Developments in Plant Pathology

# Induced Resistance to Disease in Plants

Edited by

Raymond Hammerschmidt and Joseph Kuć

Springer-Science+Business Media, B.V.

INDUCED RESISTANCE TO DISEASE IN PLANTS

Developments in Plant Pathology

VOLUME 4

*The titles published in this series are listed at the end of this volume.*

# Induced Resistance to Disease in Plants

*Edited by*

RAYMOND HAMMERSCHMIDT
*Department of Botany and Plant Pathology,*
*Michigan State University,*
*East Lansing, Michigan, U.S.A.*

and

JOSEPH KUĆ
*Department of Plant Pathology,*
*University of Kentucky,*
*Lexington, Kentucky, U.S.A.*

Springer-Science+Business Media, B.V.

A C.I.P. Catalogue record for this book is available from the Library of Congress.

ISBN 978-90-481-4488-4 ISBN 978-94-015-8420-3 (eBook)
DOI 10.1007/978-94-015-8420-3

---

*Printed on acid-free paper*


Originally published by Kluwer Academic Publishers in 1995
Softcover reprint of the hardcover 1st edition 1995

## Table of Contents

## Preface

Induced or acquired resistance to disease in plants has been known for many years. However, until only about 10 years ago, this phenomenon has only been studied in a few laboratories around the world. Since the mid 1980's, there has been increasing interest in induced resistance as a new, environmentally safe means of disease control as well as a model for the study of genes involved in host defense and the signals that control these genes.

Because of the interest in induced resistance, we felt that the time was right to try to collect much of the current as well as older literature on this topic in one book. Although many reviews on this subject exist, none has covered or could attempt to cover this topic in a very broad fashion. To attain this goal, each of the authors was asked to cover their topic as comprehensively as possible so that each chapter would serve as a solid introduction to the literature. In addition, each author was also asked to present their own views on the state of research in their area and to address where future research might head. The book addresses the biology of induced resistance in four plant families that have received the most attention, the molecular basis of induced resistance, the genetic and evolutionary significance of this phenomenon, and the practical application of induced resistance in disease control.

Study of induced resistance is rapidly expanding. We hope that this book will provide background for those interested in beginning work in this area as well as serve as a source of information for established workers who wish to learn about other areas of induced resistance.

# INDUCED RESISTANCE IN LEGUMES

B.J. DEVERALL AND E.K. DANN
*Department of Crop Sciences*
*The University of Sydney NSW 2006*
*Australia*

## 1. Introduction

Induced resistance is taken to mean heightened resistance in a plant towards pathogens as a result of a previous treatment with a pathogen, an attenuated pathogen or a chemical that is not itself a pesticide. This review chapter is concerned with induced resistance in leguminous plants. It particularly seeks and evaluates evidence for induced resistance to fungal and bacterial pathogens, but makes occasional reference to induced resistance towards viruses and pests where this is related to its main theme. For more extensive coverage of induced resistance to viruses, see Fraser (1985) and Ponz and Bruening (1986). For coverage of induced resistance to pests, see Tallamy and Raupp (1991).

Induced resistance may be localized at the site of the inducing treatment or it may be systemic and thereby effective in all or some parts of the plant distant from the site of induction. Systemic induced resistance attracts the greatest interest from the viewpoint of understanding the underlying mechanisms and by providing potential for its use in new methods of crop protection. Use of systemic induced resistance as part of programs for integrated pest and disease management is an exciting prospect.

The Chapter starts by evaluating the evidence for the occurrence and underlying mechanisms in bean (*Phaseolus* spp.), where most has been done. It then assesses evidence in soybean, pea and other legumes. A great deal of recent research has been carried out in legumes on molecular bases of expression and elicitation of resistance to avirulent strains of pathogenic species, and, although not directed to explaining induced resistance, it is categorized here for its future contribution to understanding parts of the process of induced resistance. The Chapter ends by summarizing the extent of knowledge of the biology and physiology of the process of induced resistance in the legumes, and emphasizing the great deal that needs to be done.



*R. Hammerschmidt and J. Kuć (eds.), Induced Resistance to Disease in Plants,* 1–30.

# 2. Biological Evidence for Induced Resistance in Bean (*Phaseolus*)

## 2.1. LOCALIZED INDUCTION OF RESISTANCE

The first demonstration of localized induced resistance in bean was against *Colletotrichum lindemuthianum*, the cause of anthracnose disease (Rahe *et al.*, 1969). Using etiolated hypocotyls and sprayed conidial inocula, three plant pathogenic fungi that were not capable of causing disease in a particular bean cultivar were each effective in protecting that cultivar against a virulent strain of *C. lindemuthianum* applied 24 h later. Observations suggested that the protection was effective several cell distances from the sites of attempted infection by the first inoculum.

Similar findings were obtained by Skipp and Deverall (1973) using several different parts of light-grown bean plants. Droplet application of an avirulent strain of *C. lindemuthianum* prevented the development of a virulent strain when this was applied to the same site 1 to 3 days later. The protection was effective on leaves and hypocotyls and in the seed cavities on the insides of pods. Mixing conidia of the two strains in an infection droplet greatly diminished the virulence of the virulent strain. Observations by microscopy revealed that the two types of conidia germinated and gave rise to infections in host cells quite readily, discounting the possibility that they interfered directly with each other. Continued observations of this type suggested that the interference was through a changed metabolism of the plant cells and that responses to the avirulent strain caused cells some cell-widths away to become abnormally sensitive to the virulent strain so that a rapid resistance was brought about.

Following the observation that the interference was caused after interactions with plant cells it was found that the avirulent strain would also exert its effect when it was added one day after the virulent strain. By this means, it was possible to see that the virulent hyphae grew into host cells before the avirulent germ-tubes caused nearby cells to undergo hypersensitivity. Once the latter had happened, cells containing virulent hyphae also underwent hypersensitivity. This occurrence, even at a distance of several cells from the avirulent appressorium, suggested a change in the sensitivity of cells to virulent hyphae.

Further evidence for a change in the sensitivity of bean cells around those which had undergone hypersensitivity to avirulent germ-tubes came from the use of heat treatments. Four days after inoculation of hypocotyls with the avirulent race, they were given a heat shock of 50°C for 30 seconds. As a result, all neighboring cells became necrotic whereas those at a distance from the penetrated cells remained healthy. Thus the physiology of bean cells around hypersensitive cells had changed so that they had become abnormally sensitive to either the presence of virulent hyphae or to heat.

Localized induction of resistance in bean against the rust fungus *Uromyces appendiculatus* by an amino acid derivative has been reported (Tyihak *et al.*, 1989). Aqueous solutions of N-trimethyl-L-lysine (TML) at various concentrations were sprayed onto the undersurface of primary leaves of bean seedlings, and leaves were challenged 1, 6 or 8 days later by inoculation with a suspension of urediospores ($6 \times 10^4$ spores/ml)

of *U. appendiculatis*. Pustule densities were assessed ten days later and results varied enormously depending on the concentration of TML and the interval between induction and challenge. Maximum protection was observed as 36.5% of the infection density on control plants when $10^{-9}$ mol/l TML was applied 6 days before the challenge inoculation with *U. appendiculatus*. There was no protecting effect by the TML treatment when the interval between induction and challenge was only 1 day. Application of TML was shown not to inhibit germination of urediospores or formation of appressoria over stomata, suggesting that it acted through the physiology of the bean plant.

## 2.2 LOCALIZED INTERFERENCE BETWEEN RUST PATHOGENS

Yarwood (1954) showed that volatile emanations from rusted bean leaves inhibited germination of urediospores and impaired development of other rust infections. Yarwood (1956) compared the efficacy of urediospores with fungicides as protectants against rusts. Thus he showed that 1-3 mg dry weight of urediospores of bean rust per $dm^2$ of the leaf of the sunflower *Helianthus annuus* gave 50% control of uredial production of the sunflower rust *Puccinia helianthi*. Conversely, 4 mg of urediospores of sunflower rust gave similar control of bean rust. The effect was considered to be caused by self-inhibitors. However, unless self-inhibition is much stronger between urediospores of different species than between those of the same species, it is surprising that there was no self-limiting effect of increasing the dosage of a pathogenic rust alone to 10 $mg/dm^2$. This inoculum gave rise to so many uredia that it was impossible to count them.

Inoculations of bean leaves with a mixture of urediospores of avirulent and virulent strains of the bean rust fungus, *Uromyces appendiculatus*, resulted in changes in the development of the strains only where contrasting infection sites were less than 1 mm apart (Ye and Deverall, 1989). Under these circumstances, avirulent strains progressed a little further than usual inside the leaves whereas the virulent ones gave smaller than normal uredial pustules.

Observations by optical microscopy revealed that intercellular hyphae of virulent strains grew much less abundantly towards nearby infection sites occupied by avirulent strains. Interference between the two strains appeared to have occurred at a much later stage than germination of urediospores and ingress into the leaves. It appeared to have occurred after haustorial formation in mesophyll cells and therefore after metabolic interaction with the host cells. The reduced growth of the virulent strain may have been caused by competition between haustoria for cellular infection sites or by an induced resistance in the host, perhaps involving defensive products of cells affected by the avirulent strain.

## 2.3 MORE DISTANT INDUCTION OF RESISTANCE

Induction of resistance at a more distant site was first achieved in the bean/*C. lindemuthianum* system by Elliston *et al.*, (1971) using droplet inoculation on etiolated hypocotyls. Complete protection against a virulent strain was shown when a droplet of

conidia of an avirulent strain had been placed at a site 0.5 cm distant 18 or 36 hours earlier. Partial protection was achieved when the droplets were put down at the same time. Observations by microscopy showed that the virulent strain penetrated plant cells from appressoria and then grew as primary mycelia from cell to cell in the normal way before stopping and the infected cells then underwent an hypersensitive reaction.

A number of different species and strains of *Colletotrichum* were tested in the same type of experiment by Elliston *et al.*, (1976a), seeking capacities to cause localized and more remote protection against *C. lindemuthianum*. Two species failed to give localized protection and two others and a race of *C. lagenarium* did so in an inconsistent way. *C. trifolii* and two other races of *C. lagenarium* gave localized protection. Only these two races of *C. lagenarium* caused the more remote protection, increasing in their effectiveness with time elapsed from 0 to 24 hours between their placement and that of the pathogen.

A special feature of these experiments using *C. lagenarium* was that resistance could be induced in bean cultivars susceptible to all races of *C. lindemuthianum*. The presence of-strain-specific resistance genes was not needed for a cultivar to express induced resistance.

Induced resistance in cultivars lacking resistance genes was examined under microscopy of epidermal strips by Elliston *et al.*, (1976b). Protection caused by *C. lagenarium* from a site 0.5 cm distant 24 hours earlier became evident 84 to 96 hours after inoculation with *C. lindemuthianum,* when the latter stopped growing from an extensive primary mycelium and the contents of the invaded plant cells became granular and brown.

Using the same system to investigate localized and more remote protection, Elliston *et al.*, (1977a) applied a temperature of 37°C for 12 hours before inoculation. The heat treatment did not affect local protection where pathogen followed inducer on the same site by 24 hours. The heat treatment did prevent the more remote protection achieved over the same time period in controls where sites were separated by 0.5 cm. These results suggested that the more remote protection was based on a different mechanism than localized protection, which had been associated with phytoalexin accumulation in parallel work (Elliston *et al.*, 1977b).

## 2.4 SYSTEMIC INDUCTION OF RESISTANCE TO DISEASE

Induction of systemic resistance was first demonstrated in the bean/*C. lindemuthianum* system by Sutton (1979) using droplet inoculation on leaves of young plants. Substantial protection of the second leaves (first trifoliate) was achieved by inoculation of the first leaves (unifoliates) twelve days earlier. Symptom development at the protected site was much diminished and as few as one inoculum droplet on the first leaf was effective in achieving this. Preliminary tests also indicated the effectiveness of gently smearing conidial inoculum onto the first leaves in largely protecting the entire plants one week later from spray inoculation with the pathogen. Eleven of twelve-pre-treated plants

survived and grew on to flowering and pod formation whereas all twelve control plants died within a week.

Sutton (1982) reported the results of a limited field trial after first leaves of seedlings had been dipped in conidial suspensions. The seedlings were planted out twelve days later and sprayed to run-off with inoculum. Seven days later there was light disease compared with extensive damage on control plants. Five weeks later, there was greater survival accompanied by flowering and pod set on pre-treated plants than on controls.

Cloud and Deverall (1987) repeated the earlier experiments of Sutton (1979) confirming the effectiveness of droplet inoculation on the first leaves in greatly diminishing symptom development on the second leaves when these were inoculated a week later. Observations by microscopy of inoculated sites on second leaves showed that penetrations from appressoria were fewer on protected leaves. In some experiments, hyphal growth inside penetrated cells was seen to be less. At attempted penetration sites in protected leaves, appositions on the inside of the cell walls and encroachment of nuclei were observed indicating a number of cellular responses to infection.

Some protection of upper parts of seedlings was achieved also by inoculating the cotyledonary node and the hypocotyl with the pathogen, but not by inoculating cotyledons perhaps because this caused the rapid abscission of the cotyledons. Substantial protection was also brought about by injecting, into the first leaves, culture filtrates and dialysis retentates of these filtrates from the pathogen. The results were consistent with the idea that cellular derangement in the first leaf by the inducing treatments caused a signal to move through the plant heightening the activity of resistance mechanisms to challenge inoculation.

The most recent development on systemic induced resistance in the bean/*C. lindemuthianum* system comes from the availability from Ciba-Geigy AG of 2,6-dichloroisonicotinic acid (CGA-41396). This compound was reported to induce local and systemic resistance in cucumber against *C. lagenarium* and some other pathogens (Métraux *et al.*, 1990a) and in tobacco against a broad range of pathogens including a virus, two bacteria and three fungi (Ahl Goy *et al.*, 1990). In cucumber, it was shown to move systemically following injection into the first leaf and to distribute to the youngest leaf, growing points and roots of seedlings; it also caused major accumulations of proteins including chitinase and of chitinase mRNA in the injected and the second leaves (Métraux *et al.*, 1991).

Tests on the bean system showed that application of a formulation with 20 $\mu$g ml$^{-1}$ a.i. as a spray to first leaves not only protected the second leaves against *C. lindemuthianum*, but also against *Pseudomonas syringae* pv. *phaseolicola*, the cause of halo blight disease, and *Uromyces appendiculatus*, the cause of bean rust disease (Dann, 1991). Protection against *C. lindemuthianum* was similar at the three time intervals tested between application and challenge, 2, 4 and 7 days. Further work with similar treatments to first leaves of bean has shown that the third and fourth leaves are protected against *C. lindemuthianum* at 12 and 17 day intervals between inducing treatments and challenge respectively, and the third leaves are protected against the rust fungus at 12 days. Protection against halo blight disease was similar at the time intervals tested, 4 and 7

days. The amount of protection against *C. lindemuthianum* was similar to that obtained by treating the first leaves with the same pathogen, as in previous work. The active formulation had no effect on conidial germination and mycelial development of *C. lindemuthianum* and of the fungus *Botrytis fabae*, thus corroborating results of Métraux *et al.*, (1991) using *C. lagenarium* and *Pyricularia oryzae*.

Resistance can, therefore, be induced systemically in bean plants against several fungal and bacterial pathogens of leaves above the leaf that was treated with the inducing agent. It is clearly effective in seedlings under controlled environment conditions and there are indications of its effectiveness in older plants under field conditions. Whether it can be brought about by treating seeds or germinating seeds is unknown. Research has yet to be done in bean on induction of systemic resistance in roots or in the vascular system after treatment of seedling leaves or any other part of the plant.

The only inducing agents used to-date are the local lesion-causing pathogen *C. lindemuthianum*, uncharacterized macromolecular products of this pathogen and 2,6-dichloroisonicotinic acid. There is great potential for using systemic induced resistance in bean in crop protection but much further research and development is needed in order to realize the potential.

## 3. Failure to Reveal Systemic Induced Resistance to Spider Mites

Although concerning the reactions of plants with spider mites and not diseases, it is important to note that English-Loeb and Karban, (1991) obtained no clear evidence for systemic induced resistance in third leaves of bean plants to the two-spotted spider mite, *Tetranychus urticae*, following-mite-feeding injury to the first and second leaves. This negative result contrasted with the demonstration that feeding injury to cotton cotyledons caused later cotton leaves to be more resistant to the same species of mite (Karban and Carey, 1984).

In the bean experiments, the mites were present on the lower leaves at the same time as the assay was performed on the upper leaf, whereas, in the cotton experiments, induced resistance in the leaves was recorded 14 days after the feeding mites were removed from the cotyledons. Feeding injury to the bean leaves caused them to turn yellow and senesce prematurely, and English-Loeb and Karban (1991) suggested that the bean plants may have translocated nutrients to upper leaves making them more nutritious for mites, perhaps confounding any induction of resistance.

These experiments emphasize the need for comprehensive testing of changes in resistance throughout the development of a plant following potentially inducing treatments and warn about making generalized conclusions at this early stage of understanding the reactions of plants.

## 4. Mechanisms of Induced Resistance in Bean (*Phaseolus*)

### 4.1 THE ACTIVATION PROCESS AT THE INDUCING SITE

4.1.1 *Activation of Systemic Resistance.* Three types of treatment have been shown to induce systemic resistance in bean.

One is the use of virulent strains of the pathogenic fungus, *C. lindemuthianum*. These cause local lesions at the site of application and, therefore, considerable cellular and local tissue derangement.

The second is culture filtrates of one of these strains or macromolecular fractions of the filtrates prepared as dialysis retentates. The filtrates and the dialysis retentates also cause major cellular and tissue derangement in the injected leaves (Cloud and Deverall, 1987).

It is likely that decompartmentalization within cells or the general stress brought about by these treatments causes the release of endogenous activating agents in the leaves. As a consequence, it is envisaged that a signal moves into the petiole and up the stem. Whether the signal is the same as the hypothetical endogenous activating agent is unknown. Also unknown is the nature of the macromolecular component(s) in the dialysis retentates. One or several components from the pathogen may be involved in activation.

The third type of treatment is 2,6-dichloroisonicotinic acid, which does not appear to cause cellular or tissue derangement at the site of application. Presumably it moves rapidly and systemically in the plant as it does in cucumber (Métraux *et al.*, 1991). It may share the physical properties of salicylic acid suggested by Yalpani *et al.*, (1991), on the basis of modelling, to confer capacities for long-distance transport in the phloem. 2,6-dichloroisonicotinic acid is envisaged as an artificial signal molecule, not activating release of a plant signal from the point of application, but moving into the petiole and stem in the same way as the hypothetical plant signal activated by the other two treatments discussed.

4.1.2 *Activation of Localized Resistance.* Several types of treatment have been shown to induce localized resistance in bean. The most frequently used are avirulent strains of fungal pathogens of bean acting on resistant cultivars or fungal pathogens of other plant species that do not cause disease in bean. There are some indications that some of these treatments cause very local cellular derangement at the inducing site, visible as hypersensitivity, and therefore that they may act locally through similar mechanisms discussed above for activation of systemic resistance.

One series of experiments with local resistance brought about by avirulent strains of a pathogen against virulent strains of the same pathogen implicate some very special factors as the activating agents.

Specific factors causing localized induced resistance were revealed by Berard *et al.*, (1972) using infection droplets containing spores of *C. lindemuthianum* on the surfaces of bean hypocotyls. Droplets containing spores of an avirulent race were incubated for

60 hours, collected, sterilized by micro-filtration, concentrated and termed 'diffusates'. Diffusates were placed on surfaces of new hypocotyls of the same cultivar and then overspotted with spore suspensions of a virulent race 18 hours later. As a consequence, no lesions developed. The diffusates changed the usual course of the host-parasite interaction so that growth of the virulent race was stopped in the penetrated, normally susceptible, cells. Diffusates from infection drops containing virulent spores were ineffective. The specificity of the factor in the diffusates from incompatible interactions was shown by its effectiveness against virulent races only on the same cultivar from which it was derived.

A further refinement was introduced into this work by the use of a larger range of cultivars. It was then found that the protection factor was also effective on different cultivars provided that they possessed the same genes for resistance to two different races shown to produce two protection factors which were distinguished by their effects on cultivars carrying the genes separately (Berard *et al.*, 1973). The specific cross-protection factors were uncharacterized.

This intriguing work has not been followed up. Whether it has wider relevance to research and development on inducing resistance in bean and other legumes remains to be revealed.

## 4.2 THE ROLE OF PHYTOALEXINS

4.2.1 *Phytoalexins and Induced Resistance to Pathogens.* Phytoalexins are antimicrobial substances that are synthesized by plants in response to attempted infection. Many are known in the legumes.

Bailey and Deverall (1971) showed the rapid accumulation of the phytoalexin phaseollin to antifungal levels in bean cells expressing resistance to avirulent strains of *C. lindemuthianum*. Phaseollin was restricted to small areas of necrotic tissue at infected sites, as revealed by extractions of excised sections of stems, but its location inside necrotic cells and/or their immediate neighbors was not established. If the phaseollin was in the necrotic cells alone, its concentration six days after inoculation would have been more than 3000 $\mu$g/ml, which greatly exceeds the 10 $\mu$g/ml which prevented germ-tube growth in vitro. Phaseollin accumulated in the two to three day period after the first symptoms of cell death appeared and this coincided with the period when germ-tubes of the fungus were seen to slow in growth rate and to become restricted inside the necrotic cells (Skipp and Deverall, 1972).

Phaseollin was not the only phytoalexin to accumulate in hypersensitive tissue, Bailey (1974) having shown that at least three other active compounds form in substantial amounts at the same time. These additional bean phytoalexins were phaseollidin (Perrin, Whittle and Batterham, 1972), phaseollinisoflavan (Burden, Bailey and Dawson, 1972) and kievitone (Burden *et al.*, 1972; Smith *et al.*, 1973). Rahe (1973a, b) following earlier work by Rahe and Kuć (1970) and using heat treatments for different periods after inoculation also implicated major increases in phaseollin in resistance to avirulent strains.

Phaseollin and other phytoalexins also accumulated rapidly when bean cells expressed resistance to avirulent strains of the bean rust fungus, *U. appendiculatus* (Bailey and Ingham, 1971; Elnaghy and Heitefuss, 1976). These phytoalexins were also shown to accumulate after application of a glucan-rich elicitor obtained from cell walls of germ-tubes from the rust urediospores (Hoppe *et al.*, 1980). The elicitor preparation also induced resistance to the bean rust fungus in treated leaves.

In further experiments, first leaves of bean plants were vacuum-infiltrated with the partially purified and carbohydrate-rich preparation from germ-tube walls and then inoculated with the rust fungus 5 days later (Ebraham-Nesbat *et al.*, 1982). The fungus germinated and produced appressoria in a normal way but many sub-stomatal vesicles were destroyed in intercellular spaces of treated plants. Some intercellular hyphae grew, albeit in diminished amount, and appeared to be undamaged but failed to give rise to haustoria in the cells of treated leaves. The absence of haustoria was associated with the presence of deposits of electron-opaque material inside plant cell walls and opposite to the intercellular hyphae. No such deposits were observed in control plants where haustoria developed normally. It was suggested that the preparation from urediospores elicited phytoalexin formation and that this contributed to the deterioration of-sub-stomatal vesicles, and secondly that the preparation-affected fungus stimulated the depositions inside host cells. Prevention of rust development to the uredial stage, thus, was attributed to the combined effect of phytoalexin accumulation and impeded haustorial development.

A simple explanation of the types of change in host plants which increase their resistance would be the diffusion of anti-microbial compounds from the host tissues at the sites of inoculation with the protectant organism. However, there is no evidence that any of the known phytoalexins diffuse from sites of formation in live cells, except into neighboring dead cells (Hargreaves and Bailey, 1978; Hargreaves, 1979).

Systemic fungicides move for considerable distances in healthy plant tissue, and some phytoalexins may be able to move through the same routes taken by systemic fungicides, but this has not been shown. What is not possible at present is to give any evidence that the known examples of induced resistance are caused by the movement of phytoalexins between live cells, although diffusion from dead cells into nearby intercellular spaces affecting intercellular hyphae, such as of rust fungi, is conceivable.

4.2.2 *Phytoalexins and Induced Protection Against Pests.* A possible protective effect of phytoalexin accumulation was examined in bean leaves that had been coated with extracts of glyceollin from soybean (Fischer, *et al.*, 1990). Deterrence of two arthropod pests of legumes (the Mexican bean beetle, *Epilachna varivestis*, and the southern corn rootworm, *Diabrotica undecimpunctata howardi*) from feeding on the leaves increased with the concentration of glyceollin applied. Feeding was not deterred, however, in the bean leaf beetle *Certorma trifurcata*, even by very high concentrations of glyceollin. This beetle was considered to be better adapted than the other two pests to soybean and, thus, to have evolved greater tolerance of soybean defenses. The results indicate that a

phytoalexin can be an anti-feeding factor for some pests but do not prove a natural role for endogenous phytoalexins as induced factors for resistance to pests.

## 4.3 THE TRANSLOCATABLE SIGNAL FOR SYSTEMIC INDUCED RESISTANCE

Interpretation of systemic induced resistance in bean at present is that pathogenesis or injection of components of culture filtrates of *C. lindemuthianum* into lower parts of the seedling (Cloud and Deverall, 1987) causes local derangement of bean cells. This triggers the release of a signal molecule(s) that distributes throughout the upper parts of the seedling. As discussed earlier, 2,6-dichloroisonicotinic acid may be regarded as an artificial signal molecule. The signal molecules may activate cells in the upper parts to switch on defence mechanisms much more rapidly than they normally do in response to virulent strains of pathogens. If 2,6 dichloroisonicotinic acid acts in bean as it does in cucumber by causing the accumulation of chitinase and other proteins (Métraux *et al.*, 1991), then it predisposes the plant cells to resist pathogenesis before challenge infection. Whether the hypothetical natural signal molecules resulting from pathogenesis in the first leaf act in this way remains to be investigated.

It also remains for investigation whether salicylic acid increases in induced leaves, systemically protected leaves and in the phloem of bean plants as it does in tobacco reacting hypersensitively to tobacco mosaic virus (Malamy *et al.*, 1990; Yalpani *et al.*, 1991) and in cucumber reacting hypersensitively to tobacco necrosis virus or forming anthracnose lesions after *C. lagenarium* inoculation (Métraux *et al.*, 1990b). There is marked evidence for salicylic acid as an endogenous signal for induction of systemic induced resistance moving in the phloem and being closely associated with the accumulation of pathogenesis-related proteins in these two plants. Salicylic acid in bean expressing systemic induced resistance needs examination.

Application of 3 mM sodium salicylate to stem cuttings of the asparagus bean (*Vigna sequipedalis* Fruhw.) decreased the areas of local lesions caused by tobacco necrosis virus in primary leaves (Pennazio *et al.*, 1987). The salicylate also caused the accumulation in low concentrations of one protein that corresponded in electrophoretic behavior with one of several bands of pathogenesis-related proteins produced much more abundantly in response to the virus inoculation, a result similar to one reported for bean leaves (Redolfi and Cantisani, 1981).

## 4.4 THE ROLE OF CHITINASES AND GLUCANASES IN INDUCED RESISTANCE

Attention has been drawn to greatly enhanced chitinase and glucanase activities as a possible component of defence of plants against pathogens following numerous demonstrations of the existence of pathogenesis-related proteins (see review by Linthorst, 1991) and the discoveries that some of these proteins have chitinase and glucanase activities.

Some of the pathogenesis-related proteins in bean have been described (De Tapia *et al.*, 1986) and genes encoding for endochitinase in bean have been cloned (Broglie *et al.*,

1986). At least twelve genes encoding for pathogenesis-related proteins in bean cells have been distinguished by Walter *et al.*, (1990), who also deduced from elicitor-induced transcripts the existence of two novel and related proteins (PVPR1 and PVPR2). The cellular sites of action, function and significance of these two proteins in bean remains to be demonstrated.

Following the demonstrations of an exochitinase and a ß-1,3-glucanase in extracts from bean leaves (Abeles *et al.*, 1971), Boller *et al.*, (1983) showed that ethylene caused marked increases in exochitinase, an endochitinase and ß-1,3-glucanase activities in bean leaves. A purified preparation of the endochitinase liberated oligomers of chitin from isolated cell walls of the plant pathogenic fungus, *Fusarium solani* f. sp. *phaseoli*.

Immunogold staining of sections of ethylene-treated bean leaves viewed by transmission electron microscopy revealed that chitinase and ß-1,3-glucanase accumulated in vacuoles and also that a minor site of accumulation of ß-1,3-glucanase was the cell wall (Mauch and Staehelin, 1989). The accumulation of the enzymes in vacuoles was confirmed after isolation of organelles from leaves, and only ß-1,3-glucanase was found in fluids washed from intercellular spaces. It was suggested that the glucanase activity might affect the walls of fungal hyphae invading intercellular spaces but that the chitinase and most of the glucanase would be released to affect hyphae after rupture of protoplasts in hypersensitive responses to infection.

Infection of bean leaves from conidia of *C. lindemuthianum* caused marked increases in extractable ß-1,3-glucanase and exochitinase activities (Daugrois *et al.*, 1990). The increases occurred soon after infection by avirulent strains and were associated with the expression of resistance. Increases occurred later after infection by virulent strains and were associated with the disruption of leaf tissues in the formation of anthracnose lesions. The infection induced glucanase was a basic form of the enzyme with endo-action on laminarin (a ß-1,3-glucan) and cell walls of *Colletotrichum* only. The extracts would not have distinguished sites at and distant from infections in the leaves and no search was made for changes in the enzyme activities in younger parts of the bean plants.

Several unrelated investigations on these enzymes may have relevance in future work on mechanisms. A fungal elicitor caused rapid induction of mRNA for chitinase when applied to suspension cultures of bean cells (Hedrick *et al.*, 1988). Spraying the oligosaccharide, chitosan, onto first leaves of bean induced resistance seen as a reduction in numbers of local lesions in the second leaves when these were challenged with alfalfa mosaic virus, suggesting that it may have been acting through the general active defence systems of the plant (Pospieszny *et al.*, 1991). Chitinase and ß-1,3-glucanase activities increased greatly in bean leaves that had been sprayed with 0.2% mercuric chloride or inoculated with alfalfa mosaic virus (Awade *et al.*, 1989). The enzymes were shown to have stronger serological relationships to maize and tobacco enzymes than to other similar enzymes in bean. As discussed earlier, intercellular chitinase and extracellular ß-1,3-glucanase may play different roles in resistance. Endochitinases have lysozymal activity and are known to cause lysis of bacteria, as well as acting with endoglucanases to degrade fungal walls (Mauch *et al.*, 1988b).

It remains to be discovered whether 2,6-dichloroisonicotinic acid in bean causes any or all of these enzymes to become active throughout the plant as it does in cucumber (Métraux *et al.*, 1991). It also remains to be discovered whether resistance-inducing infections or fungal preparations cause natural signals to be released in bean that either enhance activities of these enzymes before infection or render remote cells more responsive in generating these activities upon challenge infection.

### 4.5 HYDROXYPROLINE-RICH GLYCOPROTEINS

Localized induced resistance in bean is associated with the accumulation of mRNAs specific for hydroxyproline-rich glycoprotein synthesis on cell walls (Showalter *et al.*, 1985), and enhanced activities of key enzymes involved in this synthesis (Bolwell *et al.*, 1985a, b).

## 5. Evidence for Induced Resistance and Underlying Mechanisms in Other Legumes

### 5.1 INDUCED RESISTANCE IN RED CLOVER

King, *et al.*, (1964) reported that infection of leaves of red clover, *Trifolium pratense*, by bean yellow mosaic virus affected their susceptibility to the powdery mildew fungus, *Erysiphe polygoni*. Virus infection altered metabolism of the leaves so that they underwent hypersensitivity to the normally compatible powdery mildew. The visible responses of the host cells were then the same as those reported by Smith (1938) for varieties of red clover genetically resistant to *E. polygoni*.

### 5.2 LOCALIZED INDUCED RESISTANCE IN SOYBEAN

5.2.1 *Evidence in Favor.* The hypocotyls of soybean seedlings were wound-inoculated with *Phytophthora cactorum* and thereby protected from the normal killing effect of *P. megasperma* when this was wound-introduced to a nearby site on the hypocotyls (Paxton and Chamberlain, 1967). This protection persisted for a 15 day period as shown when *P. megasperma* was introduced at any daily interval after *P. cactorum*, and it was associated with a persistently high level of phytoalexin concentration in the protected sites (Svoboda and Paxton,1972). A less complete form of protection was revealed using inoculation with an avirulent strain of *P. megasperma* against a virulent strain.

5.2.2 *Questioning Evidence.* Less convincing evidence for localized induced resistance in the soybean/ *P. megasperma* f. sp. *glycinea* system was obtained in experiments using zoospores as inocula applied to one unwounded site on etiolated hypocotyls (Ward, 1983). When mixtures of zoospores of two strains were applied, a high proportion of the avirulent strain (20:1) slowed the rate of lesion development by the virulent strain but

did not prevent it. A proportion that favored the avirulent strain by 2:1 did not slow the rate of lesion development by the virulent strain. Application of the avirulent strain several hours before the virulent strain prevented the development of the latter at some sites but not all, but even this prevention was overcome by increasing the proportion of zoospores of the virulent strain to less than parity. It was not possible to retard the development of the virulent strain at all by adding the avirulent strain after the virulent one. This study not only questioned the effectiveness of localized induction of resistance in this system but also provided some evidence that it was caused by direct interference between the strains and some evidence against a role for the phytoalexin glyceollin in restriction of lesion development.

5.2.3 *Possible Underlying Mechanisms.* Introduction of a glucan-rich elicitor obtained from walls of *P. megasperma* f. sp. *glycinea* into wounds on soybean hypocotyls 10 hours before inoculation with the pathogen markedly prevented disease development (Ayers *et al.*, 1976). Immersion of roots of soybean seedlings in solutions of the ß-1,3-glucan laminarin caused the accumulation of the phytoalexin glyceollin and of callose and also provided variable protection against a virulent strain of *P. megasperma* f. sp. *glycinea* (Bonhoff and Grisebach, 1988).

Ethylene applied exogenously to soybean hypocotyls increased the activity of host ß-1,3-endoglucanase (Yoshikawa *et al.*, 1990). Partial resistance, observed as a decrease in hyphal colonisation and associated with an increase in glyceollin levels, was conferred in hypocotyls against a compatible race of *P. megasperma* f. sp. *glycinea* when inoculated 24h after ethylene treatment. As there was no direct fungitoxic effect of the purified glucanase on the fungus, it was suggested to act indirectly by releasing soluble glucans from fungal walls as elicitors of glyceollin. Exogenously applied purified glucanase also resulted in resistance of soybean hypocotyls to *Pmg*.

The localization of phytoalexin accumulation to infection sites in soybean responding to an avirulent strain of *P. megasperma* f.sp. *glycinea* was shown with the aid of a radioimmunoassay for glyceollin I applied to fine sections of roots (Hahn *et al.*, 1985). These results confirm more precisely the fact that pterocarpanoid phytoalexins such as phaseollin and glyceollin are not widely distributed in plants where resistance has been induced.

The concentrations of the phytoalexin glyceollin and of the glucosides of the isoflavones, daidzein and genistein, were measured in the necrotic lesion and in the pale green border zone around sites of resistance to *P. megasperma* f. sp. *glycinea* in soybean leaves (Morris *et al.*, 1991). Glyceollin was the only one of the compounds to accumulate in the necrotic center but interestingly glyceollin and four glucosides of the isoflavones accumulated in the surrounding tissue. More remote parts of the infected leaves were not examined in this study, but Graham and Graham (1991) in confirming that glyceollin accumulated locally showed that the glucosides also accumulated in distal parts of soybean tissues remote from sites of elicitor application. No measurements appear to have been made in even more remote tissue, such as the next leaf.

## 5.3 APPARENT SYSTEMIC INDUCED RESISTANCE IN SOYBEAN

Injection of cotyledons of soybean seedlings with conidial suspensions of *C. truncatum* or *C. lagenarium* rendered the epicotyls much more resistant to lesion development from inoculations of *C. truncatum* made 24 to 96 hours later but not to coincident inoculation (Wrather and Elrod, 1990). Injection into the cotyledon with *C. truncatum* caused abscission 10 days later, compared with 28 days in controls, and permitted isolation of the pathogen from the cotyledonary node but not from elsewhere. Injection of heat-killed conidia of *C. lagenarium* into the cotyledons rendered the epicotyls even more resistant. A distance of 2.5 cm separated the points of injection and inoculation. This report is the first indication of systemic induced resistance to a pathogen in soybean, albeit over the rather short distances checked and in young seedlings.

Infestation of soybean cotyledons with small numbers of the two-spotted spider mite (*Tetranychus urticae*) caused apparent resistance of later developing leaves to the same mite (Brown *et al.*, 1991). The effect was shown to be on the fecundity of the second-added mites. The design of the experiment allowed the first mites to be retained on the cotyledons for 5 days before they were killed and then for the elapse of two weeks before female mites were introduced to the most recently expanded leaves. Fecundity of the second batch of mites was recorded after another two weeks. The sequence of events in this successful demonstration of systemic induced resistance to a pest in soybean was similar to that in the cotton experiments mentioned earlier and in contrast to that in the bean experiments discussed in the same section (English-Loeb and Karban, 1991). These results encourage further experimentation on systemic induced resistance in soybean.

## 5.4 INDICATIONS OF PROTECTION AND INDUCED RESISTANCE IN PEA

Application of chitosans to the endocarp of opened pea pods protected this tissue from infection by *Fusarium solani* f. sp. *pisi* (Kendra *et al.*, 1989). The protection remained for at least 3 days and was associated with an induced formation of phenylalanine ammonia lyase. Treatment of pea plants with a 0.1% solution of chitosan protected many of the plants from systemic infection by peanut stunt virus and alfalfa mosaic virus, in a manner suggesting action through activation of general defence mechanisms rather than affecting virus replication (Pospieszny *et al.*, 1991). Chitosan application, wounding and infection with *Fusarium solani* each caused increases in chitinase and ß-1,3-glucanase activities as apparent defence mechanisms in pea pods (Mauch *et al.*, 1988a). Chitinase and ß-1,3-glucanase extracted from infected pea pods and used in combination were shown to lyse hyphal tips of *Ascochyta pisi* and other fungi and thereby inhibit fungal growth (Mauch *et al.*, 1988b). Some protection of pea against *Pseudomonas syringae* pv. *pisi* and against multiplication of bacterial cells in leaves and stems was achieved when heat-killed cells were introduced along with live bacteria as inocula (Akpa and Archer, 1991).

Some evidence for a wound signal in pea plants was obtained by Davies and Schuster (1981) when they found rapid formation of polysomes (responsible for protein synthesis)

in stems and other tissues after wounding of the hypocotyl or epicotyl region. They concluded that a signal was transmitted to uninjured cells above and below the site of injury.

## 5.5 INDUCED RESISTANCE IN BROAD BEAN

Inoculation of the lowest two leaves of broad bean (*Vicia faba*) with urediospores of the rust fungus, *Uromyces viciae-fabae*, when plants also had two other fully developed leaves and two young developing leaves, caused these upper leaves to become resistant to challenge-inoculation with the same rust fungus 1, 3, 6 and 9 days later. The resistance was seen as diminished infected areas on the leaves and as fewer uredia per standard area for up to 29 days from challenge. The resistance was very high when 1 day separated the two inoculations but had disappeared when 12 days separated the two (Murray and Walters, 1992).

In further experiments with plants at the same stage and using the same isolate of the rust fungus, Walters and Murray (1992) found that treatment of the first two leaves with either 10 mM tripotassium phosphate or 5 mM EDTA in place of rust inoculation also caused significant increases in resistance of the upper leaves to challenge-inoculation with the rust fungus. The chemical treatments were effective 1 to 12 days before challenge-inoculation and the induced resistance was seen for 21 days after challenge.

The mechanism of chemical induction was suggested to be due to sequestration of calcium thereby affecting cell membranes and causing release of a signal for induction of systemic resistance. This suggestion received some support from additional experiments in which lower leaves were treated with 10 mM calcium nitrate 30 minutes after treatment with phosphate or EDTA. Systemic induced resistance did not occur under these conditions, whereas application of calcium nitrate alone had no effect.

## 5.6 LIMITED KNOWLEDGE OF INDUCED RESISTANCE IN OTHER LEGUMES

Research on the induction of resistance to pathogens as a result of earlier biological or chemical treatments has not been reported except for the experiments with red clover, soybean and pea described above. Understanding of the underlying mechanisms is much less advanced for these types of plants than it is for bean. Furthermore, experiments on systemic induced resistance to fungal and bacterial pathogens in legumes other than bean, and in one case in soybean, do not appear to have been reported.

In contrast to studies on inducing resistance to later attempted infections, a great deal of work has been done on the expression of resistance at the first site of attempted infection in many legumes. This is summarized below where it indicates insights into mechanisms that are likely to be important particularly for systemic induced resistance.

## 6. Additional Insights to Possible Mechanisms for Induced Resistance

A large amount of information has been obtained about metabolic pathways, key enzymes and activation of genes that are closely associated with the expression of resistance to pathogens in legumes. This has come from studies of resistant cultivars of plants inoculated with avirulent strains of pathogens and from work with a range of elicitors applied to these cultivars or to cells derived from them and cultured in liquid suspensions.

### 6.1 METABOLISM INVOLVED IN AROMATIC SYNTHESES

Knowledge of the biosynthetic pathway from shikimic acid to the pterocarpanoid phytoalexins and of the enzymes involved was reviewed by Ebel (1986). Advances in this knowledge continue. Metabolic steps and activation of enzymes and mRNAs in bean were subjects of papers by Edwards *et al.*, (1990), Mavandad *et al.*, (1990), Tepper *et al.*, (1989), Ellis *et al.*, (1989) and Blyden *et al.*, (1991). Parallel type of work in soybean has been done by Biggs *et al.*, (1990), Fischer *et al.*, (1990a, b), Kochs and Grisebach (1989) and Welle and Grisebach (1989, 1991) with respect to enzymes and by Akada *et al.*, (1990) and Wingender *et al.*, (1989) with respect to genes and their activation. A series of papers on alfalfa concern metabolites (Kessman *et al.*, 1990b), enzymes (Choudhary *et al.*, 1990b; Edwards and Dixon, 1991; Kessman *et al.*, 1990a; Paiva *et al.*, 1991) and gene expression (Choudhary *et al.*, 1990a; Dalkin *et al.*, 1990; Harrison *et al.*, 1991). Metabolic steps, enzymes and gene expression have also been studied in pea by Preisig *et al.*, (1990), Sweigard *et al.*, (1986), Sun *et al.*, (1991) and Chiang and Hadwiger (1990). Metabolism, enzymes and induction of mRNAs in chickpea have been reported by Mackenbrock and Barz (1991), Daniel *et al.*, (1990), Gunia *et al.*, (1991) and Daniel and Barz (1990).

In some cases, these studies deal with early steps in biosynthesis involving phenylalanine and cinnamic acid and are thus relevant also to lignin synthesis. A key enzyme involved in a later step in lignin synthesis has been studied as a defensive component of alfalfa by Gowri *et al.*, (1991).

Most of this recent work used elicitors applied to cells in suspension culture. Attempted infections of plant parts by the pathogens from which the elicitors were derived also caused similar rapid activations in resistant tissues (Bell *et al.*, 1984; Bonhoff *et al.*, 1986; Ryder *et al.*, 1984; Schmelzer *et al.*, 1984). The localization of these rapid responses related to resistance in infected tissues is shown by the work of Hahn *et al.*, (1985) using soybean and *P. megasperma* f. sp. *glycinea*. The results are all consistent with early, rapid and localized activation of key components of resistance when plant cells respond to elicitors and infection.

## 6.2 ELICITORS OF DEFENCE MECHANISMS

Elicitors from pathogens grown in culture include glucans from *P. megasperma* f. sp. *glycinea* (Ayers *et al.*, 1976) and carbohydrate-rich preparations from *C. lindemuthianum* (Tepper and Anderson, 1986). The elicitors are highly active and non-specific in their action, not only with respect to host cultivars but also to plant species, although some cultivar-selectivity has been reported for preparations from *C. lindemuthianum* (Tepper *et al.*, 1989).

Future research on elicitation of resistance in legumes will be aided by progress towards obtaining gene-specific elicitors from the bacterial pathogen of soybean, *P. syringae* pv. *glycinea*. This progress came from cloning avirulence genes of the bacterium, and particularly of the *avrD* gene from *P. syringae* pv. *tomato* which elicited resistance conferred by the *Rpg4* gene in soybean (Keen and Buzzell, 1991). The *avrD* gene was expressed in both *Escherichia coli* and *P. syringae* pv. *glycinea* causing expression of resistance in soybean carrying the *Rpg4* gene. The gene also caused *E. coli* to release an elicitor into culture media *in vitro* and the elicitor was shown to be active specifically on soybean carrying the *Rpg4* gene (Keen *et al.*, 1990). The uncharacterized elicitor of low molecular weight caused symptoms characteristic of resistance expression and also accumulation of the phytoalexin glyceollin. The elicitor, apparently specific for the complementary avirulence and resistance genes in pathogen and host, seems more certainly to be implicated *in planta* during pathogen-host interactions than the earlier known-non-specific elicitors. Its use will permit assessment of the involvement of components implicated as being involved in expression of local resistance in soybean.

## 6.3 MODIFICATION OF PLANT METABOLISM

Ways in which inoculations with pathogens or applications of chemicals can modify plant metabolism, indirectly or directly affecting resistance mechanisms, have been shown by several experiments with legumes. Application of such treatments to soybean caused rapid localized changes in abscisic acid (Cahill and Ward, 1989) and it was argued that these changes were likely to have diverse and extensive effects on more remote parts of the plant, causing changes in responses to subsequent attempted infections. Application of ethylene to soybean hypocotyls increased ß-1,3-endoglucanase activities (Yoshikawa *et al.*, 1990), thus enhancing the potential to release glucans from walls of invading fungi. The glucans, acting as elicitors of phytoalexin synthesis, were argued to be the cause of enhanced glyceollin levels and greater resistance of hypocotyls during infection by *P. megasperma* f. sp. *glycinea*.

Indoleacetic acid and 2,4-D augmented the action of elicitors from *C. lindemuthianum* on phytoalexin synthesis in bean leaves (Hughes and Dickerson, 1990) and ethylene also enhanced this eliciting activity on chitinase and ß-1,3-glucanase under some circumstances (Hughes and Dickerson, 1991). Glutathione caused a major induction of transcription of genes encoding for a variety of defence-related responses in suspension

cultures of cells of bean (Wingate *et al.*, 1988). Heat-killed and UV-killed cells of both virulent and avirulent strains of *P. syringae* pv. *phaseolicola* caused increased synthesis of chitinase in bean, whereas live cells of only the avirulent strain did this rapidly after inoculation (Voisey and Slusarenko, 1989). Several elicitors caused the expression of four chitinase genes in cells of peanut (*Arachis hypogaea*) in suspension culture but only cell wall components of *P. megasperma* f. sp. *glycinea* and not other elicitors activated one of the genes (Herget *et al.*, 1990). The results indicated that this gene might be controlled by a pathogen-specific promoter. Application of chitosan-derivatives to seeds of several plants including the legumes, milk-vetch (*Astragalus sinicus*) and soybean, enhanced chitinase activities in the seeds and resulting seedlings as part of a possible defence mechanism against future fungal attack (Hirano *et al.*, 1990).

Although the role of proteinase inhibitors in systemic resistance to pathogens is unknown, it has been shown that pectic oligomers induce accumulation of proteinase inhibitors in alfalfa plants (Ryan *et al.*, 1985).

Retention of leaves or cotyledons rendered hypocotyls of bean less susceptible to lesion development by *C. lindemuthianum* than where these organs had been removed (Dunn *et al.*, 1990). It was found that abscisic acid concentrations were much higher in the more resistant hypocotyls. It was also shown that application of abscisic acid to hypocotyls increased their resistance somewhat and that application of fluridone (an inhibitor of abscisic acid synthesis) decreased their resistance. The degree of resistance was considered to be related to abscisic-acid-induced changes in the physiology of the host tissues.

### 6.4 WOUND RESPONSES

Responses of legumes to mechanical wounding have also been reported. In peanut the phytoalexins resveratrol and arachidins III and IV accumulated in cotyledons in response to being cut aseptically into thin slices (1-2 mm) and incubated for 48 h and 25°C in the dark (Arora and Strange, 1991). Polyribosome formation (responsible for protein synthesis) increased in soybean hypocotyls after excision, and in pea tissue following excision, abrasion or puncture (Davies and Schuster, 1981). It was suggested that wounded cells generated a signal which can be rapidly transported both acropetally and basipetally. Although the signal was not identified, several possibilities were proposed such as ethylene or other hormones, protease-inhibitor inducing factor and an action (variation) potential transmitted via membranes. For reviews on signals being transmitted in plants as action potentials, see Pickard (1973) and Chessin and Zipf (1990).

## 7. Remaining Biological Questions and Problems

Past research has shown that resistance can be induced systemically in bean by biological and chemical means. It is clearly effective in seedling plants under controlled environmental conditions. Further work is needed to confirm its effectiveness against

a range of foliar pathogens and to assess its action against root-infecting and vascular pathogens. The duration of protection conferred by a single induction or repeated inductions needs to be determined. In doing so, induction caused by biological and chemical treatments should be compared. A biological treatment is likely to involve interaction between a pathogen or an attenuated pathogen and the host tissue, and, therefore, to act over a period of time and to initiate transmission of signals over this period. A chemical treatment could be the application of an artificial signal, such as 2,6-dichloroisonicotinic acid, that might have a transient effect in the plant, or it could be the application of activating molecules, paralleling the component of culture filtrates of pathogens, that might have protracted effects setting-off endogenous signals over a period.

