Maier T, Davis EL, Baum TJ, Hussey RS (2003) The parasitome of the phytonematode *Heterodera glycines*. Mol Plant Microbe Interact 16:720–726

Gao B, Allen R, Davis EL, Baum TJ, Hussey RS (2004) Developmental expression and biochemical properties of a beta-1,4-endoglycanase family in the soybean cyst nematode, *Heterodera glycines*. Mol Plant Path 5:93–104

Gheysen G, Fenoll C (2002) Gene expression in nematode feeding sites. Annu Rev Phytopathol 40:191–219

Gheysen G, Mitchum MG (2008) Molecular insights in the susceptible plant response to nematode infection. Plant Cell Monogr., doi:10.1007/7089_2008_35

Gilbert J, McGuire D (1956) Inheritance of resistance to severe root-knot from *Meloidogyne incognita* in commercial type tomatoes. Proc Am Soc Hort Sci 68:437–442

Gleason CA, Liu QL, Williamson VM (2008) Silencing a candidate nematode effector gene corresponding to the tomato resistance gene *Mi-1* leads to acquisition of virulence. Mol Plant Microbe Interact 21:576–585

Goddijn OJ, Lindsey K, van der Lee FM, Klap JC, Sijmons PC (1993) Differential gene expression in nematode-induced feeding structures of transgenic plants harbouring promoter-*gusA* fusion constructs. Plant J 4:863–873

Goellner M, Wang X, Davis E (2001) Endo-beta-1,4-glucanase expression in compatible plant–nematode interactions. Plant Cell 13:2241–2255

Goggin FL, Jia L, Shah G, Hebert S, Williamson VM, Ullman DE (2006) Heterologous expression of the *Mi*-12 gene from tomato confers resistance against nematodes but not aphids in eggplants. Mol Plant Microbe Interact 19:383–388

Gonczy P, Echeverri C, Oegema K, Coulson A, Jones SJ, Copley RR, Duperon J, Oegema J, Brehm M, Cassin E, Hannak E, Kirkham M, Pichler S, Flohrs K, Goessen A, Leidel S, Alleaume AM, Martin C, Ozlu N, Bork P, Hyman AA (2000) Functional genomic analysis of cell division in *C elegans* using RNAi of genes on chromosome III. Nature 408:331–336

Goverse A, Biesheuvel GJ, Gommers FJ, Bakker J, Schots A, Helder J (1998) In planta monitoring of the activity of two constitutive promoters, CaMV 35S and TR2, in developing feeding cells induced by *Globodera rostochiensis* using green fluorescent protein in combination with confocal laser scanning microscopy. Physiolog Mol Plant Path 52:275–284

Griffitts JS, Whitacre JL, Stevens DE, Aroian RV (2001) Bt toxin resistance from loss of a putative carbohydrate-modifying enzyme. Science 293:860–863

Griffitts JS, Haslam SM, Yang T, Garczynski SF, Mulloy B, Morris H, Cremer PS, Dell A, Adang MJ, Aroian RV (2005) Glycolipids as receptors for *Bacillus thuringiensis* crystal toxin. Science 307:922–925

Hammes UZ, Schachtman DP, Berg RH, Nielsen E, Koch W, McIntyre LM, Taylor CG (2005) Nematode-induced changes of transporter gene expression in Arabidopsis roots. Mol Plant Microbe Interact 12:1247–1257

Haydock PPJ, Woods SR, Grove IG, Hare MC (2006) Chemical control of nematodes. In: Perry RN, Moens M (eds) Plant nematology. CAB International, Wallingford, pp 392–410

Hilliard MA, Bargmann CI, Bazzicalupo P (2002) *C elegans* responds to chemical repellents by integrating sensory inputs from the head and the tail. Curr Biol 12:730–740