The durability of systemic induced resistance in bean needs to be tested under the environmental stresses that might be experienced under natural conditions. The ultimate tests of effectiveness will come in many field trials that take account of seasonal variations, a wide incidence of pest and pathogen attacks and natural stresses. The optimum stage of growth of plants for application of inducing agents needs to be worked out in relation to the objective of producing high-yielding bean plants and pods.

Localized induced resistance can be achieved in soybean but there is a clear need for more experiments to test for systemic induced resistance in soybean. There is a similar need for experiments on induction of systemic resistance in pea, on which rather little has been done about localized induced resistance. There is a need also for experiments on inducing resistance to pathogens in the remaining legumes, on which nothing appears to have been done on localized or systemic induced resistance. All of the types of tests specified for bean would be needed to follow up successful demonstrations in these other legumes.

Because of the absence of relevant data, it is impossible to state that resistance can be induced systemically in all or most legumes. In the light of experience with bean reported in this Chapter and with other plant families reported in other Chapters, it would be surprising if systemic induced resistance can not be revealed as a phenomenon widely in the family. If well-conducted experiments show that the phenomenon does not occur, a major contribution would have been made to plant science. It would counter the reasonable hypothesis that systemic induced resistance is a universal process in the plant kingdom and one that can be used to advantage in plant production and protection.

## 8. Outstanding Questions on Mechanisms and Their Manipulation

Research on underlying mechanisms of induced resistance in plants is not advanced greatly as yet by work on the legumes. Interpretation of a number of incidental experiments and observations is consistent with the following hypothesis.

(1) Attempted infection or injection of certain macromolecular products of fungi that leads to localized change in plant cells causes these cells to release signals to other parts of the plant.

(2) Movement of endogenous signals or of an artificial signal, such as 2,6-dichloroisonicotinic acid, changes metabolism in more distant parts of the plant.

(3) These signals cause the synthesis of defensive agents, such as chitinases and glucanases, to ward-off attempted infections.

(4) These signals may also predispose cells to react to attempted infection more rapidly than would normally be the case. The reaction involves general defence mechanisms of plants including pathogenesis-related proteins, aromatic biosyntheses leading to phytoalexins and lignin and formation of structural barriers, such as cell-wall features including papillae.

(5) The signals thereby systemically activate the general defence mechanisms of plants that are normally activated locally when the well-known recognitional genes (the single genes for resistance) are triggered by avirulent strains of pathogens (through products of the complementary resistance and avirulence genes).

Future work will test this hypothesis. This work will be aided by the substantial amount of research that has been done on legumes, particularly on bean and soybean, on the local responses of cells to elicitors and to attempted infection by avirulent strains of pathogens and also by recent research on the ways in which avirulence genes and their products elicit defence mechanisms.

It is essential to carry out research on underlying mechanisms of systemic induced resistance in parallel with attempts to use this resistance in new procedures in crop protection. The information and understanding gained from the basic research will be needed to provide public confidence and to permit registration of novel methods of protection based on inducing systemic resistance.

## Acknowledgments

Mrs V.F. Moschione is thanked for her work in preparing the text on a word-processor.

## References

Abeles, F.B., Bosshart, R.P., Forrence, L.E. and Habig, W.H. (1971) 'Preparation and purification of glucanase and chitinase from bean leaves', Plant Physiology 47, 129-134.

Ahl Goy, P., Métraux, J.P., Speich, J. and Staub, Th. (1990) 'Derivatives of isonicotinic acid induce resistance to viruses, bacteria and fungi in tobacco', Proceedings of the 5th International Symposium on the Molecular Genetics of Plant-Microbe Interactions, Interlaken, Switzerland, p. 366.

Akada, S., Kung, S.D. and Dube, S.K. (1990) 'Nucleotide sequence of one member of soybean chalcone synthase multi-gene family', Nucleic Acids Research 18, 3398.

Akpa, A.D. and Archer, S.A. (1991) 'Effect of heat-killed bacteria on the interaction of pea with *Pseudomonas syringae* pv. *pisi*', Phytopathology 132, 237-244.

Arora, M.K. and Strange, R.N. (1991) 'Phytoalexin accumulation in groundnuts in response to wounding', Plant Science 78, 157-163.

Awade, A., De Tapia, M., Didierjean, L. and Burkard, G. (1989) 'Biological function of bean pathogenesis-related (PR3 and PR4) proteins', Plant Science 63, 121-130.

Ayers, A.R., Ebel, J., Valent, B. and Albersheim, P. (1976) 'Host-pathogen interactions. X. Fractionation and biological activity of an elicitor isolated from the mycelial walls of *Phytophthora megasperma* f. sp. *sojae'*, Plant Physiology 57, 760-765.

Bailey, J.A. and Deverall, B.J. (1971) 'Formation and activity of phaseollin in the interaction between bean hypocotyls (*Phaseolus vulgaris*) and the physiological races of *Colletotrichum lindemuthianum*, Physiological Plant Pathology 1, 435-449.

Bailey, J.A. and Ingham, J.L. (1971) 'Phaseollin accumulation in bean (*Phaseolus vulgaris*) in response to infection by tobacco necrosis virus and the rust *Uromyces appendiculatus*', Physiological Plant Pathology 1, 415-456.

Bailey, J.A. (1974) 'The relationship between symptom expression and the phytoalexin concentration in hypocotyls of *Phaseolus vulgaris* infected with *Colletotrichum lindemuthianum*, Physiological Plant Pathology 4, 477-488.

Bell, J.N., Dixon, R.A., Bailey, J.A., Rowell, P.M. and Lamb, C.J. (1984) 'Differential induction of chalcone synthase mRNA at the onset of phytoalexin accumulation in compatible and incompatible interactions', Proceedings of the National Academy of Science U.S.A. 81, 3384-3388.

Berard, D.F., Kuć, J. and Williams, E.B. (1972) 'A cultivar specific protection factor from incompatible interactions of green bean with *Colletotrichum lindemuthianum*, Physiological Plant Pathology 2, 123-127.

Berard, D.F., Kuć, J. and Williams, E.B. (1973) 'Relationship of genes for resistance to protection by diffusates from incompatible interactions of *Phaseolus vulgaris* with *Colletotrichum lindemuthianum*, Physiological Plant Pathology 3, 51-56.

Biggs, D.R., Welle, R. and Grisebach, H. (1990) 'Intracellular localization of prenyltransferases of isoflavonoid phytoalexin biosynthesis in bean and soybean', Planta 181, 244-248.

Blyden, E.R., Doerner, P.W., Lamb, C.J. and Dixon, R.A. (1991) 'Sequence analysis of a chalcone isomerase cDNA of *Phaseolus vulgaris* L.', Plant Molecular Biology 16, 167-169.

Boller, T., Gehri, A., Mauch, F. and Vogeli, U. (1983) 'Chitinase in bean leaves: induction by ethylene, purification, properties, and possible function', Planta 157, 22-31.

Bolwell, G.P., Robbins, M.P. and Dixon, R.A. (1985a) 'Metabolic changes in elicitor-treated bean cells. Enzymic responses associated with rapid changes in cell wall components', European Journal of Biochemistry 148, 571-578.

Bolwell, G.P., Robbins, M.P. and Dixon, R.A. (1985b) 'Elicitor-induced prolyl hydroxylase from French bean (*Phaseolus vulgaris*). Localization, purification and properties', Biochemical Journal 229, 693-699.

Bonhoff, A., Loyal, R., Ebel, J. and Grisebach, H. (1986) 'Race:cultivar-specific induction of enzymes related to phytoalexin biosynthesis in soybean roots following infection with *Phytophthora megasperma* f. sp. *glycinea*', Archives of Biochemistry and Biophysics 246, 149-154.

Bonhoff, A. and Grisebach, H. (1988) 'Elicitor-induced accumulation of glyceollin and callose in soybean roots and localized resistance against *Phytophthora megasperma* f. sp. *glycinea*', Plant Science 54, 203-209.

Broglie, K.E., Gaynor, J.J. and Broglie, R.M. (1986) 'Ethylene-regulated gene expression: molecular cloning of the genes encoding an endochitinase from *Phaseolus vulgaris*', Proceedings of the National Academy of Science of the U.S.A., 83, 6820-6824.

Brown, G.C., Nurdin, F., Rodriguez, J.G. and Hildebrand, D.F. (1991) 'Inducible resistance of soybean (var 'Williams') to two-spotted spider mite (*Tetranychus urticae* Koch)', Journal of the Kansas Entomological Society 64, 388-393.

Burden, R.S., Bailey, J.A. and Dawson, G.W. (1972) 'Structures of three new isoflavonoids from *Phaseolus vulgaris* infected with tobacco necrosis virus', Tetrahedron Letters 1972, 4175-4178.

Cahill, D.M. and Ward, E.W.B. (1989) 'Rapid localized changes in abscisic acid concentrations in soybean in interactions with *Phytophthora megasperma* f.sp. *glycinea* or after treatment with elicitors', Physiological and Molecular Plant Pathology 35, 483-493.

Chessin, M. and Zipf, A.E. (1990) 'Alarm systems in higher plants', The Botanical Review 56, 193-235.

Chiang, C.C. and Hadwiger, L.A. (1990) 'Cloning and characterization of a disease resistance response gene in pea inducible by *Fusarium solani*', Molecular Plant-Microbe Interactions 3, 78-85.

Choudhary, A.D., Kessmann, H., Lamb, C.J. and Dixon, R.A. (1990a) 'Stress responses in alfalfa (*Medicago sativa* L). 4. Expression of defense gene constructs in electroporated suspension cell protoplasts', Plant Cell Reports 9, 42-46.

Choudhary, A.D., Lamb, C.J. and Dixon, R.A. (1990b) 'Stress responses in alfalfa (*Medicago sativa* L). 6. Differential responsiveness of chalcone synthase induction to fungal elicitor or glutathione in electroporated protoplasts', Plant Physiology 94, 1802-1807.

Cloud, A.M.E. and Deverall, B.J. (1987) 'Induction and expression of systemic resistance to the anthracnose disease in bean', Plant Pathology 36, 551-557.

Dalkin, K., Jorrin, J. and Dixon, R.A. (1990) 'Stress responses in alfalfa (*Medicago sativa* L). 7. Induction of defence related messenger RNAs in elicitor-treated cell suspension cultures', Physiological and Molecular Plant Pathology 37, 293-307.

Daniel, S. and Barz, W. (1990) 'Elicitor-induced metabolic changes in cell cultures of chickpea (*Cicer arietinum* L) cultivars resistant and susceptible to *Ascochyta rabiei*.

2. Differential induction of chalcone-synthase-messenger RNA activity and analysis of *in vitro*-translated protein patterns', Planta 182, 279-286.

Daniel, S., Tiemann, K., Wittkampf, U., Bless, W., Hinderer, W. and Barz, W. (1990) 'Elicitor-induced metabolic changes in cell cultures of chickpea (*Cicer arietinum* L) cultivars resistant and susceptible to *Ascochyta rabiei*. 1. Investigations of enzyme activities involved in isoflavone and pterocarpan phytoalexin biosynthesis', Planta 182, 270-278.

Dann, E.K. (1991) 'Studies in inducing systemic resistance in beans and broad beans by biological and chemical means', BScAgr thesis, University of Sydney.

Daugrois, J.H., Lafitte, C., Barthe, J.P. and Touze, A. (1990) 'Induction of ß-1,3-glucanase and chitinase activity in compatible and incompatible interactions between *Colletotrichum lindemuthianum* and bean cultivars', Journal of Phytopathology 130, 225-234.

Davies, E. and Schuster, A. (1981) 'Intercellular communication in plants: Evidence for a rapidly generated, bidirectionally transmitted wound signal', Proceedings of the National Academy of Science U.S.A. 78, 2422-2426.

De Tapia, M., Bergmann, V., Awade, A. and Burkar, D.G. (1986) 'Analysis of acid extractable bean leaf proteins induced by mercuric chloride treatment and alfalfa mosaic virus infection. Partial purification and characterization', Plant Science 45, 167-177.

Dunn, R.M., Hedden, P. and Bailey, J.A. (1990) 'A physiologically-induced resistance of *Phaseolus vulgaris* to a compatible race of *Colletotrichum lindemuthianum* is associated with increases in ABA content', Physiological and Molecular Plant Pathology 36, 339-349.

Ebel, J. (1986) 'Phytoalexin synthesis: the biochemical analysis of the induction process', Annual Review of Phytopathology 24, 235-264.

Ebraham-Nesbat, F., Hoppe, H.H. and Heitefuss, R. (1982) 'Ultrastructural studies on the development of *Uromyces phaseoli* in bean leaves protected by elicitors of phytoalexin accumulation', Phytopathologische Zeitschrift 103, 261-271.

Edwards, R., Mavandad, M. and Dixon, R.A. (1990) 'Metabolic fate of cinnamic acid in elicitor treated cell suspension cultures of *Phaseolus vulgaris*', Phytochemistry 29, 1867-1873.

Edwards, R. and Dixon, R.A. (1991) 'Stress responses in alfalfa (*Medicago sativa* L). 9. Isoflavone O-methyltransferase activities in elicitor-treated cell suspension cultures of *Medicago sativa*', Phytochemistry 30, 2597-2606.

Ellis, J.S., Jennings, A.C., Edwards, L.A., Mavandad, M., Lamb, C.J. and Dixon, R.A. (1989) 'Defence gene expression in elicitor-treated cell suspension cultures of french bean cv. imuna', Plant Cell Reports 8, 504-507.

Elliston, J., Kuć, J. and Williams, E.B. (1971) 'Induced resistance to bean anthracnose at a distance from the site of the inducing interaction', Phytopathology 61, 1110-1112.

Elliston, J., Kuć, J. and Williams, E.B. (1976a) 'Protection of *Phaseolus vulgaris* against anthracnose by *Colletotrichum* species nonpathogenic to bean', Phytopathologische Zeitschrift 86, 117-126.

Elliston, J., Kuć, J. and Williams, E.B. (1976b) 'A comparative study of the development of compatible, incompatible, and induced incompatible interactions between *Colletotrichum* spp. and *Phaseolus vulgaris*', Phytopathologische Zeitschrift 87, 289-303.

Elliston, J., Kuć, J. and Williams, E.B. (1977a) 'Effect of heat treatment on the resistance of *Phaseolus vulgaris* to *Colletotrichum lindemuthianum* and *Colletotrichum lagenarium*', Phytopathologische Zeitschrift 88, 43-52.

Elliston, J., Kuć, J., Williams, E.B. and Rahe, J.E. (1977b) 'Relationship of phytoalexin accumulation to local and systemic protection of bean against anthracnose', Phytopathologische Zeitschrift 88, 114-130.

Elnaghy, M.A. and Heitefuss, R. (1976) 'Permeability changes and production of antifungal compounds in *Phaseolus vulgaris* infected with *Uromyces phaseoli*. II. Role of phytoalexins', Physiological Plant Pathology 8, 269-277.

English-Loeb, G.M. and Karban, R. (1991) 'Consequences of mite feeding injury to beans on the fecundity and survivorship of the two-spotted mite (Acari: Tetranychidae)', Experimental and Applied Acarology 11, 125-136.

Fischer, D., Ebenaujehle, C. and Grisebach, H. (1990a) 'Phytoalexin synthesis in soybean- purification and characterisation of NADPH-2'-hydroxydaidzein oxixoreductase from-elicitor-challenged soybean cell cultures', Archives of Biochemistry and Biophysics 276, 390-395.

Fischer, D., Ebenaujehle, C. and Grisebach, H. (1990b) 'Purification and characterization of pterocarpan synthase from elicitor-challenged soybean cell cultures', Phytochemistry 29, 2879-2882.

Fischer, D.C., Kogan, M. and Paxton, J. (1990) 'Effect of glyceollin, a soybean phytoalexin, on feeding by three phytophagous beetles (Coleoptera: Coccinellidae and Chrysomelidae): Dose versus response', Environmental Entomology 19, 1278-1282.

Fraser, R.S.S. (1985) 'Mechanisms of induced resistance to virus disease', in R.S.S. Fraser (ed.), Mechanisms of Resistance to Plant Diseases, Martinus Nijhoff/Dr W. Junk Publishers, Dordrecht, pp. 373-404.

Gowri, G., Bugos, R.C., Campbell, W.H., Maxwell, C.A. and Dixon, R.A. (1991) 'Stress responses in alfalfa (*Medicago sativa* L). 10. Molecular cloning and expression of S-adenosyl-L-methionine-caffeic acid 3-O-methyltransferase, a key enzyme of lignin biosynthesis', Plant Physiology 97, 7-14.

Graham, T.L. and Graham, M.Y. (1991) 'Glyceollin elicitors induce major but distinctly different shifts in isoflavonoid metabolism in proximal and distal soybean cell populations', Molecular Plant-Microbe Interactions 4, 60-68.

Gunia, W., Hinderer, W., Wittkampf, U. and Barz, W. (1991) 'Elicitor induction of cytochrome-P-450 monooxygenases in cell suspension cultures of chickpea (*Cicer arietinum* L) and their involvement in pterocarpan phytoalexin biosynthesis', Zeitschrift fur Naturforschung C 46, 58-66.

Hahn, M.G., Bonhoff, A. and Grisebach,H. (1985) 'Quantitative localization of the phytoalexin glyceollin I in relation to fungal hyphae in soybean roots infected with *Phytophthora megasperma* f.sp. *glycinea*', Plant Physiology 77, 591-601.

Hargreaves, J.A. and Bailey, J.A. (1978) 'Phytoalexin production by hypocotyls of *Phaseolus vulgaris* in response to constitutive metabolites released by damaged bean cells', Physiological Plant Pathology 13, 89-100.

Hargreaves, J.A. (1979) 'Investigations into the mechanism of mercuric chloride stimulated phytoalexin accumulation in *Phaseolus vulgaris* and *Pisum sativum*', Physiological Plant Pathology 15, 279-287.

Harrison, M.J., Choudhary, A.D., Dubery, I., Lamb, C.J. and Dixon, R.A. (1991) 'Stress responses in alfalfa (*Medicago sativa* L). 8. Cis-elements and transacting factors for the quantitative expression of a bean chalcone synthase gene promoter in electroporated alfalfa protoplasts', Plant Molecular Biology 16, 877-890.

Hedrick, S.A., Bell, J.N., Boller, T. and Lamb, C.J. (1988) 'Chitinase cDNA cloning and mRNA induction by fungal elicitor, wounding and infection', Plant Physiology 86, 182-186.

Herget, T., Schell, J. and Schreier, P.H. (1990). 'Elicitor-specific induction of one member of the chitinase gene family in *Arachis hypogaea*', Molecular and General Genetics 224,-469-476.

Hirano, S., Yamamoto, T., Hayashi, M., Nichida, T. and Inui, H. (1990) 'Chitinase activity in seeds coated with chitosan derivatives', Agricultural and Biological Chemistry 54, 2719-2720.

Hoppe, H.H., Hümme, B. and Heitefuss, R. (1980) 'Elicitor induced accumulation of phytoalexins in healthy and rust infected leaves of *Phaseolus vulgaris*', Phytopathologische Zeitschrift 97, 85-88.

Hughes, R.K. and Dickerson, A.G. (1990) 'Auxin regulation of the response of *Phaseolus vulgaris* to a fungal elicitor', Plant Cell Physiology 31, 667-675.

Hughes, R.K. and Dickerson, A.G. (1991) 'Modulation of elicitor-induced chitinase and ß-1,3-glucanase activity by hormones in *Phaseolus vulgaris*', Plant and Cell Physiology 32, 853-861.

Hümme, B., Hoppe, H.H. and Heitefuss, R. (1981) 'Glucane aus Zellwänden der Uredosporenkeimschlaüche von *Uromyces phaseoli* als Elicitoren der Phytoalexinanreicherung in *Phaseolus vulgaris*', Phytopathologische Zeitschrift 101, 51-64.

Karban, R. and Carey, J.R. (1984) 'Induced resistance of cotton seedlings to mites', Science 225, 53-54.

Keen, N.T., Tamaki, S., Kobayashi, D., Gerhold, D., Stayton, M., Shen, H., Gold, S., Lorang, J., Thordal-Christensen, H., Dahlbeck, D. and Staskawicz, B. (1990) 'Bacteria expressing avirulence gene D produce a specific elicitor of the soybean hypersensitive reaction', Molecular Plant-Microbe Interactions 3, 112-121.

Keen, N.T. and Buzzell, R.I. (1991) 'New disease resistance genes in soybean against *Pseudomonas syringae* pv. *glycinea*: evidence that one of them interacts with a bacterial elicitor', Theoretical and Applied Genetics 81, 133-138.

Kendra, D.F., Christian, D. and Hadwiger, L.A. (1989) 'Chitosan oligomers from *Fusarium solani*/pea interactions, chitinase/ß-glucanase digestion of sporelings and from fungal wall chitin actively inhibit fungal growth and enhance disease resistance', Physiological and Molecular Plant Pathology 35, 215-230.

Kessman, H., Choudhary, A.D. and Dixon, R.A. (1990a) 'Stress responses in alfalfa (*Medicago sativa* L). 3. Induction of medicarpin and cytochrome P450 enzyme activities in elicitor-treated cell suspension cultures and protoplasts', Plant Cell Reports 9, 38-41.

Kessman, H., Edwards, R., Geno, P.W. and Dixon, R.A. (1990b) 'Stress responses in alfalfa (*Medicago sativa* L). 5. Constitutive and elicitor-induced accumulation of isoflavonoid conjugates in cell suspension cultures', Plant Physiology 94, 227-232.

King, L., Hampton, R.E. and Diachun, S. (1964) 'Resistance to *Erisyphe polygoni* of red clover infected with bean yellow mosaic virus', Science 146, 1054-1055.

Kochs, G. and Grisebach, H. (1989) 'Phytoalexin synthesis in soybean - purification and reconstitution of cytochrome P450 3,9-dihydroxypterocarpan 6A-hydroxylase and separation from cytochrome P450 cinnamate 4-hydroxylase', Archives of Biochemistry and Biophysics 273, 543-553.

Linthorst, H.J.M. (1991) 'Pathogenesis-related proteins of plants', Critical Reviews in Plant Sciences 10, 123-150.

Mackenbrock, U. and Barz, W. (1991) 'Elicitor-induced formation of pterocarpan phytoalexins in chickpea (*Cicer arietinum* L) cell suspension cultures from constitutive isoflavone conjugates upon inhibition of phenylalanine ammonia lyase', Zeitschrift fur Naturforschung C 46, 43-50.

Malamy, J., Carr, J.P., Klessig, D.F. and Raskin, I. (1990) 'Salicylic acid: a likely endogenous signal in the resistance response of tobacco to viral infection', Science 250, 1002-1004.

Mauch, F., Hadwiger, L.A. and Boller, T. (1988a) 'Antifungal hydrolases in pea tissue. I. Purification and characterisation of two chitinases and two ß-1,3-glucanases differentially regulated during development and in response to fungal infection', Plant Physiology 87, 325-333.

Mauch, F., Mauch-Mani, B. and Boller, T. (1988b) 'Antifungal hydrolases in pea tissue. II. Inhibition of fungal growth by combinations of chitinase and ß-1,3-glucanase', Plant Physiology 88, 936-942.

Mauch, F. and Staehelin, L.A. (1989) 'Functional implications of the subcellular localization of ethylene-induced chitinase and ß-1,3-glucanase in bean leaves', The Plant Cell 1, 447-457. American Society of Plant Physiologists.

Mavandad, M., Edwards, R., Liang, X.O., Lamb, C.J. and Dixon, R.A. (1990) 'Effects of trans-cinnamic acid on expression of the bean phenylalanine ammonia-lyase gene family', Plant Physiology 94, 671-680.

Métraux, J.P., Ahl Goy, P., Staub, Th., Speich, J., Steinemann, A., Ryals, J. and Ward, E. (1990a) 'Induced systemic resistance in cucumber in response to 2,6-dichloroisonicotinic acid and pathogens', Proceedings of the 5th International

Symposium on the Molecular Genetics of Plant Microbe Interactions, Interlaken, Switzerland.

Métraux, J.P., Signer, H., Ryals, J., Ward, E., Wyss Benz, M., Gaudin, J., Raschdorf, K., Schmid, E., Blum, W. and Inverardi, B. (1990b) 'Increases in salicylic acid at the onset of systemic acquired resistance in cucumber', Science 250, 1004-1006.

Métraux, J.P., Ahl Goy, P., Staub, Th., Speich, J., Steinemann, A., Ryals, J. and Ward, E. (1991) 'Induced systemic resistance in cucumber in response to 2,6-dichloroisonicotinic acid and pathogens', in H. Hennecke and D.P.S. Verma (eds.), Advances in Molecular Genetics of Plant-Microbe Interactions, Kluwer Academic Publishers, Dordrecht.

Morris, P.F., Savard, M.E. and Ward, E.W.B. (1991) 'Identification and accumulation of isoflavonoids and isoflavone glucosides in soybean leaves and hypocotyls in resistance responses to *Phytophthora megasperma* f. sp. *glycinea*', Physiological and Molecular Plant Pathology 39, 229-244.

Murray, D.C. and Walters, D.R. (1992) 'Increased photosynthesis and resistance to rust infection in upper, uninfected leaves of rusted broad bean (*Vicia faba* L.)', New Phytologist 120, 235-242.

Paiva, N.L., Edwards, R., Sun, Y.J., Hrazdina, G. and Dixon, R.A. (1991) 'Stress responses in alfalfa (*Medicago sativa* L.). 11. Molecular cloning and expression of alfalfa isoflavone reductase, a key enzyme of isoflavonoid phytoalexin biosynthesis', Plant Molecular Biology 17, 653-657.

Paxton, J.D. and Chamberlain, D.W. (1967) 'Acquired local resistance of soybean plants to *Phytophthora* spp.', Phytopathology 57, 351-353.

Pennazio, S., Coloracio, D., Roggero, P. and Lenzi, R. (1987) 'Effect of salicylate stress on the hypersensitive reaction of asparagus bean to tobacco necrosis virus', Physiological and Molecular Plant Pathology 30, 347-357.

Perrin, D.R., Whittle, C.P. and Batterham, T.J. (1972) 'The structure of phaseollidin', Tetrahedron Letters 1972, 1673-1676.

Pickard, B.G. (1973) 'Action potentials in higher plants', The Botanical Review 39, 172-201.

Ponz, F. and Bruening, G. (1986) 'Mechanisms of resistance to plant viruses', Annual Review of Phytopathology 24, 355-381.

Pospieszny, H., Chirkov, S. and Atabekov, J. (1991) 'Induction of antiviral resistance in plants by chitosan', Plant Science 79, 63-68.

Preisig, C.L., Bell, J.N., Sun, Y.J., Hrazdina, G., Matthews, D.E. and VanEtten, H.D. (1990) 'Biosynthesis of the phytoalexin pisatin - isoflavone reduction and further metabolism of the product sophorol by extracts of *Pisum sativum*', Plant Physiology 94, 1444-1448.

Rahe, J.E. and Kuć, J. (1970) 'Metabolic nature of the infection-limiting effect of heat on bean anthracnose', Phytopathology 60, 1005-1009.

Rahe, J.E. (1973a) 'Occurrence and levels of the phytoalexin phaseollin in relation to delimitation at sites of infection of *Phaseolus vulgaris* by *Colletotrichum lindemuthianum*, Canadian Journal of Botany 51, 2423-2430.

Rahe, J.E. (1973b) 'Phytoalexin nature of heat-induced protection against bean anthracnose', Phytopathology 63, 572-577.

Rahe, J.E., Kuć, J., Chuang, Chien-mei and Williams, E.B. (1969) 'Induced resistance in *Phaseolus vulgaris* to bean anthracnose', Phytopathology 59, 1641-1645.

Redolfi, P. and Cantisani, A. (1981) 'Protein changes and hypersensitive reaction in virus infected bean leaves', Proceedings of the Fifth International Congress of Virology, Strasbourg (France), August 1981, (abstract).

Ryan, C.A., Bishop, P.D., Walker-Simmons, M., Brown, W.E. and Graham, J.S. (1985) 'Pectic fragments regulate the expression of proteinase inhibitor genes in plants', in J.L. Key and T. Kosuge (eds.), Cellular and Molecular Biology of Plant Stress, New York: Liss.

Ryder, T.B., Cramer, C.L., Bell, J.N., Robbins, M.P., Dixon, R.A. and Lamb, C.J. (1984) 'Elicitor rapidly induces chalcone synthase mRNA in *Phaseolus vulgaris* cells at the onset of the phytoalexin defence response', Proceedings of the National Academy of Science U.S.A. 81, 5724-5728.

Schmelzer, E., Börner, H., Grisebach, H., Ebel, J., and Hahlbrock, K. (1984) 'Phytoalexin synthesis in soybean (*Glycine max*). Similar time courses of mRNA induction in hypocotyls infected with a fungal pathogen and in cell cultures treated with fungal elicitors', FEBS Letters 172, 59-63.

Showalter, A.M., Bell, J.N., Cramer, C.L., Bailey, J.A., Varner, J.R. and Lamb, C.J. (1985) 'Accumulation of hydroxyproline-rich glycoprotein mRNA in response to fungal elicitor and infection', Proceedings of the National Academy of Sciences of the U.S.A. 82, 6551-6555.

Skipp, R.A. and Deverall, B.J. (1972) 'Relationships between fungal growth and host changes visible by light microscopy during infection of bean hypocotyls (*Phaseolus vulgaris*) susceptible and resistant to physiologic races of *Colletotrichum lindemuthianum*, Physiological Plant Pathology 2, 357-374.

Skipp, R.A. and Deverall, B.J. (1973) 'Studies on cross-protection in the anthracnose disease of bean', Physiological Plant Pathology 3, 299-314.

Smith, D.A., VanEtten, H.D., Serum, J.W., Jones, T.M., Bateman, D.F., Williams, T.H. and Coffen, D.L. (1973) 'Confirmation of the structure of kievitone, an antifungal isoflavone isolated from *Rhizoctonia*-infected bean tissues', Physiological Plant Pathology 3, 293-297.

Smith, O.F. (1938) 'Host-parasite relations in red clover plants resistant and susceptible to powdery mildew, *Erysiphe polygoni*', Journal of Agricultural Research 57, 671-682.

Sun, Y.J., Wu, Q.D., VanEtten, H.D. and Hrazdina, G. (1991) 'Stereoisomerism in plant disease resistance - induction and isolation of the 7,2'-dihydroxy-4',5'-methylenedioxyisoflavone oxidoreductase, an enzyme introducing chirality during synthesis of isoflavonoid phytoalexins in pea (*Pisum sativum* L)', Archives of Biochemistry and Biophysics 284, 167-173.

Sutton, D.C. (1979) 'Systemic cross protection in bean against *Colletotrichum lindemuthianum*', Australasian Plant Pathology 8, 4-5.

Sutton, D.C. (1982) 'Field protection of bean against *Colletotrichum lindemuthianum* by *Colletotrichum lindemuthianum*', Australasian Plant Pathology 11, 50-51.

Svoboda, W.E. and Paxton, J.D. (1972) 'Phytoalexin production in locally cross-protected Harosoy and Harosoy-63 soybeans', Phytopathology 62, 1457-1460.

Sweigard, J.A., Matthews, D.E. and VanEtten, H.D. (1986) 'Synthesis of the phytoalexin pisatin by a methyltransferase from pea', Plant Physiology 80, 277-279.

Tallamy, D.W. and Raupp, M.J. (1991) Phytochemical Induction by Herbivores, John Wiley & Sons, New York.

Tepper, C.S. and Anderson, A.J. (1986) 'Two cultivars of bean display a differential response to extracellular components from *Colletotrichum lindemuthianum*', Physiological and Molecular Plant Pathology 29, 411-420.

Tepper, C.S., Albert, F.G. and Anderson, A.J. (1989) 'Differential mRNA accumulation in three cultivars of bean in response to elicitors from *Colletotrichum lindemuthianum*', Physiological and Molecular Plant Pathology 34, 85-98.

Tyihák, E., Steiner, U. and Schönbeck, F. (1989) 'Induction of disease resistance by N-trimethyl-l-lysine in bean plants against *Uromyces phaseoli*', Journal of Phytopathology 126, 253-256.

Voisey, C. and Slusarenko, A.J. (1989) 'Chitinase mRNA and enzyme activity in *Phaseolus vulgaris* (L.) increase more rapidly in response to avirulent than to virulent cells of *Pseudomonas syringae* pv. *phaseolicola*', Physiological and Molecular Plant Pathology 35, 403-412.

Walter, M.H., Liu, J.W., Grand, C., Lamb, C.J. and Hess, D. (1990) 'Bean pathogenesis-related (PR) proteins deduced from elicitor-induced transcripts are members of a ubiquitous new class of conserved PR proteins including pollen allergens', Molecular and General Genetics 222, 353-360.

Walters, D.R. and Murray, D.C. (1992) 'Induction of systemic resistance to rust in *Vicia faba* by phosphate and EDTA: effects of calcium', Plant Pathology 41, 444-448.

Ward, E.W.B. (1983) 'Effect of mixed and consecutive inoculations on the interactions of soybeans with races of *Phytophthora megasperma* f. sp. *glycinea*', Physiological Plant Pathology 23, 281-294.

Welle, R. and Grisebach, H. (1989) 'Phytoalexin synthesis in soybean cells - elicitor induction of reductase involved in biosynthesis of 6′-deoxychalcone', Archives of Biochemistry and Biophysics 272, 97-102.

Welle, R. and Grisebach, H. (1991) 'Properties and solublization of the prenyltransferase of isoflavonoid phytoalexin biosynthesis in soybean', Phytochemistry 30, 479-484.

Wingate, V.P.M., Lawton, M.A. and Lamb, C.J. (1988) 'Glutathione causes a massive and selective induction of plant defence genes', Plant Physiology 87, 206-210.

Wingender, R., Rohrig, H., Horicke, C., Wing, D. and Schell, J. (1989) 'Differential regulation of soybean chalcone synthase genes in plant defence, symbiosis and upon environmental stimuli', Molecular and General Genetics 218, 315-322.

Wrather, J.A. and Elrod, J.M. (1990) 'Apparent systemic effect of *Colletotrichum truncatum* and *C. lagenarium* on the interaction between soybean and *C. truncatum*', Phytopathology 80, 472-474.

Yalpani, N., Silverman, P., Wilson, T.M.A., Kleier, D.A. and Raskin, I. (1991) 'Salicylic acid is a systemic signal and an inducer of pathogenesis-related proteins in virus-infected tobacco', The Plant Cell 3, 809-818.

Yarwood, C.E. (1954) 'Mechanisms of acquired immunity to a plant rust', Proceedings of the National Academy of Science U.S.A. 40, 374-377.

Yarwood, C.E. (1956) 'Cross protection with two rust fungi', Phytopathology 46, 540-544.

Ye, X.-S. and Deverall, B.J. (1989) 'Effects of heat-treatment or mixed inoculation on the development of compatible and incompatible bean rust infections', Physiological and Molecular Plant Pathology, 34, 427-437.

Yoshikawa, M., Takeuchi, Y. and Horino, O. (1990) 'A mechanism for ethylene-induced disease resistance in soybean: enhanced synthesis of an elicitor-releasing factor, ß-1,3-endoglucanase', Physiological and Molecular Plant Pathology 37, 367-376.

# INDUCED RESISTANCE IN THE SOLANACEAE

OLGA L. OZERETSKOVSKAYA
*A.N. Bakh Institute of Biochemistry*
*Leninsky PR 33*
*Moscow 117071*
*RUSSIA*

## 1. Introduction

Current concern about the environment indicates a need to limit application of chemicals for plant disease control. A broad and often over use of pesticides is ecologically harmful, toxic to many vertebrates, and may lead to a development of pesticide resistance in pathogens. To produce pesticide-free produce and improve the environment, one needs to utilize alternative, pollution-free methods for disease control. One of the most encouraging techniques for plant protection is the method of induced resistance. This method is based not on direct pathogen suppression as it occurs in the application of pesticides, but on stimulating natural defense mechanisms in plant tissues.

From my viewpoint, there is one more reason for using induced resistance. When plants are placed under stress, their defense responses may be suppressed. A broad application of classical chemical pesticides to protect plants and maintain yield in crops cannot replace the natural defenses of plants. Furthermore, in some cases chemical pesticides may suppress plant immunity when the adaptation threshold is exceeded.

There are certain situations when the plant immunity system itself requires protection. We are conscious about our immune system, but we do not focus proper attention on the fact that the immune system in plants may suffer under conditions of ecological stress that influence our own immune system (at least, in some ecologically unfavorable regions in the world). Therefore, a development of various methods for immunocorrection is presently of vital importance for controlling and enhancing the immune response in plants.

Induced resistance is frequently referred to as immunization, sensitization, vaccination, acquired immunity and sometimes cross-protection. The terminology is not yet firmly established in this relatively new branch of phytoimmunology. In principle,



*R. Hammerschmidt and J. Kuć (eds.), Induced Resistance to Disease in Plants,* 31–62.

all these terms denote the same phenomenon that is an artificial activation of defense mechanisms in plants in the course of their ontogenesis.

Several ways exist for inducing resistance in plants, including the plants of the *Solanaceae*. Among them are:

- inoculation with pathogens
- inoculation with avirulent races or nonpathogenic strains
- inoculation with inactivated pathogens
- inoculation with nonpathogens
- treatment with microbial metabolites, i.e. biogenic elicitors
- treatment with chemicals (abiotic elicitors), which are signals of induced resistance or release such signals

Two types of induced resistance are known: local and systemic. Local induced resistance develops within a limited area of plant tissue, i.e. at the site of induction. Systemic induced resistance develop in the plant tissues away from the site of induction.

The present chapter contains an analysis of induced resistance in plants of the *Solanaceae* family which have been extensively studied. Attention will be primarily on the induced resistance in potato and tobacco. Tomato will also be discussed, but this crop has not received as much study. Due to the author's special interest in potato as a major target of her studies, the section of this chapter concerned with this plant will be somewhat larger than the others.

## 2. Induced Resistance in *Solanum tuberosum*

### 2.1 LOCAL AND SYSTEMIC RESISTANCE INDUCED WITH INCOMPATIBLE PATHOGENS

Local resistance in potato tubers was discovered by Müller and Borger (1940). These investigators hypothesized the existence of phytoalexins based on their studies (Müller and Borger, 1940). A suspension of spores of an incompatible race of *Phytophthora infestans* was applied to the surface of potato tuber section in the form of a cross. One day later, the entire surface of the section was inoculated with a compatible race of the same parasite. After several days, fungal mycelium appeared on the surface of the section except the for sites previously infected with the incompatible race (where a hypersensitive reaction (HR) had developed). This lack of disease appeared as a black cross of necrotic tissues against the white background of mycelium. Development of compatible races of *P. infestans* as well as the tuber rot pathogen *Fusarium caeruleum* and certain saprophytes was also inhibited in tissues previously inoculated with an incompatible race of *P. infestans*. Defense response was localized in necrotic tissue and neighboring cells and did not spread over the entire tuber surface. Thus, a limited infection with an incompatible race of *P. infestans* induced local resistance against further infection by a compatible race.

The systemic induction of resistance in potato plants is illustrated by the work of Stromberg and Brishammar (1991). Several lower leaves of potato were infected with spores of *P. infestans* (race 1.3.4.7.8) or with *P. cryptogea*, a nonpathogen of potato isolated from wheat. Localized infection of the lower leaves induced systemic resistance in upper leaves that resulted in 30-70% protection against disease caused by *P. infestans*. The nonpathogen of potato, *P. cryptogea*, induced a higher degree of resistance than did *P. infestans*. The initially infected lower leaves produced a systemic signal or signals which induced resistance in the upper leaves.

## 2.2 INDUCTION OF RESISTANCE IN POTATO WITH BIOGENIC ELICITORS

Local and systemic induced resistance can not only be induced in potato by limited infection with pathogens or nonpathogens, but also by treating plant tissues with chemical elicitors produced by microorganisms. The use of fungal elicitors and other metabolites is the topic of this section

In the mid-1970's, studies of induction of resistance in potato with biogenic elicitors isolated from phytopathogens were initiated in the Laboratory of Induced Resistance (A.N.Bakh Institute of Biochemistry, Russian Academy of Sciences, Moscow) (Metlitskii and Ozeretskovskaya, 1973, 1985; Metlitskii *et al.*, 1978, 1985; Berezin, 1984; Ozeretskovskaya *et al.*, 1986: Metlitskii, 1987; Il'inskaya *et al.*, 1991).

2.2.1 *Elicitors of P. infestans*. Two types of elicitors were isolated from *P. infestans* mycelium. One of them was identified as a high-molecular weight ß-1.3-ß-1.6-glucan from pathogen cell walls (Chalova *et al.*, 1976). The other one was a lipoglycoprotein complex (the LGP-complex), which contained lipid (57-59%), carbohydrate (34-38%), protein (5-7%) and phosphorus (Chalova *et al.*, 1977). The ability of the LGP-complex to induce rishitin in potato tubers (cv. Temp [$R_1$]) appeared to be 3-5 times higher than the corresponding activity of glucans from cell walls. These two types of elicitors also differed in the relative proportion of rishitin and lubimin that accumulated after treatment. Specifically, the LGP-complex induced 10 times more rishitin than lubimin while the glucan elicitors induced only slightly more rishitin than lubimin.

Both elicitors exhibit non-specific inducing activity. Although *P.infestans* races differ with respect to cultivar specificity, these differences could not be accounted for by differences in the elicitors produced by each race. The elicitors induce rishitin without any correlation to combinations of virulence genes in the race from which the elicitor is extracted and R-genes in the potato varieties tested. Induction of a high level of rishitin can even be achieved in potato varieties susceptible to all races of *P. infestans* (i.e., potato varieties without R-genes). This provides further evidence that all potato varieties have inducible resistance mechanisms.

Further studies demonstrated that lipids are an active component of the LGP-complex, and protein and carbohydrate components do not determine elicitor activity (Chalova *et* al., 1988). Although one can hypothesize that protein and carbohydrate components may be solubilizing agents for lipid components of the complex, a role for these polymers has not been established.

Both neutral and polar lipids from the LGP-complex have the capability to induce rishitin. The fact that this is observed for both types of lipids allowed us to suggest that some common component (probably, fatty acids) was responsible for this function. Saponification of common lipid extract confirmed that the fatty acid fraction was the active elicitor for production of rishitin, whereas the non-saponifiable fraction had practically no activity.

Examination of fatty acids in the LGP-complex and in polar and neutral lipids extracted from the LGP-complex revealed a high level of unsaturated fatty acids. After separation of the fatty acids into saturated, unsaturated and polyunsaturated fatty acid classes, it was found that the fraction containing polyenic acids had the ability to induce rishitin. This fraction contained linoleic, linolenic, eicosatrienoic, arachidonic (AA) and eicosapentaenoic (EPA) acids. Among them only AA and EPA appeared active in elicitation of rishitin (Ozeretskovskaya *et al.*, 1987). This confirmed earlier work of Bostock *et al.* (1981, 1982).

Study of the biological activity of AA and EPA revealed that the amounts of these fatty acids needed to elicit rishitin in potato tuber tissue was far less than the amount of LPG needed to elicit the same response. This could be expected, because the a more highly purified and homogenous elicitor would be expected to have a higher specific eliciting activity. Based on our results and the earlier reports by Bostock *et al.* (1981, 1982), it can be inferred that AA and EPA are the active components of the LGP-complex extracted from *P. infestans* mycelium (Ozeretskovskaya *et* al., 1987; Chalova *et* al., 1988). Both polyenic acids were also found to be absent in potato tissues (Ozeretskovsaya *et al.*, 1987; Chalova *et al.*, 1988).

2.2.2 *Local and systemic resistance caused by elicitors.* The term "biogenic elicitors" usually denotes metabolites produced by microorganisms or plants, which induce production of phytoalexins in plant tissues. However, it is well-known that plant resistance is based on a combination of defense responses of which phytoalexin accumulation is only one component (Metlitskii and Ozeretskovskaya, 1985). For this reason, the ability of a particular compound to induce phytoalexin accumulation in plants does not definitely confirm that this compound can protect plant tissues against diseases by inducing resistance.

We studied the ability of several concentrations the LGP-complex to induce local resistance in potato tuber tissue (cv. Temp [R1]) against *P. infestans* (Ozeretskovskaya and Chalova, 1989). The experiments were carried out using tuber disks, and the elicitor preparations were applied to the upper surface of the disks at various concentrations. After a specific period of time, the elicitor-treated surface was inoculated with a suspension of zoospores of a compatible race of *P. infestans.* The results of infection were evaluated microscopically.

We were able to conclude from these experiments that there are two concentration ranges of elicitor that suppressed disease development (Figure 1). The higher concentration of elicitor (100 $\mu$g LGP $ml^{-1}$) was effective in inducing resistance only if the challenge inoculation occurred at 48 or more hours after elicitor treatment. At this

time, elicitor-induced necrosis has developed on the surface of the disk and fungitoxic levels of phytoalexins had accumulated in the tuber tissue. Based on this result, it is probable that the phytoalexins are at least partly responsible for the resistance to the pathogen. If tissues were inoculated shortly after elicitor application and phytoalexins were not yet formed, the development of the pathogen in infected disks did not differ from control ones or was even slightly stimulated (Ozeretskovskaya and Chalova, 1989).

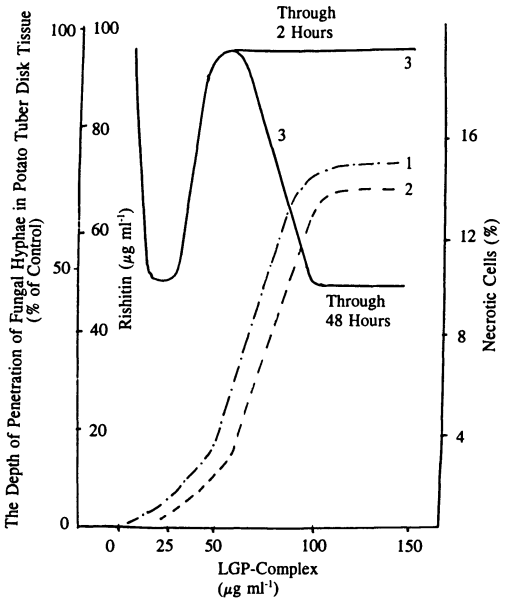


**Figure 1.** The ability of LGP-complexes of various concentration to induce necrosis (1), rishitin (2), and suppress the development of *P. infestans* race 1 (3) in potato tuber tissue discs from the variety Temp ($R_1$).

The second concentration range where resistance can be obtained by means of elicitors corresponds to low concentrations (5-10 $\mu$g LGP $ml^{-1}$), at which phytoalexins are not induced (Figure 1). Despite the lack of necrosis and phytoalexin accumulation, the treated tissues are still protected against *P. infestans*.

The effect of the elicitors on the induction of resistance was then examined with respect to its durability and systemic nature in the tubers. In these experiments, the LGP-complex at 5 and 100 $\mu$g $ml^{-1}$ was applied to the upper surface of cylinders cut from potato tubers (Ozeretskovskaya *et* al., 1986; Il'inskaya, 1991). The cylinders were incubated under high relative humidity and then were cut into disks. These disks were infected with a suspension of zoospores of a compatible race of *P. infestans*. The results

of infection were evaluated microscopically by taking into account the position of each disk with respect to cylinder end treated with elicitor (Figure 2).

It was found that treating the upper surface of the cylinder with the LGP-complex at 100 $\mu$g ml$^{-1}$ resulted in a delayed development of the fungus only within the tissue that had been in contact with the elicitor. In this layer, necrotic cells were formed and phytoalexins, which suppress development of fungus, accumulated. There was no induction of resistance in the tissues at a distance from the site of treatment with elicitor.

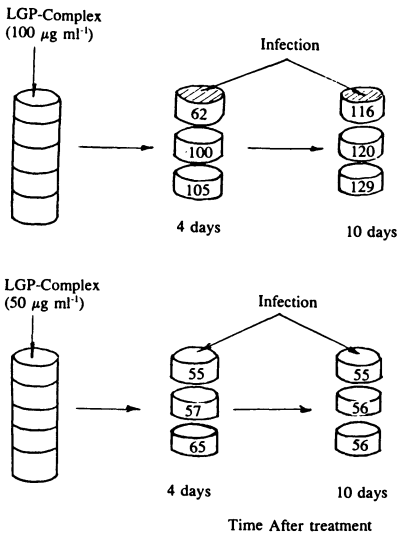


**Figure 2.** The penetration of *P. infestans* race 1 hyphae (as % of control indicated in each disc) in potato (cv. Temp) tuber tissue cylinders whose upper surface was treated with LPG-complex. At 4 or 10 days after treatment with the LPG-complexes, the cylinders were cut into discs, and the upper surface of each disc was inoculated with *P. infestans* to assess the degree of systemic protection.

Furthermore, by 10 days after infection, the necrotic tissues of the upper layer of the cylinder also lost resistance. The experiments showed that the decrease of phytoalexins in these tissues, possibly because of transformation into non-toxic compounds, corresponded with the loss of induced resistance.

A very different result was observed in studying protection caused by the elicitor at low concentrations that do not elicit necrosis or phytoalexin accumulation. Application of the LGP-complex at the concentration of 5 $\mu$g ml$^{-1}$ to the upper cylinder surface

resulted in enhanced resistance against *P. infestans* in all tissues of a cylinder. This induced state of resistance was retained for up to ten days after treatment with elicitor.