Hoth S, Schneidereit A, Lauterbach C, Scholz-Starke J, Sauer N (2005) Nematode infection triggers the de novo formation of unloading phloem that allows macromolecular trafficking of green fluorescent protein into syncytia. Plant Physiol 138:383–392

Huang G, Allen R, Davis EL, Baum TJ, Hussey RS (2006a) Engineering broad root-knot resistance in transgenic plants by RNAi silencing of a conserved and essential root-knot nematode parasitism gene. Proc Natl Acad Sci USA 103:14302–14306

Huang G, Dong R, Allen R, Davis EL, Baum TJ, Hussey RS (2006b) A root-knot nematode secretory peptide functions as a ligand for a plant transcription factor. Mol Plant Microbe Interact 19:463–470

Huang G, Gao B, Maier T, Allen R, Davis EL, Baum TJ, Hussey RS (2003) A profile of putative parasitism genes expressed in the esophageal gland cells of the root-knot nematode *Meloidogyne incognita*. Mol Plant Microbe Interact 16:376–381

James C (2005) Executive summary of global status of commercialized biotech/GM crops: ISAAA briefs. International service for the acquisition of agri-biotech applications, Ithaca, NY, pp 1–12

Jammes F, Lecomte P, de Almeida Engler J, Bitton F, Martin-Magniette M-L, Renou JP, Abad P, Favery B (2005) Genome-wide expression profiling of the host response to root-knot nematode infection in Arabidopsis. Plant J 44:447–458

Jaubert S, Ledger TN, Laffaire JB, Piotte C, Abad P, Rosso MN (2002) Direct identification of stylet secreted proteins from root-knot nematodes by a proteomic approach. Mol Biochem Parasitol 121:205–211

Jaubert S, Milac AL, Petrescu AJ, de Almeida Engler J, Abad P, Rosso MN (2005) In planta secretion of a calreticulin by migratory and sedentary stages of root-knot nematode. Mol Plant Microbe Interact 12:1277–1284

Jobling S, Jarman C, Teh M, Holmberg N, Blake C, Verhoeyen M (2003) Immunomodulcation of enzyme function in plants by single-domain antibody fragments. Nat Biotechnol 21:77–80

Johnson AW (1985a) The role of nematicides in nematode management. In: Sasser JN, Carter CC (eds) An advanced treatise on *Meloidogyne*. North Carolina State University Graphics, Raleigh, North Carolina, pp. 249–267

Johnson AW (1985b) Specific crop rotation effects combined with cultural practices and nematicides. In: Sasser JN, Carter CC (eds) An advanced treatise on *Meloidogyne*. North Carolina State University Graphics, Raleigh, North Carolina, pp. 283–301

Juergensen K, Scholz-Starke J, Sauer N, Hess P, van Bel AJE, Grundler FMW (2003) The companion cell-specific Arabidopsis disaccharide carrier AtSUC2 is expressed in nematode-induced syncytia. Plant Physiol 131:61–69

Jung C, Cai D, Kleine M (1998) Engineering nematode resistance in crop species. Trends Plant Sci 3:266–271

Kamath RS, Fraser AG, Dong Y, Poulin G, Durbin R, Gotta M, Kanapin A, Le Bot N, Moreno S, Sohrmann M, Welchman DP, Zipperlen P, Ahringer J (2003) Systematic functional analysis of the *Caenorhabditis elegans* genome using RNAi. Nature 421:231–237

Kaplan DT, Keen NT (1980) Mechanisms conferring plant incompatibility to nematodes. Revue de Nematologie 3:123–134

Klink VP, Alkharouf N, MacDonald M, Matthews B (2005) Laser capture microdissection (LCM) and expression analyses of *Glycine max* (soybean) syncytium containing root regions formed by the plant pathogen *Heterodera glycines* (soybean cyst nematode). Plant Mol Biol 59:965–979

Koenning SR, Overstreet C, Noling JW, Donald PA, Becker JO, Fortnum BA (1999) Survey of crop losses in response to phytoparasitic nematodes in the United States for 1994. J Nematol 31:587–618