However, the potato induced resistance model using cylinders of tuber tissue described above does not allow us to define the duration of resistance in tuber tissues. Longer term studies, however, can be accomplished by using "intact tubers" as the model tissue system (Ozeretskovskaya and Chalova, 1989). In these experiments, the surface of intact potato tubers was treated with the LGP-complex (10 $\mu$g ml$^{-1}$). After a specific incubation period, cylinders were cut from LPG-treated and control tubers. The cylinders were cut into disks, taking into account the positions of each disks with respect to tuber surface. Each of the disks was then infected with zoospores of a compatible *P. infestans* race. By detecting development of the pathogen into tissues in the disk to which elicitor was not directly applied, one can evaluate the systemic effect of protection (Figure 3).

It was found that two days after treatment with elicitor, the surface tuber tissues acquired resistance to *P. infestans*. After that time, tissue deeper in the tuber subsequently became resistant. Within a week after elicitor treatment all tuber tissues became uniformly protected against *P. infestans* (Figure 3).

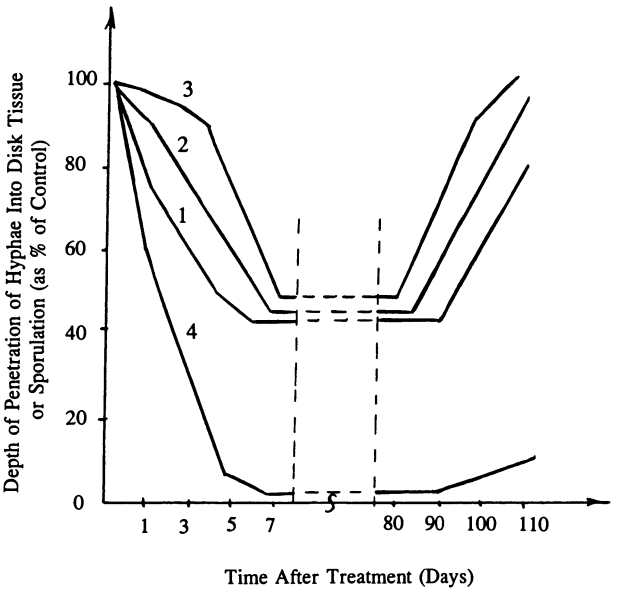


**Figure 3.** Resistance induction in various parts of whole potato tubers (cv. Temp) after treatment with 10 $\mu$g/ml LPG-complex. Penetration of *P. infestans* race 1,3 hyphae into tissue discs prepared from surface tissues (external to the phloem) (1), tissue internal to phloem (2) and pith (3). Sporulation of pathogen in pith tissue (4).

Knowing the distance between the layer of tissue being examined for resistance and the surface of the tuber and the time interval needed for these tissues to acquire resistance, the rate of transmission of the systemic immune signal (or the effect of the signal) can be estimated. We have calculated that this signal (or effect) moves through the tissue at 0.2-0.25 mm (i.e.,2-3 cells) per hour (unpublished results).

Resistance of whole tubers treated with elicitor was retained for at least 3 months. After this time, resistance declined. Initially, resistance in internal tuber layers disappeared, but protection of surface tissues still remained. The protection period against disease in tubers can be lengthened by treatment with additional elicitor at the time resistance decreases.

The protective action of the LGP-complex dramatically effects the sporulation of *P. infestans*, and this appears to be directly related to the establishment of infection hyphae in the tuber tissue. Specifically, if penetration of hyphae into tuber cells was suppressed by approximately 50%, the process of sporulation was inhibited almost completely (Figure 3). Inhibition of sporulation continues into the period when the inhibition of fungal growth is no longer evident. Suppression of *P. infestans* sporulation is very significant, because it suggests that induced plants can prevent the spread of infections in the field and in storage.

Thus, depending on the concentration of biogenic elicitor applied, two types of resistance, local and systemic, can be induced. The first type of protection (local induced resistance) based on a formation of necrosis and phytoalexins is local and short-term. In this case, protection is caused by phytoalexins that temporarily accumulate in necrotic cells. Most of all, this type of protection resembles a suppression of parasite by means of fungicidal activity. The difference is that these fungicides are not exogenously applied but are endogenously formed compounds. Phytoalexins soon disappear from tuber tissue by being metabolized by plant tissue. As a result, tuber cells which utilize their resources for formation of phytoalexins not only lose their resistance to pathogen but may become even more susceptible. This is because phytoalexins can only suppress a disease when they are synthesized at fungitoxic concentration at an appropriate time and place in response to pathogen invasion (Metlitskii, 1987). The prior formation of phytoalexins by elicitors temporarily protects plants against disease, but this may ultimately weaken plant tissues.

The second type of resistance (systemic induced resistance) can be implemented by treating plant tissues with elicitors at low concentration. This resistance does not cause necrosis of plant tissues and phytoalexins do not accumulate at the site of elicitor treatment. This type of protection is associated with an increase in resistance of living plant tissues and has systemic and long-term character. With further investigation of induced resistance in mind, this type of protection seems the more important.

We established that both types of tuber protection can also be obtained at various concentrations of AA and EPA (Chalova *et* al., 1989), and a biogenic elicitor extracted from the nonpathogen of potato *Fusarium culmorum* Sacc. (Yurganova *et* al., 1989). However, for brevity of presentation, we have only presented the work on the LGP-complex.

Thus, the elicitors at low concentrations provide systemic protection in the potato tuber. Subsequent experiments demonstrated that whole plants could be systemically protected not only against *P. infestans* but a number of other fungal and bacterial diseases after treatment of tubers with elicitor (Metlitskii *et al.*, 1978, 1985; also see Section. 2.4). In these experiments, the lower plant leaves were treated with fatty acids at different concentrations. Within five days, protection against *P. infestans* reached a maximum level in the upper untreated leaves. AA and EPA were found to be the most effective inducers of resistance (providing 94-97% protection). Linoleic, linolenic, and oleic acids provided 82%, 39%, and 42% protection, respectively. The fact systemic protection could be induced by AA, EPA, and three other fatty acids (i.e., linoleic, linolenic, and oleic acids) was later confirmed by Cohen and colleagues (Cohen *et* al., 1991).

Systemic induced resistance against *P. infestans* in potato can be also be induced by treating the lower leaves with hyphal wall components of *P. infestans* in combination with carborundum (Doke *et* al., 1987). Penetration by *P. infestans* into cells from leaves exhibiting induced resistance was accompanied by the expression of hypersensitive cell death. The pathogen was unable to develop out of these necrotic host cells.

2.2.3 *Race specific suppressor from P. infestans.* Are there mechanisms by which a pathogen can block or suppress the expression of genes responsible for resistance or induced resistance? During the purification of the LGP-complex, it was observed that an unpurified alcohol extract from the mycelium of a race (race 1.3) compatible with the potato variety under study (cv. Temp [$R_1$]) elicited less phytoalexin accumulation than the corresponding extract obtained from the mycelium of an incompatible fungus race (race 3.4). It appeared that compounds preventing induction were in the extracts of the compatible race. The compound inhibiting elicitation was isolated and identified as a fraction containing ß-1,3 and ß-1,6 glucans with a molecular weight of about 3 kDa (Chalova *et* al., 1980; Ozeretskovskaya *et* al., 1982, 1983). This glucan was equivalent to the race specific suppressor from *P. infestans* searched for from the beginning of the 70's in the A.N.Bakh Institute of Biochemistry (Metlitskii *et* al., 1979; Leont'eva *et* al., 1979) and simultaneously by Garas, Doke, and Kuć (Garas *et* al., 1979; Doke *et* al., 1979, 1980).

Race specificity of the suppressor was found with various "potato variety - *P. infestans* race" combinations. Suppression by glucans was only observed if the fungal race from which the suppressor was isolated was compatible with the potato variety tested. In this case, treatment with glucans enhanced disease in disks of potato tubers inoculated with *P. infestans*. This effect was not detected for glucans from incompatible races, which, in some cases, even reduced development of the fungus.

Race specificity of glucan-suppressors was supported by data indicating that the glucans isolated from two pathogenic races with different virulence genes differed with respect to the number and location of ß-1,6-linked glucose residues in the glucan. These glucans differed also by the number and distribution of fragments in the chain from

which di-, tri- and tetra-saccharides were formed by the action of an ß-exo-1,3-glucanase (Vasyukova *et al.*, 1987).

The immunosuppressors specifically attenuate not only varietal resistance to *P. infestans* in potato but also its general immunity. For example, even pathogens for which potato is not a host start to develop in tuber tissues treated with glucans from fungal races compatible with the varieties being tested (Leont'eva *et al.*, 1989). It is likely that the glucans of compatible *P. infestans* races, which inhibit potato immunity, thereby enhance development of secondary pathogens which frequently accompanying *P. infestans* development.

Glucan-suppressors from fungal races compatible with given potato varieties also suppress wound repair in tubers (Medvedeva *et al.*, 1985). Treatment with suppressors apparently disturbs the ability of tuber parenchymal cells to recognize wound signals and to begin meristematic activity. However, a suppression of wound repair is temporal, and after 2-3 days the cells on the wound surface start dividing and form peridermal complexes. It is possible that because of suppressor, tuber cells temporarily lose their capability to recognize non-self, including potato pathogens and non-pathogen elicitors.

Thus, glucan-suppressors do not irreversibly suppress, but merely temporarily delay the immune response in potato. This delay ensures a spread of compatible *P. infestans* race outside of the cell regions, wherein defense response occurs with a time lag. It is likely that this is similar to the ability of biotrophic parasites to delay the immune response of plant cells without killing them.

It was found that the glucans isolated from compatible fungal races also suppress resistance induced by the LGP-complex in potato tubers and promoted the development of infection. (Figure 4a.) Glucans from incompatible races did not have such properties (Ozeretskovskaya *et* al., 1982).

These results would suggest that inducing potato resistance to *P. infestans* in the field by using biogenic elicitors might not be successful. Under field conditions the spores of *P. infestans* races compatible with a given potato variety might produce glucan-suppressors which block elicitor action. However, although the suppressors suppress the induction of induced resistance in potato, they do not affect the resistance already elicited (Figure 4b). An induction of resistance may be linked with rearrangement of cell ultrastructure that requires a certain time interval, around 72-96 hours (Platonova *et* al., 1982). Pre-treating with suppressor prevents this ultrastructural rearrangement. However, once the cellular changes occur, the glucans cannot reverse these changes.

2.2.4 *Inducing properties of AA, EPA and the products of their oxidation.* The role of AA and EPA in inducing resistance in potato and an extensive body of literature on this subject necessitates the inclusion of a special section in the present chapter devoted to these fatty acids.

Bostock *et al.* (1981, 1982) reported that AA and EPA, localized in *P. infestans* mycelium, were extremely active elicitors of sesquiterpene phytoalexins in potato tuber tissue. Other fatty acids had low or no elicitor activity. For example, the fatty acid 20:3

had low activity, whereas 20:2 and 20:1 were completely inactive. Other fatty acids were also found to be inactive such as 14:0, 16:0, 18:0, 20:0, 22:0, 16:1, 18:1, 18:2,

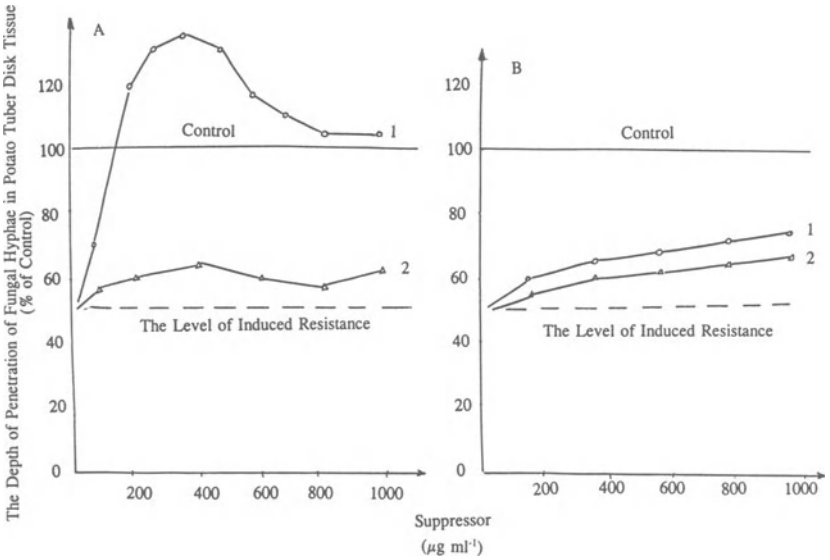


**Figure 4.** (A) Anti-elicitor effects of *P. infestans* glucan suppressors in potato (cv. Temp) tuber tissue treated simultaneously with LPG-complex and glucans. (B). Absence of suppressor effect when tubers were treated with glucans within one week after treatment with the LPG-complex. Glucans isolated from compatible race 1.3 (1) and incompatible race 3.4. LPG-complex was applied at 10 μg/ml and glucans at 300 ug/ml.

18:3 (Preisig and Kuć, 1985). Furthermore, AA and EPA exhibit some host specificity in that they only elicit accumulation of phytoalexins in potato and pepper (Bloch *et al.*, 1984). What was surprising was the inability of AA to induce rishitin in tomato since rishitin is probably formed in tomato by the same pathway as in potato.

The inducing action of AA and EPA becomes more pronounced on addition of ß-1,3-ß-1,6-glucans of *P. infestans* (Maniara *et al.*, 1984). AA applied at a concentration of 0.1 μg per ml does not induce rishitin in potato. However, addition of appropriate glucans to this low level of AA does result in rishitin accumulation. The fatty acids 20:2 and 20:1, which do not elicit rishitin in potato by themselves, elicit in the presence of the glucans (Preisig and Kuć, 1985).

AA is quickly metabolized by potato tissues. Immediately after adding AA to tuber tissue, its concentration falls by 2-3%. Within one day its concentration is halved (Preisig and Kuć, 1988). AA was incorporated in different classes of lipids in potato, such as triglycerides, diglycerides, phospholipids etc. The fungal lipid, phosphoglycerolceramidaminoethylphosphonate, is especially AA-rich (Bostock *et al.*, 1985).

The capacity of exogenously applied AA and EPA to induce disease resistance in potato (Bostock *et al.*, 1986) and give rise to systemic effects (Chalova *et al.*, 1989; Cohen *et al.*, 1991) does not establish the role of these acids in the potato-*P. infestans* induced resistance system. Involvement of AA in inducing resistance in potato was recently supported by the elegant experiments done by Ricker and Bostock (1992). Potato leaves were infected with *P. infestans* sporangia containing radioactive AA. It was seen from microradiographs that label from AA was localized in the cytoplasm, cell walls and lipid bodies of the sporangia. After infection, AA was liberated from sporangia and detected in few neighboring potato cells adjacent to those infected by the fungus. The distribution of AA released from the sporangia was the same as that observed upon exogenous application of AA to potato, i.e. AA was incorporated in the fractions of neutral and polar lipids.

The mechanism of AA liberation from fungal sporangia is unknown. It is possible that activation of a of phospholipase and other acid hydrolases in potato tissue or *P. infestans* may be responsible for release of this fatty acid. Regardless of how arachidonic acid is released, it is likely that AA liberated from the pathogen successively elicits cells surrounding the infection hyphae. This possibility was demonstrated by Ricker and Bostock (1992) who were able to differentiate the compatible and incompatible host-parasite interaction based on movement of arachidonic acid in potato tissue. In the case of an incompatible interaction, AA is found in the potato cells surrounding the pathogen within 9 hour after infection, whereas in the case of compatible interaction it is found within 12 hours. The authors assume that AA or its metabolites are quickly liberated from spores, and in the incompatible case, are capable of restricting pathogen development at early stages of infection. Our experiments showed that even a concentration of AA of 0.003-0.03 $\mu$g ml$^{-1}$ of AA elicits systemic induced resistance in potato tuber (Chalova *et al.*, 1989), and these concentrations may be similar to or greater than that released by *P. infestans* during infection.

It is known that AA and EPA are the precursors of a series of biologically active compounds. In animal tissues, oxidation of AA by cycloxygenase results in the formation of prostaglandins (PGs) and thromboxanes. A series of hydroxyderivatives of AA and EPA, the leukotrienes, are formed by means of lipoxygenase.

In general, the division *Oomycota*, to which *P. infestans* belongs, is extremely rich in eicosanoids that have the potential for biological activity. Utilizing several independent methods for analysis, the PGs of the groups F and A+B+E were found in *P. infestans* mycelium and the LGP-complex (Ozeretskovskaya *et al.*, 1988). Identification of specific PGs was plagued by their low content in the fungus.

PG fractions isolated from the LGP-complexes are capable of inducing systemic induced resistance against *P. infestans* in potato tubers. The tests showed that certain synthesized PGs and hydroxyderivatives of AA and EPA have analogous properties (Avdiushko *et al.*, 1987a, 1987b). These compounds were able to induce resistance at concentrations as low as $10^{-10}$ M. Thus, PGs and hydroxyderivatives of AA and EPA are able to induce systemic resistance in potato against disease (0zeretskovskaya *et al.*, 1988).

## 2.3 BIOCHEMICAL MECHANISMS OF LOCAL AND SYSTEMIC INDUCED RESISTANCE IN POTATO

In 1941 Müller and Borger laid a biochemical foundation for local induced resistance (Müller, Borger, 1941). Most commonly, local induced resistance is associated with HR in potato tuber tissue, and it is known that phytoalexins, which inhibit development of fungi, accumulate in the necrotic potato tissues that result from the hypersensitive response. Since phytoalexins turnover rather quickly and disappear from the hypersensitive response-reacting tissue, local induced resistance is generally short-term in potato tuber tissue.

Local induced resistance against *P. infestans* in potato may also be related to a reduction in host sterols. *P. infestans* is a sterol auxotroph, and it obtains sterols from the tuber tissue during pathogenesis. A lack of sterols decreases pathogenic properties of *P. infestans* and the process of its reproduction, particularly the formation of zoospores. Exogenous application of sterols can restore these processes (Metlitskii *et al.*, 1976; Vasyukova *et al.*, 1977). The concentration of sterols declines in necrotic potato tissues after infection with an incompatible race of *P. infestans* or after treatment with AA or high concentration of the LGP-complex. It is likely that the lack of sterols and corresponding accumulation of phytoalexins, to which sterol-free fungus becomes highly susceptible, make up a series of defense responses, ensuring local induced resistance against *P. infestans* in potato (Metlitskii *et al.*, 1980).

The mechanisms for systemic induced resistance are different than those for local induced resistance. Systemically protected tissues have no visible necrosis and are not dissimilar macroscopically from control tissues. Systemic induced resistance appears to be based on a rapid and intense plant response to subsequent infection, which results from rapid expression of defense genes. Specifically, the rate and intensity of necrotization of potato cells and production of phytoalexins in response to infection with *P. infestans* are found to be essentially higher in potato tubers systemically induced by biogenic elicitors as compared with controls (Ozeretskovskaya *et al.*, 1986)).

The concentration of sterols was almost halved in potato tubers systemically immunized by AA, and this condition lasted for the entire period of immunization (3 months). After the end of immunization, the concentration of sterols gradually rose and eventually reached the levels observed in control tubers (Kaneva *et al.*, 1991). It seems possible that an increased rate and intensity of phytoalexin production in response to challenge together with reduced concentration of sterols in immunized tuber tissues

determine a systemic induced resistance against *P. infestans* in tubers immunized by AA.

A significant rearrangement of ultrastructure takes place in the cells of potato tubers induced by the LGP-complex at low concentration. The number of leucoplasts having differentiated stroma, the volume of smooth endoplasmic reticulum, and the number of mitochondria increase (Platonova *et al.*, 1982). It is possible that the parenchymal tuber cells, which previously stored starch, acquired enhanced respiratory and synthetic capacities. This metabolic change could provide an explanation for enhanced reactivity in immunized tubers.

It is worth noting that in addition to induction of resistance against disease in potato tissues, systemic immunization by biogenic elicitors enhances the capability of tuber tissue to form periderm at wounded sites. The promotion of wound repair in potato tubers is of crucial importance during crop harvest and storage. The activity of peroxidase, polyphenoloxidase, and lipoxygenase and the content of phenolic compounds increase in potato tubers immunized by biogenic elicitors. However, among all characteristics mentioned, only the activity of peroxidase and lipoxygenase has correlated with the spread of systemic induced signal (or effect of the signal) in tuber tissues (Chalova *et al.*, 1985; Yurganova *et al.*, 1989). Bostock and colleagues also detected a local activation of peroxidase, accumulation of lignin, evolution of ethylene, and ethane in potato tubers treated with AA (Bostock *et al.*, 1986).

Chai and Doke found that $O_2^-$ was systemically induced in potato plants and superoxide dismutase and peroxidase were activated in response to treatment of the three lower leaves with hyphal wall components of *P. infestans* (Chai and Doke, 1987). The authors suggested that these changes could be related to the mechanisms of systemic induced resistance. Enhanced expression of the genes which code for hydroxyproline-rich glycoproteins in response to stress is also greater in potato tubers induced by elicitor than in controls (Gorbacheva *et al.*, 1990).

The process of transmission of a signal from elicitor-treated tissue into a potato cell is also poorly investigated. In this context, it is reasonable to consider the possible mechanism of induction of immune responses by AA.

It is known that AA is absent in potato tissues. In the course of induction of the phytoimmune response, AA is incorporated into phospholipids (such as phosphatidylinosidiphosphate) and substitutes for linoleic and linolenic acids (Bostock *et al.*, 1985; Preisig and Kuć, 1988). As a result of different stresses, a quick decomposition of phosphatidylinosidiphosphate contained in the lipids of cell membranes takes place. As a result, a secondary messenger (diacylglycerol), which contains AA and is not a normal constituent of potato, is produced. It is possible that changes in diacylglycerol composition may alter its regulating capacity.

It is also possible that hydroxyderivatives of AA and EPA, which are formed due to the action of lipoxygenase and induce systemic immune response, may also participate in intracellular induction of protective genes (Avdiushko *et al.*, 1987b). This was demonstrated by treating potato tubers with lipoxygenase inhibitors which suppressed a systemic immunization of plant tissue (Avdiushko *et al.*, 1987b).

Unfortunately, the ideas presented above remain speculative. However, there are some indications that intracellular messengers are involved in intracellular regulation in potato cells in response to treatment with elicitors. It was found that a short-term increase in the concentration of cAMP occurred within 30 minutes after treating the disks of potato tubers with AA and the LGP-complex (at the concentration of $10^{-8}$ M or 0.0005%, respectively) (Ocheretina *et al.*, 1991). By contrast, treating disks with the elicitors at high concentrations, which cause necrosis, reduced the content of cAMP.

Zook and Kuć (1987) showed that $Ca^{2+}$ enhanced the production of rishitin, but had no influence on formation of lubimin in potato tuber tissues treated with AA. By acting as a secondary messenger, $Ca^{2+}$ may control the mobilization of substrates for phytoalexin synthesis and/or activate the enzymes for their synthesis.

However, in terms of the signal of systemic induced resistance in potato, this problem remains unsolved. It is possible that molecular signals are transmitted in the vascular system in plants. We recently demonstrated that endogenous oligosaccharides, liberated from the cell walls of potato tuber, are capable of inducing systemic induced resistance against *P. infestans* in tubers (unpublished results). It is likely that ethylene and methyl jasmonate can be viewed as systemic signals, which are transmitted by air and induce protective systems in plants (Farmer and Ryan, 1990), and this possibility was recently demonstrated in potato by Cohen *et al.* (1993). Nevertheless, the nature of the signals which induce long-term systemic induced resistance in potato are still unknown.

## 2.4 POSSIBILITIES FOR PRACTICAL IMPLEMENTATION OF THE METHOD OF POTATO RESISTANCE INDUCTION WITH ELICITORS

Laboratory tests of any resistance-inducing compound do not provide all the data required for practical use of this compound in agriculture. For this reason, the use of LPG for the induction of resistance in potato has been tested under field condition for 14 years (from 1977 up to 1990).

The LGP-complex, preparations containing arachidonic acid, and the elicitor isolated from a non-toxicogenic *F. culmorum* strain were assessed in the field using a range of concentrations determined by laboratory experiments. The levels of elicitor used were very low. Depending on the specific type of elicitor, these values ranged from milligram to gram quantities per hectare when used as treatments on seed tubers. Each elicitor was tested in several various geographical regions and each test was conducted with several different potato cultivars. Standard commercial pesticides were used as controls.

We considered the effects of natural and artificial plant infection with a series of fungal and bacterial pathogens on foliar symptoms and storage of tubers. We also examined crop quality and total harvest. By means of elicitors, we induced resistance in foliage and tubers against *P. infestans, Macrosporium solani, Alternaria solani, Rhizoctonia solani, Streptomyces scabies, Pectobacterium phytophthorum* (Metlitskii *et al.*, 1978). Treatment of potato tubers with elicitors before sowing, resulted in greater resistance of the tubers of the new crop to storage diseases (Ivanyuk *et al.*, 1985). One of the possible reasons for this enhanced resistance is the enhanced ability of the tubers

from the induced plants to repair wounds. This, in turn, prevents parasite penetration into plant tissues.

Apical dominance in potato tubers is suppressed by elicitor treatment, and this results in an increase in the numbers of stolons and, therefore, the number of tubers per plant. Crop gain provided by treatment with elicitor varies from 10-40%, depending on variety, the level of infection, climatic factors, and the number of treatments. The tubers from the elicited plants do not have enhanced levels of phytoalexins and have the same steroid alkaloid content as the controls. It is very important that the LGP-complexes and preparations, incorporating their active component suppress *P. infestans* sporulation. By this means, planting immunized potato tubers would impede the spread of infection, preventing the beginning of epiphytotics.

By means of elicitors, potato plants and tubers can be protected against a series of pathogenic fungi and bacteria. Some data concerning induction of resistance against viruses in treated plants have also been obtained (unpublished results). Such comprehensive plant protection seems evident, because the principle of plant resistance induction involves a general increase of non-specific resistance. Proper application of biogenic elicitors of plant resistance, is as effective for disease control as is treatment of plants with some pesticides,

## 3. Induced Resistance in *Nicotiana* spp

Tobacco is frequently used for studying the phenomenon of induced resistance. The early work of Ross and co-workers followed by that of Kuć and colleagues made a major contribution to this field. Much of the molecular biology of induced resistance has been done with tobacco and the reader is referred to the chapter by Stermer for further details. This section describes work on the systemic induction of resistance in tobacco.

### 3.1 RESISTANCE INDUCTION

3.1.1 *Resistance induction by TMV.* In 1961, Ross established that local infection of N gene tobacco with TMV resulted in local induced resistance to subsequent challenge inoculation with TMV. (Ross, 1961a). In later work, inoculation of one leaf with TMV resulted in resistance being expressed in other non-inoculated leaves of the same plant (Ross, 1961b).

Local and systemic TMV-induced resistance was found to be effective against many parasites, such as fungi, viruses, and bacteria. As an example, N-gene tobacco infected with TMV acquired systemic induced resistance to TNV (Ross, 1966), *Phytophthora parasitica* (McIntyre, Dodds, 1979), and *Pseudomonas tabaci* (McIntyre *et al.*, 1981). Other pathogens, which induced local necrosis in tobacco, induced resistance to TNV (among them are TMV [Ross, 1961a,b], *Chalara elegans* [Hecht and Bateman, 1964], *Pseudomonas syringae* [Ahl *et al.*, 1981]). This indicates that TMV induces resistance

in tobacco against various parasites and that one can induce resistance in tobacco not only by TMV, but also by other pathogens that cause local necrosis.

Kuć and Tuzun (1990) also induced systemic immunization of tobacco by inoculation of several lower leaves with TMV. Leaf inoculation of tobacco with TMV as well as stem-injection with *P. tabacina* induced systemic induced resistance and provided further evidence that tobacco immunization with fungus as well as virus induces multi-defensive mechanisms against fungi and virus. However, this does not imply that the defense mechanisms induced are the same.

3.1.2 *Resistance induction by P. tabacina.* Blue mold causes a serious economic loss in tobacco crops. The induced resistance phenomenon in tobacco inoculated with *P. tabacina* was originally observed by Pont under field conditions (Pont, 1959). Later, this phenomenon was described by other authors (Cruickshank and Mandryk, 1960). Induced resistance in tobacco was frequently accompanied by stunted tobacco growth and other abnormal effects. Consequently, this fact led to development of a special immunization technique (Tuzun and Kuć, 1985a). The technique involved the injection of tobacco stem with sporangiospore suspension of *P. tabacina* (when the plants are ca. 20cm tall). Such immunization of tobacco provided 90-99% protection and stimulated plant growth. The protection lasted for the entire vegetative period, and the crop yield was increased as much as 25%. Immunized plants were as well protected as those treated with the systemic fungicide metalaxyl.

The movement of a factor responsible for immunization in tobacco was reported by Tuzun and Kuć (1985b). They demonstrated that the plants girdled above the site of stem injection remained non-immunized, while girdling below the site of injection had no impact on immunization above. Immunization has been accomplished via grafts from immunized rootstock to nonimmunized scion and vice-versa. A full-size immunized plant developed from immunized buds grafted on non-immunized rootstocks.

In 1987 Kuć and Tuzun presented evidence that induced resistance persisted in plants derived via tissue culture from tobacco leaves and midribs injected with *P. tabacina* (Kuć and Tuzun, 1987). Regenerants developed via tissue culture were systemically protected against diseases both in field and greenhouse experiments. However plants developed from the seeds of regenerants as well as immunized plants were not systemically induced. Thus, the induced resistance state is not heritable, but remains highly stable in vegetative tobacco tissues. This phenomenon is of specific practical interest because it demonstrates the possibility of growing plants with systemic induced resistance. At the same time it is worth noting that Lucas (Lucas *et al.*, 1985) failed in some experiments in transferring induced resistance to *P. tabacina* from protected tobacco explants.

## 3.2 MECHANISMS OF INDUCED RESISTANCE

3.2.1 *Mechanisms of induced resistance against P. tabacina.* Induction of systemic induced resistance after stem inoculation with *P. tabacina* was followed by an increase

in the concentration of PR-proteins with the molecular mass in the range of 14,500-65,000 (Tuzun *et al.*, 1989; Stermer, this volume). Two proteins were exclusively indicative of immunized plants. One of them was found before and after challenge, whereas the second appeared only 6 days after challenge, i.e. when the development of infection was limited.

A close association was reported between induced resistance to *P. tabacina* in tobacco and increased activities of chitinases and glucanases, which relate to PR-proteins of the II and III group of tobacco (Kuć, Tuzun, 1990). Increased activity of the enzymes is systemic. ß-Glucanases and chitinases are capable of hydrolyzing pathogen cell walls with a release of oligosaccharide molecules, having elicitor properties. Since ß-glucans are contained in cell walls of all *Oomycetes* (including *P. tabacina*), an activation of ß-glucanases in immunized tissues may be of special importance in protecting plants against blue mold. At the same time, it is unlikely that chitinases can affect *P. tabacina*, whose cell walls contain no chitin. It appears that systemic activation of chitinases help protect immunized tobacco plants against fungi containing chitin. It is important to note that, similar to most PR-proteins, certain induced chitinases and ß-glucanases are localized in intercellular space in plant tissues. This allows contact of these enzymes with penetrating hyphae of pathogenic fungi (Parent and Asselin, 1984; Ye *et al.*, 1989).

Induction of systemic induced resistance to blue mold by TMV is associated with a series of systemic changes in cell walls of immunized tobacco plants, such as activation of two anionic peroxidases localized in intercellular spaces, accumulation of hydroxyproline-rich glycoproteins, and accumulation of four salt-soluble proteins in cell walls, which are not related to hydroxyproline-rich glycoproteins or PR-proteins (Ye *et al.*, 1990a; Ye *et al.*, 1991). It is known that in addition to oxidation of phenols, peroxidase polymerizes lignin precursors, and also participates in cross-linking of cellulose, pectin, lignin, and extensin in plant cell walls. It is not inconceivable that systemic activation of peroxidase in immunized tobacco tissues is connected with modification of its cell walls that promote the localization of infection.

One could assume that inducing resistance to blue mold in tobacco is linked with activation of phytoalexin formation. However, experiments showed that in response to challenge with *P. tabacina*, the tobacco tissues immunized with this pathogen accumulated phytoalexins at the same rate and concentration as control plants (Stolle *et al.*, 1988). It is the conclusion of the authors that phytoalexin accumulation is not the initial agent for inhibiting *P. tabacina* development in immunized tobacco tissues.

It has been demonstrated that systemic protection against blue mold is followed by an increase in the concentration of soluble carbohydrates, mainly, of glucose and in smaller extent of fructose (Salt *et al.*, 1988). Accumulation of monosaccharides was not associated with starch hydrolysis and it is unlikely to be responsible for induced resistance in tobacco.

Induction of systemic induced resistance due to stem injection with *P. tabacina* presumes the existence of some molecular signals having a capacity for translocation and systemic sensitization of plant tissue. It is possible that ß-ionone and its derivatives, either synthesized or formed in the course of degradation of carotenoids, play the role

of a systemic signal (Salt *et al.*, 1986). It is known that ß-ionone is related to the family of substances, some of which have growth-control properties (abscisic acid in plants, trisporic acid in fungi). Salt and colleagues reported that the amount of ß-ionone was increased by 50-600 times in immunized tobacco tissues.

ß-Ionone and, specifically, its esters with fatty acids exhibit extremely high fungitoxity against *P. tabacina* (Leppik *et al.*, 1972). Injecting tobacco stem with exogenous ß-ionone resulted in growth and protective phenomena being close to that found in immunized plants (Salt *et al.*, 1986). Thus, ß-ionone may be involved in the expression of induced resistance to *P. tabacina*.

However, ß-ionone cannot be translocated in plant tissues. Therefore, it is doubtful that this substance is responsible for inducing a systemic immune response. Probably, such phenomenon is due to ß-ionone derivatives or some endogenous signals formed by its action. The short-chain branched and straight-chain fatty acid esters of 3-hydroxy-ß-ionone, which demonstrated a high fungitoxity, were found in immunized tobacco tissues. Kuć and Tuzun suggest that some of them, having odd number of atoms in the fatty acid chain, may regulate the systemic induced resistance in tobacco (Kuć and Tuzun, 1990).

3.2.2 *Mechanism of induced resistance against TMV.* The induced resistance mechanisms against virus in tobacco differ from those characteristic against *P. tabacina*. Immunizing a variety of tobacco systemically susceptible to TMV with the blue mold fungus protected plants against *P. tabacina*, but not TMV. When TMV-induced plants are held at increased temperature after challenge, at which N gene becomes ineffective, only induced resistance to blue mold but not to TMV is observed (Ye *et al.*, 1990b) even in the presence of elevated levels of peroxidase and PR-proteins 1-5. This is not unexpected, because induced resistance mechanisms against viruses and fungi are definitely different.

White and Antoniw (1991) suggest that induced resistance to viruses in tobacco is based on two principal mechanisms, the inhibition of virus replication and formation of PR-proteins. The inhibitor of virus replication (IVR) shown to be a 23 kDa protein has been detected in the intercellular fluid in the leaves of tobacco cv Samsun NN previously systemically immunized with TMV (Spiegal *et al.*, 1989). Purified IVR protected different plants against various viruses, thereby it cannot be viewed as virus- or host-specific (Gera and Loebenstein, 1983). The biological activity of IVR and its correlation with local induced resistance and systemic induced resistance in tobacco presume that it may be involved in these processes.

The second mechanism of induced resistance against virus infection incorporates production of PR-proteins (see chapter by Stermer). The majority of data obtained support the conclusion that PR-proteins are associated with induction of local induced resistance as well as systemic induced resistance to viruses in tobacco. It is established that PR-proteins are accumulated most intensively near the sites of local virus infection, i.e. precisely in those tissues, where resistance is induced (Antoniw and White, 1986). The content of the same PR-proteins found in infected leaves increases in systemically

induced tobacco leaves (White and Antoniw, 1991). In the varieties having temperature sensitive N gene, PR-proteins are produced at 20°C, when infection is local, but they do not appear at 32°C, when systemic spread of infection is in progress (White *et al.*, 1983).

The relation between PR-proteins and resistance to viruses has been confirmed by studying the interspecific hybrid *N. glutinosa x N. debnevi*, which contains PR-proteins and is highly resistant to TMV (Ahl and Gianinazzi, 1982).

However, some doubts are cast upon the involvement of the PR-proteins in induced resistance to viruses in tobacco (Fraser, 1982). Specifically, PR-protein is accumulated in large quantities in healthy tobacco plants during flowering that bears no connection with virus infection (Fraser, 1981).

It is worth noting that a correlation between the presence of a substance and resistance can not considered as evidence for its role in resistance. One can help clarify the role of PR-proteins in induced resistance to viruses by studying transgenic plants which constitutively express these proteins. It should be add that preliminary investigations in this field do not provide an answer to this question (Harpster *et al.*, 1989).

Local virus leaf infection with TMV leads to systemic activation of ß-glucanase and chitinase. However, this may explain the resistance of immunized tobacco tissue to fungi and bacteria rather than viruses (Ye *et al.*, 1990b; Pan *et al.*, 1991). Although the relation between activation of ß-glucanase and chitinase and induced resistance to blue mold in tobacco is apparent, Ye and colleagues consider the role of these enzymes and PR protein in general in inducing resistance to TMV to be highly unlikely (Ye *et al.*, 1990b).

There are indications for a systemic increase in the activity of peroxidase (Ye *et al.*, 1990a), phenylalanine ammonia-lyase and of the process of lignification (McMaster and Huang, 1984) in systemically protected with TMV tobacco plants.

White reported that acetylsalicylic acid (aspirin) induced resistance to TMV in tobacco (White, 1979). Later salicylic acid (SA) was found in TMV-inoculated tobacco varieties, having the N gene. The concentration of SA in necrotic tissues and near the sites of infection is 50 times larger than in control plants. In noninfected immunized leaves the concentration of SA increases 10 fold (Malamy *et al.*, 1990). An increase in the concentration of SA exclusively took place in the resistant tobacco variety with N gene, whereas this quantity remained constant upon infecting susceptible variety (nn). Increased temperature (32°C), which suppresses the activity of the N gene, also inhibits an increase in the concentration of SA (Yalpani *et al.*, 1991; Malamy *et al.*, 1992). Further investigations by these authors suggested that SA can be transported in the phloem and accumulate in virus-free leaves.

It is of interest to point out that in parallel with inducing systemic induced resistance, SA also causes a systemic expression of nine classes of mRNA, which code PR-proteins (Ward *et al.*, 1991). The same genes are expressed in response to TMV-inoculation of tobacco. The authors believe that induction of these gene families can be viewed as a molecular marker for recognizing systemic induced resistance. A strong correlation was

found between induced resistance and the enhanced systemic expression of PR-1 protein (Yalpani *et al.*, 1991; Malamy *et al.*, 1992).

Mobility of SA in combination with its inducing activity suggests that SA can serve as a translocated signal in resistance induction (Malamy *et al.*, 1990; Metraux *et al.*, 1990; Ward *et al.*, 1991; Yalpani *et al.*, 1991; Enyedi *et al.*, 1992). It is also well known that SA can conjugate with saccharides in plants (Enyedi *et al.*, 1992; Malamy *et al.*, 1992). Such conjugates in the form of O-ß-D-glucosyl-SA were reported to be present in the inoculated tobacco leaves (Enyedi *et al.*, 1992). Glucosyl-SA was found inside and outside of the site of infection, but not in phloem and immunized virus-free leaves (Enyedi *et al.*, 1991). Thus, it is unlikely that SA is delivered to phloem in the form of glucoconjugates. However, the balance between free salicylic acid and the glucoside may be important in the regulation of induced resistance (Hennig *et al.*, 1993).

Gaffney *et al* (1993) provided evidence of the need of salicylate for the expression of induced resistance in tobacco. They blocked the accumulation of salicylate by expression of the salicylate hydroxylase gene (*nah*G) from *Pseudomonas putida* in transgenic tobacco. They observed that the plants containing *nah*G were no longer able to express induced systemic resistance to TMV and that the size of the TMV lesions was larger in the transgenic as compared to the non-transformed controls.

SA is produced from trans-cinnamic acid which is produced by the action of phenylalanine ammonia-lyase (PAL) on phenylalanine. Based on a recent paper, cinnamic acid is converted to benzoic acid, and the benzoic acid is then 2-hydroxylated to form salicylic acid (Leon *et al*, 1993; Yalpani *et al.*, 1993). Understanding the biosynthesis SA in immunized tissues of tobacco will give us a better understanding of the role of SA in induced resistance and may provide insight into the role phenol metabolism in inducing plant resistance against viruses and other pathogens.

Finally, it is not inconceivable that the increased concentration of SA associated with induced resistance may result from the action of some preliminary molecular signal, which precedes SA in transduction of systemic induced resistance. It is unclear whether SA is capable of activating not only PR-proteins, but other defense mechanisms in tobacco, such as phytoalexins, proteinase inhibitors, thickening and lignification of cell walls. The observation that a salicylate binding protein from tobacco (Chen *et al.*, 1993) is a catalase that is inhibited by SA suggests that SA may act by increasing the levels of active oxygen species that may trigger defense responses (Chen *et al.*, 1994). Further studies should offer an explanation for the relation between SA and induced resistance not only in tobacco and cucumber (Metraux *et al.* 1990), but in other plants that exhibit induced resistance to disease.

## 4. Induced Resistance in *Lycopersicon*

Induced resistance to diseases in tomato has not been thoroughly investigated. However, there is evidence that induced resistance also occurs in this plant.

Inoculating the lower leaves of tomato with *P. infestans* increased the resistance to this pathogen (Heller and Gessler, 1986). The resistance was characterized by smaller and fewer necrotic lesions, reduced sporulation, and penetration into the epidermis was delayed or inhibited. When the fungus did not penetrate into epidermal cells, a papilla-like structure was formed. Penetration by hyphae was never followed by sporulation.

Use of cytologic methods demonstrated that growth of *P. infestans* on the surface of induced leaves was inhibited (Kovats et al, 1991). It is possible that this was due to the presence of fungitoxic compounds on the surface of induced plants. These compounds may influence the formation of cysts from zoospores or attachment of its spores to leaf surface. In the plants exhibiting induced resistance, the host mesophyll cells reacted to infection with a very strong hypersensitive response.

The development of the disease caused by *Fusarium oxysporum f. sp. lycopersisi* was noticeably limited in tomato plants inoculated with the mycorrhiza-producing fungus *Glomus mosseae* (Dehne and Schönbeck, 1975). Chlorosis was less pronounced on the leaves of mycorrhizal plants. Additionally, the rate of ion leakage from the stem and leaf cells of induced plants was less than observed in controls after challenge inoculation with *F.oxysporum*. This report also noted that phenol metabolism had changed and this was reflected in enhanced deposition of lignin in the cell walls of the induced host .

Local induced resistance to bacterial blight caused by *Corynebacterium michiganese pv. michiganese* was obtained in tomato (Sule, 1988). The defense response was induced by preinoculating plants with various strains of *Pseudomonas syringae pv. phaseolicola*. Only living cells of *Pseudomonas* induced resistance, and cells killed by heat or ultrasound treatment did not induce resistance. Multiplication of *C. michiganense* was restricted and infection was localized in induced tissues. Since the tissues as close as 5 mm from the site of induction were not protected against challenge, this resistance was not systemic.

Immersing tomato seedlings in a spore suspension from nonpathogenic strains of *Verticillium dahliae* or of the tomato nonpathogen *F. oxysporum f. sp. cucumerinum* also suppressed the development of wilt disease caused by *F. oxysporum* f.sp. *lycoperscici* or *V. albo-altrum*. Inducing activity was found to be associated with a macromolecular fraction of culture filtrates of pathogens (Amemiya 1985; 1986).

Field tests of tomato seed pre-treated with a biogenic elicitor (e.g., the LGP-complex and the preparations involving its active component) enhanced resistance of tomato plants to *P. infestans, Alternaria solani, Septoria lycopersici, Xanthomonas vesicatoria* (IvanYuk *et al.*, 1990). It was also established that plants from tomato seeds treated as above had induced resistance to the nematode *Meloidogyne incognita*, which is a serious greenhouse disease in Russia. The number of galls was considerably reduced on the roots of immunized plants, and the capability of female nematodes to produce eggs was also dramatically suppressed. The reduction in egg production makes soil sterilization in greenhouses much easier before successive planting (Zinovieva, 1989).

Nematodes as well as *Oomycetes* require an exogenous supply of sterols. Thus, it is possible that induced resistance to nematodes in tomato due to treated seed is based on

the same principle as induced resistance to *P. infestans* in potato, i.e. an inhibition of formation of the sterols necessary for pathogen growth along with simultaneous production of rishitin (Zinovieva, 1989; Zinovieva *et al.*, 1989).

## 5. Conclusions

Induced resistance in potato, tobacco, and tomato follows the same general patterns as that observed in other plant species. Induced resistance has been established for the three species of plants. Although we have learned a great deal about the induction and expression of induced resistance in these plants, new problems are posed today. I believe that the most crucial problem is the elucidation of the nature of the systemic signal or signals that are involved in resistance induction. It is also important to understand how the induction of the signal is regulated, by what mechanism the signal is synthesized or released, and how the plant stops the synthesis and export of the signal(s). Of all plant species examined in this family, tobacco is the best studied, with much less known about induced resistance in tomato and potato.

One can anticipate that identification of the nature of systemic molecular signals should allows us to develop new techniques for disease control in *Solanaceae* plants. Such techniques can be based on inducing resistance in plants by either molecular signals or the substances (methods) triggering these signals. It is possible that this approach will provide the resistance both to diseases and environmental stresses in *Solanaceae* plants.

Induced resistance most closely resembles horizontal or polygenic resistance. While it is not considered to be genetic resistance, induced resistance is a change in the resistance phenotype. By the action of an inducing agent, the degree of expression of existing genes is changed, while the plant genome is held constant.

Similar to polygenic resistance, induced resistance imparts a relative or partial protection in plants. This is more likely to be an advantage rather than a drawback. In fact, when inducing resistance to disease in plants, one frequently seeks to obtain not an absolute but rather a more stable relative protection. The high stability of multigenic resistance is associated with expression of many defense genes, and it is unlikely that any one pathogen could adapt to all of these defenses. Induced resistance also uses multiple mechanisms, and this suggests that this type of resistance should be stable.

However, a drawback for polygenic resistance is equally inherent to induced resistance. A noticeable reduction of protective action in some Solanaceous crops under conditions of high infection pressure has been observed. We have repeatedly observed that induced resistance against *P. infestans* in potato has been less efficient during epiphytotics.

The method of resistance induction in *Solanaceae* plants is unlikely to totally replace pesticide application or use of major genes for resistance, but it is highly effective as a complementary method of control and appears to lack many of the negative "side effects" of pesticide or major gene use. The aim of scientists and agricultural experts lies in a reasonable combination of the old and new techniques for plant disease control that will

ensure necessary conditions for an activation of potential defense systems in plants. Thus, induced resistance provides one additional technique to be used in combination with other control measures.

## REFERENCES


Ahl, P., Benjams, A., Samson, R. and Glaninazzi, S.. (1981) 'Induction Chez le Tabac par *Pseudomonas syringae* de Nouveles Proteines (Proteines "b") Associees au Developpement d'une Resistance Non-specifigue a une Deuxieme Infection', Phytopathologische Zeitschriít 102, 201-207.

Ahl, P. and Glaninazzi, S.. (1982) 'b-Protein as a constitutive component in highly (TMV) resistant interspecific hybrids of *Nicotiana glutinosa* x *Nicotiana debneyi*', Plant Science 26, 173-181.

Amemiya, Y., Hirano, K. and Jida, W.. (1985) 'Induction of resistance to Verticillium wilt in tomato', Technical Bulletin. Faculty of Horticulture. Chiba University., 36, 135-139.

Amemiya, Y., Yamaguchi, K., Hirano, K. and Jida, W., (1986) 'Suppression of Fusarium wilt of tomato by use of cross-protection', Technical Bulletin. Faculty of Horticulture Chiba University 37, 79-83.

Antoniw, J.F. and White, R.F., 'Changes with time in the distribution of virus and Pr-protein around single local lesions of TMV infected tobacco', Plant Molecular Biology 6, 145-149.

Avdyushko, S.A., Chalova, L.I., Ozeretskovskay, O.L., Chalenko, G.I., Bezuglov, V.V. and Bergelson, (1987a) 'Capability of prostaglandins to induce resistance to *Phytophthora infestans* in potato', Doklady Akademii Nauk SSSR 294, 982-985.

Avdyushko, S.A., Chalova, L.I., Ozeretskovskay, O.L., Chalenko, G.I., Imbs, A.B., Latyshev, N.A., Bezuglov, V.V. and Bergelson, L.D., (1987b) 'The products of lipoxygenase oxidation of polyenic acids induce resistance to *Phytophthora infestans* in potato', Doklady Akademii Nauk SSSR 296, 1012-1014.

Berezin, I.V. (ed) (1984)Biochemistry of Plant Immunity, 'Dormancy and Aging', (Moscow: Nauka), 264 pages (in Russian).

Bloch, C.B., Dewit, P.J.G.M. and Kuć, J., (1984) 'Elicitation of phytoalexins by arachidonic and eicosapentaenoic acid: A host survey', Physiological Plant Pathology 25, 199-208.