Li X-Q, Wei J-Z, Tan A, Aroian RV (2007) Resistance to root-knot nematode in tomato roots expressing a nematicidal *Bacillus thuringiensis* crystal protein. Plant Biotech J 5:455–464

Li L, Fester T, Taylor CG (2008) Transcriptomic analysis of nematode infestation. Plant Cell Monogr., doi:10.1007/7089_2008_36

Lilley CJ, Devlin P, Urwin PE, Atkinson HJ (1999a) Parasitic nematodes, proteinases and transgenic plants. Parasitol Today 15:414–417

Lilley CJ, Urwin PE, Atkinson HJ (1999b) Characterization of plant nematode genes: identifying targets for a transgenic defence. Parasitol 118:S63–S72

Lilley CJ, Goodchild SA, Atkinson HJ, Urwin PE (2005) Cloning and characterization of a *Heterodera glycines* aminopeptidase cDNA. Int J Parasitol 35:1577–1585

Liu B, Hibbard JK, Urwin PE, Atkinson HJ (2005) The production of synthetic chemodisruptive peptides in planta disrupts the establishment of cyst nematodes. Plant Biotech J 3:487–496

Luc M, Bridge J, Sikora RA (2005a) Reflections on nematology in subtropical and tropical agriculture. In: Luc M, Sikora RA, Bridge J (eds) Plant-parasitic nematodes in subtropical and tropical agriculture. CAB International, Wallingford, pp. 1–10

Luc M, Sikora RA, Bridge J (2005b) Plant-parasitic nematodes in subtropical and tropical agriculture. CAB International, Wallingford

Maeda I, Kohara Y, Yamamoto M, Sugimoto A (2001) Large-scale analysis of gene function in *Caenorhabditis elegans* by high-throughput RNAi. Curr Biol 11:171–176

Mazarei M, Lennon KA, Puthoff DP, Rodermel SR, Baum TJ (2003) Expression of an Arabidopsis phosphoglycerate mutase homologue is localized to apical meristems, regulated by hormones, and induced by sedentary plant-parasitic nematodes. Plant Mol Biol 53:513–530

Mazarei M, Lennon KA, Puthoff DP, Rodermel SR, Baum TJ (2004) Homologous soybean and Arabidopsis genes share responsiveness to cyst nematode infection. Mol Plant Path 5: 409–423

McCarter JP (2004) Genomic filtering: an approach to discovering novel antiparasitics. Trends Parasitol 20:462–468

McCarter J, Dautova Mitreva M, Martin J, Dante M, Wylie T, Rao U, Pape D, Bowers Y, Theising B, Murphy CV, Kloek AP, Chiapelli BJ, Clifton SW, Bird DMcK, Waterston R (2003) Analysis and functional classification of transcripts from the nematode *Meloidogyne incognita*. Genome Biol. 4:R26:1–19

McCarter JP, Bird DM, Mitreva M (2005) Nematode gene sequences: update for December 2005. J Nematol 37:417–421

McClure MA (1988) Lectin binding sites on the amphidial exudates of *Meloidogyne*. J Nematol 20:321–326

McDonald AH, Nicol JM (2005) Nematode parasites of cereals. In: Luc M, Sikora RA, Bridge J (eds) Plant-parasitic nematodes in subtropical and tropical agriculture. CAB International, Wallingford, pp. 131–191

McPherson MJ, Urwin PE, Lilley CJ, Atkinson HJ (1997) Engineering plant nematode resistance by antifeedants. In: Fenoll C, Grundler FMW, Ohl SA (eds) Cellular and molecular aspects of plant–nematode interactions. Kluwer, Dordrecht, pp 237–249.