Bostock, R.M., Kuć, J.A. and Laine, R.A., (1981) 'Eicosapentaenoic and arachidonic acids from *Phytophthora infestans* elicit fungitoxic sesquiterpenes in the potato', Science 212, 67-69.

Bostock, R.M., Laine, R.A. and Kuć, J.A. (1982) 'Factors affecting the elicitation of sesquiterpenoid phytoalexin accumulation by eicosapentaenoic and arachidonic acis in potato', Plant Physiology 70, 1417-1424.

Bostock, R.M., McCue, K. and Creamer, J.R., (1985) 'Incorporation and distribution of 3H-arachidonic acid in the mycelial lipids of *Phytophthora infestans*', Phytopathology 75, 1297,

Bostock, R.M., Schaeffer, D.A. and Hammerschmidt, R., (1986) 'Comparison of elicitor activities of arachidonic acid, fatty acids, and glucans from *Phytophthora infestans* in hypersensitivity expression in potato tuber', Physiological and Molecular Plant Pathology 29, 349-360.

Chai, H.B. and Doke, N., (1987) 'Systemic activation of $O_2^-$ generating reaction, superoxide dismutase, and peroxidase in potato plants in relation to induction of systemic resistance to *Phytophthora infestans*', Annals of the Phytopathological Society of Japan 53, 645-649.

Chalova, L.I., Avdyushko, S.A., Chalenko, G.I., Daravaeva, K.A. and Ozeretskovskaya, O.L., (1989) 'Arachidonic and eicosapentaenoic acids as systemic long-term immunizators in potato', Doklady Akademii Nauk SSSR 303, 1262-1265.

Chalova, L.I., Avdyushko, S.A., Daravaeva, K.A., Yurganova, L.A. and Ozeretskovaskaya, O.L., (1988) 'Active component of the inductor of defense reactions in potato', Prikladnaya Biokhimiya i Mikrobiologiya 24, 789-794.

Chalova, L.I., Baramidze, V.G., Yurganova, L.A., Dyakov, Yu.T., Ozeretskovskaya, O.L., and Metlitskii, L.V. (1977) 'Isolation and characteristics of inductors of defense responses in potato from cytoplasm of *Phytophthora infestans*', Doklady Akademii Nauk SSSR 235, 1215-1216.

Chalova, L.I., Chalenko, G.I., Karapetyan, T.E., Leont'eva, G.V. and Ozeretskovskaya, O.L., (1980) 'Cytoplasmic glucans from *Phytophthora infestans* mycelium and their possible role in race specificity of this fungus', Doklady Akademii Nauk SSSR 255, 1012-1015.

Chalova, L.I., Nogaideli, D.E., Karavaeva, K.A. and Ozeretskovskaya, O.L., (1985) 'Activities of peroxidase and polyphenoloxydase as markers of systemic sensiblilization in potato tubers', Mikologiya i Fitopatologiya 19, 495-498.

Chalova, L.I., Ozeretskovskaya, O.L., Yurganova, L.A., Baramidze, V.G., Protsendo, M.A., D'yakov, Yu.T. and Metlitskii, L.V., (1976) 'Metabolites of phytopathogenic fungi as inductors of defense responses in plants', Doklady Akademii Nauk SSSR 230, 722-725.

Chen, Z., Ricigliano, J.W., and Klessig, D.F. (1993) 'Purification and characterization of a soluble salicylic acid binding protein from tobacco', Proceedings of the National Academy of Sciences 90, 9533-9537.

Chen, Z., Silva, H., and Klessig, D.F. (1994) 'Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid', Science 262, 1883-1886.

Cohen, Y., Jisi, U. and Mosinger, E., (1991) Systemic resistance of potato plants against *Phytophthora infestans* induced by unsaturated fatty acids', Physiological and Molecular Plant Pathology 88, 255-263.

Cohen, Y., Gesi, U., and Niderman, T. (1993) 'Local and systemic protection against *Phytophthora infestans* induced in potato and tomato plants by jasmonic acid and jasmonic-methyl ester', Phytopathology 83, 1054-1062.

Cruickshank, I.A.M. and Mandryk, M., (1960) 'The effect of stem infection of tobacco with *Peronospora tabacina* on foliage reaction to blue mold', Journal of Australian Institute of Agricultural Research 26, 369-372.

Dehne, H.W. and Schönbeck, F., (1975) ' Untersuchungen uber den Einfluss der Endotrophen Mycorrihiza auf die Fusarium-Welke der Tomate' Pflkrankheiten, 10, 75, 630-632.

Doke, N., Garas, N.A. and Kuć, J.A. (1979) 'Partial characterization and aspects of a hypersensitivity-inhibitory factor (HIF) isolated from *Phytophthora infestans*', Physiological Plant Pathology 15, 127-140.

Doke, N., Garas, N.A. and Kuć, J.A., (1980) 'Effect on host hypersensitivity of the suppressors released during germination of *Phytophthora infestans* cystospores', Phytopathology 70, 35-39.

Doke, N., Ramirez, A.V. and Tomiyama, K., (1987) 'Systemic induction of resistance in potato plants against *Phytophthora infestans* by local treatment with hyphal wall components of the fungus', Journal of Phytopathology 119, 232-239.

Enyedi, A.J., Yalpani, N., Silverman, P. and Raskin, I., (1992) 'Localization, conjugation, and function of salicylic acid in tobacco during the hypersensitive reaction to tobacco mosaic virus', Proceedings of the National Academy of Sciences (USA) 89, 2480-2482.

Farmer, E. and Ryan, C.A., (1990) 'Interplant communication: Airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves', Proceedings of the National Academy of Sciences (USA) 87, 7713-7716.

Fraser, R.S.S., (1981) 'Evidence for the occurrence of the pathogenesis related proteins in leaves of healthy tobacco plants during flowering', Physiological Plant Pathology 19, 69-76.

Fraser, R.S.S., (1982) 'Are pathogenesis related proteins involved in systemic acquired resistance of tobacco plants to tobacco mosaic virus?', Journal of General Virology 58, 305-313.

Gaffney, T., Friedrich, L., Vernooij, B., Negrotto, D., Nye, G., Uknes, S., Ward., E., Kessmann, H., and Ryals, J. (1993) 'Requirement of salicylic acid for the induction of systemic acquired resistance', Science 261, 754-766.

Garas, N.A., Doke, N.A. and Kuć, J.A., (1979) 'Suppression of the hypersensitive reaction in potato tubers by mycelial components from *Phytophthora infestans*', Physiological Plant Pathology 15, 117-126.

Gera, A. and Loebenstein, G., (1983) 'Further studies of an inhibitors of virus replication of a local lesion-responding tobacco cultivar', Phytopathology 73, 111-115.

Gorbacheva, L.A., Dudareva, N.A., Salganik, R.I., Chalova, L.I. and Ozeretskovskaya, O.L., (1990) 'Changing in the expression of the gene of hydroxyproline-rich glycoproteins in potato on the action of biogenic elicitors', Doklady Akademii Nauk SSSR 313, 1267-1269.

Harpster, M.H., Cutt, J.R., Dixon, D.C., Carr, J.P., Dunsmuir, P. and Klessig, D.F., (1989) 'Disease response of transgenic tobacco plants, which constitutively express

PR1b to tobacco mosaic virus (TMV)', Fundacion Juan March. Serie Universitaria 247, 91-98.

Hecht, E.I. and Bateman, D.F., 'Nonspecific acquired resistance to pathogens resulting from localized infection by *Thielaviopsis basicola* or viruses in tobacco Leaves', Phytopathology 54, 523.

Hennig, J., Malamy, J., Grykiewcz, G., Indulski, J., and Klessig, D. 1993) 'Interconversion of the salicylic acid signal and its glucoside', Plant Journal 4, 593-600.

Heller, W.I. and Gessler, C., (1986) 'Induced systemic resistance in tomato plants against *Phytophthora infestans*, Phytopathology 116, 323-328.

Ivanyuk, V.G., Chalova, L.I., Chalenko, G.I., Daravaeva, K.A. and Ozeretskovskaya, O.L., (1985) 'Increases in potato resistance at storage due to induction of protective responses from *Phytophthora infestans*, Vestnik Sel'skokhozyaistvennoi Nauki 10, 103-107.

Ivanyuk, V.G., Chalova, L.I., Yurganova, L.A., Karavaeva, K.A. and Ozeretskovskaya, O.L., (1990) 'Immunization of tomato plants by biogenic inducers of defense responses, Vestnik Sel'skokhozyaistvennoi Nauki 5, 144-146.

Il'inskaya, L.I., (1991) 'Biochemistry of resistance induction in potato plants', Doctorate thesis, Moscow, 320 pages (in Russian).

Il'inskaya, L.I., Vasyukova, N.I. and Ozeretskovskaya, O.L., (1991) 'Biochemical aspects of resistance induction and susceptibility in plants', VINITI. Reviews on Science and Technology. Plant Disease Control 7, 192 pp. (in Russian).

Kaneva, I.M., Chalenko, G.I., Vasyukova, N.I. and Ozeretskovskaya, O.L., (1991) 'Sterols of immunized potato tubers', Fiziologiya Rastenii (Moscow) 38, 1188-1197.

Kovats, K., Binder, A. and Hohl, H.R., (1991) 'Cytology of induced systemic resistance of tomato to *Phytophthora infestans*', Planta 183, 491-496.

Kuć, J. and Tuzun, S., (1990) 'Metabolic regulation of resistance genes in tobacco for the control of blue mold', In: Blue Mold Disease of Tobacco, *Proceedings of Symposium*, Releigh N.C., Feb. 14-17, (1988) (Main, C. and Spurr, H. [Eds.] The Tobacco Found, N.C. State University, Raleigh, NC).

Leon, J., Yalpani, N., Raskin, I., Lawton, M. (1993) 'Induction of benzoic acid 2-hydroxylase in virus-inoculated tobacco', Plant Phsyiology 103, 323-328.

Leont'eva, G.V., Chalenko, G.I., Medvedeva, T.E., Vasyukova, N.I. and Ozeretskovskaya, O.L., (1989) 'Inhibition of potato resistance by immunosuppressors of *Phytophthora infestans*', Prikladnaya Biokhimiya i Mikrobiologiya 25, 532-539.

Leont'eva, G.V., Yurganova, L.A., Chalenko, G.I, Chalova, L.I., Scherbakova, L.A., Vasyukova, N.I. and Ozeretskovskaya, O.L., 'Factor of compatibility in *Phytophthora infestans* cell walls in potato plants', Doklady Akademii Nauk SSSR 249, 1012-1016.

Leppik, R.A., Holloman, D.W. and Bottomley, W., (1972) 'Quiesone: An inhibitor of germination of *Peronospora tabacina* conidia', Phytochemistry 11, 2055-2063.

Lucas, J.A., Dolan, T.E. and Coffey, M.D., (1985) 'Nontransmissibility to regenerants from protected tobacco explants of induced resistance to *Peronospora hyoscyami*', Phytopathology 75, 1222-1225.

Malamy, J., Hennig, J., and Klessig, D.F. (1992) 'Temperature-dependent induction of salicylic acid conjugates during the resistance response to tobacco mosaic virus infection', The Plant Cell 4, 359-366.

Malamy, J., Carr, J.P., Klessig, D.F. and Raskin, I., (1990) 'Salicylic acid: A likely endogenous signal in the resistance response of tobacco to viral infection', Science 250, 1002-1004.

Maniara, G., Laine, R. and Kuć, J., (1984) 'Oligosaccharides from *Phytophthora infestans* enhance the elicitation of sesquiterpenoid stress metabolites by arachidonic acid in potato', Physiological Plant Pathology 24, 177-188.

McIntyre, J.L. and Dodds, J.A., (1979) 'Induction and systemic protection against *Phytophthora parasitica* var. *nicotianae* by tobacco mosaic virus infection of tobacco hypersensitivity to the virus', Physiological Plant Pathology 15, 321-330.

McIntyre, J.L., Dodds, J.A. and Here, J.D., (1981) 'Effects of localized resistance against diverse pathogens and insects', Phytopathology 71, 297-301.

McMaster, B.J. and Huang, B.-F., (1984) 'Phenylalanine ammonia lyase activation and lignification as an induced resposnse in tobacco plants' Phytopathology 74, 877.

Medvedeva, T.E., Vasyukova, N.I., Chalenko, G.I. and Ozeretskovskaya, O.L., (1985) 'The effect of suppressor of *Phytophthora infestans* on wound repair process in potato tubers', Doklady Akademii Nauk SSSR 283, 764-768.

Metlitskii, L.V., (1987) 'Immunity control in plants 45 Timiryazev Reading' (Moscow: Nauka), 69 pages (in Russian).

Metlitskii, L.V. and Ozeretskovskaya, O.L., (1973) Phytoalexins (Moscow: Nauka), 176 pages (in Russian).

Metlitskii, L.V. and Ozeretskovskaya, O.L., (1985) How plants protect against diseases' (Moscow: Nauka), 189 pages (in Russian).

Metlitskii, L.V., Ozeretskovskaya, O.L.., Chalova, L.I., Ivanyuk, V.G., Chalenko, G.I. and Platonova, T.A., (1985) 'Plant immunization by biogenic inducer of systemic defense responses', Doklady Akademii Nauk SSSR 283, 253-256.

Metlitskii, L.V., Ozeretskovskaya, O.L., Dorozhkin, N.A., Ivanyuk, V.G., Chalova, L.I., Yurganova, L.A. and Baramidze, V.G., (1978) 'Induction of resistance against pathogenic fungi in potato plants', Prikladnaya Biokhimiya i Mikrobiologiya 14, 262-270.

Metlitskii, L.V., Ozeretskovskaya, O.L. and Vasyukova, N.I., (1980) 'Phytosterols and their role in plant pathogenic fungi system, Illustrated by an Example of the Fungus of Pythiaceae', Uspekhi Sovremennoi Biologii 89, 28-40.

Metlitskii, L.V., Ozeretskovskaya, O.L., Vasyukova, N.I., Davydova, M.A. and Segal', G.M., (1976) 'The role of sterols in potato *Phytophthora infestans* system', Doklady Akademii Nauk SSSR 227, 244-247.

Metlitskii, L.V., Ozeretskovskaya, O.L., Yurganova, L.A., Chalenko, G.I. and Leont'eva, G.V., (1979) 'Factor of compatibility and its possible role in the

interaction between potato plants and *Phytophthora infestans*', Doklady Akademii Nauk SSSR 245, 479-483.

Métraux, J.P., Signer, H., Ryals, J., Ward, E., Wyass-Benz, M., Guadin, J., Raschdorf, K., Schmid, E., Blum, W. and Inverardi, B., (1990) 'Increase in salicylic acid at the onset of systemic acquired resistance in cucumber', Science 250, 1004-1006.

Müller, K.O. and Borger, H., (1940) 'Experimentelle Untersuchungen uber die Phytophtora - Resistanz der Kartoffel', Arbeiten Biologischen Anst. Reichsanstalt. (Berlin) 23, 189-231.

Ocheretina, O.Yu., Chalova, L.I., Karavaeva, K.A., Sokolovskii, V.Yu., Ozeretskovskaya, O.L. and Kritskii, M.S., (1991) 'The effect of biogenic inductors on the system of cyclic nucleotides in potato tubers', Prikladnaya Biokhimiya i Mikrobiologiya 27, 890-897.

Ozeretskovskaya, O.L. and Chalova, L.I., (1989) 'Induction of plant resistance against diseases (Molecular and Genetic Mechanisms of Interaction Between Microorganisms and Plants)', Puschnio, USSR, 178-184.

Ozeretskovskaya, O.L., Chalova, L.I., Avdyshko, S.A., Chalenko, G.I. and Yurganova, L.A., (1987) 'Arachidonic and eicosapentaenoic acids as an active component of inductor from *Phytophthora infestans*', Doklady Akademii Nauk SSSR 292, 738-741.

Ozeretskovskaya, O.L., Chalova, L.I., Avdyushko, S.A., Chalenko, G.I. and Karavaeva, K.A., (1988) 'Eicosanoid compounds as inducers of resistance in potato', Fiziologiya Rastenii 35, 175-183.

Ozeretskovskaya, O.L., Chalova, L.I., Chalenko, G.I., Karavaeva, K.A. and Avdyushko, S.A., (1986) 'Induction of systemic resistance against diseases by biogenic elicitors of protective response in potato plants', Izvestiya Akademii Nauk SSSR, Ser.Biol. 1, 24-31.

Ozeretskovskaya, O.L., Chalova, L.I., Karapetyan, T.E., Chalenko, G.I. and Metlitskii, L.V., (1983) '*Phytophthora infestans* glucans as anti-inducers of defense responses in potato plants', Doklady Akademii Nauk SSSR 269, 1006-1009.

Ozeretskovskaya, O.L., Vasyukova, N.I., Leont'eva, G.V., Chalova, L.I., Chalenko, G.I., Yurganova, L.A., Karapetyan, T.E. and Metlitskii, L.V., (1982) 'Factor of race specificity of *Phytophthora infestans*', Izvestiya Akademii Nauk SSSR, Ser. Biol. 6, 852-866.

Pan, S.Q., Ye, X.S. and Kuć, J., (1991) 'Association of ß-1,3-glucanase activity and isoform pattern with systemic resistance to blue mold in tobacco infected with *Peronospora tabacina* or leaf inoculation with tobacco mosaic virus', Physiological Molecular Plant Pathology 39, 25-39.

Parent, J.G. and Asselin, A., (1984) 'Detection of pathogenesis-related proteins (PR or b) and of other proteins in intercellular fluid of hypersensitive plants infected with tobacco mosaic virus', Canadian Journal of Botany 62, 564-569.

Platonova, T.A., Akenshina, G.V. and Ozeretskovskaya, O.L., (1982) 'The effect of the LGP-complex form *Phytophthora infestans* mycelium on cellular ultrastructure of potato tuber', Prikladnaya Biokhimiya i Mikrobiologiya 18, 607-614.

Pont, M., (1959) 'Blue mold (*Peronospora tabacina* Adam) of tobacco in North Queensland. Some aspects of chemical control', Queensland Journal of Agricultural Science 16, 299-327.

Preisig, C. and Kuć, J., (1985) 'Arachidonic acid related elicitors of the hypersensitive response in potato and enhancement of their activities by glucans from *Phytophthora infestans*', Archives of Biochemistry and Biophysics 236, 379-389.

Preisig, C. and Kuć, J., (1988) 'Metabolism by potato tuber of arachidonic, an elicitor of hypersensitive resistance', Physiological Molecular Plant Pathology 32, 77-88.

Ricker, K.E. and Bostck, R.M., (1992) 'Evidence for release of the elicitor arachidonic acid and its metabolites from sporangium of *Phytophthora infestans* during infection of potato', Physiological Molecular Plant Pathology 41, 61-72.

Ross, A.F., (1961a) 'Localized acquired resistance to plant virus infection in hypersensitive hosts', Virology 14, 329-339.

Ross, A.F., (1961b) 'Systemic acquired resistance induced by localized virus infections in plants', Virology 14, 340-358.

Ross, A.F., (1966) 'Systemic effects of local lesion formation in viruses of plants', 127, In: Beemster, A.B.R. and Dijkstra, J. [Eds.]. North Holland, Amsterdam, 1966.

Salt, S.D., Pan, S.Q., and Kuć, J., (1988) 'Carbohydrate changes in tobacco systemically protected against blue mold by stem infection with *Peronospora tabacina*', Phytopathology 78, 733-738.

Salt, S.D., Tuzun, S. and Kuć, J., (1986) 'Effects of ß-ionone and abscisic acid on the growth of tobacco and resistance to blue mold. Mimicry of effects of stem infection by *Peronospora tabacina* Adam', Physiological and Molecular Plant Pathology 28, 287-297.

Splegel, S., Gera, A., Salomon, R., Ahl., P., Harlap, S. and Loebenstein, G., (1989) 'Recovery of an inhibitor of virus replication from the intercellular fluid of hypersensitive tobacco infected with tobacco mosaic virus and from induced-resistant tissue', Phytopathology 79, 258-267.

Stolle, K., Zook, M., Shain, L., Hebard, F. and Kuć, J., (1988) 'Restricted colonization of *Peronospora tabacina* and phytoalexin accumulation in immunized tobacco leaves', Phytopathology 78, 1193-1197.

Stromberg, A. and Brishammar, S., (1991) 'Induction of systemic resistance in potato (*Solanum tuberosum*) plants to late blight by local treatment with *Phytophthora infestans*, *Phytophthora cryptogea* or dipotassium phosphate', Potato Research 34, 219-225.

Sule, S., (1988) 'Induced resistance in tomato against *Corynebacterium michiganense* pv. *michiganense* by prior inoculation with *Pseudomonas syringae* pathovars', Phytopathology 122, 343-350.

Tuzun, S. and Kuć, J., (1985a) 'A modified technique for inducing systemic resistance to blue mold and increasing growth of tobacco', Phytopathology 75, 1127-1129.

Tuzun, S. and Kuć, J., (1985b) 'Movement of a factor in tobacco infected with *Peronospora tabacina* Adam which systemically protects against blue mold', Physiological Molecular Plant Pathology 26, 321-330.

Tuzun, S. and Kuć, J., (1987) 'Persistence of induced systemic resistance to blue mold in tobacco plants derived via tissue culture', Phytopathology 77, 1032-1035.

Tuzun, S. and Kuć, J., (1989) 'Induced systemic resistance to blue mold of tobacco, 177-200, In: Blue Mold of Tobacco', McKeen, W.E. [Ed.] (American Phytopathological Society Press: St. Paul, Minnesota).

Tuzun, S., Rao, M.N., Vogeli, U., Scharde, C.H., and Kuć, J., (1989) 'Induction of systemic resistance to blue mold: Early induction and accumulation of ß-1,3-glucanases, chitinases, and other pathogenesis-related proteins (b proteins) in immunized tobacco', Phytopathology 79, 979-983.

Vasyukova, N.I., Davydova, M.A., Ozeretskovskaya, O.L., Metlitskii, L.V., and Segaĺ, G.M., (1977) 'Nutrition-inhibition hypothesis for phytoimmunity illustrated by an example of potato - *Phytophthora infestans* system', Mikologiya i Fitopatologiya 11, 488-497.

Vasyukova, N.I., Ozeretskovskaya, O.L., Leont'eva, G.V.,Chalenko, G.I., Elyakova, L.A., Zvyagintzeva, T.N. and Shirokova, N.I., (1987) 'Differences in the structure of ß-glucans of two *Phytophthora infestans* races', Bioorganicheskaya Khimiya 13, 825-832.

Ward, E.R., Uknes, S.J., Williams, S.C., Dincher, S.S., Wiederhold, D.L., Alexander, D.C., Ahl, P., Metraux, J.-P. and Ryals, J.A., 'Coordinate gene activity in response to agents that induce systemic acquired resistance', The Plant Cell 3, 1085-1094.

White, R.F., (1979) 'Acetylsalicylic acid (aspirin) induces resistance to tobacco mosaic virus in tobacco', Virology 99, 410-412.

White, R.F. and antoniw, J.F., (1991) 'Virus-induced resistance responses in plants', Plant Sciences 9, 443-455.

White, R.F., Antoniw, J.F., Carr, J.P. and Woods, R.D., (1983) 'The effect of aspirin and polyacrylic acid on the multiplication and spread of TMV in different cultivars of tobacco with and without the N-gene', Phytopathologische Zeitschrift 107, 224-232.

Yalpani, N., Leon, J., Lawton, M., Raskin, I. (1993) 'Pathway of salicylic acid biosynthesis in health and virus-infected tobacco', Plant Physiology 103, 315-321.

Yalpani, N., Silverman, P., Wilson, T.M.A., Kleier, D.A. and Raskin, I., (1991) 'Salicylic acid is a systemic signal and an inducer of pathogenesis-related proteins in virus-infected tobacco', The Plant Cell 3, 809-819.

Ye, X.S., Jarlfors, U., Tuzun, S., Pan, S.Q. and Kuć, J., (1991) 'Biochemical changes in cell walls and cellular responses of tobacco leaves related in systemic resistance to blue mold inducted by tobacco mosaic virus', Canadian Journal of Botany 70, 49-57.

Ye, X.S., Pan, S.Q. and Kuć, J., (1989) 'Pathogenesis-related proteins and systemic resistance to blue mold and tobacco mosaic virus induced by tobacco mosaic virus', Physiological and Molecular Plant Pathology 35, 161-175.

Ye, X.S., Pan, S.Q. and Kuć, J., (1990a) 'Activity, isozyme pattern, and cellular localization of peroxidase as related to systemic resistance of tobacco to blue mold (*Peronospora tabacina*) and to tobacco mosaic virus', Phytopathology 80, 1295-1299.

Ye, X.S., Pan, S.Q., and Kuć, J., (1990b) 'Association of pathogenesis-related proteins and activities of peroxidase, ß-1,3-glucanase, and chitinase with systemic induced resistance to blue mold but not to systemic tobacco mosaic virus', Physiological and Molecular Plant Pathology 36, 523-531.

Yurganova, L.A., Chalova, L.I., Nogaideli, D.E., Chalenko, G.I. and Ozeretskovskaya, O.L., (1989) 'Inducing activity of *Fusarium culmorum* Sacc.', Prikladnaya Biokhimiya i Mikrobiologiya 25, 73-79.

Yurganova, L.A., Nogaideli, D.E., Chalova, L.I., Chalenko, G.I., and Ozeretskovskaya, O.L. (1989) 'Activity of lipoxygenase in potato tubers after immunization', Mikol. Fitopatolol. 23, 73-79.

Zinovieva, S.V., (1989) 'Induction of plant resistance to parasitic nematodes by metabolites of phytopathogenic fungi', Part II, 264, Proceedings of All-Union Conference on Production and Application of Microbiological Approach to Disease Control, Moscow, USSR, (in Russian).

Zinovieva, S.V., Vasyukova, N.I. and Ozeretskovskaya, O.L., (1989) 'The role of sterols in the interaction between plants and parasitic nematodes', Doklady Akademii Nauk SSSR 309, 1273-1275.

Zook, M.N., Rush, J.S., and Kuć, J., (1987) 'A role for $Ca^{+2}$ in the elicitation of rishitin and lubimin accumulation in potato tuber tissue', Plant Physiology 84, 520-525.

# INDUCED RESISTANCE IN CUCURBITS

R. HAMMERSCHMIDT AND P. YANG-CASHMAN
*Department of Botany and Plant Pathology*
*Michigan State University*
*East Lansing, MI 48824-1312*
*USA*

## 1. Introduction

Induced or acquired resistance to disease has been intensively studied in three families of dicotyledonous plants. In the previous two chapters, the research with Legumes and Solanaceous plants was reviewed. In this chapter, the third dicot family, the Cucurbitaceae, will be examined.

Similar to studies of other dicotyledonous plants, induced resistance to disease in cucurbits can be either local or systemic in nature. In addition, many of the same features associated with induced resistance in Legumes or members of the Solanaceae are seen in cucurbit induced resistance: Requirement for necrotic lesion development to trigger the induced state; non-specificity of the induced resistance to fungi, bacteria and viruses; and systemic induction of defense-related proteins and the potential to express active defense upon challenge.

In this chapter, evidence for the existence of induced resistance will be examined in *Cucumis sativus, Cucumis melo*, and *Citrullus lunatus*. In addition, the nature of the resistance response and its regulation will be discussed, and avenues for future research suggested.

## 2. Biological Evidence for Induced Resistance in Cucurbits

### 2.1 CUCUMBER (*CUCUMIS SATIVUS*)

In the mid-1970's, Kuć and co-workers presented the first of a long series of papers on induced resistance in cucumber Kuć 1983, 1987). Hammerschmidt *et al.* (1976)



*R. Hammerschmidt and J. Kuć (eds.), Induced Resistance to Disease in Plants*, 63–85.

reported that inoculation of entire seedlings of scab (*Cladosporium cucumerinum*) susceptible plants with the bean anthracnose fungus *Colletotrichum lindemuthianum* resulted in resistance to a subsequent challenge inoculation with *C. cucumerinum*. In this same study, it was also reported that prior inoculation of entire scab resistant/anthracnose susceptible seedling with *C. cucumerinum* resulted in resistance to the anthracnose fungus, *Colletotrichum lagenarium*. These results showed that local resistance to disease could be induced in cucumber. Of more significance, however, was the 1975 report of Kuć *et al.* that described systemic resistance in cucumber. In this "classic" paper, the authors demonstrated that prior inoculation of one leaf of a cucumber plant with *C. lagenarium* resulted, within one week, in the systemic protection of the entire plant against subsequent challenge by *C. lagenarium*. This resistance was characterized by a decrease in the size and the numbers of lesions that developed. What was of great interest was not only the systemic nature of the resistance, but also that a compatible, necrotic lesion response could induce the resistance. This was clearly different from what had been reported in the bean-*C. lindemuthianum* system (see chapter by Deverall and Dann).

In a subsequent study, Kuć and Richmond (1977) described a number of parameters that were involved in the expression of systemic resistance of cucumber that was induced by and against *C. lagenarium*. They reported that systemic resistance could be elicited by inoculation of either a true leaf or one or both cotyledons. The induced resistance state was found to last at least 6 weeks after the initial inducing inoculation, and they also found that induced resistance was present in leaves that had not expanded at the time of the inducing inoculation. The final level of systemic induced resistance was found to be dependent upon the concentration of inoculum used to induce and number of lesions that developed on the leaf used to induce resistance. Inoculum concentrations as low as $10^3$ conidia per ml resulted in a significant increase in resistance. By varying the number of inoculation droplets containing conidia of *C. lagenarium*, the final level of systemic resistance could also be manipulated. Only one anthracnose lesion on the first leaf of a plant was sufficient to induce resistance in the second true leaf. Increasing the number of lesions on the first true leaf resulted in increasing the resistance of the plant to subsequent challenge by *C. lagenarium* on the second true leaf of the plants. All cultivars of cucumber tested were found to be inducible, and systemic increases in resistance could also be induced in cultivars that were genetically resistant to anthracnose.

Dean and Kuć (1986) showed that induced resistance could not be totally overcome even at high concentrations of challenge inoculum. In these experiments, plants were induced on one leaf with *C. lagenarium* and then challenged with droplets of inoculum on the second leaf. The concentration of challenge inoculum varied from $10^3$ to $10^7$ conidia per ml. Clear differences in the number and size of lesions were evident up to $10^6$ conidia per ml. At $10^7$ conidia per ml, all plants had similar numbers of lesions (based on counting both chlorotic and necrotic lesions). However, the induced plants had significantly fewer necrotic lesions and the total area of necrotic tissue per leaf was reduced.

Over the next several years, Kuć's group expanded our knowledge of the biological base for induced resistance in cucumber. Through these studies they demonstrated that a number of pathogens were capable of inducing resistance, and that resistance was non-specific and effective against a broad range of pathogens.

Building on the observations of Hammerschmidt *et al.* (1976), Staub and Kuć (1980) reported that *C. lagenarium* infection of one leaf would systemically protect scab susceptible plants against subsequent infection by *C. cucumerinum*. In this study, both anthracnose resistant and susceptible cultivars were used, suggesting that the development of a spreading necrotic lesion was not a prerequisite for induction of resistance. In addition, Staub and Kuć also demonstrated that *C. cucumerinum* infection of the first true leaf would protect the second leaf against infection by *C. lagenarium*. Induced resistance to *C. cucumerinum* was also found in plants that were pre-treated with a brief heat shock (Stermer and Hammerschmidt, 1988).

Jenns and Kuć (1977) were the first to demonstrate that prior inoculation of cucumber with tobacco necrosis virus (TNV), a pathogen that causes local necrotic lesions on cucumber, would induce systemic resistance to TNV and to *C. lagenarium*. Similar to studies of induced resistance by and against *C. lagenarium*, the TNV induced resistance was also characterized by smaller and fewer lesions. This study was significant as it showed that a fungal pathogen could induce systemic resistance to a virus and *vice versa*. However, the need for the development of a necrotic lesion prior to the elicitation of resistance was again demonstrated as non-necrosis inducing viruses such as tobacco mosaic virus did not induce systemic resistance.

Coutts and Wagih (1983) confirmed the results of Jenns and Kuć (1978). They found that prior infection of cotyledons with TNV resulted in increased resistance to TNV in adjacent cotyledons as well as in the first leaf. The resistance was characterized by a decrease in both the number and size of TNV lesions.

The ability of one pathogen type to induce resistance against another was further demonstrated by Caruso and Kuc (1979). They found that *C. lagenarium* would also induce systemic resistance against the bacterium *Pseudomonas syringae* pv. *lachrymans* (Psl) and that Psl was an effective inducer of resistance. The resistance induced by Psl against Psl and *C. lagenarium* was also found to be effective in all cultivars of cucumber tested. Varying the concentration of Psl cells used to induce resistance also had an effect on the final level of resistance. Low concentrations of Psl induced the lowest degree of systemic resistance while increasing the concentration resulted in higher level of resistance. Psl appeared to be an effective inducer because, like other effective inducers of resistance, this pathogen resulted in the development of necrotic lesions. Doss and Hevesi (1981) and Smith *et al.* (1991) subsequently confirmed that induced resistance could be elicited against and by Pseudomonads. In a later study, Jenns *et al.* (1979b) demonstrated the ability of TNV, Psl and *C. lagenarium* to act as inducers and challenge agents. As could be predicted by previous work, all three pathogens induced resistance and all three could be protected against. In addition, induced resistance against *C. cucumerinum* was also confirmed.

Bergstrom (1981) also demonstrated that cucumber plants could be protected against another fungal pathogen, *Mycopsphaerella melonis,* the cause of gummy stem blight. Resistance was observed in both foliage and in stem tissue of plants previous inoculated with *C. lagenarium.*

Prior inoculation with resistance-inducing pathogens has also been reported to induce resistance to cucumber mosaic virus (Bergstrom *et al.*, 1982). This disease is different from the other diseases as the symptoms of cucumber mosaic infection are not necrotic in nature. *C. lagenarium*, Psl or TNV infection of the first two leaves of cucumber plants induced resistance against CMV that was inoculated onto the plants by mechanical rubbing. The induced resistance was seen as a reduction in the number of chlorotic primary CMV lesions on the challenged leaf and a delay in the appearance of systemic mosaic symptoms throughout the plant. Bergstrom *et al.* (1982) also used CMV infested melon aphids to challenge inoculate induced and control plants. Although they did not observe any differences in primary symptom development on the aphid-infested leaves, the systemic spread of CMV (based on symptom expression) was greatly delayed in the induced plants.

Induced resistance to vascular wilts has also been reported. Bergstrom (1981) demonstrated that induction of resistance with *C. lagenarium* or TNV would protect the plants against wilt caused by *Erwinia tracheiophila.* The induced resistance was characterized by a delay in the development of wilt symptoms.

Gessler and Kuć (1982) provided more evidence for induced resistance to vascular wilts by inducing resistance to the root pathogen *Fusarium oxysporum* f.sp. *cucumerina* by inoculation of one leaf with *C. lagenarium.* This work not only demonstrated that roots could be induced, but the work also provided more evidence that the resistance inducing signal(s) moved to tissues below the inducing leaf as well as above. However, local induced resistance against *f.o. cucumerina* has also been demonstrated in cucumber by prior infection with *F.o. niveum* (a pathogen of watermelon) (Michail *et al.* 1989). The resistance reported in this latter study may include some systemic effects as plants that were preinoculated with resistance-inducing Fusaria exhibited resistance to anthracnose

Induced resistance has also been reported to be effective against obligate fungal pathogens. Infection of one leaf with TNV resulted in the systemic expression of resistance to the powdery mildew *Sphaerotheca fuliginea* (Bashan and Cohen, 1982; Conti *et al.*, 1990). The level of resistance was dependent upon the number of TNV lesions that developed on the leaf used for induction. The resistance was characterized by a significant reduction of powdery mildew colonies and a reduction in conidia produced. Induced resistance to the downy mildew fungus-like protist *Pseudopenonospora cubense* has also been reported to occur (Okuno *et al.*, 1991) and to not occur (Bashan and Cohen, 1982).

In summary, the induced resistance response in cucumber is a very non-specific response with regard to the type of pathogen used to induce resistance as well as the type of pathogen that is being protected against. As will be discussed later, the act of inducing resistance results in myriad systemic biochemical changes in the host. Each of

these changes may not play a role in the defense to a given pathogen (very different pathogens representing viruses, bacteria, fungi and protists were tested), but the fact that so many changes are induced or primed may help to explain why resistance is so broad-based.

## 2.2 MELON (*CUCUMIS MELO*)

Compared to the literature on induced resistance in cucumber, much less is know about induced resistance in other cucurbits such as melon. Caruso and Kuć (1977a, 1977b) reported that prior inoculation of anthracnose susceptible melon plants with *C. lagenarium* resulted in expression of systemic resistance to *C. lagenarium* under both greenhouse and field conditions. The timing and general characteristics of the resistance was very similar to what was seen in cucumber (Caruso and Kuć, 1977a).

Taking a different strategy, Esquerré-Tugayé and co-workers (Esquerré-Tugayé *et al.*, 1979) found that pre-exposure of melon plants to high levels of ethylene also induced resistance to a subsequent challenge inoculation with *C. lagenarium*. In these experiments, prior exposure if anthracnose-susceptible melons to 500 ppm ethylene gas resulted in the expression of resistance. This type of resistance was correlated with the enhancement of hydroxyproline-rich glycoproteins in the cell walls of the host. Roby *et al.* (1988) reported that fungal infection (thus confirming the work of (Caruso and Kuć, 1977a]) or treatment with a fungal elicitor would also induce systemic resistance in melon to *C. lagenarium*.

## 2.3 WATERMELON (*CITRULLUS LUNATUS*)

One of the first reports of induced resistance in cucurbits was the protection of watermelon seedlings against *Fusarium oxysporum* by prior inoculation of the roots with the pathogen of corn, *Helminthosporium carbonum* (Shimostsuma *et al.*, 1972). In these experiments, roots of susceptible watermelon seedlings were dipped into a spore suspension of *H. carbonum* prior to transplanting into *Fusarium*-infested soil. Resistance was characterized by a decrease in the severity of wilt symptoms.

More recently, Biles and Martyn (1989) have demonstrated that watermelon seedlings can be protected against virulent isolates of *F. oxysporum* f.sp. *niveum* by prior root inoculation with less virulent or avirulent isolates of the pathogen or by inoculation with the cucumber pathogen *F. oxysporum* f.sp. *cucumerina*. The resistance was most effectively induced by avirulent strains of the watermelon pathogen. The resistance was expressed both locally and systemically.

Similar to cucumbers and melons, inoculation of one leaf of watermelon with spores of the anthracnose fungus also resulted in systemic protection of the entire plant against subsequent infection by the same pathogen under both greenhouse (Caruso and Kuć, 1977a) and field (Caruso and Kuć, 1977b) conditions.

## 3. Effect of Induced Resistance on Arthropod Herbivores

One of the hall marks of systemic induced resistance in cucumber and other plants is the non-specificity of the resistance to a broad range of pathogens. Because of these observations, it has been suggested that the induction of systemic resistance to pathogens in cucumber may also result in increased resistance to arthropod herbivore feeding. Systemic increases in resistance to arthropod herbivore attack have been reported to occur in plants after mechanical damage or feeding injury and this includes cucurbits (Tallamy 1985).

Potter and co-workers carried out a series of experiments to test whether or not cucumber plants that were expressing systemic induced resistance were more resistance to attack by a number of arthropod herbivores. In the first study, Apriyanto and Potter (1990) induced cucumber plants by inoculation of one leaf with TNV. They confirmed that the plants were induced by challenge inoculations with *C. lagenarium*. Although the plants exhibited a high degree of resistance to *C. lagenarium*, no effect on the growth, development or behavior of two-spotted mites, fall army worm larvae or white flies was observed. Somewhat surprisingly, striped cucumber beetles fed more consistently on the induced as compared to control plants. Cucurbitacins, which act as feeding stimulants for these beetles, are know to increase after injury. However, TNV infection had no effect on cucurbitacin content. Thus, the increase in feeding by the beetles was the result of another factor.

In a subsequent study, Ajlan and Potter (1991) reported that induction of resistance in cucumber with *C. lagenarium* had no effect on population dynamics of the two-spotted spider mite, growth of fall army worm larvae or reproduction of melon aphids. Thus, these results confirmed their previous work. The authors were able to consistently induce systemic resistance to *C. lagenarium* by prior infection with this fungus. They also reported that feeding injury caused by the fall army worm larvae or spider mites did not induce systemic resistance to *C. lagenarium*.

It is probably not surprising that there was no reciprocal induction of resistance between pathogens and the arthropod herbivores. As discussed by Hammerschmidt (1993), it is probable that the types of signals that result in increased pathogen resistance and the mechanisms used for resistance are different than the signals and mechanisms involved in induced resistance to arthropod herbivores.

## 4. Mechanisms of resistance

In general, it can be assumed that the actual mechanisms of defense in plants exhibiting induced resistance should be very similar, if not identical to, the mechanisms used by plants exhibiting resistance that is controlled by resistance genes or exhibiting non-host resistance. Unlike legumes or the solanaceous plants, relatively little is known about the nature of defense in cucurbits that is controlled by resistance genes. In fact, much of our

current knowledge, as illustrated in this section, of cucurbit defense processes has been revealed by studies on the mechanisms expressed by induced plants.

## 4.1 FUNGAL DISEASES

4.1.1. *Colletotrichum lagenarium*. The first studies aimed at understanding the nature of resistance in induced resistance involved a histological investigation of the infection of cucumber by *Colletotrichum lagenarium*. Using light microscopy, Richmond *et al.* (1979) studied the efficiency of penetration of *C. lagenarium* into leaves of control cucumber plants or those that were previously induced by infection with *C. lagenarium*. Spore germination and appressorium formation was found to be similar on both control and induced plants. This observation indicated that the resistance to anthracnose was not the result of a chemical inhibitor that accumulated in or on the cuticle of induced plants. However, the number of successful penetrations of the pathogen into the epidermal cells of control plants was found to be much greater that into induced plants. There was no obvious hypersensitive response associated with the lack of infection into the induced plants. It was noted, however, that there was a difference in the ability of the appressoria to stain with cotton blue, although the reason for this was not apparent. Jenns and Kuć (1979a) subsequently reported that cucumber plants induced with TNV also allowed fewer penetrations by *C. lagenarium*. The observation that induced plants appear to stop penetration prior to infection has subsequently been confirmed by other researchers (Hammerschmidt and Kuć, 1982; Stumm and Gessler, 1986); Kovats *et al.*, 1990;)

A possible mechanism for the inhibition of penetration into induced leaves was first reported by Hammerschmidt and Kuć (1982). This report confirmed the previous observations of Richmond et al. (1979) and Jenns and Kuć (1979a) that induced plants were able to reduce penetration into the epidermis. The important role of the epidermis was shown by observing the decreased rate of infection of *C. lagenarium* into epidermal strips that were inoculated after removal from the plant. Hammerschmidt and Kuć (1982) also demonstrated that a much higher proportion of appressoria on petioles of induced plants that had no visible sign of penetration into the epidermis were associated with the deposition of a lignin-like polymer deposited in a halo-shaped structure under the appressoria. Comparison of time course data of penetration into control and induced plants revealed that the frequency of penetration (with no lignification) into controls was nearly identical with the frequency of non-penetration and lignification into the induced plants. These results suggested that resistance was being expressed at some early time during attempted penetration of the fungus into the host.

The lack of penetration into induced host tissue was later found to be very similar (anatomically and histochemically) to the non-host resistance of several species of cucurbits to non-pathogenic *Colletotrichum* species (Hammerschmidt *et al.*, 1985). These authors reported that *C. lagenarium* that was pathogenic on cucumber could not infect pumpkin or squash, and this lack of infection was correlated with a failure of penetration of the pathogen from appressoria into host epidermal cells and the appearance of a lignified halo around and under the appressoria. Attempts to infect cucumber with the

non-pathogen *C. atramentarium* revealed a similar response. This pathogen of potato was capable of germinating and producing appressoria on cucumber, but infection into the host was apparently blocked by the deposition of lignin in the outer epidermal cell wall of the plants. In fact, histologically and histochemically, the non-host response appeared identical to the induced resistance response to attempted infection.

The role for lignification as a component of induced resistance in leaves was supported by Dean and Kuć (1985). They found leaf discs from induced leaves would incorporate label from radioactive cinnamic acid more rapidly after challenge with *C. lagenarium* than did challenged uninduced discs. This report also indicated that induced plants have a greater capacity to deposit lignin after wounding, and this may be related to the rapid host responses to attempted infection.

Stumm and Gessler found that the failed infection of *C. lagenarium* into induced leaves was associated with the appearance of autofluorescent papillae under appressoria. These fluorescent papilla were associated with appressoria that did not successfully penetrate the epidermal cell wall. Although the chemical nature of these structures was not presented, the autofluorescence suggests that phenolic materials were present.

Kováts *et al.* (1991) recently reported on the cytological aspects of induced resistance in cucumber to *C. lagenarium*. These authors reported, unlike previous authors, that somewhat fewer appressoria were produced on leaves of induced as compared to control plants. In addition, Kováts et al. found that glucan-containing papilla were produced under appressoria. this suggested that glucans as well as lignins may be important in blocking penetration of *C. lagenarium* into induced tissues. These authors also reported that in leaves, lignification occurred primarily in the entire epidermal cell and, thus, may be involved in slowing pathogen development after infection into the host cell. This is different than the results of Hammerschmidt and Kuć(1982) who found lignified structures in the upper epidermal wall beneath appressoria. However, as pointed out by Kováts *et at.* (1991), they used leaf tissue while Hammerschmidt and Kuć (1982) examined epidermal strips from petioles. Thus, one plant organ may respond to induction in a slightly different manner than another.

In addition to the light microscopy studies, two ultrastructural studies have been reported by Xuie *et al.* (1988) and Stein *et al.* (1993). Xuei *et al.* reported, using both light and electron microscopy, that successful penetration occurred earlier and to a greater extent in control as compared to induced plants. Ultrastructural studies revealed that under the appressoria of *C. lagenarium* on induced plants was an electron dense material both within the cell wall and as a granular material in between the wall and the plasmamembrane. These changes could be observed as early as 24 hours after inoculation, and they appeared to be associated with the failure of the pathogen to penetrate through the wall. Infection hyphae in the outer epidermal cell wall of induced plants were constricted in size as compared to those seen in controls. The encasement of the fungal infection peg in dense cell wall materials and papilla was also observed.

In a later study by Stein *et al.* (1993), these ultrastructural changes reported by Xuei *et al.* (1988) were confirmed and expanded upon. In this study, ultrastructural cytochemistry of the cell wall changes was carried out. Using potassium permangante

as a stain for lignin (lignin reacts with soluble permanganate to form insoluble, electron-dense manganese dioxide), the areas of the wall showing alterations reacted positively for lignin. That the observed staining was from manganese deposition was confirmed using energy dispersive X-ray analysis. Only where lignin was presumed to be deposited was a strong signal for manganese found. Since the reports of both Stein *et al.* (1993) and Xuei *et al.* (1993) both used leaf tissue, the report by Kovats *et al* (1991) that no lignin was found under appressoria on induced cucumber plants may need to be re-examined. The size of the lignified area, as seen by transmission electron microscopy, was often smaller or the same size as the appressorium (Stein *et al.*, 1993). Thus, observation of the lignified part of the papilla formed on leaves may not be visible by light microscopy under the darkly pigmented appressorium.

Using energy dispersive X-ray analysis, Stein *et al.* (1993) also reported the presence of silicon under the appressoria that exhibited no penetration into the tissue. Silicon (as silicon oxides) has been reported to act as a general cell wall strengthening material in other host defense responses. Thus, it appears that induced cucumber plants use at least three means of strengthening cell walls to prevent infection:lignin, callose and silicon oxides.

4.1.2 *Cladosporium cucumerinum.* Hammerschmidt and Kuć (1982) reported that induced resistance of cucumber to *C. cucumerinum* was also associated with the deposition of lignin in the outer epidermal cell walls of the host at sites of attempted infection. This resistance response was histologically and histochemically identical to the response of resistant cultivars of cucumber to this pathogen. Unlike the response seen with *Colletotrichum*, entire epidermal cells around the infection site appeared to lignify after attempted infection by *C. cucumerinum*. This may be a reflection of the different mode of penetration by the two pathogens.

The association of lignification of the epidermis as a mechanism for resistance of cucumber to *C. cucumerinum* was previously reported by Hijwegen (1963) and Hammerschmidt *et al.* (1984). Histologically, the resistant and induced resistant host responses in cucumber to *C. cucumerinum* appear to be identical.

Heat shock induced resistance, unlike biologically induced resistance, appears to have a different mode of action. Stermer and Hammerschmidt (1987) reported that heat shock of scab susceptible varieties resulted in enhanced local resistance to infection by *C. cucumerinum*. However, unlike systemic resistance to this pathogen, no enhanced lignification was observed upon challenge with this fungus. The heat shocked plants did, however, express a higher content of hydroxyproline-rich glycoproteins in their cell walls. Because the hypocotyl cell walls of the heat shocked seedlings were less susceptible to degradation by cell wall degrading enzymes, it is possible that strengthening the walls with hydroxyproline rich glycoproteins may account for the enhanced resistance. The possible association of hydroxyproline rich glycoproteins in resistance of cucumber to *C. cucumerinum* had previously been reported by Hammerschmidt *et al.* (1984).

4.1.3 *Sphaerotheca fuliginea*. Infection of cotyledons or a lower true leaf of cucumber with TNV resulted in the induction of systemic resistance to the powdery mildew fungus, *S. fuligenia* (Bashan and Cohen, 1982; Conti *et al.*, 1990). Both groups reported an association of lignification with decreased penetration by the pathogen into induced host tissue. In the work reported by Conti *et al.* (1990), the germination rate of conidia was identical on both control and induced plants suggesting that the mechanism of protection was not based on a failure of the conidia to germinate.

## 4.2 BACTERIAL DISEASES

Although induced resistance against two bacterial disease, *Pseudomonas syringae* pv. *lachrymans* and *Erwinia tracheiphila* has been described, very little is know about the mechanisms used by the host plant to inhibit pathogen development. Caruso and Kuć (1979) reported that growth of *P. s. lachrymans* was inhibited in cumber plants previously induced with either the bacterium or with *C. lagenarium*. They found up to a three log decrease in the populations of bacteria in the induced as compared to the control plants. On the contrary, Doss and Hevesi (1982) found that cucumber plants that had been induced with either *C. lagenarium* or mercuric chloride exhibited a decrease in angular leaf spot symptoms in induced plants, but there was no effect of resistance induction on growth of the bacterium in the plant. They concluded that induced resistance was the result of a lack of symptom expression rather than a direct inhibition of bacterial growth.

More recently, Yang-Cashman and Hammerschmidt (Hammerschmidt *et al.*, 1993) have confirmed the results of Caruso and Kuć (1979). In the study by Yang-Cashman and Hammerschmidt, antibiotic-marked strains of *P.s. lachrymans* were used to monitor the populations of the bacterium in induced and control cucumber plants. They found that there was up to a 100-fold decrease in populations of the bacterium in the induced plants. They also found that at least part of the inhibition may be the result of some growth retardation factor(s) that were present in the plants as a result of induction, because re-isolation of bacteria from induced plants immediately after inoculation frequently resulted in a lower yield of viable cells than were found in control plants. The nature of the inhibitory substance(s), however, has not been identified.

Bergstrom (1981) found that resistance in cucumber plants could also be induced against the vascular wilt bacterium *E. tracheiphila*. Although no mechanisms for the resistance have been reported, Bergstrom did report on the presence of a bacterial agglutinating factor in xylem fluids that was present at higher titer in the induced as compared to the control plants. The possible role of this bacterial agglutinin in resistance has not been studied further. However, based on the mode of pathogenesis by this *Erwinia*, this agglutinin may be important.

## 4. Systemic Biochemical Changes Associated with the Induced Resistance State

The systemic nature of induced resistance implies that some types of biochemical or physiological changes must occur in the plant as a result of resistance induction. The purpose of this section is to review these types of changes and to relate them to the observed mechanisms of resistance described above.

### 4.1 PEROXIDASE

One clear resistance response that is known to occur in induced plants is the ability to more rapidly lignify at the point of attempted fungal infection. The last enzymatic step of lignification utilizes peroxidase, an enzyme that can generate lignin polymers by catalyzing the formation of free radicals of the lignin monomer precursors. In the early 1970's, Ross and co-workers reported that tobacco expressing systemic induced resistance to TMV had higher levels of peroxidase activity than did the non-induced controls. This observation in tobacco and the observation that induced cucumber will lignify faster than controls suggested that there may be an increase in peroxidase in cucumber after resistance induction.

Hammerschmidt *et al.* (1982) reported that prior inoculation of cucumber plants with *C. lagenarium* did result in a several-fold increase in peroxidase activity in the leaves above the induction site. Wounding or damage with dry ice had no systemic effect on peroxidase activity. The increase in peroxidase activity was found to parallel the amount of resistance that was induced. For example, inducing the plants with only one anthracnose lesion on leaf one resulted in a small, but significant, increase in total peroxidase activity and resistance in leaf two. Increasing the number of lesions from one to thirty on leaf one also resulted in a significant increase in peroxidase activity and resistance that paralleled the increase in the number of inducing lesions. Similarly, giving a second inducing inoculation was also found to increase not only the level of resistance, but also the total peroxidase activity. In time course studies, the appearance of enhanced peroxidase activity was also in agreement with the time of resistance induction.

Hammerschmidt *et al.* (1982) found that the increase in peroxidase activity was associated with an enhancement of three acidic peroxidase isoforms. Increases in these isoforms was found in all tissue types analyzed, and these isoforms appeared to be localized in the apoplast. Smith and Hammerschmidt (1988) followed up on these observations. They reported that a similar set of acidic peroxidases were also systemically induced in watermelon and muskmelon. The isoforms from these three cucurbits were found to range in size from 30 to 33kD. Serological analysis using antibodies raised against the cucumber peroxidases also indicated that the peroxidases from the three cucurbits were related. Genetic analysis of the segregation of these acidic peroxidases indicate that the isoforms are modifications of one gene or are part of a closely linked set of genes at a single locus (Dane 1983).

Smith (1988) purified the 33 kD peroxidase, and this preparation was used to obtain sequence information about the protein. Rasmussen *et al.* (1994) were able to clone a cDNA for the 33 kD peroxidase. This clone was different from a previously characterized peroxidase cDNA from cucumber (Morgens *et al.*, 1990). Using a portion of the clone as a probe, Rasmussen *et al.* (1994) were also able to demonstrate that the message for the peroxidase was induced systemically, and that the appearance of the message preceded the increase in peroxidase activity.

Stein *et al* (1993) used diaminobenzidine staining to attempt to localize peroxidase activity in induced leaf tissue. Possibly because of the high solubility of the induced isoforms, no increase in diaminobenzidine positive material was found in induced, but not challenged leaves. In induced leaves that were challenged with *C. lagenarium*, these authors found intense diaminobenzidine staining in the outer host epidermal wall that was directly under appressoria that had not penetrated. The pattern of diaminobenzidine staining was identical to that of lignin deposition. This indicated strong peroxidase activity in the wall where cell wall modifications were occurring. No diaminobenzidine staining was observed where successful penetrations had occurred.

In addition to pathogen attack, systemic accumulation of peroxidase can be induced by treatment of leaves with $K_2HPO_4$ (Irving and Kuć 1990). Di- and tri-basic potassium salts of phosphates were previously shown to induce systemic resistance in cucumber (Gottstein and Kuć, 1989), and thus this result was probably not unexpected. The phosphates did, however, induce the peroxidase activity more rapidly and to a higher level of activity than did infection with *C. lagenarium.*

## 4.2 CHITINASE

Chitinases have been hypothesized to play a role in active defense because of their potential antifungal activity. Métraux and Boller (1986) first reported that inoculation of one leaf of cucumber plants with *C. lagenarium, P.s. lachrymans* or TNV resulted in the local and systemic expression of chitinase activity. Most of the induced activity was found to be associated with an acidic chitinase. Subsequent work by Métraux *et al.* (1988) revealed that the acidic chitinase was one of the PR proteins of cucumber. Métraux and Boller (1988) further demonstrated that the chitinase was extracellular. This is important as most of the pathogens that could be affected by chitinase develop outside of the protoplast. Métraux *et al.* (1989) later cloned this chitinase and found strong systemic expression of the message after inoculation of one leaf with TNV. The sequence information indicated that the chitinase also had homology to bifunctional chitinase/lysozyme. The regulation of this chitinase was reported by Lawton *et al.* (1994). They found that the gene was developmentally regulated as well as being strongly induced by salicylate. Interestingly, the gene was slightly induced by cycloheximide (although cycloheximide treatment would block the strong induction by salicylate). This is similar to the results of Rutter (1987) who found that cycloheximide would block the heat shock-induced accumulation of acidic peroxidases, but that low levels of cycloheximide would also induce peroxidase activity.

Similar to the observations with peroxidase activity, Irving and Kuć (1990) also found that dibasic potassium phosphate would induce a systemic increase in chitinase activity. The rate of appearance and total activity of chitinase was more strongly induced by the phosphate treatment than by *C. lagenarium*. This, however, may be a reflection of the more rapid reaction of the host tissue that is seen after treatment with phosphates than is observed after inoculation with the fungus.

In addition to cucumber, Roby *et al.* (1988) reported that muskmelon plants inoculated with *C. lagenarium* on one leaf also exhibited systemic increases in chitinase activity. Fungal and endogenous elicitors were capable of inducing local increases in chitinase, but only the fungal elicitors could induce a systemic increase. The increase in chitinase was correlated with an increase in resistance to subsequent challenge by *C. lagenarium*.

## 4.3 CALLOSE SYNTHASE

As described above, the induced resistance response is associated with the inhibition of penetration by *C. lagenarium* into host tissue. This inhibition has been correlated with the deposition of lignin under the appressoria (Hammerschmidt and Kuć, 1982). In addition, callose (a ß-1,3-glucan) has also been found in papilla under the appressoria (Kováts *et al.* 1991). Callose, like lignin, has been thought to be part of the general cell wall strengthening that is involved in active defense.

Because of the increased ability of the induced cucumber plants to deposit callose, Schmele and Kauss (1990) examined the effect of systemic resistance induction on the activity of a plasmamembrane callose synthase. The activity of the cucumber enzyme was greater in systemically induced tissue as well as locally in tissue infected with TNV. Thus, and similar to the work with peroxidase and cinnamyl CoA ligase, systemic expression of resistance is correlated with a change in metabolism that appears to favor more rapid cell wall changes in response to attempted infection.

## 4.4 LIPOXYGENASE

The role of lipid peroxidation in defense responses has achieved increasing attention in recent years. The potential role of the enzyme lipoxygenase in systemic resistance of cucumber was recently reported by Avdiushko *et al.* (1993). In this report, the infection of one leaf of cucumber resulted in both a local and systemic increase in lipoxygenase activity. Although the role of lipoxygenase in the systemic resistance response is not clear, the observations that lipoxygenase can act to produce both potential signal molecules (e.g. jasmonic acid) and antimicrobial compounds indicates that this enzyme may play an important role in the induced resistance response.

## 4.5 PROTEINASE INHIBITORS

Roby *et al.* (1987) reported that infection of one leaf of melon resulted in a systemic increase in proteinase inhibitors. Although the role of proteinases in pathogen attack is unclear, it has been hypothesized that proteinase inhibitors may function in protecting the plants against arthropod herbivores (Ryan, 1991). This type of protection has not been evaluated in melons, but the lack of pathogen induction of resistance in cucumber to arthropod herbivores (see section 3 of this chapter) suggests that proteinase inhibitors may not play a role in systemic defense to insects or mites.

## 5.0 Systemic signals for resistance

The observation that infection of one leaf of cucumber results in the systemic expression of resistance in tissues both above and below the point of inoculation indicates that the infection results in the production of a signal or signals that induce the state of resistance. Early work by Kuć provided strong evidence for the presence of such signals, and this part of the chapter will review what is known about systemic signals for resistance in cucumber.

Since resistance is induced both above and below the point of inoculation, it has been hypothesized that the translocated signal for resistance is phloem mobile (Guedes and Kuć, 1980). Steam girdling of the inoculated leaf prior to inoculation results in a lack of resistance expression in other parts of the plant even though normal disease lesions develop on the inoculated leaf. Similarly, girdling the petiole of a leaf above the leaf used for resistance induction results in a lack of induced resistance in that leaf. Because xylem function was maintained through the steam-girdled areas, these results indicated that the signal was phloem (or at least symplast) mobile.

Jenns and Kuć (1979b) provided further evidence for a translocated signal by showing that the signal could move through successful graft unions. This work also demonstrated that cucumber, muskmelon and watermelon all use the same or similar signal(s) for resistance because grafting of watermelon or muskmelon scions onto cucumber rootstocks that were infected with *C. lagenarium* resulted in resistance induction in these other two cucurbits. The wounding and wound repair processes associated with the formation of the graft union had no obvious effect on resistance expression.

Dean and Kuć (1986a, 1986b) provided further evidence for the presence of a translocated signal and demonstrated that the inoculated leaf was the source of this signal. In the first paper (Dean and Kuc, 1986a), the inoculated leaf was shown to be the origin of the translocated signal by grafting uninduced scions onto induced rootstocks that still had the inoculated leaf intact. If the inoculated leaf was detached prior to making the graft union, no resistance was found in the scion. However, if the inoculated leaf remained on the rootstock, the scion did become resistant to infection by *C. lagenarium*.

In this same paper, Dean and Kuć also showed that induced, but not infected, leaves could not serve as a systemic signal source.

In a subsequent paper, Dean and Kuc (1986b) excised the *C. lagenarium* infected leaf at daily intervals after inoculation. By this procedure, the translocation of the signal was found to occur by 72 hours after inoculation. Using grafting experiments, these authors also provided evidence that functional phloem was needed for transport of the signal. This was based on the observation that removing the infected leaf from the rootstock prior to establishment of translocation through the graft resulted in no induced resistance in the scion.

Smith *et al.* (1991) demonstrated that systemic resistance to *C. lagenarium* and systemic increases in the acidic, apoplastic peroxidases could be rapidly induced by the hypersensitive response elected by *P.s. syringae*. In this work, resistance and peroxidase was systemically induced within 24 hours of inoculation of leaf one. Using the same leaf detaching strategy used by Dean and Kuc (1986b), Smith *et al.* (1991) showed that the first true leaf needed to be on the plant for only 6 hours for the systemic expression of a small, but significant increase in systemic resistance. This was prior to the visible appearance of the hypersensitive response, but clearly at a time when the events leading to hypersensitive death are underway. Thus, from both the work of Dean and Kuć (1986a, 1986b) and Smith *et al.* (1991), it is clear that the inoculated leaf was the source of the signal and that the generation of the signal was correlated with the initial stages of cell death.

Taking advantage of the fact that wounded stems and petioles readily exude phloem exudate, Métraux *et al.* (1990) were the first to report on a putative translocated signal for induced resistance in cucumber. These authors found that increased amounts of salicylic acid could be found in phloem exudates from the stem internode above the induced leaf. The increase in salicylic acid was found to just precede the appearance of induced resistance. Since salicylic acid was already known to have resistance-inducing activity in both tobacco (White, 1979) and cucumber (Mills and Wood, 1986), the results of Métraux *et al.* (1990) supported a role for salicylic acid as a signal molecule in cucumber.

Injection of salicylic acid into cucumber leaves or petioles was found to induce chitinase gene expression (Métraux *et al.*, 1989) as well as peroxidase activity (Rasmussen *et al.*, 1991) and acidic peroxidase gene expression (Rasmussen *et al.*, 1994). Reduction in anthracnose lesion size, total necrotic lesion area and penetration of *C. lagenarium* into salicylic acid treated cucumber cotyledons was also reported (Rasmussen *et al.*, 1991). These results support the possible role of salicylic acid as a factor in the expression of induced resistance in cucumber.

The role of salicylic acid as the translocated signal was, however, questioned by Rasmussen *et al.* (1991). In this report, the first true leaf was inoculated with *Pseudomonas syringae* pv. *syringae*. This bacterium rapidly induces systemic resistance (Smith *et al.*, 1991). In addition, after inoculation with this bacterium, the inoculated leaf only needed to be on the plant for as little as 6 hours to result in the expression of a low level of systemic resistance. Rasmussen and co-workers tested whether or not

salicylic acid was the translocated signal by detaching the inoculated leaf at intervals after inoculation and measuring the amount of salicylic acid in the phloem exudate of the inoculated leaf. They found that no increases in salicylic acid in the phloem of the first leaf were observed until eight hours after inoculation. Despite this, leaving the first leaf on the plant for only four hours resulted in increased levels of salicylic acid in the second true leaf. The amount of salicylic acid was measured at 24 hours after inoculation - 20 hours after the source leaf was removed. These results suggested that another signal was the primary, translocated signal, and that this signal resulted in the induction of salicylic acid. The nature of the primary signal, however, is unknown.

## 6. Natural and synthetic inducers of resistance

The use of exogenously applied chemicals for the induction of resistance in cucumber has a long history. In 1963, Hijwegen confirmed the previous work of vanAndel (1958) that treatment of scab susceptible cucumber seedlings with phenylserine resulted in protection of the seedlings against the scab pathogen, *C. cucumerinum*. He demonstrated that the phenylserine treated plants contained a higher amount of lignin, based on histochemical tests, but he did not conclude that enhanced lignification was part of the induced resistance response.

Mills and Wood (1983) found that pretreatment of cucumber plants with salicylic, acetylsalicylic or polyacrylic acids induced local, and to a lesser extent systemic, resistance to subsequent infection with *C. lagenarium*. Exogenous application of salicylic acid was also effective in inducing resistance to the downy mildew pathogen *Pseudoperonospora cubensis* (Okuno *et al.*, 1991).

In another study, Coutts and Wagih (1984) reported the induction of resistance to TNV by prior application of polyacrylic acid.

Growth regulators may also play a role in acquired resistance. Mills *et al.* (1986) found that application of the synthetic cytokinin, 6-benzylaminopurine, resulted in the expression of induced resistance to *C. lagenarium*. This suggests the changes in endogenous growth regulators may play a role in the expression of induced resistance.

Taking another approach, Doubrava *et al.* (1986) fractionated leaf tissue of spinach to determine if this plant contained chemicals that were capable of inducing resistance in cucumber. After a series of fractionations, they isolated a water soluble material that was able to effectively induce resistance to *C. lagenarium* in cucumber. The active material in this fraction was oxalic acid. These authors also found that the active resistance-inducing factor in rhubarb leaves was also oxalic acid. Modifications of the oxalate molecule (i.e. esterification of the acid groups) eliminated the resistance inducing activity. The induction of systemic resistance by oxalate was associated with the induction of a local chlorotic stippling of the treated leaves.

The efficacy of oxalate as an inducing agent was subsequently confirmed (Gottstein and Kuć, 1989). These authors also reported that di or tri-basic potassium phosphate

salts were also effective inducers of resistance to *C. lagenarium*. Similar to oxalate, the phosphate salts also induced a localized chlorotic stippling on the treated leaves.

The biological spectrum of phosphate and oxalate-induced resistance was reported by Mucharromah and Kuć (1991). In this study, the authors used both chemicals to induce resistance to the following fungal diseases: anthracnose, scab, gummy stem blight, and powdery mildew. Good resistance was also induced against the bacterium *P. syringae* pv. *lachrymans* and tobacco necrosis virus (TNV). Only small increases in resistance were seen in experiments using cucumber mosaic virus or the bacterial wilt pathogen, *E. tracheiophila*.

Synthetic resistance-inducing chemicals have also been reported. Application of the nicotinic acid derivative, 2,6-dichloroisonicotinic acid (INA), resulted in induced resistance to infection by *C. lagenarium,* and *P.s. lachrymans* (Métraux *et al.* 1991). INA appears to function by inducing resistance and is not converted to any antimicrobial compounds by the plant (Métraux *et al.*, 1991). Control of angular leaf spot by INA has also been observed in field trials using several cucumber cultivars (Hammerschmidt, unpublished results).

## 7. Future perspectives

Although a large body of literature on the induced resistance response in cucurbits has been generated over the last 20 years, there is still much to be learned. Several areas need to be addressed to help develop of full picture of induced resistance in this family. Work needs to be done to critically evaluate the putative defense mechanisms that have been described as part of the resistance response. Questions need to be asked and answered to determine the relative contribution of cell wall changes, chitinases, etc. in the restriction of pathogen development in induced tissues. The molecular regulation of the defense responses is also poorly characterized, and this type of information is needed to help fully understand the resistance mechanisms.

The work on cucurbit induced resistance has largely been confined to cucumber and, to a lesser extent, muskmelon and watermelon. Many of the members of the genus *Cucurbita* are important food crops in many parts of the world. Induced resistance, once understood in these species, could provide a safe, economical alternative or supplement for disease control in these crops.

More work is needed on the practical applications of induced resistance. Good disease control has been demonstrated using induced resistance as the sole control factor. However, much work needs to be done to find proper implementation procedures, the effectiveness of induced resistance under a variety of environmental conditions and pathogen pressures, and how induced resistance can be integrated into an overall scheme of disease control.

## References

Avdiushko, S.A., Ye, X.S., Hildebrand, D.F., and Kuć, J. (1993) 'Induction of lipoxygenase activity in immunized cucumber plants', Physiological and Molecular Plant Pathology 42, 83-95.

Avdiushko, S.A., Ye, X.S., and Kuć, J. (1993). 'Detection of several enzymatic activities in leaf prints of cucumber plants', Physiological and Molecular Plant Pathology 42, 441-454.

Ajlan, A.M. and Potter, D.A. (1991) 'Does immunization of cucumber against anthracnose by *Colletotrichum lagenarium* affect host suitability for arthropods', Entomologia et Experimentia Applicata 58, 83-91

Andebrhan, R., Coutts, R.H.A., Wagih, E.E. and Wood, R.K.S. (1980)'Induced resistance and changes in the soluble protein fractions of cucumber (*Cucumis sativus*) cultivar Baton Vert leaves locally infected with *Colletotrichum lagenarium* or tobacco necrosis virus', Phytopathologische Zeitschrift 98, 47-52.

Apriyanto, D. and Potter, D.A. (1990) 'Pathogen-activated induced resistance of cucumber: response of arthropod herbivores to systemically protected leaves', Oecologia 85, 25-31.

Bashan, B. and Cohen, Y. (1983) 'Tobacco necrosis virus induces systemic resistance in cucumbers against *Spaerotheca fuliginea*', Physiological Plant Pathology 23, 137-144.

Bergstrom, G.C. (1981) 'Biological induction of phsyiological resistance in cucurbits to *Erwinia tracheiphila, Mycospherella melonis,* and cucumber mosaic virus', PhD. Dissertation, University of Kentucky, Lexington.

Bergstrom. G.C., Johnson, M.C. and Kuć, J. (1982) 'Effects of local infection of cucumber by *Colletotrichum lagenarium, Pseudomonas lachrymans*, or tobacco necrosis virus on systemic resistance to cucumber mosaic virus', Phytopathology 72, 922-926.

Biles, C.L. and Martyn, R. (1989) 'Local and systemic resistance induced in watermelons by formae specialis of Fusarium oxysporum', Phytopathology 79, 856-860.

Boller, T. and Metraux, J.P. (1988) 'Extracellular localization of chitinase in cucumber', Physiological and Molecular Plant Pathology 33, 11-16.

Caruso, F.L. and Kuć, J. (1979) 'Induced systemic resistance of cucumber to anthracnose and angular leaf spot by *Pseudomonas lachrymans* and *Colletotrichum lagenarium*', Physiological Plant Pathology 14, 191-201.

Caruso, F.L. and Kuć, J. (1977) 'Protection of watermelon and muskmelon against *Colletotrichum lagenarium* by *Colletotrichum lagenarium*', Phytopathology 67, 1285-1289.

Caruso, F.L. and Kuć, J. (1977) 'Field protection of cucumber,watermelon and muskmelon against *Colletotrichum lagenarium* by *Colletotrichum lagenarium*', Phytopathology 67, 1290-1292.

Conti, G., Bassi, M., Carminucci, D., Gatti, L. and Bocci, A.M. (1990) 'Preinoculation with tobacco necrosis virus enahnces peroxidase activity and lignification in cucumber as a resistance response to *Sphaerotheca fuliginea*', Journal of Phytopathology 128, 191-202.

Coutts, R.H.A. and Wagih, E.E. (1983) 'Induced resistance to viral infection and soluble protein alterations in cucumber (*Cucumis sativus*) and cowpea (*Vigna sinensis*)', Phytopathologische Zeitschrift 107, 57-69.

Coutts, R.H.A. and Wagih, E.E. (1983) 'Induced resistance to viral infection and soluble protein alterations in cucumber (*Cucumis sativa*) and cowpea (*Vigna sinensis*) plants', Phytopathologische Zeitschrift 107, 57-69.

Dane F.(1983) 'Cucurbits', In, Isozymes in Plant Genetics and Breeding, Part B. Ed. by Tanksley SD, Orton TJ. Elsevier, Amsterdam. pp 369-390.

Dean, R.A. and Kuć, J. (1986a) 'Induced systemic protection in cucumber: Effects of inoculum density on symptom development caused by *Colletotrichum lagenarium* in previously infected and uninfected plants', Phytopathology 76, 186-189.

Dean, R.A. and Kuć, J. (1986b) 'Induced systemic protection in cucumber: The source of the "signal"', Physiological and Molecular Plant Pathology 28, 227-233.

Dean, R.A. and Kuć, J. (1986c) 'Induced systemic protection in cucumber: Time of production and movement of the signal', Phytopathology 76, 966-970.

Dean, R.A. and Kuć, J. (1987) 'Rapid liginifcation in response to wounding and infection as a mechanism for induced systemic protection in cucumber', Physiological and Molecular Plant Pathology 31, 69-81.

Doss, M. and Hevisi, M. (1981) 'Systemic acquired resistance of cucumber to *Pseudomonas lachrymans* as expressed in suppression of symptoms, but not in multiplication of bacteria', Acta Phytopathologica Scientarum Hungaricae 16, 269-272.

Doubrava, N.S., Dean, R.S. and Kuć, J. (1988) 'Induction of systemic resistance to anthracnose caused by *Colletotrichum lageanrium* in cucumber by oxalate and extracts from spinach and rhubarb leaves' Physiological and Molecular Plant Pathology 33, 69-79.

Esquerré-Tugayé, M.T., Lafitte, C., Mazau, D., Toppan, A., Touze, A., (1979) 'Cell surfaces in plant-microorganism interactions II. Evidence for the accumulation of hydroxyproline-rich glycoproteins in the cell wall of plants as a defense mechanism', Plant Physiology 64, 320.

Gessler, C. and Kuć, J. (1982) 'Induction of resistance to Fusarium wilt in cucumber by root and foliar pathogens', Phytopathology 72, 1439-1441.

Gottstein, H. and Kuć, J. (1989) 'Induction of systemic resistance to anthracnose in cucumber by phosphates', Phytopathology 79, 176-179.

Guedes, M.E.M., Richmond, S. and Kuć, J. (1980) 'Induced systemic resistance in cucumber to anthracnose as influenced by the location of the inducer inoculation with *Colletotrichum lagenarium* and the onset of flowering and flowering', Physiological Plant Pathology 17, 229-233.

Hammerschmidt, R. (1993) 'Nature and generation of systemic signals induced by pathogens, arthropod herbivores, and wounds', Advances in Plant Pathology 10, 303-337.

Hammerschmidt, R., Acres, S. and Kuć, J. (1976) 'Protection of cucumber against *Colletotrichum lagenarium* and *Cladosporium cucumerinum*', Phytopathology 66, 790-793.

Hammerschmidt, R., Bonnen, A.M., Bergstrom, G.C. and Baker, K.K.(1985) 'Association of lignification with nonhost resistance of cucurbits to fungi', Canadian Journal of Botany 63, 2393-2398.

Hammerschmidt, R., Nuckles, E.M. and Kuć, J. (1982) 'Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*', Physiological Plant Pathology 20, 73-82.

Hammerschmidt, R. and Kuć, J. (1982) 'Lignification as a mechanism for induced systemic resistance in cucumber', Physiological Plant Pathology 20, 61-71.

Hammerschmidt, R., Lamport, D.T.A. and Muldoon, E.P. (1984) 'Cell wall hydroxyproline enhancement and lignin deposition as an early event in the resistance of cucumber to *Cladosporium cucumerinum*', Physiological Plant Patholology 24,43-47.

Hammerschmidt, R., Yang-Cashman, P., Zook, M.N., and Rasmussen, J. (1994) 'Bacterial induced resistance to disease', in *Biotechnology and Plant Protection - Bacterial Pathogenesis and Disease Resistance* D.D. Bills and S-d Kung, eds. World Scientific, Singapore. pp. 315-326.

Irving, H.R. and Kuć, J. (1990) 'Local and systemic induction of peroxidase, chitinase and resistance in cucumber plants by potassium phosphate monobasic', Physiological and Molecular Plant Pathology 37, 355-366.

Jenns. A.E. and Kuć, J. (1977) 'Localized infection with tobacco necrosis virus protects cucumber against *Colletotrichum lagenarium*', Physiological Plant Pathology 11, 207-212.

Jenns, A.E. and Kuć, J. (1979) 'Graft transmission of systemic resistance of cucumbers to anthracnose induced by *Colletotrichum lagenarium* and tobacco necrosis virus', Phytopathology 69, 753-756.

Jenns, A.E, Caruso, F.L. and Kuć, J. (1979) 'Non-specific resistance to pathogens induced systemically by local infection of cucumber with tobacco necrosis virus, *Colletotrichum lagenarium*, or *Pseudomonas lachrymans*', Phytopathologica Mediterrnaia 18, 129-134.

Jenns, AE. and Kuć, J. (1980) 'Characteristics of anthracnose resistance induced by localized infection of cucumber with tobacco necrosis virus', Physiological Plant Pathology 17, 81-91.

Kovats, K., Binder, A. and Hohl, H.R. (1991) 'Cytology of induced systemic resistance of cucumber to *Colletotrichum lagenarium*', Planta 183, 484-490.

Kuć, J. (1983) 'Induced systemic resistance in plants to disease caused by fungi and bacteria', in *The Dynamics of Host Defense*, J. Bailey and B. Deverall, eds. Academic Press, Sydney, pp191-221.

Kuć, J. (1987) 'Plant immunization and its applicability for disease control', in *Innovative Approaches to Plant Disease Control*, I. Chet, ed. John Wiley and Sons, New York. pp 255-274.

Kuć, J., Shockley, G. and Kearney, K. (1975) 'Protection ofcucumber against *Colletotrichum lagenarium* by *Colletotrichum agenarium*', Physiological lant Pathology 7, 195-199.

Kuć, J. and Richmond, S. (1977) 'Aspects of the protection of cucumber against *Colletotrichum lagenarium* by *Colletotrichum lagenarium*', Phytopathology 67, 533-536.

Lawton, K.A., Beck, J., Potter, S., Ward, E., and Ryals, J. (1994) 'Regulation of cucumber class III chitinase gene expression', Molecular Plant-Microbe Interactions 7, 48-57.

Malamy, J., Carr J.P., Klessig, D.F. and Raskin, I. (1990) 'Salicylic acid: a likely endogenous signal in the resistance response of tobacco to viral infection', Science 250, 1001-1004.

Martyn, R.D., Biles, C.L. and Dillard, E.A. (1991) 'Induced resistance to fusarium wilt of watermelon under simulated field conditions', Plant Disease 9, 874-877.

Métraux, J.P., Signer, H., Ryals, J., Ward, E., Wyss-Benz, M., Gaudin, J., Raschdorf, K., Schmid, E., Blum, W. and Inverardi, . (1990) 'Increase in salicylic acid at the onset of systemic acquired resistance in cucumber', Science 250, 1004-1006.

Métraux, J.P., Burkhart, W., Moyer, M., Dincher, S., Middlesteadt, W., Williams, S., Payne, G., Carnes, M., Ryals, J. (1989) 'Isolation of a complementary DNA encoding a chitinase with structural homology to a bifunctional lysozyme/chitinase', Proceedings of the National Academy of Sciences, USA. 86, 896-900.

Métraux, J.P., Streit, L. and Staub, T, (1988). 'A pathogenesis related protein in cucumber is chitinase', Physiological and Molecular Plant Pathology 33, 1-10.

Michail, S.H., Sheir, H.M. and Rasmy, M.R. (1989) 'Cross protection of watermelon and cucumber plants against wilt by prior inoculation with an irrespective forma specialis of *Fusarium oxysporum*', Acta Phytopathologica et Entomologica Hungarica 24, 301-309.

Mills, P.R., Gussin, E.J. and Wood, R.K.S. (1986) 'Induction of resistance in cucumber to Colletotrichum lagenarium by 6-benzylaminopurine', Journal of Phytopathology 116, 11-17.

Mills, P.R. and Wood, R.K.S. (1984) 'The effects of polyacrylic acid, acetylsalicylic acid and salicylic acid on resistance of cucumber to *Colletotrichum lagenarium*', Phytopathologische Zeitschrift 111, 209-216.

Morgens, PH., Callahan, A.M., Dunn, L.J. and Abeles, F.(1990)'Isolation and sequencing of cDNA clones encoding ethylene-induced putative peroxidases from cucumber cotyledons', Plant Molecular Biology 14, 715-725.

Mucharromah, E. and Kuć, J. (1991) 'Oxalate and phosphates induce systemic resistance agaisnt diseases caused by fungi, bacteria and viruses in cucumber', Crop Protection 10, 265-270.

Okuno, T., Nakayama, M., Okajima, N. and Furusawa, I. (1991) 'Systemic resistance to downy mildew and appearance of acid soluble proteins in cucumber leaves treated with abiotic and biotic elicitors', Annals of the Phytopathological Society of Japan 57, 203-211.

Peng, M. and Kuć, J. (1992) 'Peroxidase-generated hydrogen peroxide as a source of antifungal activity in vitro and on tobacco leaf disks', Phytopathology 82, 696-699.

Rasmussen, J.B., Hammerschmidt, R., Zook, M.N. (1991) 'Systemic induction of salicylic acid accumulation in cucumber after inoculation with *Pseudomonas syringae* pv. *syringae*', Plant Physiology. 97, 1342-1347.

Rasmussen, J.B., Smith, J.A., Williams, S., Burkhardt, W., Ward, E., Somerville, S., Ryals, J. and Hammerschmidt, R. (1994) 'cDNA cloning and systemic expression of a peroxidase associated with systemic acquired resistance in cucumber', Physiological and Molecular Plant Pathology (in press).

Richmond, S., Elliston, J. E. and Kuć, J. (1979) 'Penetration of cucumber leaves by *Colletotrichum lagenarium* is reduced in plants systemically protected by previous infection with the pathogen', Physiological Plant Pathology 14, 329-338.

Roby, D. Toppan, A. and Esquerre-Tugaye, M.-T. (1987) 'Cell surfaces in plant-microorganism interactions. VII. Increased proeteinase inhibitor activity in melon in response to infection by Colletotrichum lagenarium or to treatment with an elicitor from this fungus', Physiological and Molecular Plant Pathology 30, 453-460.

Roby, D., Toppan, A. and Esquerre-Tugaye, M.-T. (1988) 'Systemic induction of chitinase activity and resistance in melon plants upon fungal infection or elicitor treatment', Physiological and Molecular Plant Pathology 33, 409-418.

Rutter, S. (1987) 'Studies on induced resistance in cucumber', MS Thesis. Michigan State University, East Lansing.

Ryan, C.A. (1991) 'Proteinase inhibitors in plants: Genes for improving defense against insects and pathogens', Annual Review of Phytopathology 28, 425-449.

Schmele, I. and Kauss, H. (1990) 'Enhanced activity of the plasmamembrane localized callose synthase in cucumber leaves with induced resistance', Physiological and Molecular Plant Pathology 37, 221-228.

Shimotsuma, M, Kuć, J. and Jones, C.M. (1972) 'The effect of prior inoculation with non-pathogenic fungi on Fusarium wilt of watermelon', HortScience 7, 72-73.

Smith, J.A. and Hammerschmidt, R. (1988) 'Comparative study of acidic peroxidases associated with induced resistance in cucumber, muskmelon and watermelon', Physiological and Molecular Plant Pathology 33, 255-261.

Smith, J.A., Fulbright, D.W. and Hammerschmidt, R. (1991) 'Rapid induction of systemic resistance in cucumber by *Pseudomonas syringae* pv. *syringae*', Physiological and Molecular Plant Pathology 38, 223-235.

Stein, B.D., Klomparens, K. and Hammerschmidt, R. (1993) 'Histochemistry and ultrastructure of the induced resistance response of cucumber plants to *Colletotrichum lagenarium*', Journal of Phytopathology 137, 177-188.

Stermer, B.A. and Hammerschmidt, R. (1987) 'Association of heat shock induced resistance with increased accumulation of insoluble extensin and ethylene synthesis', Physiological and Molecular Plant Pathology 31, 453-461.

Stermer, B.A. and Hammerschmidt, R. (1984) 'Heat shock induces resistance to *Cladosporium cucumerinum* and enhances peroxidase activity in cucumber', Physiological Plant Pathology 25, 239-249.

Stumm, D. and Gessler, C. (1986) 'Role of papillae in the induced systemic resistance of cucumber against *Colletotrichum lagenarium*', Physiological and Molecular Plant Pathology 29, 405-410.

Tallamy, D.W. (1985) 'Squash beetle (*Epilachna borealis*) feeding behavior: an adaptation against induced cucurbit defenses', Ecology 66, 1545-1579.

Tallamy, D.W. and Raupp, M.J. (eds). (1991) '*Phytochemical Induction by Herbivores*', John Wiley and Sons, NY, 431 pp.

Xuei, X.L., Jarlfors, U. and Kuć, J. (1988) 'Ultrastructural changes associated with induced systemic resistance of cucumber to disease: host response and development of *Colletotrichum lagenarium* in systemically protected leaves', Canadian Journal of Botany 66, 1028-1038.

# INDUCED DISEASE RESISTANCE IN MONOCOTS

ULRIKE STEINER AND F. SCHÖNBECK
*Institut fur Pflanzenkrankheiten und Pflanzenschutz*
*Universitat Hannover*
*Herrenhauser Str. 2*
*30418 Hannover*
*GERMANY*

## 1. Introduction

The major agronomic crops wheat, rice, barley and corn are all classified as graminaceous monocots. They are cultivated worldwide on an area of 578 million hectares and annually produce 1769 million tons of grain. Thus, these crops play a prominent role in production of food for the growing world population. Although diseases and pests have always accompanied agricultural plant production, only a few complete solutions for plant protection have been developed. In most cases, the disease problems could be solved only for a limited time. In spite of the increasing use of pesticides, yield losses caused by diseases and pests increased in the major crops in absolute as well as in relative numbers in the last 25 years (Oerke and Schönbeck, 1992). The reasons for this increase are the intensive agricultural practices with increased fertilizer use, a higher susceptibility of high-yielding cultivars and insufficient plant protection procedures. Therefore, phytomedical research must continuously search for new possibilities and methods for improving plant protection. These efforts are a necessary supplement to existing procedures to maintain the productivity and health of plants, and thereby ensure a longterm productivity of agriculture.

Most of the conventional chemical and biological plant protection procedures, as well as the use of disease-resistant cultivars, tend towards the direct control of pests and diseases by their elimination. However, and especially in monoculture crops like wheat, rice, barley and corn, these practices raise problems. For example, the risk for development of pesticide resistant populations of pathogens is enhanced by long term use of the same chemical. In addition, it is well known that resistance genes of the plants may also be overcome by changes in the pathogens. Obviously, there is a demand for procedures, which supplement the use of resistance breeding and chemical control in



*R. Hammerschmidt and J. Kuć (eds.), Induced Resistance to Disease in Plants,* 86–110.

plant protection with new strategies that may replace or complement the traditional methods.

To a certain degree, susceptible plants can be altered in their resistance against pathogens without genetic manipulation . This enhancement in resistance in response to an extrinsic stimulus without a known alteration of the genome is called induced resistance. The protection is based on the stimulation of defense mechanisms by metabolic changes that enable the plants to defend themselves more efficiently. Through induced resistance, an increased level of resistance in plants is accessible in a short time whereas conventional breeding is a lengthy procedure which often influences other positive properties negatively (i.e., yield and quality).

The phenomenon of induced resistance has been known since the beginning of the century. Since publication of the first review by Chester (1933) on alteration of resistance after infection with pathogens, numerous publications with different host-parasite systems have proven the efficacy of induced resistance against viruses, bacteria and fungi. Most of this work, however, was with dicotyledonous plants. Considering the economic importance of monocotyledonous crop plants, the number of investigations of plants in this Order is comparatively small. The aim of this chapter is to provide an overview of induced resistance in monocots discussing the following aspects: procedures of induction, characteristics and criteria, the changes in plant metabolism accompanying the impaired development of the pathogens, and the significance of induced resistance as a practical means for plant protection with special regard to the yield response.

## 2. Biotic and abiotic inducers

Inducing agents can be either biotic or abiotic. As demonstrated in dicotyledonous plants, two kinds of procedures are also used to induce resistance in monocots: preinoculations with nonpathogens, incompatible races, saprophytes or symbionts; pretreatments of the plants with naturally-occurring metabolites or chemically defined substances.

Preinoculations with avirulent or virulent pathogens induced local resistance against powdery mildew in barley and wheat (Ouchi *et al*, 1976; Kunoh *et al*, 1985; Sahashi and Shishiyama 1986; Schweizer *et al*, 1989; Smedegaard-Petersen, 1990) and for blast disease in rice (Iwano, 1987). Gregersen und Smedegaard-Petersen (1989) induced resistance in barley with previous inoculations with the saprophytic fungus *Cladosporium macrocarpum*. Hwang und Heitefuß (1982) systemically protected spring barley against powdery mildew by prior infection with compatible and incompatible races of the fungus.

The formation of necrosis as a prerequisite of induced resistance have been described frequently (Doke *et al*, 1987). This has lead to the conclusion that metabolic reactions of the plants involved in this process act as the inducer (Kuc 1983). In barley, an increase of inoculum density of the avirulent mildew isolate used for induction caused a higher degree of necrotization of the plant tissue and this was correlated with the degree of induced resistance (Chaudhary *et al*, 1983; Cho and Smedegaard-Petersen,

1986; Thordal-Christensen and Smedegaard-Petersen, 1988). Nevertheless, a general requirement of necrotic lesions is questionable. Although protection was higher after preinoculation with an incompatible race, Hwang and Heitefuß (1982) also induced systemic resistance in spring barley by preinoculation with a compatible race of *Erysiphe graminis* f. sp. *hordei* that did not cause a hypersensitive reaction or necrotization of the plant tissue. Preinoculations with *Erysiphe graminis* f. sp. *avenae* and *Puccinia coronata* f.sp. *avenae* on barley decreased the susceptibility against *Erysiphe graminis* f. sp. *hordei* and preinoculations with *Erysiphe graminis* f.sp. *hordei* on oat resulted in disease reductions caused by *Erysiphe graminis* f. sp. *avenae* (Villich-Meller und Weltzien,1990).

Reports are available on the effectiveness of naturally-occurring and synthetic chemical compounds as abiotic inducers of resistance in susceptible plants. Rathmell (1984) has described a wide range of synthetic chemicals that can stimulate resistance in rice against *Pyricularia oryzae* and in wheat against *Puccinia recondita*. Isonicotinic acid and its derivatives have been patented as substances for the enhancement of plants resistance reactions (Kunz *et al*, 1988). Salicylic acid is also known as a resistance-inducing substance in various dicotyledonous plants against virus and fungal diseases. Walter *et al* (1993) showed recently that besides treatments with salicylic acid, sodium salicylate and acetysalicylic acid, application of the phenolic acids vanillic acid, iso-vanillic acid and syringic acid also led systemically to reductions in mildew infection of barley.

Plant metabolites have also been shown to effectively induce resistance. Barley treated with extracts from wild and ornamental plant species reduced disease severity of powdery mildew up to 90% when challenge inoculated three days after the application of the inducers (Klingauf und Herger, 1985). Extracts from infected barley cv. Bigo leaves, applied to susceptible barley cv. Xenia seedlings, reduced susceptibility to *Puccinia striiforminis* infection (Reiss 1986). Yamada *et al* (1990) und Hiramoto *et al* (1992) systemically induced resistance in juvenile leaves against powdery mildew with extracts from seeds of barley and wheat.

Kristensen *et al* (1993) prepared crude extracts from conidia of *Erysiphe graminis* f. sp. *hordei* which had the ability to induce local protection in barley against powdery mildew. Treatments of rice seedlings with spores and toxins of *Pyricularia oryzea* increased the resistance of the plants against these fungi (Ouyang *et al* 1987). Fujiwara *et al* (1987) hypothesized that wounded barley plants synthesized volatile substances that decreased the susceptibility of undamaged plant parts.

Metabolites of microorganisms have only been studied to limited extent, and we have yet to exhaust these organisms as a source of resistance-inducing substances. Systemic resistance against different biotrophic fungi like rusts, powdery and downy mildew was induced in plants of various families by the application of microbial metabolites or of a purified protein enriched fraction isolated from a culture filtrate of a *Bacillus subtilis* isolate selected in a screening of more than 100 bacteria and 100 fungi (Schönbeck *et al*, 1980; Steiner 1990). Reiss *et al* (1988) showed that the culture filtrates of isolates of *Bacillus pumilus* and of *Erwinia herbicola*, applied 3 days prior to inoculation, induced

resistance against *Puccinia striiformis, P. hordei* and *Erysiphe graminis* on barley. The great number of known abiotic resistance inducing agents, not all are listed here, and the variability in their specificity suggests that many inducers can be found for induced protection of plants.

There currently exists more information about induced resistance against biotrophic fungi in monocots as compared to dicots for which the number of articles concerning the protection against perthotrophic fungi is higher. One reason may be that biotrophic fungi, i.e. powdery mildew and rust, cause the most important diseases of cereals. Thus, most of the literature reviewed in this chapter will be concerned with induced resistance in graminaceous monocots against biotrophic fungi.

## 3. Markers for induced resistance

Induced resistance is distinguished from conventional chemical as well as biological procedures in plant protection by the lack of toxicity of the inducing agents towards the pathogens. The protection of the plants is not based on the elimination of the pathogens but rather on the activation of plant defense mechanisms or on the enhancement of their activity. The basic idea behind induced resistance is that genes for resistance or defense reactions exist in all plants. These genes are not expressed until after a resistance-inducing treatment activates or enhances their expression, or that changes in the plant metabolism modify the activity of such genes. Induced resistance is considered to be a biological plant protection procedure in which the plant is the target of the procedure, not the pathogens.

The criteria listed below can contribute to the verification of induced resistance. Induced resistance is more difficult to prove for abiotic inducers than for induction by previous infections. Biochemical markers that specifically correlate with induced resistance are yet not available for monocots or dicots.

- Absence of toxic effects of the inducing agents on the pathogens
- Suppression of the induced resistance by a previous application of specific inhibitors, such as actinomycin D, which affect gene expression of the plant.
- Necessity of a time interval between application of the inducer and the onset of protection in the plant
- Absence of a typical dose-response correlation known for toxic compounds.
- Nonspecifity of protection
- Local as well as systemic protection
- Dependence on the plants genotype causing significant differences in level and type of protection in different cultivars.

In monocots, the induction of resistance by microbial metabolites is characterized by the fulfillment of all of these criteria (Schönbeck *et al*,1993). The application of metabolites enhanced resistance against diseases caused by fungal species from the

Peronosporales, Erysiphales and Uredinales under greenhouse and field conditions. Thus, the induced resistance showed nonspecifity by protecting plants against pathogenic fungi of different phylogenetic origin. However, the protection was effective only against biotrophic parasites which form haustoria in their host cells. Perthotrophic fungi and bacteria were not affected. For establishing the resistance response in plants, an interval between inducer application and inoculation with the pathogen of at least 2 days is required. With the metabolites of *B. subtilis*, the optimal time interval for induction of resistance in various barley and wheat cultivars was 48 hours. If the plants were treated with the transcription inhibitor actinomycin D (10 ppm) before or at the same time as treatment with the inducer, resistance was not established. This indicated that transcriptional processes are involved (Steiner, unpublished). Characteristically, the protection induced by microbial metabolites was not complete, but the degree of protection could not be increased by longer time intervals between induction and challenge, by higher concentrations of the inducer, or by more frequent applications of the inducer. As shown with barley cultivars, environmental factors and the genotype of the plants determined the effectiveness of the plant defense mechanisms (Steiner *et al*, 1988; Oerke *et al*, 1989). The application of the microbial inducer led to a systemic protection of the upper plant parts indicating that the inducer or a signal triggering the induced protection is xylem transmissible.

For use in plant protection, the types of inducers that would be ideal are those which lead within a short time interval, to a long lasting protection. For example, induction of resistance in tobacco was an irreversible process. Buds from protected plants grafted on non-protected rootstocks developed into fully grown induced resistant plants (Tuzun and Kuć, 1989). The irreversibility of this phenomenon in tobacco suggest that gene expression can be permanently changed leading to more disease resistance. The genetic and regulatory implications of these observations are profound, as are the possibilities they present for the economic control of plant disease in the field.

## 4. Mechanisms of induced resistance

Induced resistance in monocots appears to be the result of complex processes in plant metabolism. The following theoretical conception of the sequence of events occurring after application of the inducer and the challenge inoculation is derived from the knowledge about single reactions and may be taken as a summary of the present state of knowledge (Figure 1). How the mechanisms involved in signal triggering and transduction and in defense gene activation in monocots differ from that in dicots is not known.

### 4.1. SIGNALING

In spite of the remarkable progress about the key events in the resistance of plants

against pathogens, knowledge of the reception, recognition and transport of microbial or abiotic signals is fragmentary. This is also true for induced resistance where the basic mechanisms are not yet sufficiently understood in any system. The resistance inducing agents may be the signal itself or it may trigger the synthesis of yet unknown signal(s)

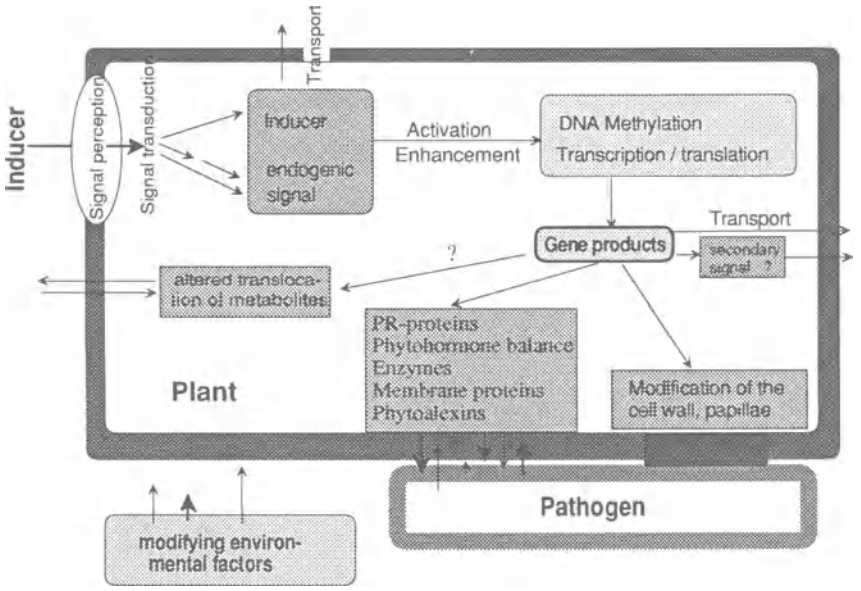


**Figure 1.** Sequence of metabolic changes in plant cells after induction of resistance and challenge infection (modified from Schönbeck, *et al.*, 1993).

that are translocated systemically in the plant. The signal has attributes of a plant hormone. It is produced in one part of the plant and acts in another and is presumably produced in small quantities since it has not yet been isolated or identified. Independent of the kind of the inducing agent (biotic or abiotic origin), signal transduction can be divided hypothetically into an intracellular and an intercellular transfer which lead at least to the systemic protection.