McSorley R, Arnett JD, Bost SC, Carter WW, Hafez S, Johnson AW, Kirkpatrick T, Nyczepir AP, Radewald JD, Robinson AF, Schmitt DP (1987) Bibliography of estimated crop losses in the United States due to plant-parasitic nematodes. Ann Appl Nem 1:6–12

Mello CC, Conte D (2004) Revealing the world of RNA interference. *Nature* 431:338–342

Milligan SB, Bodeau J, Yaghoobi J, Kaloshian I, Zabel P, Williamson VM (1998) The root-knot nematode resistance gene *Mi* from tomato is a member of the leucine zipper, nucleotide binding, leucine-rich repeat family of plant genes. Plant Cell 10:1307–1319

Mitreva M, Elling AA, Dante M, Kloek AP, Kalyanaraman A, Aluru S, Clifton SW, Bird DMcK, Baum TJ, McCarter JP (2004) A survey of SL1-spliced transcripts from the root-lesion nematode *Pratylenchus penetrans*. Mol Gen Genomics 272:138–148

Mitreva M, Blaxter ML, Bird DMcK, McCarter JP (2005) Comparative genomics of nematodes. Trends Genet 21:573–581

Mitreva-Dautova M, Roze E, Overmars H, de Graaff L, Schots A, Helder J, Goverse A, Bakker J, Smant G (2006) A symbiont-independent endo-1,4-beta-xylanase from the plant-parasitic nematode *Meloidogyne incognita*. Mol Plant Microbe Interact 19:521–529

Monson M, Schmitt DP (2004) Economics. In: Schmitt DP, Wrather JA, Riggs RD (eds) Biology and management of soybean cyst nematode. Schmitt and Associates, Marceline, pp 41–53

Ohl SA, van der Lee FM, Sijmons PC (1997) Anti-feeding structure approaches to nematode resistance. In: Fenoll C, Grundler FMW, Ohl SA (eds) Cellular and molecular aspects of plant–nematode interactions. Kluwer, Dordrecht, pp 250–261

Olsen AN, Skriver K (2003) Ligand mimicry? Plant-parasitic nematode polypeptide with similarity to CLAVATA3. Trends Plant Sci 8:55–57

Opperman CH, Taylor CG, Conkling MA (1994) Root-knot nematode directed expression of a plant root-specific gene. Science 263:221–223

Opperman CH, Bird DMcK, Schaff JE (2008) Genomic analysis of the root-knot nematode genome. Plant Cell Monogr., doi:10.1007/7089_2008_37

Paal J, Henselewski H, Muth J, Meksem K, Menendez CM, Salamini F, Ballvora A, Gerbhardt C (2004) Molecular cloning of the potato *Gro1-4* gene conferring resistance to pathotype Ro1 of the root cyst nematode *Globodera rostochiensis*, based on a candidate gene approach. Plant J 38:285–297

Padgham JL, Duxbury JM, Mazid AM, Abawi GS, Hossain M (2004) Yield loss caused by *Meloidogyne graminicola* on lowland rainfed rice in Bangladesh. J Nematol 36:42–48

Parkinson J, Mitreva M, Whitton C, Thomson M, Daub J, Martin J, Hall N, Barrell B, Waterston RH, McCarter JP, Blaxter M (2004) A transcriptomic analysis of the phylum Nematoda. Nature Genetics 36:1259–1267

Perry RN (1998) The physiology and sensory perception of potato cyst nematodes, *Globodera* species. In: Marks RJ, Brodie BB (eds) Potato cyst nematodes, biology, distribution and control. CAB International, Wallingford

Perry RN, Moens M (2006) Plant nematology. CAB International, Wallingford

Popeijus H, Overmars HA, Jones JT, Blok VC, Goverse A, Helder J, Schots A, Bakker J, Smant G (2000) Degradation of plant cell walls by a nematode. Nature 406:36–37

Prins M, Lohuis D, Schots A, Goldbach R (2005) Phage display-selected single-chain antibodies confer high levels of resistance against tomato spotted wilt virus. J Gen Virology 86:2107–2113

Prot JC (1980) Migration of plant-parasitic nematodes toward plant roots. *R*evue de Nematologie 3:305–318

Qin L, Overmars H, Helder J, Popeijus H, van der Voort JR, Groenink W, van Koert P, Schots A, Bakker J, Smant G (2000) An efficient cDNA-AFLP-based strategy for the identification of putative pathogenicity factors from the potato cyst nematode *Globodera rostochiensis*. Mol Plant Microbe Interact 13:830–836

Qin L, Kudla U, Roze EH, Goverse A, Popeijus H, Nieuwl J, Overmars H, Jones JT, Schots A, Smant G, Bakker J, Helder J (2004) Plant degradation: a nematode expansin acting on plants. Nature 427:30

Rasmann S, Kollner T, Degenhardt J, Hiltpold I, Toepfer S, Kuhlmann U, Gershenzon J, Turlings T (2005) Recruitment of entomopathogenic nematodes by insect-damaged maize roots. Nature 434:732–737

Rich JR, Kavitha PG (2006) Evaluation of Avicta and N-Hibit seed treatments for management of reniform nematodes on cotton in north Florida, 2005. Fungicide and Nematicide Tests 61

Rich JR, Keen NT, Thomason IJ (1977) Association of coumestans with the hypersensitivity of lima bean roots to *Pratylenchus scribneri*. Physio Plant Path 10:105–116

Riddle DL, Bird AF (1985) Responses of the plant-parasitic nematodes *Rotylenchulus reniformis*, *Anguina agrostis*, and *Meloidogyne javanica* to chemical attractants. Parasitol 91:185–195

Risher JF, Mink FL, Stara JF (1987) The toxicologic effects of the carbamate insecticide aldicarb in mammals: a review. Environ Health Perspect 72:267–281

Roberts PA (2002) Concepts and consequences of resistance. In: Starr JL, Cook R, Bridge J (eds) Plant resistance to parasitic nematodes. CAB International, Wallingford, pp 23–41

Robinson AF (1995) Optimal release rates for attracting *Meloidogyne incognita*, *Rotylenchulus reniformis*, and other nematodes to carbon dioxide in sand. J Nematol 27:42–50

Robinson AF (2002) Reniform nematodes: *Rotylenchulus* species. In: Starr JL, Cook R, Bridge J (eds) Plant resistance to parasitic nematodes. CAB International, Wallingford, pp 153–174

Rosso M, Schouten A, Roosien J, Borst-Vrenssen T, Hussey RS, Gommers F, Bakker J, Schots A, Abad P (1996) Expression and functional characterization of a single chain Fv antibody directed against secretions involved in plant nematode infection process. Biochem Biophys Res Commun 220:255–263

Rosso MN, Dubrana MP, Cimbolini N, Jaubert S, Abad P (2005) Application of RNA interference to root-knot nematode genes encoding esophageal gland proteins. Mol Plant Microbe Interact 18:615–620

Ruben E, Jamai A, Afzal J, Njiti VN, Triwitayakorn K, Iqbal MJ, Yaegashi S, Bashir R, Kazi S, Arelli P, Town CD, Ishihara H, Meksem K, Lightfoot DA (2006) Genomic analysis of the *rhg1* locus: candidate genes that underlie soybean resistance to the cyst nematode. Mol Genet Genomics 276:503–516

Sankula S, Marmon G, Blumenthal E (2005) Biotechnology-derived crops planted in 2004 - Impacts on US Agriculture. National Center for Food and Agricultural Policy, Washington DC

Sasser JN, Freckman DW (1987) A world perspective on nematology: the role of the society. In: Veech JA, Dickson DW (eds) Vistas on nematology. Society of Nematology, Hyattsville, Maryland, pp 7–14