Possible mediators in intracellular signal transduction could be at the membrane level ion fluxes and/or at the cytoplasmatic level. Membrane level phenomena include changes in ion fluxes while cytoplasmic changes may include changes in concentration of ions like calcium or potassium or cyclic nucleotides (Dixon *et al*, 1990). The significant increase in $K^+$-concentration and alkalization of the intercellular space of barley leaves which occurred a few minutes after application of resistance-inducing microbial metabolites and was sustained for a few hours indicate also that ion fluxes may be mediators in signal transduction on the membrane level (Steiner, unpublished).

Preinoculation of barley coleoptiles with *Erysiphe pisi* preconditioned the plant cells to defend themselves more efficiently against *Erysiphe graminis* f. sp. *hordei*. Kunoh *et*

*al* (1988, 1990) and Kobayashi *et al* (1990) proposed that a signal which induced resistance in single epidermal cells was released by the nonpathogen during the early stages of appressoria formation.

It has been proposed that resistance is induced systemically by an endogenous signal produced in the inducer treated leaf and transported to other parts of the plant (Kloepper *et al*, 1992). An increase in the concentration of salicylic acid was reported to be associated with induced resistance in dicotyledonous plants. This compound induces resistance after exogenous application, and Malamy *et al* (1990) and Metraux *et al* (1990) suggest that salicylic acid may be an endogenous signal for systemic induced resistance. However, recent work by Rasmussen et al (1991) suggested that salicylic acid was not the primary signal. In barley, the uptake of $^{14}C$-labelled salicylic acid into the treated first leaves was rapid, but very little was systemically translocated. Within 24 hours only 1.4% of the salicylic acid applied to the first leaf was found in the second leaf (Walter *et al*,1993).

During the past few years, several new plant encoded signaling molecules possibly involved in plant-pathogen interaction have been described. These include jasmonic acid and systemin (Farmer und Ryan 1992, Van Loon und Pennings 1993). Recent results by Wastemack *et al* (1993) showed that in monocots jasmonic acid and its methyl ester were not involved in resistance mechanisms mediated by systemic induced resistance. In addition, application of the resistance inducer 2,6-dichloro-isonicotinic acid did not elevate the level of endogenous jasmonic acid or methyl jasmonate acid in barley and wheat.

## 4.2. DEFENSE GENE ACTIVATION

The role of gene activation and changes in protein synthesis are poorly understood for most models of induced resistance (See chapter by Stermer). Changes in the activity of genes in barley with induced resistance against powdery mildew are indicated by a reduced methylation of the genomic DNA after the application of trigonelline, isonicotinic acid methyl ester and a purified inducer fraction of the metabolites produced by a *Bacillus subtilis* isolate (Kraska and Schönbeck, 1993). A reduced level of DNA methylation is in general correlated with higher gene activity. The first changes occurred 1 day after application of isonicotinic acid methyl ester and 2 days after application of trigonelline and the bacterial metabolites. In time course experiments, the total genomic DNA methylation could be reduced by 5.1% for isonicotinic acid methyl ester, 4.5% for trigonelline and 3.6% for the microbial metabolites. These results are in accordance with observed decreases in infection densities after induced resistance for different time intervals. It is possible that the resistance inducer affected the chromatin structure by reducing the level of DNA methylation. However, from these experiments it is not clear which genes could be affected by the inducer and whether they are directly involved in defense mechanisms. However, the experiments support the idea that resistance reactions in plants can be altered by a modifying chromatin structure and gene regulation.

Biochemical analysis have suggested that peroxidases, chitinases (Irving and Kuć, 1990) and ß-1,3-glucanases (Fischer *et al*, 1988) are involved in the mechanisms of

induced resistance of dicotyledonous plants against perthotrophic fungi. This was confirmed by first molecular investigations of protein synthesis in induced resistant plants. In wheat, a m-RNA which was present in higher a concentration after inoculation with an nonpathogenic organism was cloned and the sequence was analyzed. It was the m-RNA of a peroxidase (Schweizer *et al*, 1989). Also in barley, m-RNA synthesis was induced after preinoculation but this message could not be attached to known genes or enzymes (Gregersen *et al* 1990). In wheat, Rebmann *et al* (1991) were able to identify, after induction of local resistance by preinoculation with *Erysiphe graminis* f. sp. *hordei*, an induced cDNA-clone encoding for a thaumatin-like protein. These investigations represent the first steps in research on isolation and activation of genes in monocotyledonous plants after induction of resistance against fungal pathogens.

## 4.3. PLANT DEFENSE RESPONSES

Morphological changes in the host plants have not been found during the interval between induction of resistance and challenge inoculation. Only after inoculation, are enhanced cytoplasmic aggregations, papillae formation and lignification (Asada and Matsumoto, 1987; Ride and Barber, 1987) visible signs of increased resistance. Ultrastructural studies suggest that induced resistance in monocots and dicots is associated with a reduction of pathogen penetration into the plant and rapid formation of larger papillae (Sahashi and Shishiyama 1986; Ebrahim-Nesbat *et al*, 1983). As shown with barley cultivars, however, enhanced papilla formation was not the only cause for the reduced infection density. Depending on the genotype of the plant, it was observed that the number of successful penetration sites was diminished without cell wall modifications after the induction of resistance with metabolites of *Bacillus subtilis*. The reduction in disease severity was also not correlated with a higher occurrence of hypersensitive reactions of the plant cells before or after haustoria development (Steiner 1989).

Yokoyama *et al* (1991) extracted a so- called papilla-regulating extract from healthy barley leaves which induced resistance against powdery mildew in barley and wheat after application to coleoptiles. This induced resistance was correlated with the formation of oversized papillae which accumulated autofluorescing metabolites.

Although increased activities of chitinases and glucanases are often correlated with systemic induced resistance in dicots (Roby *et al*, 1988; Binder *et al*, 1989; Tuzun *et al*, 1989; Metraux *et al*, 1991; Pan *et al*, 1992), these correlations could not be shown clearly for monocots. Smith and Metraux (1991) induced systemic resistance in rice against *Pyricularia oryzea* by preinoculation with *Pseudomonas syringae*, but found no correlation between enhanced resistance and increased activity of these enzymes.

Enhanced formation of phytoalexins and the activation of enzymes of the phenylpropanoid pathway (PAL, TAL, catechol-methyltransferase) was found in Oryzemate-treated susceptible rice plants after infection with *Pyricularia oryzae* indicating that plant defense mechanisms were activated (Sekizava and Watanabe, 1981). More evidence that the phenylpropanoid pathway is involved in induced resistance in rice

against *Pyricularia oryzae* was given by Ouyang *et al* (1987). They measured increasing activities of the phenylalanine ammonia-lyase and p-coumarate:CoA ligase.

New reports on the induction of local resistance in wheat and barley induced by preinoculation with the nonpathogens *Erysiphe graminis* f. sp. *tritici* or Erysiphe graminis f. sp. hordei described the sequences of peroxidases coding cDNA-clones (Schweizer *et al*, 1989; Thordal-Christensen et. al., 1992). Their possible function for an enhanced formation of papillae is yet unclear.

Treatment of wounded wheat leaves with chitin or non-pathogenic isolates of *Botrytis cinerea* caused rapid lignification and protected the leaves against subsequent infection by either *Fusarium graminearum* or *Penicillium oxalicum* (Ride and Barber 1987). The degree of protection correlated with the lignin-inducing capacities of the pretreatments, but their was no significant protection against subsequent challenge by the appressoria-forming pathogens *Pyricularia oryzae* or *Erysiphe graminis* f. sp. *tritici*. These results support the proposal that lignification can be involved in induced resistance of monocots only towards certain fungi (Vance and Sherwood 1977).

In monocots, with resistance induced against biotrophic fungi by the metabolites of a *Bacillus subtilis* isolate, the enhancement of enzyme activities involved in secondary metabolism like peroxidases, phenylalanine ammonialyases (PAL), and polyphenoloxidases (PPO), the synthesis of lignin and soluble phenols, and the production of chitinases and ß-1,3-glucanases do not appear to have any key function. Time course studies showed that the application of inducers enhanced the peroxidase activity in the treated wheat and barley leaves but not in the systemically protected upper plant parts (Steiner, unpublished)

## 4.4. IMPAIRED NUTRITION AS RESISTANCE FACTOR AGAINST BIOTROPHIC FUNGI

Induction of resistance by the microbial metabolites of *B. subtilis* against the biotrophic fungi neither affect conidial germination nor the formation of appressoria on the leaf surfaces as proven with rust and powdery mildew on wheat and barley (Stenzel *et al*, 1985; Steiner, 1989). Fungal development became affected at the time of contact with the host cells. The formation of primary haustoria was reduced by about 50%, and this correlated with the lower number of colonies which subsequently developed on the protected leaves (Table 1). The colonies of *Erysiphe graminis*, on barley as well as on wheat, were smaller and developed less mycelium than the colonies on untreated plants. Fewer conidiophores per unit area of colony and fewer conidia per conidiophore were produced, and this resulted in drastically reduced sporulation rates of the fungus. Additionally, the infectivity of the conidia as well as of ascospores was reduced up to 30% compared to those formed on untreated plants (Steiner, 1989, Dehne *et al*, 1984) indicating an impaired nutrition of the fungi. From these observations it appears that if once the parasitic relationship is in disorder the subsequent stages of pathogen development were also affected by induced resistance.

The impaired growth and sporulation of powdery mildew colonies on induced resistant plants not only depends upon the reduced number of secondary haustoria, but also on reduced efficiency of these fungal structures (Table 2). The fewer haustoria in induced resistant barley remained smaller (but without any signs of malformation). Therefore, the surface for nutrient uptake was reduced. The specific efficiency of these haustoria, quantified by the formation of secondary mycelium per $um^2$ haustorial surface, was diminished. This indicated that the haustoria had an impaired ability for nutrient uptake.

The ultrastructure of the haustoria in the induced plants was not altered, but the reduced haustorial efficiency was associated with an enlargement of the extrahaustorial matrix (Stenzel *et al*, 1985). This matrix is probably the cause of the impaired nutrient flow into the haustoria. More frequently, the extrahaustorial membranes were invaginated into the extrahaustorial matrix and appeared as single tubes and branched clusters of vesicles. This indicated that secretory processes of the plant were participating in the

Table 1. Effect of resistance induced by metabolites of *Bacillus subtilis* on the different developmental stages of colonies of *Erysiphe graminis* f.sp. *hordei* on winter barley (Steiner 1989, modified).

| Fungal structures | Untreated Plants | Treated Plants | %Change |
|---|---|---|---|
| Primary haustoria[1] | 46 | 25* | -46 |
| Colony number/leaf | 27 | 14* | -48 |
| Colony size ($mm^2$) | 6.3 | 4.1* | -35 |
| Haustoria number | | | |
| per colony | 1443 | 767* | -47 |
| per $mm^2$ | 229 | 187* | -18 |
| Conidiophores/$mm^2$ | 79 | 49* | -38 |
| Conidia/conidiophore | 2.8 | 1.7* | -40 |
| Conidia volume ($\mu m^3$) | 3671 | 2898* | -21 |

[1]per 100 germinated conidia

*significantly different from control (p=0.05)

**Table 2.** Effect of resistance induced by metabolites of *Bacillus subtilis* on the efficiency of haustoria of *Erysiphe graminis* f.sp. *hordei* in winter barley (Steiner, 1989, modified)

| Fungal Structures | Untreated plants | Treated plants | %Change |
|---|---|---|---|
| $\mu m^2$ haustorial surface | 5918 | 5351 | -11 |
| nm mycelium/ $\mu m^2$ haustorial surface | 506 | 360* | -29 |
| $10^{-4}$ conidia/ $\mu m^2$ haustorial surface | 2.2 | 1.3* | -41 |

*Significantly different from untreated control

resistance by increasing the matrix. Additionally, in the plant cytoplasm, osmiophilic material accumulated at the extrahaustorial membrane. Such structural changes could inhibit transport processes at the haustorial membrane (Figure 2). The enlargement of the extrahaustorial matrix as well as the accumulation of osmiophilic material seems to be an indicator for the altered compatibility between host cell and biotrophic fungi. Differences in the composition of the extrahaustorial membrane during haustoria development were found with fluorescent dyes showing alterations in the pattern and amount of protein-, ion- and $Ca^{+2}$ -binding sites. However, as in susceptible plants, the extrahaustorial membrane of the haustoria possessed no ATPases activity. The local disturbance in the physiology of the membrane may lead to an altered permeability, which caused a reduced nutrient supply of the fungus and subsequently the poorer pathogen development.

The impaired nutrition of fungi on induced plants was reflected by an altered carbohydrate supply. The extent starch accumulation under young powdery mildew colonies on induced resistant plants was smaller compared to colonies on susceptible plants. This correlated with the lower number of haustoria and the smaller size of green islands, which represent areas of high sink activity, that eventually formed. Additionally, the haustoria in induced resistant plants showed, at the end of the photoperiod, a reduced glycogen content compared to that in susceptible plants (Steiner, 1989). Besides an altered permeability of the extrahaustorial membrane, the lower sink activity induced by the developing colonies (indicated by smaller green islands) could account for a lower content of storage carbohydrates.

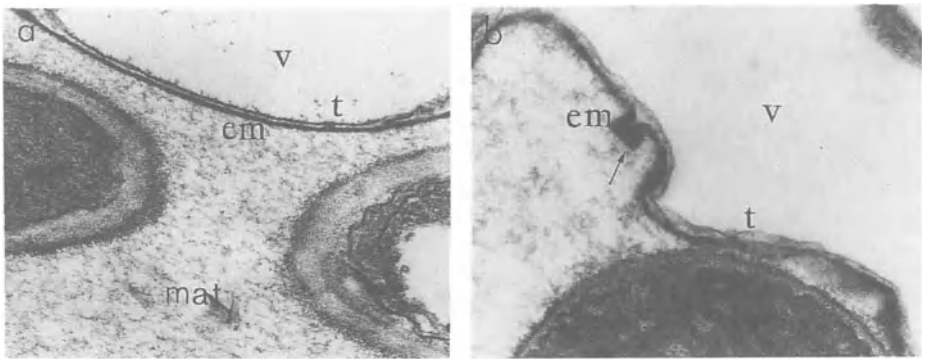


**Figure 2.** Effect of resistance inducing metabolites on the structure of the extrahaustorial membrane of secondary haustoria of *Erysiphe graminis* in winter barley 7 days after inoculation. A). Untreated plants; B). induced plants. Note osmiophilic deposits. (em=extrahaustorial membrane; t=tonoplast; mat=extrahaustorial matrix; v=vacuole).

Reduced nutrient supply and reduced haustorial efficiency are considered to be related to partial resistance (Carver *et al*, 1978). This type of resistance also leads to restricted colony development of obligate biotrophic fungi. Because of the physiological and cytological similarities of induced resistance to the partial resistance, induced resistance may possibly activate or enhance mechanisms occurring in partial resistance. The potential for the resistance mechanisms are obviously present in susceptible plants. The mode of action of induced resistance, resulting in an impairment of fungal nutrition by reduced haustorial efficiency, may explain the limitation of this type of resistance to obligate biotrophic fungi.

## 5. Applicability to disease control

Although induced resistance offers a significant potential for the control of plant diseases, it currently plays only a minor role in practical disease control. One reason is the very limited number of investigations under practical conditions to determine the efficacy and stability of induced resistance under natural infection pressure. Induced resistance will have to be brought from the laboratory to the field and greenhouse. Under those conditions, induced resistance will have to be proven to be efficient and reliable This is especially true with monocots because of their importance in food production.

According to Kuć (1987), a major problem encountered with the use of induced resistance for the control of plant disease in the field is not its effectiveness but rather the economics of the application technology and the delivery of the immunizing agent.

In spring barley, Pelcz (1989) induced resistance in the field with an avirulent race of *Erysiphe graminis* to subsequent challenge by virulent races in the natural population of *E. graminis*. However, restricted foliar inoculation with avirulent or virulent pathogens or injecting inoculum into stems (as is successfully done in tobacco, (Tuzun and Kuć, 1989) are clearly not practical means of inducing resistance in major crops with high numbers of individual plants. The preparation and costs of inoculum as well as the risks of its delivery makes induced resistance practiced this way noncompetitive with current pesticides. Furthermore, knowledge about interactions between the preinoculum and other pathogens and pests is not sufficient at present. For example, some investigations have shown an induced susceptibility against avirulent pathogens by prior inoculations with virulent pathogens (Ouchi *et al*,1974; Kunoh *et al*,1985; Wainwright *et al*, 1986). Preinoculations to induce resistance are economically worthwhile only in long lasting and valuable cultures. A more practical way for the use of induced resistance is the application of abiotic resistance inducers which can be applied like conventional pesticides with existing equipment.

## 5.1. EFFECTS ON DISEASE SEVERITY

Induced resistance caused by prior infections could have practical importance in agriculture growing polycultures of cereals or mixtures of cultivars (Stolen *et al*, 1981; Ibenthal *et al*, 1985). The induced resistance could be assumed to be one factor for the reduction of disease incidence especially with powdery mildew under these conditions. As reported by Villich-Meller und Weltzien (1989) a 50% reduction in incidence of powdery mildew and *Drechslera avenae* occurred in field investigations with mixtures of spring barley and oat.

In monocots, induction of resistance by applying microbial metabolites of a *Bacillus subtilis* isolate as inducers have been demonstrated under field conditions by Schönbeck and coworkers. Repeated treatments of winter wheat with the microbial inducers reduced disease severity comparable to the treatments with highly effective systemic fungicides (Schönbeck et al, 1982; Dehne *et al*, 1984). The effectiveness of induced resistance increased with increasing size of treated areas. This is due to the influence of induced resistance to epidemiological important parameters like colony size and especially sporulation. The reduction in sporulation of *Erysiphe graminis* on wheat by about 96% per leaf represents a pronounced restriction on the development of the mildew epidemic (Table 3). Similar reduction in disease was obtained against powdery mildew on winter and summer barley (Steiner *et al*, 1988; Oerke *et al*, 1989). The extent of protection by induced resistant was dependent on the host cultivar, but was not clearly correlated with the presence of known resistance genes. Wang and Heitefuß (1982) observed that cultivars expressing adult plant resistance may be more suitable for induced resistance as a type of plant protection. Extreme high nitrogen fertilization supply, which increased the susceptibility of the plants, diminished the efficacy of the protection by induced resistance.

Table 3. Effect of resistance induced by *Bacillus subtilis* metabolites on the sporulation of *Erysiphe graminis* f.sp. *tritici* on flag leaves of winter wheat 'Caribo' under field conditions (n=50, EC) (Stenzl 1985. modified).

| Sporulation | untreated plants | treated plants | %change |
|---|---|---|---|
| (Conidia/day) X colony | 3493 | 870* | -75 |
| (Conidia/day) X $mm^2$ colony area | 1027 | 458* | -56 |
| (Conidia/day) X leaf | 150,480 | 6320* | -96 |

*Significantly different from untreated controls ($p < 0.05$)

## 5.2. EFFECTS ON YIELD RESPONSE

Induced resistance can only be of interest for practical plant protection if the plants yield response is not influenced negatively due to additional energy consumption of the resistance reactions. Induced resistant plants should have the similar yield as pesticide-treated ones. Very little information has been generated on the effects of induced resistance on yield capacity of induced plants.

The risk of plant damage or yield reduction caused by pathogen development after preinoculation with low-virulent strains can be avoided by the use of abiotic inducers. Disease-yield loss relationship were determined for induction of resistance by *B. subtilis* metabolites in winter wheat and in winter and spring barley (Dehne *et al*, 1984; Steiner *et al*,1988; Oerke *et al*,1989).

The effect of the induced resistance on grain yield was examined with winter barley cultivars differing in disease susceptibility and productiveness. Compared to fungicide treatments, the degree of disease reduction was lower in all varieties with induced resistance. Although the induced resistance did not completely prevent development of powdery mildew, the yield enhancement of two cultivars exceeded that of the fungicide treatments (Table 4). The yield efficiency of the induced resistant barley plants was independent of the nitrogen fertilization level under the conditions of the test. However, in comparison to fungicide-treated plants, higher yields occurred mainly at low fertilization (Table 5).

The treatment with the resistance inducers altered host susceptibility as well as influenced the yield. The increase in number of kernels per ear of barley could be accounted for by the level of disease reduction during early plant development. The high increase of thousand kernel weight can not only be explained by a an increased assimilation rate of the upper leaves. This could be based on an increase in ribulose-1,5-bisphosphate-carboxylase content or activity and a delay in senescence (Falkhof 1988; Steiner 1989), However, the increase appeared to be caused by an influence on sink and source relationships in the plant which led to a more efficient incorporation of assimilates into kernels in induced resistant plants. The carbohydrate content of kernels of induced resistant barley was also increased in comparison to kernels of untreated or fungicide treated plants. The induced resistant barley plants, radiolabelled with $^{14}CO_2$, showed an unimpaired translocation of assimilates from the flag leaf into the ear in comparison to noninfected barley (Kehlenbeck *et al*, 1993). Similar results were reported by Kern (1985) with a barley cultivar expressing plant adult resistance. In this report, the infection with powdery mildew resulted in fairly strong fungal development, but this had only a slight effect on translocation of assimilates.

**Table 4.** Effect of fungicide applications and resistance induced by metabolites of *Bacillus subtilis* on the yield and disease severity of different winter barley cultivars (dt/ha: n=4)(Steiner *et al.*, 1988, modified).

| Cultivar treatment | Yield (dt/ha) | Powdery mildew EC 69[1] |
|---|---|---|
| 'Birgit' | | |
| untreated | 58 | 14.2 |
| fungicide[2] | 67 (16%)[3] | 0.9 |
| inducer[4] | 73 (26%) | 0.4 |
| 'Mammut' | | |
| untreated | 68 | 28.6 |
| fungicide | 76 (12%) | 0.3 |
| inducer | 82 (21%) | 4.5 |

[1] Median value from the sporulating area of the colonies on the flag first leaves at the end of flowering.
[2] Bayfidan®, 0.5 l/ha.
[3] % increase over control.
[4] *B. subtilis* metabolites.

The induction of resistance by microbial metabolites seems to stimulate the ability of the plants to compensate the damaging effects of pathogens on plant metabolism. This leads to a prolonged maintenance of assimilation rates. Induced resistant barley tolerated higher infection densities, suggesting that for induced resistant plants, disease-yield loss models have validity. Besides the effect on disease severity, the influence on yield may result from reduced damage caused by the pathogen.

Similar results were recorded by Newton *et al* (1993) using elicitor active extracts derived from yeast cell walls as resistance inducer in barley against powdery mildew. In field trials, the plants sprayed with yeast extracts had crops of approximately the same yield and quality as those treated with fungicide even though the plants treated with yeast extract had more disease. The efficacy of induced resistance was significantly effected by the formulation of the inducers. The best results were obtained when the extracts were used as prophylactic sprays or in conjunction with reduced fungicide inputs.

Practical experience with combining resistance inducers and fungicide applications for the integration of induced resistance into established programs of plant protection is lacking. Preliminary investigations in winter wheat have shown that the resistance

**Table 5.** Effect of fungicide treatment and resistance inducing metabolites of *Bacillus subtilis* on the yield and disease severity of spring barley 'Carina' at different nitrogen fertilization intensities (dt/ha: n=4) (Oerke *et al.*, 1989; modified).

| Fertilization treatment | Yield (dt/ha) | Powdery mildew EC 69[1] |
|---|---|---|
| 90 kg N/ha | | |
| untreated | 57 | 27.2 |
| fungicide[2] | 61 (7%) | 1.1 |
| inducer (4X)[4] | 65 (14%) | 7.7 |
| 120 kg N/ha | | |
| untreated | 56 | 49.2 |
| fungicide | 63 (13%) | 3.2 |
| inducer (4X) | 61 (9%) | 26.2 |

[1] Median value from the sporulating area of the colonies on the flag and first leaves at the end of flowering.
[2] Bayfidan®, 0.5 l/ha
[3] % increase over untreated control.
[4] *B. subtilis* metabolites.

inducing metabolites of *Bacillus subtilis* are effective against biotrophic fungi and successfully replaced fungicide applications. However, yield losses caused by soilborne pathogens and *Septoria nodorum* were controlled efficiently only with fungicides (Table 6).

The practical usefulness of induced resistance by abiotic inducers against virus diseases, which are more difficult to control than fungi, has also been shown in cereal crop production. Treatments of various barley cultivars in the field with culture filtrates of *Stachybotrys chartarum* and *B. subtilis* before inoculation with brome mosaic virus reduced both symptoms and virus content. The yield of infected, induced resistant barley plants approached that of healthy plants (Maiss 1987).

The acceptance for a new procedure in plant protection can be low if the plants are not completely disease-free. However, it has been shown that in spite of some disease on induced resistant plants, the economic threshold was not reached because of the altered disease-yield-loss relationships of these plants. Yield losses, as a result of increased energy costs because of resistance reactions, were not observed under field conditions (ThordalChristensen *et al*,1987).

**Table 6**. Effect of combined fungicide applications and resistance-inducing metabolites of *Bacillus subtilis* on grain yield of winter wheat 'Kranzler' (g/1000 grains: n=10; dt/ha: n=4) (Steiner and Schönbeck, unpublished).

| Treatment | g/1000 grains | yield (dt/ha) |
|---|---|---|
| Untreated | 39.1 | 59.4 |
| Inducer | 44.5 | 68.4 (15%)[1] |
| Sportack®, Inducer | 44.8 | 72.8 (23%) |
| Sportack®, Inducer, Dyrene® | 48.0 | 75.9 (28%) |
| Sportack®, Bayfidan®, Dyrene® | 50.4 | 76.8 (29%) |

| Applications: | | Pathogens |
|---|---|---|
| Inducer | EC 31, 35, 53, 61 | *Erysiphe graminis* |
| Sportack® 1.5l/ha | EC 31 | *Puccinia recondita* |
| Bayfidan® 0.5 l/ha | EC 39, 61 | *Puccinia striiformis* |
| Dyrene® 4 l/ha | EC 65 | *Septoria nodorum* |

## 6. Outlook for the future

The patenting of nicotinic acid derivatives as the first synthetic resistance-inducing substance for plant protection (Kunz *et al*, 1988) has drawn attention to the industrial research on such agents and has initiated extended screening procedures in several companies. Today, 2,6-dichloroisonicotinic acid and its methylester which is used intensively in research, belongs to the first generation of such agents. During the next years the spectrum of resistance-inducing substances for different applications will surely increase through intensified research efforts.

In comparison with pesticides with a toxic mode of action, the screening for resistance-inducing agents demands new and more selective testing systems. It is necessary, for example, to discriminate between susceptibility altering substances which act on the basis of sublethal herbicidal effects from true resistance inducers (Rathmell 1984). In addition, the active chemicals must also be considered in relation to epidemiological effects and yield response of the plants.

In the near future, molecular biological techniques will play an important role in research on the basic mechanisms of induced resistance. Great progress would be made by the identification of the plant's own signal substances which mediate systemic resistance or sensitize the plants to react rapidly when challenged by pathogens. Perhaps this substances could be used directly as spray or seed dressing for protection of the plants. Research on the regulation and activation of resistance genes after induction of resistance will be certainly of interest. Artificial gene constructs of such resistance genes with suitable promoters could be used in transgenic plants in which stable and effective resistance can be induced in the field by application of inducers or signal substances.

Some properties of the use of induced resistance in plant protection seem to provide some advantages when compared to the use of pesticides or breeding for resistance. The major advantage from our current perspective is that induction of resistance appears to activate multiple and complex mechanisms in the plants and therefore presents a durable type of control measure.

It is of decisive importance for the introduction of induced resistance into the practice of plant protection to demonstrate the ability of integration of such a biological procedure into established programs for production and plant protection. The critical factors for the establishment will be efficacy and stability of protection. Therefore, resistance inducers must be found which fulfill the criteria for practical use. This includes the application of inducing ag
ents by existing equipment, a formulation of the inducers that ensures optimal performance, persistence and a long shelf life under normal conditions, and compatibility with common pesticides and fertilizers. Because the plant is substantially involved in plant protection by induced resistance, further knowledge is also needed on its efficacy under different climatic and environmental conditions, and the influence of genotype, age and on nutritional status of the plant. The users (farmer, growers etc.) must also be acquainted with the new modes of action of an induced resistance-based control strategy.

Induced resistance opens the possibility to activate latent resistance mechanisms in already existing high-yielding, high-quality varieties of crops which otherwise are highly susceptible. The broad spectrum of plants in which resistance has been induced successfully supports the hypothesis that plants are provided with the appropriate genetic background for defense. The most interesting applications would be the use of resistance inducers against viruses for which pesticides are not available or against diseases which can be controlled with difficulty such as those caused by bacteria and soilborne diseases. Plant health can be obtained not only by direct control of pests and disease but also by influencing the plant's own resistance potential. There is a need for less pest orientated thinking and more on enhancing the plant's ability to protect itself.

The examples mentioned in the article have pointed to the many possibilities for the use induced resistance in controlling cereal diseases. In plant protection, the use of induced resistance appears to be realistic as a supplement to conventional methods, thus increasing the spectrum of technologies useful for disease management. This type of biological control can add to sustainable agriculture by widening the choice of agents available and by filling niches for which chemicals are not available. Through better understanding of mechanisms of induced resistance, a new promising technology for plant protection can be developed.

## 7. References

Asada, Y., and Matsumoto, J. (1987) 'Induction of disease resistance in plants by a lignification -inducing factor', in S. Nishimura (ed.) Molecular Determinants of Plant Diseases, Japanese Science Society Press, Tokyo, Springer Verlag Berlin, p. 223.

Binder, A., Baer, G., Hofmann, C., Kovats, K. (1989) 'Mechanisms in systemic induced disease resistance', in Lugtenberg, B.J.J. (ed.) Signal molecules in plants and plant-microbe interactions, Nato ASI Series Vol. 36, p. 267.

Carver, T.L.W., and Carr, A.J.H. (1978) 'Effects of host resistance on the development and colonies of oat mildew', Annals of Applied Biology 88, 171.

Chaudhary, R.C., Schwarzbach, E., Fischbeck, G. (1983) 'Quantitative studies of resistance induced by avirulent cultures of *Erysiphe graminis* f. sp. *hordei* in barley, Phytopathologische Zeitschrift 108, 80.

Chester, K.S.(1933) 'The problem of acquired physiological immunity in plants', The Quarterly Review of Biolology 8, p.129, p. 275.

Cho, B.H., Smedegaard-Petersen, V. (1986) 'Induction of resistance to *Erysiphe graminis* f. sp. *hordei* in near isogenic barley lines', Phytopathology 76, 301.

Dehne, H.W., Stenzel, K., and Schönbeck, F. (1984) 'Zur Wirksamkeit induzierter Resistenz unter praktischen Anbaubedingungen III. Reproduktion Echter Mehltaupilze auf induziert resistenten Pflanzen', Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 91, 258.

Dixon, R.A., and Lamb, C.J. (1990) 'Molecular communication in interactions between plants and microbial pathogens', Annual Review of Plant Physiology and Plant Molecular Biology 41, 339.

Doke, N., Ramirez, A.V., Tomiyama, K. (1987) 'Systemic induction of resistance in potato plants against Phytophthora infestans by local treatment with hyphal wall components of the fungus', Journal of Phytopathology 119, 232.

EbrahimNesbat, F., Balder, H., and Schönbeck, F. (1983) 'Elektronenmikroskopische Untersuchungen des Gerstenmehltaus (*Erysiphe graminis* DC f.sp. *hordei* Marchal) nach Resistenzinduktion mit mikrobiellen Stoffwechselprodukten', Phytopathologische Zeitschrift 106, 76.

Falkhof, A.G. (1988), 'Biological procedures for promoting plant health. 2. Induced resistance - Assimilation performance and shoot growth of barley plants', Mitt. Biol. Bundesanstalt für Land- und Fortswirtschaft, 245, 316

Farmer, E.E., Ryan, C.A. (1992) 'Octacecanoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase inhibitors', Plant Cell 4, 129.

Fischer, W., Schweitzer, P., Christ, U., Mosinger, E., Kovats, K, Baer, G., and Binder, A. (1988) 'Mechanisms in systemic induced disease resistance', Phytoparasitica 16, 211.

Fujiwara, M., Oku, H., Shiraishi, T. (1987) 'Involvement of volatile substances in systemic resistance of barley against *Erysiphe graminis* f. sp. *hordei* induced by pruning of leaves', Journal of Phytopathology 120, 81.

Gregersen, P.L., Collinge, D.B., SmedegaardPetersen,V. (1990) 'Early induction of new mRNAs accompanies the resistance reaction of barley to the wheat pathogen *Erysiphe graminis* f.sp. tritici', Physiological and Molecular Plant Pathology 36, 471.

Gregersen, P.L., Smedegaard-Petersen, V. (1989) 'Induction of resistance in barley against *Erysiphe graminis* f. sp. *hordei* after preinoculation with the saprophytic fungus *Cladosporium macrocarpum*', Journal of Phytopathology 124, 128.

Hiramoto, T., Tobimatsu, R., Shiraishi, T, Yamada, T., Ichinose, Y., Oku, H. (1992) 'Endogenous elicitor present in barley seeds. II. Mechanisms of the induction of the resistance in barley leaves to *Erysiphe graminis'*, Journal of Phytopathology 135, 167.

Hwang, B.K., Heitefuß, R. (1982) 'Induced resistance of spring barley to *Erysiphe graminis* f.sp. *hordei*', Phytopathologische Zeitschrift 103, 41.

Ibenthal, W.-D-, v. Meier zu Beerentrup, H., Nabizadehr, F. (1985) 'Ertragsniveau und Krankheitsbefall von Sommergerste in Sortenmischungen', Zeitschrift für Pflkrankheiten und Pflanzenschutz 92, 37.

Irving, H.R., and Kuc, J. (1990) 'Local und systemic induction of peroxidase, chitinase and resistance in cucumber plants by $K_2HPO_4$', Physiological and Molecular Plant Pathology 37, 355.

Iwano, M. (1987) 'Biological control of blast disease through resistance of rice plant induced by incompatible strain of blast fungus', Annual Report Society Plant Protection North Japan 38, 10.

Kehlenbeck, H., Krone, C., E.-C. Oerke, F. Schönbeck (1993) 'The effectiveness of induced resistance on yield of mildewed barley', Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz , in press.

Kern, M., Ibenthal, W.-D., Heitefuß (1987) 'Assimilatverteilung in Sommergerstensorten mit unterschiedlicher Resistenz gegenber dem Echten Mehltau (*Erysiphe graminis* f. sp. *hordei*)', Journal of Phytopathology 119, 319.

Klingauf, F., Herger, G. (1985) 'Die Wirkung von Pflanzenextrakten auf den Echten Mehltau an Wintergerste *Erysiphe graminis* f.sp. *hordei'*, Med. Fac. Landbouw. Rijksuniversiteit Gent 50, 1153.

Kloepper, J. W., Tuzun, S., Kuc, J. (1992) 'Proposed definitions related to induced disease resistance', Biocontrol Science and Technology 2, 349.

Kobayashi, I., Komura, T., Sakamoto, Y., Yamaoka, N., Kunoh, H. (1990) 'Recognition of a pathogen and a nonpathogen by barley coleptile cells (I) Cytoplasmatic responses to the nonpathogen, *Erysiphe pisi*, prior to its penetration', Physiological and Molecular Plant Pathology 37, 479.

Kraska, T, Schönbeck, F. (1993) 'About changes in chromatin structure after resistance induction in *Hordeum vulgare* L.', Journal of Phytopathology 137, 10.

Kristensen, H.J., Thordal-Christensen, H., Collinge, D.B., Smedegaard-Petersen, V. (1993) 'Elicitors in induced resistance in barley', in B. Fritig, M. Legrand (eds.) Mechanisms of plant defense responses, Kluwer Academic Publishers, Dordrecht Boston London, p. 168.

Kuc, J. (1983) 'Induced systemic resistance in plants to diseases caused by fungi and bacteria', in J. A. Bailey and B. J. Deverall (eds.), The dynamics of host defense, Academic Press, Sydney, p.191.

Kuc, J. (1987) 'Plant immunization and its applicability for disease control', in I. Chet (ed.), Innovative approaches to plant disease control, J. Wiley & Sons, New York, p. 255

Kunoh, H., Katsuragawa, N., Yamaoka, N., Hayashimoto, A. (1988) 'Induced accessibility and enhanced inaccessibility at the cellular level in barley coleoptiles. III. Timing and localization of enhanced inaccessibility in a single coleoptile cell and its transfer to an adjacent cell', Physiological and Molecular Plant Pathology 33, 81.

Kunoh, H., Komura, T., Kobayshi, I., Yamaoka, N. (1990) 'Induced accessibility and enhanced inaccessibility at the prepenetration stage of a nonpathogen', Physiological and Molecular Plant Pathology 37, 399.

Kunoh, H., Kuroda, K., Hayashimoto, A., Ishizaki, H. (1985) 'Induced susceptibility and enhanced resistance at the cellular level in barley coleoptiles. II. Timing and localization of induced susceptibility in a single coleoptile cell and its transfer to an adjacent cell', Canadian Journal of Botany 64, 889.

Kunz, W., Staub, T., Metraux, J.P., Hoegerle, K., Nyfeler, R., and Ahl, P. (1988) 'Verfahren zu Schutz von Pflanzen gegen Krankheiten', European Patent A1 0 268 75.

Maiss E. (1987) 'Einsatz einer Resistenzinduktion durch Kulturfiltrate von *Stachybotrys chartarum* (Ehrenb. ex Link) Hughes und *Bacillus subtilis* (Ehrenberg) Cohn against

virus diseases under commercial conditions of crop production', Archiv für Phytopathologie und Pflanzenschutz 23, 275.

Malamy, J., Carr, J.P., Klessig, D.F., and Raskin, I. (1990) 'Salicylic acid: a likely endogenous signal in the resistance response of toabacco to viral infection', Science 250, 1002.

Métraux, J.P., Ahl, P., Staub, T., Speich, J., Steinemann, A., Ryals, J., and Ward, E. (1991) 'Induced systemic resistance in cucumber in response to 2,6-dichloroisonicotinic acid and pathogens', in H. Hennecke, D.P.S. Verma, (eds.), Advances in Molecular Genetics of Plant Microbe Interactions, Vol. 1, Kluwer Academic Publishers, Netherlands, p.432.

Métraux, J.P., Signer, H., Ryals, J., Ward, E., WyssBenz, M, Gaudin, J., Raschdorf, K., Schmid, E., Blum, W., and Inveradi, B. (1990) 'Increase in salycylic acid at the onset of systemic aquired resistance in cucumber', Science 250, 1004

Oerke, E.-C., Schönbeck, F. (1992) 'Ertragsverluste und Pflanzenschutz im Getreidebau - ein berblick zur Situation weltweit', Mitt. Biol. Bundesanstalt Landw. Forstw., 283, 47.

Oerke, E.C., Steiner, U., and Schönbeck, F. (1989) 'Zur Wirksamkeit der induzierten Resistenz unter praktischen Anbaubedingungen. V. Mehltaubefall und Ertrag von Winter und Sommergerste in Abhingigkeit von der Stickstoffdngung', Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 96, 140.

Ouchi, S., Oku, H., Hibino, C., Akiyama, I. (1974) 'Induction of accessibility and resistance in leaves of barley by some races of *Erysiphe graminis'*, Phytopathologische Zeitschrift 79, 24.

Ouchi, S., Oku, H., Hibino, C., (1976) 'Localization of induced resistance and susceptibility in barley leaves inoculated with the powdery mildew fungus', Phytopathology 66, 901.

Ouyang, G.C., Ying, C.Y., Zhu, M.H., Xue, Y.L. (1987) 'Induction of disease resistance by spores and toxins of *Pyricularia oryzae* in rice and its relation to the phenylpropane pathway', Plant Physiology Communications 4, 40.

Pan, S.Q., Ye, X.S., Kuc, J. (1992) 'Induction of chitinase in tobacco plants systemically protected against blue mold by *Peronospora tabacina* or tobacco mosaic virus,, Phytopathology 82, 119.

Pelcz, J. (1989) 'Resistenzinduktion bei Gerste durch eine avirulente Rasse von *Erysiphe graminis* DC f. sp. *hordei* March. unter Feldbedingungen', Archiv für Phytopathologie und Pflanzenschutz 25, 131.

Rathmell, W.G. (1984) 'The discovery of new methods of chemical disease control: current developments, future prospects and the role of biochemical and physiological research', in D.S. Ingram and P. H. Williams P.H. (eds.), Advances in Plant Pathology 2, p. 259.

Rasmussen, J.B. Hammerschmidt, R., Zook, M.N. (1991) 'Systemic induction of salicylic acid accumulation in cucumber after inoculation with *Pseudomonas syringae* pv. *syringae*', Plant Physiology 97, 1342

Rebmann, G., Mauch, F., Dudler, R. (1991) 'Sequence of a wheat cDNA encoding a pathogen-induced thaumatin-like protein', Plant Molecular Biology 17, 283.

Reiss, E. (1986) 'Senkung des Gelbrostbefalls anflliger Gerstenpflanzen nach Applikation von Infiltraten aus gelbrostinfizierten, resistenten Gerstenblttern,, Archiv für Phytopathologie und Pflanzenschutz 22, 271

Reiss, E., Schubert, J., and Naumann, K. (1988) 'Ermittlung mikrobieller Resistenzinduktoren und deren vorlufige Charakterisierung', Archiv für Phytopathologie und Pflanzenschutz 24, 335.

Ride, J.P., and Barber, M.S. (1987) 'The effects of various treatments on induced lignification and the resistance of wheat to fungi', Physiological and Molecular Plant Pathology 31, 349.

Roby, D., Toppan, A. Esquerre-Tugaye, M.-T. (1988) 'Systemic induction of chitinase activity and resistance in melon plants upon fungal infection or elicitor treatment', Physiological and Molecular Plant Pathology 33, 409.

Sahashi, N., and Shishiyama, J. (1986) 'Increased papilla formation, a major factor of induced resistance in the barley *Erysiphe graminis* f. sp. *hordei* system', Canadian Journal of Botany 64, 2178.

Schönbeck, F., Dehne, H.W., and Balder, H. (1982) 'Zur Wirksamkeit induzierter Resistenz unter praktischen Anbaubedingungen. I. Echter Mehltau an Reben, Gurken und Weizen', Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz, 87, 177.

Schönbeck, F., Dehne, H.W., and Beicht, W. (1980) 'Untersuchungen zur Aktivierung unspezifischer Resistenzmechanismen in Pflanzen', Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 87, 654.

Schönbeck, F., Steiner, U., Kraska, T. (1993) 'Induced resistance: criteria, mechanisms, practical application and estimation', Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz, in press.

Schweizer, P., Hunziker, W., and Mosinger, E. (1989) 'cDNA cloning, in vitro transcription and partial sequence analysis of mRNAs from winter wheat (*Triticum aestivum* L.) with induced resistance to *Erysiphe graminis* f.sp. *tritici*', Plant Molecular Biology 12, 643.

Sekizawa, Y., and Watanabe, T. (1981) 'On the mode of action of probenazole against rice blast,, Journal of Pesticide Scieince 6, 247.

Smedegaard-Petersen, V. (1990) 'Induction of defense reactions against powdery mildew in barley', Proceedings Crop Protection Northern Britain 233.

Smith, J.A., Métraux, J.P. (1991) '*Pseudomonas syringae* pv. *syringae* induces systemic resistance to *Pyricularia oryzae* in rice', Physiological and Molecular Plant Pathology 39, 451.

Steiner, U. (1989) 'Zum Einfluß Induzierter Resistenz auf den Wirt-Parasit-Komplex Gerste - Echter Mehltau: Sortenabhengige Resistenzreaktionen und Befalls-Verlust-Relationen', Dissertation Universitat Hannover.

Steiner, U. (1990) 'Charakterisierung der biologisch aktiven Komponenten des Resistenz induzierenden Kulturfiltrates von Bacillus subtilis', Mitt. d. Biol. Bundesanstalt 266, 292.

Steiner, U., Oerke, E.C., and Schönbeck, F. (1985) 'Zur Wirksamkeit der induzierten Resistenz unter praktischen Anbaubedingungen. IV: Befall und Ertrag von Wintergerstensorten mit induzierter Resistenz und nach Fungizidbehandlung', Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 95, 506.

Stenzel, K. (1985) 'Untersuchungen zur Entwicklung Echter Mehltaupilze an Pflanzen mit induzierter Resistenz'. Dissertation. Universitat Hannover.

Stenzel, K., Steiner, U., and Schönbeck, F. (1985) 'Effect of induced resistance on the efficiency of powdery mildew haustoria in wheat and barley', Physiological Plant Pathology 27, 357.

Stolen,O.,Hermansen, J.E., Lohde, J. (1980) 'Varietal mixtures of barley and their ability to reduce powdery mildew and yellow rust diseases', Kgl. Vet.-og Landbohojsk. Arsskr. 1980, 109.

Thordal Christensen, H., Gregersen, P.L., Andersen J.B., Smedegaard Petersen, V. (1987) 'Induction of defense reactions in plants', Journal of Agricultural Science i. Finland 59, 231.

Thordal Christensen, H., Smedegaard-Petersen, V. (1988) 'Comparison of resistance-inducing abilities of virulent and avirulent races of *Erysiphe graminis* f. sp. and a race of *Erysiphe graminis* f. sp. tritici in barley', Plant Pathology 37, 20.

Thordal-Christensen, H., Brandt, J., Cho, B.H., Rasmussen, S.K., Gregersen, P.L., Smedegaard-Petersen, V., Collinge, D.B. (1992) 'cDNA cloning and characterization of two barley peroxidases transcripts induced differentially by the powdery mildew fungus *Erysiphe graminis'*, Physiological and Molecular Plant Pathology 40, 395.

Tuzun, S., Kuc, J. (1989) 'Induced systemic resistance to blue mold of tobacco in', M. C. Keen (ed.), Blue mold of tobacco, APS Press, St. Paul, Minnesota, p.177.

Tuzun, S., Rao, M.N., Vogeli, U., Schrade, C.L., Kuc, J. (1989) 'Induced systemic resistance to blue mold: Early induction and accumulation of ß-1,3-glucanases, chitinases, and other pathogenesis-related proteins (b-proteins) in immunized tobacco', Phytopathology 79, 979

Vance, C.P., Sherwood, R.T. (1977) 'Lignified papillae formation as a mechanism for protection in reed canarygrass', Physiological Plant Pathology 10, 247.

Van Loon, L.C., Pennings, G.G.H., (1993) 'Involvment of ethylene in the induction of systemic aquired resistance in tobacco', in B. Fritig, M. Legrand (eds.) Mechanisms of plant defense responses, Kluver Acadademic Publishers, Dordrecht, Boston London, p. 156.

Villich-Meller, V., Weltzien, H.C. (1990) 'Resistenzinduktion in Gerste und Hafer durc Vorinokulation mit Apathogenen - ein befallsmindernder Mechanismus in gemischten Getreidebestenden', Zeitschrift für Pflanzenkrankheitene und Pflanzenschutz 97, 532.

Wainwright, A., Kenny, M.A., Yarham, D.J. (1986) 'Increased susceptibility to *Septoria nodorum* in winter wheat infected by *Tilletia caries*', Plant Pathology 35, 582.

Walters, D.R., Mitchell, A.F., Hampson, McPherson, A. (1993) 'The induction of systemic resistance in barley to powdery mildew infection using salicylates and various phenolic acids', Annals of Applied Biology 122, 451.

Wastemack, C., Atzoren, R., Kogel, K.H. (1993) 'Induction of thionin, the jasmonat induced 6 KD-protein of barley by 2,6-dichloroisonicotinic acid', Journal of Phytopathology, in press.

Yamada, T., Hiramoto, T. Tobimatsu, R., Shiraishi, T., Oku, H. (1990) 'Elicitor-like substances present in barley and wheat seeds', Journal of Phytopathology 128, 89.

Yokoyama, K., Aist, J.R., Bayles, C.J. (1991) 'A papilla-regulating extract that induces resistance to barley powdery mildew', Physiological and Molecular Plant Pathology 39, 71.