Schneider SM, Rosskopf EN, Leesch JG, Chellemi DO, Bull CT, Mazzola M (2003) United States Department of Agriculture – Agricultural Research Service research on alternatives to methyl bromide: pre-plant and post-harvest. Pest Manag Sci 59:814–826

Schnepf E, Crickmore N, Van Rie J, Lereclus D, Baum J, Fieitelson J, Zeigler DR, Dean DH (1998) *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiol Molec Biol Rev 62:775–806

Schnepf EH, Schwab GE, Payne J, Narva KE, Foncerrada L (2003) Nematicidal proteins. Mycogen Corporation, USA

Scholl EH, Thorne JL, McCarter JP, Bird DMcK (2003) Horizontally transferred genes in plant-parasitic nematodes: a high-throughput genomic approach. Genome Biol 4:R39

Serageldin I (1999) Biotechnology and food security in the 21st century. *Science* 285:387–389

Shurtleff MC, Averre CW (2000) Diagnosing plant diseases caused by nematodes. American Phytopatholog Soci Press, St Paul

Sikora RA, Bridge J, Starr JL (2005) Management practices: an overview of integrated nematode management technologies. In: Luc M, Sikora RA, Bridge J (eds) Plant-parasitic nematodes in subtropical and tropical agriculture. CAB International, Wallingford, pp 793–825

Slutsky M, Levin JL, Levy BS (1999) Azoospermia and oligospermia among a large cohort of DBCP applicators in 12 countries. Int J Occup Environ Health 5:116–122

Smant G, Stokkermans JP, Yan Y, de Boer JM, Baum TJ, Wang X, Hussey RS, Gommers FJ, Henrissat B, Davis EL, Helder J, Schots A, Bakker J (1998) Endogenous cellulases in animals: isolation of beta-1, 4-endoglucanase genes from two species of plant-parasitic cyst nematodes. Proc Natl Acad Sci USA 95:4906–4911

Soriano IR, Asenstorfer RE, Schmidt O, Riley IT (2004a) Inducible flavone in oats (*Avena sativa*) is a novel defense against plant-parasitic nematodes. Phytopathol 94:1207–1214

Soriano IR, Riley IT, Potter MJ, Bowers WS (2004b) Phytoecdysteroids: a novel defense against plant-parasitic nematodes. J Chem Ecol 30:1885–1899

Starr JL, Bridge J, Cook R (2002a) Resistance to plant-parasitic nematodes: history, current use and future potential. In: Starr JL, Cook R, Bridge J (eds) Plant resistance to parasitic nematodes. CAB International, Wallingford, pp 1–22

Starr JL, Cook R, Bridge J (2002b) Plant resistance to parasitic nematodes. CAB International, Wallingford

Stepek G, Behnke JM, Buttle DJ, Duce IR (2004) Natural plant cysteine proteinases as anthelmintics? Trends Parasitol 20:322–327

Stiekema WJ, Bosch D, Wilmink A, de Boer JM, Schouten A, Roosien J, Goverse A, Smant G, Stokkermans J, Gommers FJ, Schots A, Bakker J (1997) Towards plantibody-mediated resistance against nematodes. In: Fenoll C, Grundler FMW, Ohl SA (eds) Cellular and molecular aspects of plant–nematode interactions. Kluwer, Dordrecht, pp 262–271

Stoger E, Sack M, Fischer R, Christou P (2002) Plantibodies: applications, advantages, and bottlenecks. Curr Opin Biotechnol 13:161–166

Taylor AL (1978) Nematocides and nematicides – history *http://flnemifasufledu/HISTORY/nematicide_hishtm*

The *C. elegans* Sequencing Consortium (1998) Genome sequence of the nematode *C elegans*: A platform for investigating biology. Science 282:2012–2018

Thomas CM, Cottage A (2006) Genetic engineering for resistance. In: Perry RN, Moens M (eds) Plant nematology. CAB International, Wallingford, pp 255–272