# MOLECULAR REGULATION OF SYSTEMIC INDUCED RESISTANCE

BRUCE A. STERMER
*The Samuel Roberts Noble Foundation, Inc.*
*Plant Biology Divsion*
*P.O. Box 2180*
*Ardmore, OK 73402*
*USA*

## 1. Introduction

Following the report of Ross (1961) describing the induction of systemic resistance in the virus-free leaves of tobacco plants reacting hypersensitively to tobacco mosaic virus (TMV), interest in the biochemistry and molecular biology of the phenomenon has been growing. Systemic induced resistance appears to be the result of several mechanisms which together are effective against a wide range of fungi, bacteria and viruses. This inducible resistance is exhibited by a variety of plant species. Many of the efforts to understand the components forming the basis of this phenomenon have focussed on the known and cryptic plant proteins demonstrating enhanced levels during the induction of resistance. These studies have benefited greatly from the development of molecular cloning techniques and plant transformation systems. In less than a decade considerable progress has been made in our understanding of the proteins associated with induced resistance and of the genes which encode them. Judging by the growing number of papers, this area is now receiving considerable attention from researchers interested not only in the control of plant disease but also in the opportunities the system provides for the study of plant gene expression. The purpose of this chapter is to outline research on the molecular aspects of systemic induced resistance, including the regulation of systemically expressed genes, and to point out what we know and still need to know about the molecular basis of induced resistance.



*R. Hammerschmidt and J. Kuć (eds.), Induced Resistance to Disease in Plants,* 111–140.

## 2. Systemic Changes Associated with Induced Resistance

### 2.1 PEROXIDASES

Early studies reported a systemic increase in peroxidase activity in tobacco plants with localized TMV infection (Simons and Ross, 1970; van Loon, 1976). Further investigation of changes in the enzymes of phenylpropanoid and oxidative metabolism found little difference between the uninfected tissues of infected or uninfected tobacco plants, although the activity of a number of enzymes of respiratory and phenolic metabolism did increase earlier and to a greater extent after challenge inoculation in leaves expressing induced resistance (Simons and Ross 1971a, 1971b; van Loon 1982). In cucumber plants the induction of systemic induced resistance by localized *Colletotrichum lagenarium* infections is also associated with enhanced peroxidase activity (Hammerschmidt *et al.* 1982). Similarly, induction of resistance by heat shock treatments revealed a tight correlation between peroxidase activity in cucumber and the level of resistance in the plant (Stermer and Hammerschmidt 1984). The enhanced peroxidases are acidic, extracellular and most probably localized in the cell wall (Smith and Hammerschmidt 1988). Peroxidases have several functions which could have an effect on the resistance of a plant. One is the oxidative polymerization of hydroxycinnamyl alcohols to form lignin, a process that is a commonly reported defense mechanism in plants (Vance *et al.* 1980), and has been proposed as a mechanism for induced systemic resistance in cucumber (Hammerschmidt and Kuć, 1982). Another possible role for peroxidase is the oxidative cross-linking of pre-existing hydroxyproline-rich structural proteins in the cell wall, making the cell wall more resistant to degradation by microbial enzymes (Bradley *et al.* 1992; Stermer and Hammerschmidt 1987). Also, peroxidases are implicated in an oxidative defense mechanism in elicitor treated (Apostol *et al.*, 1989) and infected (Lizzi and Coulomb 1991) plants, and peroxidase-generated hydrogen peroxide may function directly as an antimicrobial agent (Peng and Kuć, 1992). Although peroxidase activity is induced systemically in tobacco and cucumber by diverse types of pathogens it may not be an equally important factor in the resistance to every class of pathogens. Evidence suggests that enhanced peroxidase activity may not be directly involved in resistance of tobacco to TMV (Ye *et al.* 1990) or to the bacterium *Pseudomonas solanacearum* (Nadolny and Sequiera 1980). This observation supports the notion that systemic induced resistance consists of several mechanisms.

### 2.2 PATHOGENESIS-RELATED PROTEINS

In 1970 two independent groups reported that the synthesis of several proteins was induced in tobacco plants exhibiting a hypersensitive response to TMV (Gianinazzi *et al.* 1970; van Loon and van Kamman 1970). As more proteins were identified the name pathogenesis-related (PR) proteins was proposed (Antoniew 1980). These proteins accumulate in the uninfected as well as the infected tissues of inoculated plants (Métraux

*et al.* 1988; Tuzun *et al.* 1989). Although most studies have concentrated on tobacco, PR-proteins are known to occur in a wide range of species, including both monocots and dicots (Redolfi 1983; White *et al.* 1987). They were initially distinguished as acidic host-encoded proteins, resistant to proteases and secreted by plant cells into the extracellular spaces (Antoniw and White 1983; Bol 1988; Rigden and Coutts 1988). However, basic forms of PR-proteins have also been identified recently which accumulate in the vacoule of the plant (Bol *et al.* 1990). The best characterized PR proteins from TMV-infected tobacco are placed into five groups based on size, amino acid composition and serological cross-reactivity (Kauffmann *et al.* 1990; van Loon *et al.* 1987). Only in the past few years has it been possible to assign a biological function to any of the PR proteins. In 1987, Legrand and coworkers identified two PR proteins in tobacco as endochitinases (PR-2 group) and found two other serologically related basic isoforms of endochitinase in tobacco (Legrand *et al.* 1987). Proteins from the PR-3 group were found to have ß-1,3-glucanase activity (Kauffman *et al. 1987*). Soon after, it was reported that several of the PR proteins in potato are chitinases and ß-1,3-glucanases (Kombrink *et al. 1988*). Members of the PR-5 group of tobacco proteins are highly similar to thaumatin, an extremely sweet protein from a West African monocot, and are referred to as thaumatin-like proteins (Cornellissen *et al.* 1986a). A possible activity for these proteins is suggested by their homology to a maize bifunctional proteinase/amylase inhibitor, although the inhibitor function of tobacco thaumatin-like proteins has not been demonstrated (Richardson *et al.* 1987). Osmotin, a basically charged member of the PR-5 group accumulates in the vacuole of tobacco cells subjected to high salt stress (Singh *et al.* 1989). One form, osmotin II, was purified from TMV-infected tobacco during a search for anti-Phytophthora proteins; this 24 kD protein, referred to as AP24, and a similar one from tomato were potent inhibitors of the fungus (Woloshuk *et al.* 1991). The function of the PR-1 and PR-4 groups of proteins is unknown. The reader is referred to excellent reviews for a more detailed discussion on tobacco PR proteins (Bol *et al.* 1990, Rigden and Coutts 1988, Ryals *et al.* 1992, van Loon 1985).

Compared to tobacco, relatively little is known about the systemically induced PR proteins of other plants. Tomato plants infected by *Phytophthora infestans* accumulate eleven acid soluble intercellular proteins, two of which (P14 and P70) also accumulate in the uninfected leaves (Christ and Mösinger 1989). Another study of tomato reported an approximate two-fold increase in the chitinase, glucanase, peroxidase and protease activities of the upper leaves after infection of the lower leaves with *P. infestans* (Binder *et al.* 1989). In both cases the systemic increases in proteins were paralleled by a systemic induction of resistance to the fungus. Cucumber plants expressing induced resistance have systemically enhanced levels of peroxidase, chitinase and glucanase, of which the chitinase has been the best characterized (Binder *et al.* 1989, Métraux *et al.* 1989). Potato also exhibits distal accumulation of chitinase and glucanase, which corresponded to increases in the mRNA encoding these two enzymes; however, this gene activation was reported only within the infected leaf (Hahlbrock *et al.* 1989). Recently, resistance in rice against *Pyricularia oryzae*, which can be induced by chemical treatment

or *Pseudomonas syringae* pv. *syringae* infiltration, has been associated with the systemic expression of genes (Reimmann *et al.* 1991). However, the activities of phenylalanine ammonia-lyase, coniferyl alcohol dehydrogenase, peroxidase, ß-1,3-glucanase and chitinase were not systemically induced, suggesting that rice may utilize a different spectrum of mechanisms to achieve systemic induced resistance (Smith and Métraux 1991).

Chitinases and ß-1,3-glucanases may be involved in the defense of plants against fungi and bacteria by their action on the cell walls of invading pathogens (Boller 1985). Chitinase hydrolyzes the ß-1,4-N-acetylglucosamine linkages of chitin, a polymer that is a major component of the cell wall of most classes of fungi, except for the Oomycetes. In addition, the peptidoglycan polymer of bacterial cell walls is also cleaved by most of the chitinases that have been tested for this activity (Boller 1988). The ß-1,3-glucanases hydrolyze ß-1,3-glucan polymers, another major component of fungal cell walls. This enzyme acts synergistically with chitinase to attack the growth of hypal tips. Purified chitinase and ß-1,3-glucananse used against 18 different species of fungi *in vitro* were found to be more inhibitory together that was either enzyme alone (Mauch *et al.* 1988). Another defense-related function of these two hydrolases may be to release elicitor active molecules from the surface of pathogens and thereby increase the speed and magnitude of a defense response (Hadwiger and Beckman 1980, Keen and Yoshikawa 1983). Although the contribution of chitinase and ß-1,3-glucanase to systemic induced resistance is unknown, they clearly have antifungal and antibacterial potential.

## 2.3 PHENOLIC COMPOUNDS

Small molecules have been implicated in systemic induced resistance, although their role appears to be more important for signal transduction than for directly inhibiting pathogens. The best studied is salicylic acid (SA), a compound derived from cinnamic acid. The levels of SA increase several fold in the upper uninfected leaves of TMV-infected tobacco (Malamy *et al.* 1990). Similar systemic increases were observed in cucumber with localized infection by fungal, bacterial or viral pathogens (Métraux *et al.* 1990, Rasmussen *et al.* 1991). Exogenous application of SA increases the resistance of several plants, including cucumber and tobacco, to many types of pathogens, strengthening speculation that SA is the primary signal for induced resistance (Enyedi *et al.* 1992). Nevertheless, this remains to be established as some evidence suggests that although SA may have a role as an endogenous inducer of resistance, it is not the systemic signal of induced resistance (Rasmussen *et al.* 1991).

Systemic changes in the level of other phenolic compounds have been examined by Simons and Ross (1971). They measured the levels of orthodihydroxyphenols, chlorogenic acid and total phenolic compounds in the upper leaves of tobacco following inoculation of the lower leaves with a local lesion strain of TMV. The induction of systemic resistance was not accompanied by changes in the concentrations of these compounds, except for an earlier and greater *decrease* in their levels in resistant upper leaves after challenge inoculation.

## 2.4 LIPIDS

Little is known about the possible systemic changes in the spectrum or concentrations of lipids in plants expressing induced resistance to disease. A study by Ádám *et al.* (1990) examined the membranes of tobacco leaves that were systemically resistant and found no significant changes in their resistance to fusaric acid-induced electrolyte leakage, nor did they find any significant difference in the sterol, phospholipid or fatty acid composition of the membranes. In contrast, many changes in membrane lipids are known to occur in plants at the site of infection. For example, infection of cucumber leaves with *Sphaerotheca fuliginea* results in almost a three-fold increase in the synthesis of sterols (Lösel 1991). In tobacco tissues expressing a hypersensitive response to TMV, an increase in the saturation of fatty acids contained in the microsomal phospholipids was observed while the linolenic acid content decreased by 9% (Ruzicska *et al.* 1983). Interestingly, the authors credited the change in fatty acid composition to a four-fold increase in lipoxygenase activity of the infected tobacco tissues. This enzyme has a key role in converting linolenic acid to jasmonic acid, producing a signal that activates the synthesis of proteinase inhibitors in tomato, tobacco and alfalfa (Farmer and Ryan 1992).

## 2.5 CYTOKININS

Systemic increases in the cytokinin activity of tobacco have been associated with the induction of resistance. At eight days after the inoculation of the lower leaves of Xanthi tobacco with TMV, the cytokinin activity of the upper leaves was significantly higher, varying from 10 to 100% above controls (Sziráki *et al.* 1980). These researchers speculated that the increased cytokinin levels somehow caused the restriction of lesion growth; however, chromatographic separation of the extracts did not consistently link the cytokinin activity to any specific cytokinin standard. Possibly, the increased cytokinin activity observed in the systemically resistant tissue is due to increased synthesis of dehydrodiconiferyl alcohol glucosides (DCGs). These phenylpropanoid compounds, which were first described in tobacco cells, have cytokinin activity (Teutonico *et al.* 1991). Accumulation of DCGs may occur in tissues with induced resistance because it is peroxidase that catalyzes the first of two steps, the dimerization of coniferyl alcohol, required to make DCGs in plants (Orr and Lynn 1992). Thus, the increase in peroxidase activity of systemically induced tobacco may lead to greater synthesis of the DCGs. In this context it may be noteworthy that elevated cytokinin levels in tobacco have been reported to increase the expression of defense-related genes encoding extensin, chitinase, PR-1 and a PR-1-like protein (Memelink *et al.* 1987).

## 3. Genes Systemically Expressed After Infection

### 3.1 PEROXIDASES

Although there have been many studies of peroxidases, these heme-containing glycoproteins are still poorly understood (van Huystee 1987). Most plants contain a number of different peroxidase isoforms. In tobacco (cv. Xanthi) at least 12 isoforms have been detected, and have been classified into three groups; the basic (pI = 9.3, 9.2, 8.9, 8.3), the moderately acidic (pI 6.1, 5.6, 5.0, 4.8, 4.6), and the strongly acidic (pI = 3.7, 3.5) (Lagrimini and Rothstein 1987). Analysis of the expression pattern of these different tobacco isoforms revealed that only two of the peroxidases, named P61 and P56 after their pI, were induced by TMV infection (Lagrimini and Rothstein 1987). P61, and to a lesser extent P56, were also systemically induced in the uninoculated leaves of infected plants. However, a similar analysis using tobacco cultivar Ky14 found that two highly acidic, soluble isoforms, P37 and P35, were systemically induced after TMV or *Peronospora tabacina* infection (Ye *et al.* 1990). A summary of proteins systemically induced by infection is presented in Table I. Two other peroxidase isoforms that also increased systemically, but to a lesser degree, P88 and P68, were detected only in the salt extracts of cell walls from the induced leaves (Ye *et al.* 1990). The different results obtained by the two research groups indicates the difficulties in studying the role of peroxidase isoforms in disease resistance. The two strongly acidic peroxidase isoforms, P35 and P37, have been N-terminal sequenced, leading to the synthesis of a oligonucleotide probe that was used to select cDNA clones encoding the corresponding tobacco peroxidase (Lagrimini *et al.* 1987). A full length cDNA that matched the sequenced peptides encoded a preprotein containing the 302 amino acids of the mature peroxidase plus a 22 amino acid signal sequence and four potential N-glycosylation sites. Genomic analysis indicated that tobacco contains four strongly acidic peroxidase genes; the two copies of the P35 gene originated from *N. tomentosiformis* and the two copies of the P37 gene came from *N. sylvestris* (Lagrimini *et al.* 1987).

A cDNA clone encoding a pathogen-induced peroxidase from rice has been reported, but no systemic increase in its mRNA was observed (Reimmann *et al.* 1992). In addition, a cDNA encoding a highly acidic peroxidase implicated in suberization has been isolated from potato tubers (Roberts *et al.* 1988) and tomato (Roberts and Kolattukudy 1989). Also, two genomic clones encoding a basic and a neutral peroxidase have been cloned from *Arabidopsis* (Intrpruk *et al.* 1991), but their role in disease resistance in unknown.

In cucurbits, at least three peroxidase isoforms are associated with induced resistance (Hammerschmidt *et al.* 1982). Cucumber, muskmelon and watermelon plants each contain three acidic peroxidases that increase systemically after infection and are readily extracted from intercellular spaces. The isoforms from the three plant species have a similar charge and molecular weight ($\sim$30 kDa) and are immunologically related (Smith and Hammerschmidt 1988). The cloning and preliminary analysis of a cucumber cDNA

encoding a 33 kD apoplastic peroxidase associated with systemic induced resistance has recetnly been reported (Rasmusses *et al.* 1994).

## 3.2 PR-1 PROTEINS

The gene family encoding the predominant pathogenesis-related proteins of Samsun NN tobacco, PR-1a, -1b and -1c, has been well characterized. Cornelissen *et al.* (1986a) identified cDNA clones that corresponded to mRNA for PR proteins that included a complete copy of PR-1b. This mRNA contained a leader sequence of 29 nucleotides, an open reading frame of 504 nucleotides encoding a 30 amino acid long signal peptide and a 138 amino acid long mature protein, and a 3' non-coding region of 235 nucleotides. Comparison of the nucleotide sequences of PR-1a, -1b and -1c demonstrated that the genes are closely related to each other with a 90% identity in the protein coding regions and 80% in the 3' untranslated regions (Pfitzner and Goodman, 1987). Although the PR-1 gene family probably evolved by duplication from a common ancestral gene, these genes do not appear to be tightly linked in tobacco (Pfitzner and Goodman, 1987). Southern blot analysis has shown that the tobacco genome contains at least eight genes for acidic PR-1 proteins and a similar number of genes encoding basic homologs that have a 67% amino acid sequence identity to their acidic counterparts (Cornelisson *et al.* 1987). However, not all these genes are expressed; only three of the genes for the acidic proteins and at least one of the basic homologs are expressed after TMV infection (Cornelisson *et al.*, 1987). The PR-1 genes do not contain introns (Cornelisson *et al.*, 1987; Pfitzner *et al.*, 1988).

The number of genes encoding PR-1 proteins or their homologs varies depending on the plant. The radish genome seems to contain only a single copy of *din*1 (Azumi and Watanabi, 1991 and tomato contains an estimated one to three genes for each of its five extracellular PR proteins (van Kan *et al.*, 1992). A single copy gene encoding a PR-1 homolog in *Arabidopsis* has been reported (Metzler *et al.*, 1991). The larger gene family encoding PR-1 proteins in tobacco is due, at least in part, to its amphidiploid genome. Genetic studies with Samsun NN tobacco and its parents have shown that the acidic PR-1a and -1c originated from *N. sylvestris* and that PR-1b came from *N. tomentosiformis* (Gianinazzi and Ahl, 1983).

## 3.3 CHITINASES

Several structurally distinct chitinases are found in plants. Based on sequence information these enzymes can be organized into at least three classes (Shinshi *et al.*, 1990). Class I chitinases are basic enzymes with an N-terminal cysteine-rich, lectin-like domain and a highly conserved chitinolytic domain. This class includes basic isoforms of tobacco (Ryals *et al.*, 1992a), bean (Broglie *et al.*, 1986) and *Arabidopsis* (Samac *et al.*, 1990). Although infection or ethylene treatment may induce the activity of class I chitinases (Broglie *et al.*, 1986; Samac *et al.*, 1990), the systemic induction of these basic, intravacoular forms by localized infection has not been demonstrated, thus a

causative role in induced resistance is doubtful (Ryals *et al.,* 1992). Class II chitinases have a chitinolytic domain similar to class I but are acidic proteins and lack the N-terminal cysteine-rich domain. This class includes the tobacco pathogenesis-related proteins P and Q, both of which are associated with the induction of systemic resistance (Hooft van Huijsduijnen *et al.,* 1987). Class III chitinases are also acidic proteins and lack the cysteine-rich domain, but they have a chitinolytic domain with structural homologies to a bifunctional lysozyme/chitinase instead of the class I or II chitinases (Métraux *et al.* , 1989). Examples of class III include the acidic chitinases of cucumber (Métraux *et al.*, 1989), tobacco (Lawton *et al.*, 1992) and *Arabidopsis* (Samac *et al.*, 1990), of which the cucumber and tobacco enzymes are known to be systemically induced by infection. A chitinase which does not easily fit into this classification is bean PR4, an acidic protein that contains the N-terminal cysteine-rich domain of class I but is localized in the apoplast as are class II and III (Margis-Pinheira *et al.*, 1991).

Genes encoding all three classes of chitinases from tobacco have been cloned and sequenced. The gene for a tobacco class I chitinase contained a pre-enzyme of 329 amino acids with a 23 amino acid N-terminal peptide not present in the mature protein followed by a 43 amino acid cysteine-rich domain linked to a chitinolytic domain (Shinshi *et al.*, 1990). Complementary DNA clones encoding the acidic class II tobacco chitinases, PR-P and PR-Q, have been isolated (Hooft van Huijsduijnen *et al.,* 1987; Linthorst *et al.*, 1990b; Payne *et al.*, 1990a). Structural analyses of these clones showed substantial homology between PR-P and PR-Q and basic chitinases, but no significant homologies with either the bacterial chitinases or acidic cucumber chitinase (Payne *et al.,* 1990a). The tobacco acidic chitinase gene contains two introns (Linthorst *et al.,* 1990b). Complementary DNA clones encoding acidic and basic isoforms of the class III chitinase have recently been isolated from tobacco. The basic and acidic isoforms are ~65% identical to each other and are equally homologous to the class III chitinases from cucumber and *Arabidopsis* (Lawton *et al.,* 1992). Both isoforms lacked a C-terminal extension, suggesting that the basic as well as the acidic enzymes are localized extracellularly . Southern blot analysis indicated that the basic and acidic class III tobacco chitinases are each encoded by two genes. A cDNA clone encoding the class III chitinases from cucumber has been isolated (Métraux *et al.*, 1989). Genomic analysis indicated that a single gene encodes this enzyme in cucumber.

The genes encoding chitinases of *Arabidopsis* have also been isolated and characterized (Samac *et al.*, 1990). Two single copy genes were identified, one encoding a basic chitinase with a 73% amino acid sequence similarity to the tobacco class I chitinase and another encoding an acidic chitinase with a 60% amino acid similarity to the class III chitinase of cucumber. The *Arabidopsis* genes share little sequence homology (31%) between themselves. Although systemic induced resistance does occur in *Arabidopsis*, evidence indicates that the chitinases may not be as important in systemic resistance of *Arabidopsis* as they are in tobacco or cucumber (Uknes *et al.*, 1992). A high constitutive level of the basic chitinase mRNA occurs in *Arabidopsis* roots with lower levels found in leaves; the acidic chitinase mRNA was undetectable in plants treated with ethylene or salicylic acid (Samac *et al.*, 1990).

## 3.4 ß-1,3-GLUCANASES

Three distinct structural classes have been proposed for the ß-1,3-glucanases of tobacco (Payne *et al.* 1990). Class I contains at least three basic glucanases predominantly localized in the vacoule; class II consists of extracellular, acidic glucanases such as PR-2, PR-N and PR-O; class III contains extracellular, acidic glucanases represented by PR-Q. The amino acid sequences of the class II glucanase genes of tobacco are approximately 54% identical with PR-Q and approximately 51% identical with genes of class I glucanases (Ward *et al.*, 1991). The class III PR-Q is 63% identical at the amino acid level to the soybean elicitor-releasing glucanase (Payne *et al.*, 1990; Takeuchi *et al.*, 1990). The soybean ß-1,3-glucanase is able to release highly active elicitor molecules from *Phytophthora megasperma* f.sp. *glycinea*, a soybean pathogen, and thus may be a key host component involved in the induction of defense reactions (Keen and Yoshikawa, 1983). The basic class I glucanases are major components of uninfected tobacco plants, especially the roots and lower leaves, and have received considerable study (Meins and Ahl, 1989; Shinshi *et al.*, 1988; Van den Bulcke *et al.*,1989), but their response to infection is local rather than systemic (Meins and Ahl, 1989). The expression of the acidic class II and III glucanases, but not the basic class I glucanases, is associated with the systemic induction of resistance in tobacco plants (Ward *et al.*, 1991).

Treatment of *Arabidopsis* with resistance-inducing compounds or infection with *Pseudomonas syringae* pv. *tomato* induced expression of a gene encoding an acidic protein (*Arabidopsis* PR-2) that was 56% identical to the tobacco PR-Q glucanase, 51% identical to the acidic PR-2 glucanase and 52% identical to the basic glucanase (Uknes *et al.*, 1992). Systemic expression of the glucanase genes in *Arabidopsis* has not been described. However, systemic increases in glucanase activity, which coincide with the induction of resistance, have been reported in tomato and cucumber plants after localized infection by *Phytophthora infestans* and *Colletotrichum lagenarium*, respectively (Binder *et al.*, 1989). These enzymes were not characterized at the molecular level. Although glucanases have been purified from a wide variety of plant sources (Boller, 1985 and 1988), their relationship to systemic induced resistance has not been thoroughly studied.

## 3.5 PR-4 PROTEINS

Among the proteins known to accumulate systemically in TMV-infected tobacco is the acidic, extracellular PR-4. This pathogenesis-related protein, also referred to as PR-R, is reported to have two forms, of 13 and 15 kD (van Loon *et al.*, 1987). Characterization of a 13.5 kD PR-4 protein by N-terminal amino acid sequencing and subsequent cloning and sequencing of its cDNA revealed two distinct cDNA sequences, designated PR-4a and PR-4b, that differed at only six nucleotide positions in the predicted coding regions (Friedrich *et al.*, 1991). The PR-4 proteins are apparently encoded as preproteins which include a signal peptide of approximately 25 residues; Southern blot analysis indicated that the PR-4 gene family is composed of 3 to 4 members in tobacco (Friedrich *et al.*, 1991). Interestingly, the deduced amino acid

sequence of PR-4 was found to share approximately 75% identity with two wound-induced potato proteins (Win-1 and Win-2) and with hevein from rubber tree latex; however these proteins contain an additional N-terminal "lectin domain" which PR-4 lacks (Friedrich *et al.*, 1991). No function is known for PR-4.

## 3.6 THAUMATIN-LIKE PROTEINS

Members of this group are characterized by their homology to thaumatin, a sweet tasting protein from a West African shrub (Cornelissen *et al.*, 1986a). In Samsun NN tobacco, two genes that have approximately 65% identity to thaumatin are expressed in response to TMV infection (van Kan *et al.*, 1989). These two tobacco genes share 95% identity and encode the major and minor forms of the acidic PR-S proteins (Payne *et al.*, 1988; van Kan *et al.*, 1989). A cDNA encoding a thaumatin-like protein that is approximately 46% identical to the acidic and basic PR-5 proteins of tobacco has been isolated from *Arabidopsis* (Uknes *et al.*, 1992). Expression of the corresponding gene in *Arabidopsis* is induced by infection or treatment with salicyclic or 2,6-dichloroisonicotinic acids.

A basic member of the thaumatin-like protein family also occurs in tobacco. Based on serology, amino acid composition and $NH_2$-terminal sequence, osmotin, a protein that accumulates in tobacco vacoules in response to osmotic stress, is related to PR-S (Stintzi *et al.*, 1991). Osmotin is encoded in *Nicotiana tabacum* by a pair of genes, each originating from one of the parental species, *N. sylvestris* and *N. tomentosiformis* (Nelson *et al.*, 1992). The gene derived from *N. sylvestris* lacks introns and has two transcription start sites (Nelson *et al.*, 1992). Already present in healthy tissues, osmotin levels are markedly increased in tobacco leaves reacting hypersensitively to TMV (Stintzi *et al.*, 1991). Nevertheless, systemic expression of osmotin has not been reported.

Thaumatin-like proteins have been described in a wide range of plants. Zeamatin, an anti-fungal protein from corn seeds, shares considerable homology with thaumatin, PR-R and osmotin (Vigers *et al.*, 1991). The first 60 amino acids of zeamatin are identical to a bifunctional trypsin/$\alpha$-amylase inhibitor from corn seeds; however, zeamatin does not inhibit either $\alpha$-amylase or trypsin (Vigers *et al.*, 1991). Seeds of oats, sorghum and wheat also contain proteins that fall in this group (Vigers *et al.*, 1991). Tomato plants inoculated with *Phytophthora infestans* accumulate a basic protein that is highly similar to osmotin and inhibitory to the fungus (Woloshuk *et al.*, 1991), and soybean contains an acidic protein that is structurally related to thaumatin-like proteins (Graham *et al.*, 1992).

## 3.7 SAR 8.2

Evidence for a new type of pathogenesis-related protein has recently been obtained by differential screening of a cDNA library constructed from systemically induced Xanthi nc tobacco. Five distinct cDNAs deduced to encode small, basically charged proteins with N-terminal signal peptides have been identified (Alexander *et al.*, 1993). An

interesting feature of the putative proteins is the presence of a cysteine-rich domain at the C-terminus. Southern analysis indicated that the SAR8.2 gene family consists of 10 to 12 members in tobacco. No significant homology has been found between the SAR8.2 cDNAs and known sequences.

## 3.8 CHALCONE SYNTHASE

This enzyme catalyzes a key regulatory step in the synthesis of pterocarpan phytoalexins that is characteristic of many legumes (Dixon *et al.*, 1983). Analysis of the expression of bean chalcone synthase (CHS 8 and CHS 15) promoter-GUS reporter gene fusions revealed that application of oxalate, a treatment that induces disease resistance in cucumber, or infiltration of an incompatible isolate of *Pseudomonas syringae* pv. *syringae,* induces the expression of the CHS 8 but not the CHS 15 gene fusion (Stermer *et al.*, 1990). Both treatments activated the CHS 8 promoter 30 to 40 mm from localized sites of application, indicating that the CHS 8 gene is responsive to signals that act not only locally but at a distance. Although bean is reported to have the ability to respond with systemic resistance after infection, the role of CHS in the induced resistance of bean has not been examined (Cloud and Deverall, 1987).

## 3.9 GLYCINE-RICH PROTEINS

Glycine-rich proteins (GRP) are widespread in plants where they are postulated to be cell wall components (Cassab and Varner, 1988; Condit and Keller, 1990). However, a recent report implicating a GRP in the maturation of specific mRNAs in response to wounding suggests that GRPs may play more diverse roles in plants (Sturm, 1992). The systemic induction of mRNA encoding GRPs has been reported for tobacco infected with TMV (Hooft van Huijduijnen *et al.*, 1986; van Kan *et al.*, 1988). Differential hybridization of a cDNA library constructed from TMV-infected Samsun NN tobacco selected a number of clones that corresponded to systemically accumulating mRNAs; the translation products of two classes of these mRNAs were not immunologically related to previously known PR proteins (Hooft van Huijduijnen et al., 1986). Nucleic acid sequence analysis determined that one of these mRNA classes encoded GRPs (van Kan *et al.*, 1988). Of two genomic clones isolated, one (gGRP-8) represented an infection-induced gene encoding a hydrophilic protein of 109 amino acids, 25% of which were glycine, with a putative N-terminal signal peptide of 26 amino acids (van Kan *et al.*, 1988). Genomic Southern analysis indicated that the GRP mRNA corresponds to a family of approximately eight genes in tobacco. The GRP has not yet been identified in tobacco plants and may be insolubilized in the plant cell wall where it could have a structural role (van Kan *et al.* 1988).

Complementary DNA encoding a highly related GRP from petunia has been isolated, and the expression of the petunia GRP gene is also enhanced by virus infection and salicylic acid treatment (Linthorst et al, 1990a). *Arabidopsis* contains at least five distinct single copy genes encoding GRPs. Little homology exists among the five

*Arabidopsis* genes or with previously described GRP from other species (de Oliveria *et al.*, 1990). Transcript levels of the five GRPs were affected slightly but differentially by salicylic acid treatment. In addition, one class of GRP from tomato has recently been described that responds to wounding by both local and distant expression (Showalter *et al.*, 1992), and a cDNA encoding a virus-inducible GRP from rice has been reported (Fang *et al.*, 1991).

**Table 1.** Classes of proteins accumulating systemically in plants after local infection

| Class | Possible Role in Defense | Tobacco | Cucumber | Arabidopsis[1] |
|---|---|---|---|---|
| Peroxidases | Strengthen cell walls; generate toxic free radicals | P61, P65 (cv. Xanthi); P37, P35 (cv. KY 14) | 30-33 kD isoforms | ND[2] |
| PR-1 | Functions unknown | PR-1a, PR-1b, PR-1c | ND | PR-1 homolog |
| Chitinases | Antifungal | PR-P, PR-Q (PR-3a, PR-3b) | Class III chitnase | ND |
| ß-1,3-glucanases | Enhance antifungal activity of chitnases | PR-2, PR-N, PR-O (PR-2a, PR-2b, PR-2c) | Glucanase | Glucanse? |
| PR-4 | Unknown | PR-R (PR-4a, PR-4b) | ND | ND |
| Thaumatin-like proteins | Antifungal; α-amylase/protease inhibitors | PR-s (PR-5a, PR-5b) | ND | PR-5 homolog |
| SAR 8.2 | Unknown | SAR 8.2 Protein[3] | ND | ND |
| Glycine-rich proteins | Strengthen cell walls | GRP-8[3] | ND | GRP? |

[1] Transcript levels of *Arabidopsis* proteins are enhanced by salicylic acid treatment.
[2] Not demonstrated
[3] Presence deduced from systemic increase in corresponding mRNA

## 4. Control of Systemic Expression

Most of the genes involved in defense responses in plants are regulated, at least in part, at the level of transcription (Dixon and Harrison, 1990; Dixon *et al.*, 1993). This also appears to hold true for the genes that encode proteins implicated in systemic induced resistance. Carr and coworkers (Carr *et al.*, 1985) reported that the accumulation of PR-1 protein in tobacco is regulated by the abundance of translatable mRNA encoding the protein. Soon after it was demonstrated that PR-1 mRNAs, plus mRNAs corresponding to other PR proteins, accumulated systemically in tobacco plants with a localized TMV infection, providing further evidence that transcription is involved in the control of PR protein expression (Hooft van Huijduijnen *et al.*, 1986). Since then several groups have cloned and characterized cDNA and genomic sequences corresponding to the acidic and basic tobacco PR proteins (see previous section), which has permitted the analysis of PR protein expression in considerable detail.

### 4.1 EXPRESSION PATTERNS

The levels of mRNA from different PR gene families in tobacco have been examined by northern blot hybridization revealing distinctive patterns of expression (Brederode *et al.*, 1991; Memelink *et al.*, 1990; Ward *et al.*, 1991b). The genes encoding the acidic PR proteins, PR-1, glucanase, chitinase, PR-4 and thaumatin-like proteins, respond similarly to different forms of stress. Brederode *et al.* (1991) showed that these genes are highly inducible by TMV, but only moderately inducible by ethephon or UV light treatment and not inducible by wounding. In contrast, the genes for the basic forms of these PR proteins were expressed most strongly after treatment of tobacco with ethephon and to a lesser degree after wounding and TMV infection. A notable difference between the acidic and basic PR protein genes was the strong systemic expression of the former and the weak or non existent expression of the latter after TMV infection (Brederode *et al.*, 1991). Because wounding and ethephon treatments can strongly induce expression of the basic counterparts of PR-1, glucanase, chitinase and thaumatin-like proteins in tobacco, yet wounding does not induce resistance to TMV, these basic PR proteins are unlikely to play a role in induced antiviral resistance (Brederode *et al.*, 1991). This is supported by the observation that ethephon will induce resistance in N-gene tobacco to TMV without systemic induction of ß-1,3-glucanse (Ye *et al.*, 1992) and that injecting aspirin into tobacco stems protected leaves against TMV without expression of PR proteins (Ye *et al*, 1989). Instead, the expression patterns of the basic forms suggest that many have a function in normal plant development and responses to stress. For example, osmotin and the basic counterparts of ß-1,3-glucanase and chitinase are synthesized during *in vitro* culture of tobacco mesophyll protoplasts (Grosset *et al.*, 1990), and genes encoding these basic proteins are also expressed in tobacco explants during flower formation and are expressed constitutively in roots of tobacco plants (Neale *et al.*, 1990). Recently, an acidic chitinase was found to rescue a carrot embryo mutant arrested at the globular

stage, indicating that an acidic form may also have a role in development (De Jong *et al.*, 1992).

A more comprehensive study by Ward *et al.* (1991b) reported that TMV infection caused the systemic accumulation of mRNA corresponding to the acidic forms of PR-1, class II and III glucanases, class II and III chitinases, PR-4 and PR-5, plus accumulation of the basic forms of PR-1, class III chitinase and SAR8.2. The basic class I glucanase and class I chitinase and an acidic peroxidase were not systemically induced. The lack of induction of mRNA encoding the acidic peroxidase is not surprising because the probe used corresponded to a non-induced peroxidase isoform (Lagrimini and Rothstein, 1987). Salicyclic and methyl-2,6-dichloroisonicotinic acids, compounds that induce resistance in tobacco, induced expression of the same genes as did TMV infection (Ward *et al.*, 1991b). The coordinate expression of the systemically induced genes is associated temporally with the induction of resistance. The mRNAs for these genes began to accumulate to high levels in upper, uninfected leaves at 6 days after infection of lower leaves, a time when systemic induced resistance was just detected in the uninfected leaves (Ward *et al.*, 1991b).

## 4.2 PR-1 PROMOTERS

The isolation of genomic clones encoding proteins implicated in systemic induced resistance has made possible the characterization of regulatory elements that may control resistance induction. The PR-1 gene family of tobacco is one of the best studied with respect to its promoters. The 5' upstream sequence of the acidic PR-1a gene contains a consensus TATA box at position -34 and an 11 base pair imperfect direct repeat at -116 and -135 with respect to the transcription start site (Pfitzner *et al.*, 1988). In addition, the sequence C--GAA---TTC--G, which differs from the consensus heat shock regulatory element only by the insertion of one extra nucleotide, is located at position -57; the significance of this regulatory element homolog is unclear because heat shock conditions do not induce the expression of PR-1 genes (Cornelissen *et al.*, 1987; Pfitzner et al., 1988). Analysis of genomic clones encoding tobacco PR-1 genes indicated that several were not expressed due to deletions and insertions in their promoter region (Cornelissen *et al.*, 1987; Oshima *et al.*, 1987). The *cis*-acting elements of the PR-1a promoter which are involved in the induction of this gene by salicylic acid or TMV infection have been defined by functional assays in transgenic tobacco (Van de Rhee *et al.*, 1990). Upstream sequences of the PR-1a gene were fused to reporter genes and used to transform tobacco. Sequences containing at least 689 base pairs 5' of the transcription start site were sufficient for systemic induction of reporter gene expression. The TMV- and salicylic acid-responsive elements were localized between positions -643 and -689 in the PR-1a promoter (Van de Rhee, *et al.*, 1990). These results indicate that the previous reports of PR-1 mRNA accumulation in the non-infected upper leaves of tobacco plants with a localized infection are due to systemic transcriptional activation (Cornelissen *et al.*, 1986; Hooft van Huijduijnen *et al.*, 1986).

Basic members of the tobacco PR-1 gene family have very little similarity to acidic PR-1 genes in their promoter regions. A recent analysis of two basic PR-1 genes showed that homology with the upstream region of their acidic counterparts is limited to several regions in the first 150 bases of the promoter (Eyal *et al.*, 1992; Payne *et al.*, 1989). In comparison, the promoter regions of the two basic genes are approximately 70% identical up to position -654 where homology abruptly ends (Eyal *et al.*, 1992). The basic PR-1 promoter characterized by Eyal *et al.* (1992) contains, in addition to a TATA box at -44 nucleotides upstream, a CCAAT box at -93, a G-box consensus core sequence (CACGTG) at -185 and two AT-1 like sequences (AATATTTTTATT) at -467 and -767. No heat shock consensus sequence was reported. As would be expected by the different promoter composition, expression of basic PR-1 genes does not follow the pattern of acidic PR-1 genes. In the light, 18 ppm ethylene rapidly induced accumulation of basic but not acidic PR-1 transcripts and the transcripts of the basic genes were also induced, up to 50-fold, during 48 h of darkness (Eyal *et al.*, 1992). Interestingly, the *din*1 gene of radish (*Raphanus sativus*), which appears to be a member of the PR-1 family, undergoes a 100-fold increase in its transcripts after 24 h of darkness, suggesting a regulatory pattern in common with basic PR-1 genes (Azumi and Watanabi, 1991).

## 4.3 CHITINASE PROMOTERS

Analysis of a tobacco gene encoding a basic class I chitinase revealed that the gene contained two introns and the major transcription start site was 11 base pairs upstream of the ATG codon. In addition to a TATA and CCAAT box at positions -28 and -114 respectively, the promoter contained a motif at -140 that resembles the consensus heat shock regulatory element found in the PR-1 protein genes of tobacco (Shinshi *et al.*, 1990). Expression of a chimeric gene consisting of a basic tobacco chitinase coding region downstream of a CaMV 35S promoter resulted in intracellular accumulation of the enzyme in transgenic tobacco; in contrast, a petunia acidic chitinase similarly expressed as a chimeric gene in tobacco accumulated in the extracellular fluid (Linthorst *et al.*, 1990b). Closer examination of a basic class I tobacco chitinase demonstrated that a short C-terminal amino acid sequence (GNGLLVDTM) was sufficient for the targeting of chitinases, including the normally secreted cucumber chitinase, to the vacuole (Neuhaus *et al.*, 1991b).

Transcription driven by the promoter of an acidic chitinase has also been characterized (Samac and Shah, 1991). In fusion studies where the *Arabidopsis* acidic chitinase promoter was placed upstream of the ß-glucuronidase (GUS) reporter gene, transgenic *Arabidopsis* containing the gene fusion showed only local expression of GUS activity after *Rhizoctonia solani* infection. Furthermore, transgenic tomato plants carrying the *Arabidopsis* chitinase promoter construct exhibited only local expression around necrotic lesions after infection by *Phytophthora infestans* or *Alternaria solani* (Samac and Shah, 1991). These results provide further support for the notion that chitinase may not be important in the systemically induced resistance of *Arabidopsis*. Analysis of a series of 5' deletions of the *Arabidopsis* chitinase promoter indicated that nucleotide sequences

contained in the first 192 base pairs upstream from the transcription start site were sufficient to regulate both the constitutive and induced patterns of expression. Positive and negative regulatory elements that were involved in quantitative expression of the gene were located further upstream (Samac and Shah, 1991). The promoter of the *Arabidopsis* acidic chitinase gene did not contain any obvious sequence similarities to previously identified regulatory motifs.

## 4.4 GLUCANSES PROMOTERS

The regulation of a *Nicotiana plumbaginifolia* ß-1,3-glucanase gene (*gn*1) has been analyzed in transgenic tobacco containing a glucanase promoter-GUS reporter gene fusion (Castresana *et al.*, 1990) of developmentally regulated GUS activity were observed in the roots and older leaves of the transgenic tobacco, and the induction of GUS activity in leaves by *Pseudomonas syringae* pv. *syringae* was restricted to cells surrounding the hypersensitive lesion. Similarly, a construct using the promoter of the tobacco PR-2 glucanase gene fused to GUS was activated near necrotic lesions but not distally in TMV-infected transgenic tobacco (Hennig et al, 1991). Salicylic acid treatment induced expression of both the PR-2 and *gn*1 promoter-GUS gene fusions.

## 4.5 THE GRP-8 PROMOTER

The gene encoding a GRP is coordinately expressed with the acidic PR proteins of tobacco such as PR-1; for example, the GRP-8 and PR-1 genes are both induced by TMV infection or salicyclic acid treatment (Hooft van Huijduijnen *et al.*, 1986; van Kan *et al.*, 1988). However, comparison of their 5' flanking regions revealed only minor homology between the two classes of genes (van Kan *et al.*, 1988). The GRP contained numerous direct and inverted repeats between -510 and -200 base pairs upstream of the transcription start site. Interestingly, a 64 base pair inverted repeat of the GRP-8 promoter is also present in the upstream sequence of the gene encoding the small subunit of ribulose 1,5-bisphosphate carboxylase (rubisco ss) from tobacco (van Kan *et al.*, 1988). In potato, the transcription of the rubisco ss gene is rapidly *repressed* in response to infection or elicitor treatment, an effect which occurs systemically throughout the affected leaf (Kombrink and Hahlbrock, 1990). Perhaps the 64 base pair repeat is involved in detection of defense-related systemic signals. The timing of the potato rubisco ss gene inactivation was similar to that observed for transcriptional activation of defense genes. Kombrink and Hahlbrock (1990) suggested that the induction of multicomponent defense responses requires repression of other cellular functions to ensure metabolic balance.

The upstream sequences of the GRP also contained homology with the SV40 enhancer at position -540 and homology with the activator element (CCACACAATAATG) of the *Agrobacterium* cytokinin gene at position -110 (van Kan *et al.*, 1988). The sequence similar to the SV40 core enhancer may be relevant to systemic induced resistance. Expression of PR-1a and GRP-8 promoter deletions fused to the GUS reporter gene in

transgenic plants revealed that PR-1a sequences located between -689 and -643 and GRP-8 sequences located between -645 and -400 were necessary for induction of reporter gene expression by TMV infection or salicyclic acid treatment, and the only detectable similarity between the two enabling promoter regions was the small sequence resembling the SV40 core enhancer sequence (Bol et al, 1990).

## 5. Expression of PR Protein Genes in Transgenic Plants

The ability to constitutively express proteins in transgenic plants has allowed researchers to investigate the role of these proteins in resistance. Most studies have place the coding region of genes of interest under the transcriptional control of the cauliflower mosaic virus 35S promoter and transformed the chimeric construct into tobacco. The results obtained to date suggest that, in some cases but not all, constitutive expression of a single PR protein gene can result in increased disease resistance to selected pathogens. Broglie *et al* (1991) demonstrated that transgenic tobacco seedlings expressing a basic chitinase from bean and having up to 4-, 24- and 44-fold increases in root, stem and leaf chitinase activity, respectively, over controls, also exhibited delayed symptom development and a lower percentage of seeding mortality after exposure to the soil-born fungus *Rhizoctonia solani*. However, seedlings expressing the bean chitinase were not more resistant to *Phythium aphanidermatum*, a fungus that lacks chitin in its cell wall. In another study, high level expression of a basic, class I tobacco chitinase in transgenic *Nicotiana sylvestris* did not increase resistance to the chitin-containing fungus *Cercospora nicotianae* (Neuhaus *et al.*, 1991a). Also, constitutive expression of PR-1a, PR-1b, GRP or PR-5 (thaumatin-like protein) separately in transgenic tobacco did not result in increased resistance to TMV, implying that none of these proteins is capable of antiviral activity alone (Cutt *et al, 1989*; Linthorst *et al.*, 1989).

Other research groups are taking a systematic look at the effects of constitutive expression of PR protein genes, or their antisense transcripts, on the resistance of plants to a battery of pathogens (Alexander *et al.*, 1993; Ryals *et al.*, 1992a). So far, significant increases in resistance to *Rhizoctonia solani* have been observed in transgenic tobacco expressing high levels of basic class I chitinase and acidic class II and III chitinases (Ryals *et al.*, 1992b). In addition, engineered expression of the SAR8.2 gene or the PR-1a gene in tobacco resulted in greater resistance to *Phytophthora parasitica* and *Peronospora tabacina*, respectively (Alexander *et al.*, 1993a and 1993b).

Liu *et al.* (1994) recently reported that potato plants overexpressing osmotin exhibited a delay in the onset of late blight symprtoms after inoculation with *Phytophtora infestans*. Although not conferring total immunity, as in the case of PR-1 expression in tobacco (Alexander *et al.*, 1993), this result indicated that osmotin may play a role in acquired resistance.

Peroxidase activity has also been overexpressed in *Nicotiana tabacum* and *N. sylvestris* under the regulation of the cauliflower mosaic virus 35S promoter (Lagrimini

*et al.*, 1990). The cDNA encoding the expressed peroxidase corresponded to the P35 acidic peroxidase isolated by Lagrimini *et al.* (1987) which was not associated with systemic induced resistance. Although alterations in the disease resistance of the overexpressing plants have not been reported, the transformants of both species did display an unusual developmental phenotype of severe wilting through loss of turgor in leaves initiated at the time of flowering (Lagrimini *et al.*, 1990).

## 6. Concluding Remarks

A large, rapidly growing body of information exists on the systemically expressed genes that are associated with induced resistance. This is especially true for tobacco, the plant in which the biochemistry and molecular biology of systemic induced resistance has been best studied. At least eight families of genes are known to be systemically expressed by localized infection of tobacco, and proteins corresponding to many of these families have been well characterized. Furthermore, many homologs of the systemically expressed genes of tobacco, e.g., PR-1 related, chitinases, glucanases and thaumatin-like proteins, are found in a wide range of other plants, including monocots and dicots, many of which also exhibit induced resistance. Generally, the systemically expressed genes are responsive to local and systemic signals within the plant and encode acidic proteins that are targeted extracellularly. The basic counterparts of these genes are responsive to local and developmental signals and encode intracellular proteins. As with most rules, a few exceptions do occur; transcripts encoding basic forms of tobacco PR-1 and class III chitinase appear to accumulate systemically. Also, the SAR8.2 gene family is predicted to encode a basic protein with a N-terminal signal peptide that would suggest extracellular localization. SAR8.2 transcripts are normally at elevated steady state levels in tobacco, but the levels increase further in response to systemic signals.

Despite the volume of information accumulating on systemic induced resistance, there is much that we do not know. For example, the PR-1 proteins are the most abundant induced proteins in systemically protected tobacco leaves and are highly conserved in the plant kingdom, yet their biological function is still a mystery. Similarly, no function has been assigned to the PR-4 or SAR8.2 gene products. Even in the cases where a function has been indicated, either by the detection of enzyme activity or by homology to known proteins, the role of the systemically induced proteins in resistance has not been demonstrated. Another area where our knowledge is lacking is identification of the promoter elements that control systemic gene expression. Results so far have been puzzling because genes with similar patterns of systemic expression contain promoters with very little sequence homology. No nucleic acid motifs unique to systemically induced genes have been reported as of yet. The promoter of a systemically induced gene can activate the systemic expression of a reporter gene, demonstrating that the necessary sequences to react to systemic plant signals are present, but this gives us the problem of how coordinate gene expression in achieved with dissimilar promoters. The different promoters of systemically induced genes may each detect separate systemic

signals, which would seem unlikely, or a sole systemic signal may act through several different intermediate signals which would then activate the genes.