Thurau T, Kifle S, Jung C, Cai D (2003) The promoter of the nematode resistance gene *Hs1pro-1* activates a nematode-responsive and feeding site specific gene expression in sugar beet (*Beta vulgaris* L) and *Arabidopsis thaliana*. Plant Mol Biol 52:643–660

Tilman D, Fargione J, Wolff B, D'Antonio C, Dobson A, Howarth R, Schindler D, Schlesinger WH, Simberloff D, Swackhamer D (2001) Forecasting agriculturally driven global environmental change. Science 292:281–284

Tomczak A, Koropacka K, Smant G, Goverse A, Bakker E (2008) Resistant plant responses. Plant Cell Monogr., doi:10.1007/7089_2008_39

Trudgill DL, Evans K, Phillips MS (1998) Potato cyst nematodes: damage mechanisms and tolerance in potato. In: Marks RJ, Brodie BB (eds) Potato cyst nematodes, biology, distribution, and control. CAB International, Wallingford, pp 117–133

Tytgat T, Vanholme B, De Meutter J, Claeys M, Couvreur M, Vanhoutte I, Gheysen G, Van Criekinge W, Borgonie G, Coomans A, Gheysen G (2004) A new class of ubiquitin extension proteins secreted by the dorsal pharyngeal gland in plant-parasitic cyst nematodes. Mol Plant Microbe Interact 17:846–852

Urwin PE, Atkinson HJ, Waller DA, McPherson MJ (1995) Engineered oryzacystatin-I expressed in transgenic hairy roots confers resistance to *Globodera pallida*. Plant J 8:121–131

Urwin PE, Lilley CJ, McPherson MJ, Atkinson HJ (1997a) Resistance to both cyst and root-knot nematodes conferred by transgenic Arabidopsis expressing a modified plant cystatin. Plant J 12:455–461

Urwin PE, Moller SG, Lilley CJ, McPherson MJ, Atkinson HJ (1997b) Continual green-fluorescent protein monitoring of cauliflower mosaic virus 35S promoter activity in nematode-induced feeding cells in *Arabidopsis thaliana*. Mol Plant Microbe Interact 10:394–400

Urwin PE, Lilley CJ, Atkinson HJ (2002) Ingestion of double-stranded RNA by preparasitic juvenile cyst nematodes leads to RNA interference. *Molec Plant-Microbe Interacts* 15:747–752

Vaghchhipawala ZE, Schlueter JA, Shoemaker RC, Mackenzie SA (2004) Soybean *FGAM* synthase promoters direct ectopic nematode feeding site activity. Genome 47:404–413

Valette C, Andary C, Geiger JP, Sarah JL, Nicole M (1998) Histochemical and cytochemical investigations of phenols in roots of banana infected by the burrowing nematode *Radopholus similis*. Phytopathol 88:1141–1148

van der Vossen EA, van der Voort JN, Kanyuka K, Bendahmane A, Sandbrink H, Baulcombe DC, Bakker J, Stiekema WJ, Klein-Lankhorst RM (2000) Homologues of a single resistance-gene cluster in potato confer resistance to distinct pathogens: a virus and a nematode. Plant J 23:567–576

Van Pouche K, Karimi M, Gheysen G (2001) Analysis of nematode-responsive promoters in sugar beet hairy roots. Meded Rijksuniv Gent Fak Landbouwkd Toegep Biol Wet 66:591–598

Vanholme B, De Meutter J, Tytgat T, Van Montagu M, Coomans A, Gheysen G (2004) Secretions of plant-parasitic nematodes: a molecular update. Gene 332:13–27

Vanholme B, Mitreva M, Van Criekinge W, Logghe M, Bird DMcK, McCarter JP, Gheysen G (2006) Detection of putative secreted proteins in the plant-parasitic nematode *Heterodera schachtii*. Parasitol Res 98:414–424

Veech JA, McClure MA (1977) Terpenoid aldehydes in cotton roots susceptible and resistant to the root-knot nematode, *Meloidogyne incognita*. J Nematol 9:225–229

Vishnudasan D, Tripathi MN, Rao U, Khurana P (2005) Assessment of nematode resistance in wheat transgenic plants expressing potato proteinase inhibitor (*PIN2*) gene. Transgenic Res 14:665–675

Vogel J, Raab TK, Schiff C, Somerville SC (2002) *PMR6*, a pectate lyase-like gene required for powdery mildew susceptibility in Arabidopsis. Plant Cell 14:2095–2106

Vos P, Simons G, Jesse T, Wijbrandi J, Heinen L, Hogers R, Frijters A, Groenendijk J, Diergaarde P, Reijans M, Fierens-Onstenk J, de Both M, Peleman J, Liharska T, Hontelez J, Zabeau M (1998) The tomato *Mi*-1 gene confers resistance to both root-knot nematodes and potato aphids. Nat Biotechnol 16:1365–1369

Wang X, Mitchum MG, Gao B, Li C, Diab H, Baum TJ, Hussey RS, Davis EL (2005) A parasitism gene from a plant-parasitic nematode with function similar to *CLAVATA3/ESR* (*CLE*) of *Arabidopsis thaliana*. Mol Plant Path 6:187–191

Waterhouse PM, Graham MW, Wang MB (1998) Virus resistance and gene silencing in plants can be induced by simultaneous expression of sense and antisense RNA. Proc Natl Acad Sci USA 95:13959–13964

Watts V (1947) The use of *Lycopersicon peruvianum* as a source of nematode resistance in tomatoes. Proc Am Soc Hort Sci 49:233

Weerasinghe RR, Bird DM, Allen NS (2005) Root-knot nematodes and bacterial Nod factors elicit common signal transduction events in *Lotus japonicus* root hair cells. Proc Natl Acad Sci USA 102:3147–3152

Wei J-Z, Hale K, Carta L, Platzer E, Wong C, Fang S-C, Aroian RV (2003) *Bacillus thuringiensis* crystal proteins that target nematodes. Proc Natl Acad Sci USA 100:2760–2765

Whitehead AG (1998) Plant nematode control. CABI International, New York

Williamson VM (1999) Plant nematode resistance genes. Curr Opin Plant Biol 2:327–331

Williamson VM, Hussey RS (1996) Nematode pathogenesis and resistance in plants. Plant Cell 8:1735–1745

Williamson VM, Kumar A (2006) Nematode resistance in plants: the battle underground. Trends Genet 22:396–403

Winter MD, McPherson MJ, Atkinson HJ (2002) Neuronal uptake of pesticides disrupts chemosensory cells of nematodes. Parasitol 125:561–565

Wyss U (1997) Root parasitic nematodes: an overview. In: Fenoll C, Grundler FMW, Ohl SA (eds) Cellular and molecular aspects of plant–nematode interactions. Kluwer, Dordrecht, pp 5–22

Yadav BC, Veluthambi K, Subramaniam K (2006) Host-generated double stranded RNA induces RNAi in plant-parasitic nematodes and protects the host from infection. Mol Biochem Parasitol 148:219–222

Yoon Y, Kim E, Hwang Y, Choi C (2004) Avermectin: biochemical and molecular basis of its biosynthesis and regulation. Appl Microbiol Biotechnol 63:626–634

Young ND, Mudge J (2002) Marker-assisted selection for soybean cyst nematode resistance. In: Starr JL, Cook R, Bridge J (eds) Plant resistance to parasitic nematodes. CAB International, Wallingford, pp 241–252

Zijlstra C, Blok VC, Phillips MS (1997) Natural resistance: the assessment of variation in virulence in biological and molecular terms. In: Fenoll C, Grundler FMW, Ohl SA (eds) Cellular and molecular aspects of plant–nematode interactions. Kluwer, Dordrecht, pp 153–166

Index