Transcriptional activation, however, is not the only method of controlling gene expression. Changes in the stability of mRNA or in the efficiency of mRNA processing could also alter the amount of a particular transcript. Furthermore, regulation of gene expression could occur at the translational and post-translational levels. Thus, studies that only consider the abundance of mRNA may not necessarily reflect the activity of the promoter or indicate the accumulation or activation of the corresponding protein. Little is known about the regulation of systemic gene expression at these multiple levels. A recent study examining the expression of the osmotin gene in tobacco (cv. Wisconsin 38) reported that several strong inducers of its mRNA, ABA, TMV and wounding, did not lead to comparable protein accumulation (LaRosa *et al.*, 1992). These results indicate that changes in the level of mRNA without supporting information on the level and functional activity of the protein should be interpreted carefully.

Comparisons of various plant species that are known to express systemic resistance indicates that each may have a different spectrum of induced proteins. Tobacco, because it has been examined the most thoroughly, has the largest described set of genes and their products which are associated with induced resistance. In spite of this, the induced protease activity of tomato has not been reported for tobacco. Then there is the example of chitinase which has demonstrated antifungal activity and is induced in tobacco and cucumber but is not induced in resistant *Arabidopsis*. Thus, similar to what is seen in local induced defense mechanisms, systemically induced resistance employs an array of potential defenses, which may include mechanisms commonly used by diverse plant species and mechanisms unique to particular groups or species of plants. Because of the apparent diversity in the expression of induced resistance it will be important to study a wide range of plants to understand the many facets of this resistance. The relatively short list of systemically expressed genes in plants other than tobacco is because they have not received the same intensive study.

The future prospects for understanding the regulation and basis of systemic induced resistance are good. The technology now exists to dissect the mechanisms involved in resistance, from detection of the initial infection to expression of genes after challenge inoculation. Currently, the effect of overexpressing several PR proteins in the same tobacco plant is being examined by introducing multiple gene constructs into the plants and by genetic crosses (Alexander *et al.*, 1993a; Ryals *et al.*, 1992a). Similarly, transgenic plants expressing high levels of antisense to the PR protein genes are to be used to evaluate the role of the genes in disease resistance. The antisense approach is particularly interesting because it will create functional mutants of one or more systemically expressed genes in tobacco and could produce a tobacco plant deficient in all of the major PR proteins. Another powerful tool will be the use of *Arabidopsis* mutants that are altered in their expression of induced resistance. Researchers are developing infection systems in *Arabidopsis* which can be used to discover and analyze the components involved in systemic signaling and expression of resistance (de Maagd

*et al.*, 1993). These and other approaches should provide key insights into the mechanisms involved in systemic induced resistance.

## References


Adám, A., Barna, B., Farkas, T. and Király, Z. (1990) 'Effect of TMV induced systemic acquired resistance and removal of the terminal bud on membrane lipids of tobacco leaves', Plant Science 66, 173-179.

Alexander, D., Stinson, J., Pear, J., Glascock, C., Ward, E., Goodman, R.M., Ryals, J. (1992)'A new multigene family inducible by tobacco mosaic virus or salicylic acid in tobacco', Molecular Plant-Microbe Interactions 5, 513-515.

Alexander, D., Glascock, C., Pear, J., Stinson, J., Ahl-Goy, P., Gut-Rella, M., Ward, E., Goodman, R.M. and Ryals, J. (1993a) 'Systemic acquired resistance in tobacco: Use of transgenic expression to study the functions of pathogenesis-related proteins', Advances in Molecular Genetics of Plant-Microbe Interactions, E.W. Nester and D.P.S. Verma, eds., Kluwer Academic Publishers, Netherlands, pp 527-533.

Alexander, D., Goodman, R.M., Gut-Rella, M., Glascock, C., Weyman, K., Friedrich, L.,Maddox, D., Ahl-Goy, P., Luntz, T., Ward, E., Ryals, J. (1993b) 'Increased tolerance to two oomycete pathogens in transgenic tobacco expressing pathogenesis-related protein 1a', Proceedings of the National Academy of Sciences, USA 90, 7327-7331.

Antoniw, J.F. and White, R.F. (1983) 'Biochemical properties of the pathogenisis-related proteins from tobacco', Netherlands Journal of Plant Pathology 89, 255-264.

Antoniw, J.F., Ritter, C.E., Pierpoint, W.S. and Van Loon, L.C. (1980) 'Comparison of three pathogenesis-related proteins from plants of two cultivars of tobacco infected with TMV', Journal of General Virology 47, 79-87.

Apostol, I., Heinstein, P.F. and Low, P.S. (1989) 'Rapid stimulation of an oxidative burst during elicitation of cultured plant cells', Plant Physiology 90, 109-116.

Azumi, Y. and Watanabe, A. (1991) 'Evidence for a senescence-associated gene induced by darkness', Plant Physiology 95, 577-583.

Binder, A., Baer, G., Hofmann, C. and Kovats, K. (1989) 'Mechanisms in systemic induced disease resistance', Signal Molecules in Plants and Plant-Microbe Interactions, B.J.J. Lugtenberg, ed, Springer-Verlag, Berlin, Heidelberg, vol. H36, pp 267-272.

Bol, J.F. (1988) 'Structure and expression of plant genes encoding pathogenesis-related proteins' in D.P.S. Verma and R.B. Goldberg (eds.), Temporal and Spatial Regulation of Plant Genes, Springer-Verlag, New York, pp. 201-221.

Bol, J.F., Linthorst, H.J.M. and Cornelissen, B.J.C. (1990) 'Plant pathogensis-related proteins induced by virus infection', Annual Review Phytopathology 28, 113-138.

Boller, T. (1985) 'Induction of hydrolases as a defense reaction against pathogens' in J.L. Key and T. Kosuge (eds.), Cellular and Molecular Biology of Plant Stress, UCLA Symposia on Molecular and Cellular Biology, New Series, Volume 22, Alan R. Liss, Inc., New York, pp. 247-262.

Boller, T. (1988) 'Ethylene and the regulation of antifungal hydrolyases in plants', Oxford Surveys of Plant Molecular and Cell Biology 5, 145-175.

Bradley, D.J., Kjellbom, P. and Lamb, C.J. (1992) 'Elicitor- and wound-induced oxidative cross-linking of a proline-rich plant cell wall protein: a novel, rapid defense response', Cell 70, 21-30.

Brederode, F.T., Linthorst, H.J.M. and Bol, J.F. (1991) 'Differential induction of acquired resistance and PR gene expression in tobacco by virus infection, ethephon treatment, UV light and wounding', Plant Molecular Biology 17, 1117-1125.

Broglie, K., Chet, I., Holliday, M., Cressman, R., Biddle, P., Knowlton, S., Mauvais, C.J. and Broglie, R. (1991) 'Transgenic plants with enhanced resistance to the fungal pathogen *Rhizoctonia solani*', Science 254, 1194-1197.

Broglie, K.E., Gaynor, J.J. and Broglie, R.M. (1986) 'Ethylene-regulated gene expression: Molecular cloning of the genes encoding an endochitinase from *Phaseolus vulgaris*'', Proceedings National Academy of Science USA 83, 6820-6824.

Carr, J.P., Dixon, D.C. and Klessig, D.F. (1985) 'Synthesis of pathogenesis-related proteins in tobacco is regulated at the level of mRNA accumulation and occurs on membrane-bound polysomes', Proceedings National Academy of Science USA 82, 7999-8003.

Cassab, G.I. and Varner, J.E. (1988) 'Cell wall proteins', Annual Review of Plant Physiology and Plant Molecular Biology 39, 321-353.

Castresana, C., de Carvalho, F., Gheysen, G., Habets, M., Inze, D. and Van Montagu, M. (1990) 'Tissue-specific and pathogen-induced regulation of a *Nicotiana plumbaginifolia* ß-1,3-glucanase gene', The Plant Cell 2, 1131-1143.

Christ, U. and Mösinger, E. (1989) 'Pathogenesis-related proteins of tomato: I. Induction by *Phytophthora infestans* and other biotic and abiotic inducers and correlations with resistance', Physiological and Molecular Plant Pathology 35, 53-65.

Cloud, A.M.E. and Deverall, B.J. (1987) 'Induction and expression of systemic resistance to the anthracnose disease in bean', Plant Pathology 36, 551-557.

Condit, C.M. and Keller, B. (1990) 'The glycine-rich cell wall proteins of higher plants' in W.S. Adair and R.P. Mecham (eds.), Organization and Assembly of Plant and Animal Extracellular Matrix, Academic Press, New York, pp. 119-135.

Cornelissen, B.J.C., Hooft van Huijsduijnen, R.A.M. and Bol, J.F. (1986) 'A tobacco mosaic virus-induced tobacco protein is homologous to the sweet-tasting protein thaumatin', Nature 321, 531-532.

Cornelissen, B.J.C., Hooft van Huijsduijnen, R.A.M., Van Loon, L.C. and Bol, J.F. (1986) 'Molecular characterization of messenger RNAs for 'pathogenesis-related' proteins 1a, 1b and 1c, induced by TMV infection of tobacco', EMBO Journal 5, 37-40.

Cornelissen, B.J.C., Horowitz, J., van Kan, J.A.L., Goldberg, R.B. and Bol, J.F. (1987) 'Structure of tobacco genes encoding pathogenesis-related proteins from the PR-1 group', Nucleic Acids Research 15, 6799-6811.

Cutt, J.R., Harpster, M.H., Dixon, D.C., Carr, J.P., Dunsmuir, P. and Klessig, D.F. (1989) 'Disease response to tobacco mosaic virus in transgenic tobacco plants that constitutively express the pathogenesis-related PR1b gene', Virology 173, 89-97.

De Jong, A.J., Cordewener, J., Lo Schiavo, F., Terzi, M., Vandekerckhove, J., Van Kammen, A. and De Vries, S.C. (1992) 'A carrot somatic embryo mutant is rescued by chitinase', The Plant Cell 4, 425-433.

de Maagd, R.A., Cameron, R.K., Dixon, R.A. and Lamb, C.J. (1993) 'Screening methods for *Arabidopsis* mutants affected in the signal transduction pathways leading to defense responses', Advances in Molecular Genetics of Plant-Microbe Interactions, E.W. Nestler and D.P.S. Verma, eds, Kluwer Academic Publishers, Netherlands, pp. 445-449.

de Oliveira, D.E., Seurinck, J., Inze, D., Van Montagu, M. and Botterman, J. (1990) 'Differential expression of five *Arabidopsis* genes encoding glycine-rich proteins', The Plant Cell 2, 427-436.

Dixon, R.A. and Harrison, M.J. (1990) 'Activation, structure and organization of genes involved in microbial defense in plants', Advances in Genetics 28, 165-234.

Dixon, R.A., Bhattacharyya, M.K., Harrison, M.J., Faktor, O., Lamb, C.J., Loake, G.J., Ni, W., Oommen, A., Paiva, N., Stermer, B. and Yu, L.M. (1993) 'Transcriptional regulation of phytoalexin biosynthetic genes', Advances in Molecular Genetics of Plant-Microbe Interactions, E.W. Nester and D.P.S. Verma, eds, Kluwer Academic Publishers, Netherlands, pp 497-509.

Dixon, R.A., Dey, P.M. and Lamb, C.J. (1983) 'Phytoalexins: enzymology and molecular biology', Advances in Enzymology and Related Areas of Molecular Biology 55, 1-136.

Enyedi, A.J., Yalpani, N., Silverman, P. and Raskin, I. (1992) 'Signal molecules in systemic plant resistance to pathogens and pests', Cell 70, 879-886.

Eyal, Y., Sagee, O. and Fluhr, R. (1992) ' Dark-induced accumulation of a basic pathogenesis-related (PR-1) transcript and a light requirement for its induction by ethylene', Plant Molecular Biology 19, 589-599.

Fang, R-X., Pang, Z., Gao, D-M., Mang, K-Q and Chua, N-H. (1991) 'cDNA sequence of a virus-inducible, glycine-rich protein gene from rice', Plant Molecular Biology 17, 1255-1257.

Farmer, E.E. and Ryan, C.A. (1992) 'Octadecanoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase inhibitors', The Plant Cell 4, 129-134.

Friedrich, L., Moyer, M., Ward, E., Ryals, J. (1991) 'Pathogenesis-related protein 4 is structurally homologous to the carboxy-terminal domains of hevein, Win-1 and Win-2', Molecular and General Genetics 230, 113-119.

Gianinazzi, S. and Ahl, P. (1983) 'The genetic and molecular basis of b-proteins in the genus Nicotiana', Netherlands Journal of Plant Pathology 89, 275-281.

Gianinazzi, S., Martin, C. and Vallee, J.C. (1970) 'Hypersensibility aux virus, temperatures et proteines solubles chez le *Nicotiana* Xanthi nc. Aparition de nouvelles macromolecules lors de la repression de la synthase virale', C.R. Acad. Sci. Paris D. 270, 2383-2386.

Graham, J.S., Burkhart, W., Xiong, J. and Gillikin, J.W. (1992) 'Complete amino acid sequence of soybean leaf P21 similarity to the thaumatin-like polypeptides', Plant Physiology 98, 163-165.

Grosset, J., Meyer, Y., Chartier, Y., Kauffmann, S., Legrand, M. and Fritig, B. (1990) 'Tobacco mesophyll protoplasts synthesize 1,3-ß-glucanase, chitinases, and "osmotins" during *in vitro* culture', Plant Physiology 92, 520-527.

Hadwiger, L.A. and Beckman, J.M. (1980) 'Chitosan as a component of pea- *Fusarium solani* interactions', Plant Physiology 66, 205-211.

Hahlbrock, K., Arabatizis, N., Becker-Andre, M., Joos, H-J., Kombrink, E., Schroder, M., Strittmatter, G. and Taylor, J. (1989) 'Local and systemic gene activation in fungus-infected potato leaves', Signal Molecules in Plants and Plant-Microbe Interactions, B.J.J. Lugtenberg, ed, Springer-Verlag, Berlin, Heidelberg, vol. H36, pp. 241-249.

Hammerschmidt, R. and Kuć, J. (1982) 'Lignification as a mechanism for induced systemic resistance in cucumber', Physiological Plant Pathology 20, 61-71.

Hammerschmidt, R., Nuckles, E.M. and Kuć, J. (1982) 'Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*', Physiological Plant Pathology 20, 73-82.

Hennig, J., Dewey, R.E., Cutt, J.R. and Klessig, D.F. (1991) 'Regulation of a tobacco ß-1,3-glucanase gene promoter by TMV infection, salicylic acid and developmental signals', Proceedings Third International Congress of Plant Molecular Biology, Tucson, Abstract 1137.

Hooft van Huijsduijnen, R.A.M., Kauffmann, S., Brederode, F.T., Cornelissen, B.J.C., Legrand, M., Fritig, B. and Bol, J.F. (1987) 'Homology between chitinases that are induced by TMV infection of tobacco', Plant Molecular Biology 9, 411-420.

Hooft van Huijsduijnen, R.A.M., van Loon, L.C. and Bol, J.F. (1986) 'cDNA cloning of six mRNAs induced by TMV infection of tobacco and a characterization of their translation products', The EMBO Journal 5, 2057-2061.

Intapruk, C., Higashimura, N., Yamamoto, K., Okada, N., Shinmyo, A. and Takano, M. (1991) 'Nucleotide sequences of two genomic DNAs encoding peroxidase of *Arabidopsis thaliana*', Gene 98, 237-241.

Kauffman, S., Legrand, M., Geoffroy, P., and Fritig, B. (1987) 'Biological function of pathogenesis-related proteins. Four PR-proteins of tobacco have 1,3-ß-glucanase activity', EMBO Journal 6, 3209-3212.

Kauffmann, S., Legrand, M. and Fritig, B. (1990) 'Isolation and characterization of six pathogenesis-related (PR) proteins of Samsun NN tobacco', Plant Molecular Biology 14, 381-390.

Keen, N.T. and Yoshikawa, M. (1983) 'ß-1,3-Endoglucanase from soybean releases elicitor-active carbohydrates from fungus cell walls', Plant Physiology 71, 460-465.

Kombrink, E. and Hahlbrock, K. (1990) 'Rapid, systemic repression of the synthesis of ribulose 1,5-bisphosphate carboxylase small-subunit mRNA in fungus-infected or elicitor-treated potato leaves', Planta 181, 216-219.

Kombrink, E., Schröder, M. and Hahlbrock, K. (1988) 'Several "pathogenesis-related" proteins in potato are 1,3-ß-glucanases and chitinases', Proceedings National Academy of Science USA 85, 782-786.

Lagrimini, L.M. , Burkhart, W., Moyer, M. and Rothstein, S. (1987) 'Molecular cloning of complementary DNA encoding the lignin-forming peroxidase from tobacco: Molecular analysis and tissue-specific expression', Proceedings National Academy of Science USA 84, 7542-7546.

Lagrimini, L.M. and Rothstein, S. (1987) 'Tissue specificity of tobacco peroxidase isozymes and their induction by wounding and tobacco mosaic virus infection', Plant Physiology 84, 438-442.

Lagrimini, L.M., Bradford, S. and Rothstein, S. (1990) 'Peroxidase-induced wilting in transgenic tobacco plants', The Plant Cell 2, 7-18.

LaRosa, P.C., Chen, Z., Nelson, D.E., Singh, N.K., Hasegawa, P.M. and Bressan, R.A. (1992) 'Osmotin gene expression is posttranscriptionally regulated', Plant Physiology 00, 409-415.

Lawton, K., Ward, E., Payne, G., Moyer, M. and Ryals, J. (1992) 'Acidic and basic class III chitinase mRNA accumulation in response to TMV infection of tobacco', Plant Molecular Biology 19, 735-743.

Legrand, M., Kauffmann, S., Geoffroy, P. and Fritig, B. (1987) 'Biological function of pathogenesis-related proteins: Four tobacco pathogenesis-related proteins are chitinases', Proceedings National Academy of Science USA 84, 6750-6754.

Linthorst, H.J.M., Meuwissen, R.L.J., Kauffmann, S. and Bol, J.F. (1989) 'Constitutive expression of pathogenesis-related proteins PR-1, GRP, and PR-S in tobacco has no effect on virus infection', The Plant Cell 1, 285-291.

Linthorst, H.J.M., van Loon, L.C., Memelink, J. and Bol, J.F. (1990) 'Characterization of cDNA clones for a virus-inducible, glycine-rich protein from petunia', Plant Molecular Biology 15, 521-523.

Linthorst, H.J.M., van Loon, L.C., van Rossum, C.M.A., Mayer, A., Bol, J.F., van Roekel, J.S.C., Meulenhoff, E.J.S. and Cornelissen, B.J.C. (1990) 'Analysis of acidic and basic chitinases from tobacco and petunia and their constitutive expression in transgenic tobacco', Molecular Plant-Microbe Interactions 3, 252-258.

Liu, D., Raghothama, K.G., Hasegawa, P.M., and Bressan, R.A. (1994) 'Osmotin overexpression in potato delays development of disease symptoms', Proceedings of the National Academy of Sciences, USA 91, 1888-1892.

Lizzi, Y. and Coulomb, P.J. (1991) 'comportement des fractions peroxydasique et catalasique solubles au cours d'internactions entre le piment et le virus de la mosaique du tabac ou le Phytophthora capsici;', Canadian Journal of Botany 69, 1853-1864.

Lösel, D.M. (1991) 'Synthesis and functioning of membrane lipids in fungi and infected plants' Pesticide Science 32, 353-362.

Malamy, J., Carr, J.P., Klessig, D.F., and Raskin, I. (1990) 'Salicylic acid: a likely endogenous signal in the resistance response of tobacco to viral infections', Science 250, 1001-1004.

Margis-Pinheiro, M., Metz-Boutigue, M.H., Awade, A., de Tapia, M., le Ret, M., Burkard, G. (1991) 'Isolation of a complementary DNA encoding the bean PR4 chitinase: an acidic enzyme with an amino-terminus cysteine-rich domain', Plant Molecular Biology 17, 243-253.

Mauch, F., Mauch-Mani, B. and Boller, T. (1988) 'Antifungal hydrolases in pea tissue II. inhibition of fungal growth by combination of chitinase and ß-1,3-glucanase', Plant Physiology 88, 936-942.

Meins, Jr., F. and Ahl, P. (1989) 'Induction of chitinase and ß-1,3-glucanase in tobacco plants infected with *Pseudomonas tabaci* and *Phytophthora parasitica* va. *Nicotianae*', Plant Science 61, 155-161.

Memelink, J., Hoge, J.H.C. and Schilperoort, R.A. (1987) 'Cytokinin stress changes the developmental regulation of several defence-related genes in tobacco', The EMBO Journal 6, 3579-3583.

Memelink, J., Linthorst, H.J.M., Schilperoort, R.A. and Hoge, J.H.C. (1990) 'Tobacco genes encoding acidic and basic isoforms of pathogenesis-related proteins display different expression patterns', Plant Molecular Biology 14, 119-126.

Métraux, J.P., Burkhart, W., Moyer, M., Dincher, S., Middlesteadt, W., Williams, S., Payne, G., Carnes, M. and Ryals, J. (1989) 'Isolation of a complementary DNA encoding a chitinase with structural homology to a bifunctional lysozyme/chitinase', Proceedings National Academy of Sciences USA 86, 896-900.

Métraux, J.P., Signer, H., Ryals, J., Ward, E., Wyss-Benz, M., Gaudin, J., Raschdorf, K., Schmid, E., Blum, W. and Inverardi, B. (1990) 'Increase in salicyclic acid at the onset of systemic acquired resistance in cucumber' Science 250, 1004-1006.

Métraux, J.P., Streit, L. and Staub, T (1988) 'A pathogenesis-related protein in cucumber is a chitinase', Physiological and Molecular Plant Pathology 33, 1-9.

Metzler, M.C., Cutt, J.R. and Klessig, D.F. (1991) 'Isolation and characterization of a gene encoding a PR-1-like protein from *Arabidopsis thaliana*', Plant Physiology 96, 346-348.

Nadolny, L. and Sequeria, L. (1980) ' Increases in peroxidase activities are not directly invovled in induced resistance in tobacco', Physiological Plant Pathology 16, 1-8.

Neale, A.D., Wahleithner, J.A., Lund, M., Bonnett, H.T., Kelly, A., Meeks-Wagner, D.R., Peacock, W.J. and Dennis, E.S. (1990) 'Chitianse, ß-1,3-glucanase, osmotin, and extensin are expressed in tobacco explants during flower formation', The Plant Cell 2, 673-684.

Nelson, D.E., Raghothama, K.G., Singh, N.K., Hasegawa, P.M. and Bressan, R.A. (1992) 'Analysis of structure and transcriptional activation of an osmotin gene', Plant Molecular Biology 19, 577-588.

Neuhaus, J-M., Ahl-Goy, P., Hinz, U., Flores, S. and Meins, Jr., F. (1991) 'High-level expression of a tobacco chitinase gene in *Nicotiana sylvestris*. Susceptibility of transgenic plants to *Cercospora nicotianae* infection', Plant Molecular Biology 16, 141-151.

Neuhaus, J-M., Sticher, L., Meins, Jr., F., Boller, T. (1991) 'A short C-terminal sequence is necessary and sufficient for the targeting of chitinases to the plant vacuole', Proceedings National Academy of Sciences, USA 88, 10362-10366.

Ohshima, M., Matsuoka, M., Yamamoto, N., Tanaka, Y., Kano-Murakami, Y., Oreki, Y., Kato, A., Harada, N. and Ohashi, Y. (1987) ' Nucleotide sequence of the PR-1 gene of *Nicotiana tabacum*', FEBS Letters 225: 243-246.

Orr, J.D. and Lynn, D.G. (1992) 'Biosynthesis of dehydrodiconiferyl alcohol glucosides: implications for the control of tobacco cell growth', Plant Physiology 98, 343-352.

Payne, G., Ahl, P., Moyer, M., Harper, A., Beck, J., Meins, Jr., F. and Ryals, J. (1990) 'Isolation of complementary DNA clones encoding pathogenesis-related proteins P and Q, two acidic chitinases from tobacco', Proceedings National Academy of Science USA 87, 98-102.

Payne, G., Middlesteadt, W., Desai, N., Williams, S., Dincher, S., Carnes, M. and Ryals, J. (1989) 'Isolation and sequence of a genomic clone encoding the basic form of pathogenesis-related protein 1 from *Nicotiana tabacum*', Plant Molecular Biology 12, 595-596.

Payne, G., Middlesteadt, W., Williams, S., Desai, N., Parks, T.D., Dincher, S., Carnes, M. and Ryals, J. (1988) 'Isolation and nucleotide sequence of a novel cDNA clone encoding the major form of pathogenesis-related protein R', Plant Molecular Biology 11, 223-224.

Payne, G., Ward, E., Gaffney, T., Ahl-Goy, P., Moyer, M., Harper, A., Meins, Jr., F. and Ryals, J. (1990) 'Evidence for a third structural class of ß-1,3-glucanase in tobacco', Plant Molecular Biology 15, 797-808.

Peng, M. and Kuc, J. (1992) 'Peroxidase-generated hydrogen peroxide as a source of antifunal activity in vitro and on tobacco leaf disks', Phytopathology 82, 696-699.

Pfitzner, U.M. and Goodman, H.M. (1987) 'Isolation and characterization of cDNA clones encoding pathogenesis-related proteins from tobacco mosaic virus infected tobacco plants', Nucleic Acids Research 15, 4449-4465.

Pfitzner, U.M., Pfitzner, A.J.P. and Goodman, H.M. (1988) 'DNA sequence analysis of a PR-1a gene from tobacco: Molecular relationship of heat shock and pathogen responses in plants', Molecular and General Genetics 211, 290-295.

Rasmussen, J.B., Hammerschmidt, R. and Zook, M.N. (1991) 'Systemic induction of salicylic acid accumulation in cucumber after inocualtion with *Pseudomonas syringae* pv *syringae*', Plant Physiology 97, 1342-1347.

Rasmussen, J.B., Smith, J.A., Williams, S., Burkhardt, W., Somerville, S.C., Ryals, J. and Hammerschmidt, R. (1994) 'cDNA cloning and systemic expression of a peroxidase associated with systemic acquired resistance in cucumber', Physiological and Molecular Plant Pathology (in press).

Redolfi, P. (1983) 'Occurrence of pathogenesis-related (b) and similar proteins in different plant species', Netherlands Journal of Plant Pathology 89, 245-254.

Reimmann, C., Mauch, F. and Dudler, R. (1991) 'Identification and characterization of systemically induced rice genes correlating with acquired resistance against the rice

blast fungus *Pyricularia oryzae*' Third International Congress of Plant Molecular Biology, Tucson, Abstract #1377.

Reimmann, C., Ringli, C. and Dudler, R. (1992) 'Complementary DNA cloning and sequencing analysis of a pathogen-induced putative peroxidase from rice', Plant Physiology 100, 1611-1612.

Richardson, M., Valdes-Rodriquez, S., and Blanco-Labra, A. (1987) 'A possible function for thaumatin and a TMV-induced protein suggested by homology to a maize inhibitor', Nature 327, 432-434.

Rigden, J. and Coutts, R. (1988) 'Pathogenesis-related proteins in plants', Trends in Genetics 4, 87-89.

Roberts, E. and Kolattukudy, P.E. (1989) 'Molecular cloning, nucleotide sequence, and abscisic acid induction of a suberization-associated highly anionic peroxidase', Molecular General Genetics 217, 223-232.

Roberts, E., Kutchan, T. and Kolattukudy, P.E. (1988) 'Cloning and sequencing of cDNA for a highly anionic peroxidase from potato and the induction of its mRNA in suberizing potato tubers and tomato fruits', Plant Molecular Biology 11, 15-26.

Ross, A.F. (1961) 'Systemic acquired resistance induced by localized virus infections in plants', Virology 14, 340-358.

Ruzicska, P., Gombos, Z. and Farkas, G.L. (1983) 'Modification of the fatty acid composition of phospholipids during the hypersensitive reaction in tobacco', Virology 128, 60-64.

Ryals, J., Ward, E., Ahl-Goy, P. and Métraux, J.P. (1992a) 'Systemic acquired resistance: an inducible defense mechanism in plants', Society for Experimental Biology Seminar Series 49, 205-229.

Ryals, J., Ward, E., Uknes, S., Lawton, K., Gaffney, T., Friedrich, L., Alexander, D., Goodman, R., Métraux, J-P., Kessmann, H., Ahl-Goy, P. and Gut-Rella, M.G. (1992b) 'The molecular biology of systemic acquired resistance', Proceedings of Davis meeting, in press.

Samac, D.A. and Shah, D.M. (1991) 'Developmental and pathogen-induced activation of the Arabidopsis acidic chitinase promoter', The Plant Cell 3, 1063-1072.

Samac, D.A., Hironaka, C.M., Yallaly, P.E. and Shah, D.M. (1990) 'Isolation and characterization of the genes encoding basic and acidic chitinase in *Arabidopsis thaliana*', Plant Physiology 93, 907-914.

Shinshi, H., Neuhaus, J-M, Ryals, J. and Meins, Jr., F. (1990) 'Structure of a tobacco endochitinase gene: evidence that different chitinase genes can arise by transposition of sequences encoding a cysteine-rich domain', Plant Molecular Biology 14, 357-368.

Shinshi, H., Wenzler, H., Neuhaus, J.-M., Felix, G., Hofsteenge, J. and Meins, Jr., F. (1988) 'Evidence for N- and C-terminal processing of a plant defense-related enzyme: Primary structure of tobacco prepro-ß-1,3-glucanase', Proceedings National Academy of Science USA 85, 5541-5545.

Showalter, A.M., Butt, A.D. and Kim, S. (1992) 'Molecular details of tomato extensin and glycine-rich protein gene expression', Plant Molecular Biology 19, 205-215.

Simons, T.J. and Ross, A.F. (1970) 'Enhanced peroxidase activity associated with induction of resistance to tobacco mosaic virus in hypersensitive tobacco', Phytopathology 60, 383-384.

Simons, T.J. and Ross, A.F. (1971) 'Changes in phenol metabolism associated with induced systemic resistance to tobacco mosaic virus in Samsun NN tobacco', Phytopathology 61, 1261-1265.

Simons, T.J. and Ross, A.F. (1971) 'Metabolic changes associated with systemic induced resistance to tobacco mosaic virus in Samsun NN tobacco', Phytopathology 61, 293-300.

Singh, N.K., Nelson, D.E., Kuhn, D., Hasegawa, P.M. and Bressan, R.A. (1989) 'Molecular cloning of osmotin and regulation of its expression by ABA and adaptation to low water potential', Plant Physiology 90, 1096-1101.

Smith, J.A. and Hammerschmidt, R. (1988) 'Comparative study of acidic peroxidases associated with induced resistance in cucumber, muskmelon and watermelon', Physiological and Molecular Plant Pathology 33, 255-261.

Smith, J.A. and Métraux, J-P (1991) '*Pseudomonas syringae* pv. *syringae* induces systemic resistance to *Pyricularia oryzae* in rice', Physiological and Molecular Plant Pathology 39, 451-461.

Stermer, B.A. and Hammerschmidt, R. (1984) 'Heat shock induces resistance to *Cladosporium cucumerinum* and enhances peroxidase activity in cucumber', Physiological Plant Pathology 25, 239-249.

Stermer, B.A. and Hammerschmidt, R. (1987) 'Association of heat shock induced resistance to disease with increased accumulation of insoluble extensin and ethylene synthesis', Physiological and Molecular Plant Pathology 31, 453-461.

Stermer, B.A., Schmid, J., Lamb, C.J. and Dixon, R.A. (1990) 'Infection and stress activation of bean chalcone synthase promoters in transgenic tobacco', Molecular Plant-Microbe Interactions 3, 381-388.

Stintzi, A., Heitz, T., Kauffmann, S., Legrand, M. and Fritig, B. (1991) 'Identification of a basic pathogenesis-related, thaumatin-like protein of virus-infected tobacco as osmotin', Physiological and Molecular Plant Pathology 38, 137-146.

Sturm, A. (1992) 'A wound-inducible glycine-rich protein from *Daucus carota* with homology to single-stranded nucleic acid-binding proteins', Plant Physiology 99, 1689-1692.

Sziraki, I., Balazs, E. and Kiraly, Z. (1980) 'Role of different stresses in inducing systemic acquired resistance to TMV and increasing cytokinin level in tobacco', Physiological Plant Pathology 16, 277-284.

Takeuchi, Y., Yoshikawa, M., Takeba, G., Tanaka, K., Shibata, D. and Horino, O. (1990) 'Molecular cloning and ethylene induction of mRNA encoding a phytoalexin elicitor-releasing factor, ß-1,3-endoglucanase, in soybean', Plant Physiology 93, 673-682.

Teutonico, R., Dudley, M.D., Orr, J.D., Lynn, D.G., and Binns, A.N. (1991) 'Activity and accumulation of cell-division-promoting phenolics in tobacco tissue cultures', Plant Physiology 97, 288-297.

Tuzun, S., Rao, M.N., Vogelli, L., Schardle, C.L. and Kuc', J. (1989) 'Induced systemic resistance to blue mold: early induction and accumulation of chitinase, ß-1,3-glucanase and other pathogenisis-related (b-) proteins in immunized tobacco', Phytopathlogy 79, 979-983.

Uknes, S., Mauch-Mani, B., Moyer, M., Potter, S., Williams, S., Dincher, S., Chandler, D., Slusarenko, A., Ward, E. and Ryals, J. (1992) 'Acquired resistance in *Arabidopsis*', The Plant Cell 4, 645-656.

Van de Rhee, M.D., Van Kan, J.A.L., Gonzalez-Jaen, M.T. and Bol, J.F. (1990) 'Analysis of regulatory elements involved in the induction of two tobacco genes by salicylate treatment and virus infection', The Plant Cell 2, 357-366.

Van den Bulcke, M., Bauw, G., Castresana, C., Van Montagu, M. and Vandekerckhove, J. (1989) 'Characterization of vacuolar and extracellular ß(1,3)-glucanases of tobacco: evidence for a strictly compartmentalized plant defense system', Proceedings National Academy of Science USA 86, 2673-2677.

van Huystee, R.B. (1987) 'Some molecular aspects of plant peroxidase biosynthetic studies', Annual Review of Plant Physiology 38, 205-219.

van Kan, J.A.L., Cornelissen, B.J.C. and Bol, J.F. (1988) 'A virus-inducible tobacco gene encoding a glycine-rich protein shares putative regulatory elements with the ribulose bisphosphate carboxylase small subunit gene', Molecular Plant-Microbe Interactions 1, 107-112.

van Kan, J.A.L., Joosten, M.H.A.J., Wagemakers, C.A.M., van den Berg-Velthuis, G.C.M. and de Wit, P.J.G.M. (1992) 'Differential accumulation of mRNAs encoding extracellular and intracellular PR proteins in tomato induced by virulent and avirulent races of *Cladosporium fulvum*', Plant Molecular Biology 20, 513-527.

van Kan, J.A.L., van de Rhee, M.D., Zuidema, D., Cornelissen, B.J.C. and Bol, J.F. (1989) 'Structure of tobacco genes encoding thaumatin-like proteins', Plant Molecular Biology 12, 153-155.

van Loon, L.C. (1976) 'Systemic acquired resistance, peroxidase activity and lesion size in tobacco reacting hypersensitively to tobacco mosaic virus', Physiological Plant Pathology 8, 231-242.

van Loon, L.C. (1982) 'Regulation of changes in proteins and enzymes associated with active defence against virus infection' in R.K.S. Wood (eds.) Active Defense Mechanisms in Plants, Plenum Press, New York, pp. 247-273.

van Loon, L.C. (1985) 'Pathogenesis-related protein', Plant Molecular Biology 4, 111-116.

van Loon, L.C., Gerritsen, Y.A.M. and Ritter, C.E. (1987) 'Identification, purification, and characterization of pathogenesis-related proteins from virus-infected Samsun NN tobacco leaves', Plant Molecular Biology 9, 593-609.

van Loon, L.C. and van Kammen, A. (1970) 'Polyacrylamide disc electrophoresis of the soluble leaf proteins from *Nicotiana tabacum* var. 'Samsun' and 'Samsun NN' II. Changes in protein constitution after infection with tobacco mosaic virus', Virology 40, 199-211.

Vance, C.P., Kirk, T.K. and Sherwood, R.T. (1980) 'Lignification as a mechanism of disease resistance', Annual Review of Phytopathology 18, 259-288.

Vigers, A.J., Roberts, W.K. and Selitrennikoff, C.P. (1991) 'A new family of plant antifungal proteins', Molecular Plant-Microbe Interactions 4, 315-323.

Ward, E.R., Payne, G.B., Moyer, M.B., Williams, S.C., Dincher, S.S., Sharkey, K.C., Beck, J.J., Taylor, H.T., Ahl-Goy, P., Meins, Jr., F. and Ryals, J.A. (1991a) 'Differential regulation of ß-1,3-glucanase messenger RNAs in response to pathogen infection', Plant Physiology 96, 390-397.

Ward, E.R., Uknes, S.J., Williams, S.C., Dincher, S.S., Wiederhold, D.L., Alexander, D.C., Ahl-Goy, P., Métraux, J-P. and Ryals, J.A. (1991b) 'Coordinate gene activity in response to agents that induce systemic acquired resistance', The Plant Cell 3, 1085-1094.

White, R.F., Rybicki, E.P., Von Wechmar, M.B., Dekker, J.L. and Antoniw, J.F. (1987) 'Detection of PR1 type proteins in *Amaranthaceae*, *Chenopodiaceae*, *Gramineae* and *Solanaceae* by immunoelectroblotting', Journal of General Virology 68, 2043-2048.

Woloshuk, C.P., Meulenhoff, J.S., Sela-Buurlage, M., van den Elzen, P.J.M. and Cornelissen, B.J.C. (1991) 'Pathogen-induced proteins with inhibitory activity toward *Phytophthora infestans*', The Plant Cell 3, 619-628.

Ye, X.S., Pan, S.Q, and Kuć, J. (1989) 'Pathogenesis-related proteins and systemic resistance to blue mold and tobacco mosaic virus induced by tobacco mosaic virus, *Peronospora tabacina*, and aspirin', Physiological and Molecular Plant Pathology 35, 161-175.

Ye, X.S., Pan, S.Q. and Kuc, J. (1990) 'Association of pathogenesis-related proteins and activities of peroxidase, ß-1,3-glucanase and chitinase with sytemic induced resistance to blue mould of tobacco but not to systemic tobacco mosaic virus', Physiological and Molecular Plant Pathology 36, 523-531.

Ye, X.S., Pan, S.Q. and Kuć, J. (1990) 'Activity, isozyme pattern, and cellular localization of peroxidases as related to systemic resistance of tobacco to blue mold (*Peronospora tabacina*) and to tobacco mosaic virus', Phytopathology 80, 1295-1299.

Ye, X.S., Pan, S.Q., and Kuć, J. (1992) ' Specificity of induced systemic resistance as eleicited by ethephon and tobacco mosaic virus in tobacco', Plant Science 84, 1-9.

# THOUGHTS ON THE ROLE AND EVOLUTION OF INDUCED RESISTANCE IN NATURAL ECOSYSTEMS, AND ITS RELATIONSHIP TO OTHER TYPES OF PLANT DEFENSES AGAINST DISEASE

MICHÉLE C. HEATH
*Botany Department*
*University of Toronto*
*25 Willcocks Street*
*Toronto, Ontario M5S 3B2*
*CANADA*

## 1. A General Comparison of Different Types of Resistance

Induced resistance is only one of several types of resistance to microbial disease that flowering plants possess. The other major categories are *basic resistance*, *organ-specific resistance*, *age-related resistance* and *parasite-specific resistance*. Since comparative data for all five forms of resistance are more complete for fungal parasites than other pathogens, these organisms will be the main focus of this chapter.

Of the five categories, parasite-specific resistance is the best defined genetically, and is the type most often used by plant breeders to protect agricultural crops against pathogens. Characteristically, it occurs only in certain genotypes of a plant species and is effective only against a single parasite for which the plant species as a whole is considered a susceptible host. Commonly it is controlled by single genes, often introduced by the breeder into a basically susceptible genotype, and is expressed only if the pathogen has a "matching" gene for avirulence (resulting in a "gene-for-gene" interaction).

In contrast, the genetic basis of basic resistance is not well understood. This resistance is the resistance that protects every plant against the thousands of potential parasites for which the particular plant species is a nonhost (Heath 1991). In theory, such resistance could be achieved by each plant possessing parasite-specific resistance genes for each of these potential parasites. The alternative, and arguably the more likely (Heath 1991), hypothesis is that this type of resistance is parasite-nonspecific and depends on constitutive defenses and/or defenses inducible by a wide variety of organisms. In either case, an organism has to be able to overcome this form of resistance in its host species



*R. Hammerschmidt and J. Kuć (eds.), Induced Resistance to Disease in Plants,* 141–151.

(by means of attributes known as pathogenicity factors) before it can become a successful parasite (Heath 1991).

Induced resistance, organ-specific resistance, and age-related resistance differ from the other two types of resistance in that they all can be expressed in what, under certain circumstances, would be considered a susceptible plant. There are very few pathogens that can successfully attack all parts of their hosts, and this organ-specific resistance presumably is the result of some plant parts having components of basic resistance that are not matched by pathogenicity factors in the parasite. Even normally susceptible plant parts may not be susceptible throughout their lifetimes, resulting in age-related resistance. Such resistance may decrease (Heilbronn and Harrison 1989) or increase (Ward 1989) with age of the organ or of the plant (Reuveni et al. 1986).

Induced resistance differs from organ-specific and age-related resistance in that its expression relies on some previous treatment of the plant that "sensitizes" it so that what should be susceptible tissue resists invasion by parasites that previously were successful pathogens. A single induction treatment can elicit resistance to a wide variety of parasites (Kuć 1982; Ye et al. 1989) and in this lack of specificity, induced resistance resembles basic resistance more than parasite-specific resistance. Indeed, induced, age-related and organ-specific resistance do not require the presence of known parasite-specific genes for resistance against the parasite in question, although some forms of parasite-specific resistance may also be age-related in that they are only expressed at a certain stage of plant development (Bhattacharyya and Ward 1986).

## 2. Are Defense Mechanisms the Same for all Forms of Resistance?

Resistance of plants to colonization by microbes is the norm, and the basis of this resistance has been the subject of intensive investigation over the last 50 years. Despite the remarkable paucity of examples where resistance of a particular plant to a particular organism can be ascribed unequivocally to a particular defense mechanism, it is clear that plants have a number of features that are associated with, and most likely contribute to, their defense. In general terms, these defenses appear ubiquitous among flowering plants and include constitutive features, such as anatomical characteristics or toxic secondary metabolites, and a number of localized, parasite-inducible responses such as the modification of cell walls, the accumulation of phytoalexins and other toxic molecules, and the more widespread accumulation of a wide range of degradative enzymes and other "defense proteins". These inducible responses commonly can be elicited by a variety of microbial products, making it highly likely that they, together with constitutive factors, are the basis of basic resistance in all plants (Heath 1991). A major handicap in determining which defenses are most important in any given plant-microbe interaction is that what constitutes a defense mechanism will depend on a) how the potential parasite interacts with the plant during attempted invasion, and b) how many "hurdles" to infection the organism can overcome because of attributes (pathogenicity factors) that allow it to tolerate, negate or avoid them.

An obvious and interesting question is whether other types of resistance rely on the same defense mechanisms that seem to control basic resistance. Given that there are few examples in which the mechanisms of resistance are unequivocally known or proven, this is not an easy question to answer. Not all forms of resistance have been equally well studied, and for organ-specific resistance, in particular, few data are available. In some cases, the same types of inducible defense mechanisms have been implicated in basic, age-related and parasite-specific resistance (for an example, see work on soybean by Lazarovits *et al.* 1981; Bhattacharyya and Ward 1986; Ward 1989) suggesting that they all exploit the same, rather limited, number of responses that each plant possesses.

However, there also is good evidence to suggest that, in a single plant, the expression of different forms of resistance may involve different combinations of defenses (for refs. see Heath 1991). Constitutive features such as surface topography and constitutive toxins, in particular, may be more important in basic and organ-specific resistance than in parasite-specific resistance, since it is commonly assumed that the latter involves only active defenses elicited by some microbial factor controlled by the gene for avirulence. In the case of fungal parasites, basic resistance often is expressed earlier during infection than parasite-specific resistance (Heath 1974). In part this may be due to the greater role in basic resistance of pre-existing, constitutive resistance mechanisms. However, it also may reflect the fact that, in crop plants, parasite-specific resistance is introduced (by the plant breeder) into a susceptible host for which the parasite already has a set of pathogenicity factors that normally facilitate successful infection. Therefore, the speed at which the fungus is stopped depends on the speed at which the recognition events, controlled by the genes for avirulence and resistance, occur.

Induced resistance can be elicited in otherwise susceptible plants by the application of living organisms (Kuć 1982) or by abiotic treatments (e.g. Ye *et al.* 1989; Stevens *et al.* 1990), and may be expressed only in the treated tissue or considerably beyond the region of treatment. Localized resistance elicited by prior inoculation with incompatible pathogens can most easily be explained when these pathogens trigger defenses whose effects linger sufficiently to inhibit subsequent infection by a compatible pathogen (Elliston *et al.* 1977b). The more intriguing examples of induced resistance are those in which the inducing treatment does not directly elicit any of the defenses, such as cell death, phytoalexin accumulation, or detectable cell wall modifications, that usually are thought to account for resistance in genotypically resistant plants. These treatments must elicit other localized or systemic changes that either directly interfere with any subsequent invasion by the parasite, or alter the parasite-plant interaction in favour of the plant. As documented elsewhere in this book (see chapters by Deverall and Dann, Ozeretskovakaya, Hammerschmidt and Yang-Cashman, Steiner and Schönbeck, and Stermer) many changes have been detected in systemically protected plants including changes in cell wall structure that might make them more resistant to fungal penetration (Esquerré-Tugayé *et al.* 1979; Stermer and Hammerschmidt 1987), or increases in levels of soluble proteins that might directly inhibit fungal growth (Ye *et al.* 1990; Woloshuk *et al.* 1991). That these changes may affect subsequent fungal development is indicated by the fact that, once inside the tissue, fungal growth often appears impaired in

comparison with that in unprotected plants (Kovats *et al.* 1991; Ye *et al.* 1992); however, it has yet to be shown that these changes are more important in preventing fungal growth than antimicrobial compounds elicited during fungal infection. In addition, induced resistance also is commonly characterized by a decrease in the frequency of initial penetration of epidermal cells in association with an increased frequency of localized, modifications of the plant cell wall elicited after fungal inoculation (Cloud and Deverall 1987; Conti *et al.*, 1990; Kovats *et al.* 1991; Stein *et al.* 1992). Such rapid and localized wall modifications are typical of the expression of basic resistance and it would seem that protected plants may respond more as nonhosts than hosts towards some of their normally successful pathogens. Significantly, compared to normal cucumber plants, protected ones have higher latent activity of membrane-bound ß-1,3-glucan synthase (Schmele and Kauss 1990), which may account for the faster appearance of callose-containing papillae after fungal challenge of protected plants (Stumm and Gessler 1986; Kovats *et al.* 1991). Moreover, protected plants lignify faster than unprotected plants in response to wounding, probably because of the systemic increase in at least two enzymes, including peroxidase, essential for lignin formation (Dean and Kuć 1987). It seems likely, therefore, that some of the systemic changes induced in protected plants ensure that fungal-induced damage results in rapid wall-associated modifications that may help prevent further fungal ingress. The probable involvement in induced resistance of several pre-existing and fungal-induced defenses indicates that this form of resistance, like basic resistance (Heath 1991), is multicomponent (Ye *et al.* 1990; Kovats et al. 1991)

Interestingly, some of the systemic changes that occur in a plant after treatments that induce resistance also occur as a plant ages (e.g. Braber 1980; Fluhr *et al.* 1991; Wyatt *et al.* 1991). This raises the possibility that the mechanisms of induced resistance and some forms of age-related resistance may be similar, a hypothesis supported in bean (*Phaseolus vulgaris*) by the similar susceptibility of these forms of resistance to heat treatment, and the differing effect of heat on some examples of parasite-specific or nonhost resistance (Elliston *et al.* 1977a). Like induced resistance, age-related resistance towards fungi may be accompanied by localized responses that could account for the cessation of fungal growth. In bean infected with the bean rust fungus, these responses include silica deposition (Heath 1981) that is typical of the expression of basic, but not parasite-specific, resistance in this plant. Changes that occur in these leaves as they age include an increase in peroxidase (Braber 1980) and changes in cell wall polysaccharides (Arribas *et al.* 1991) but how or if they relate to the changes in resistance is unknown.

## 3. Role and Evolution of Induced Resistance in Natural Ecosystems

For some crop plants, induced systemic resistance has been shown to protect them against some diseases under field conditions (Oerke *et al.* 1989; Tuzun *et al.* 1992). However, to my knowledge no-one has demonstrated the existence of induced resistance in noncultivated species in natural ecosystems. Moreover, the number of plant species in which either localized or systemic induced resistance has been demonstrated is still

very small. It is often pointed out that plant diseases appear to cause less damage in natural ecosystems than in our crop plants (Browning 1981). Conceivably, this may be the result of a high incidence of induced resistance in wild plants, but it also is possible that noncultivated plants have a higher level of basic resistance because of features, such as high levels of constitutive toxins, that have been bred out of cultivated species (Heath 1991). Nevertheless, the concept of induced resistance fits the emerging picture that plants are remarkably responsive to stresses in their environment. Mechanical perturbations can cause stems to thicken (Biro *et al.* 1980), wounding and insect herbivory can result in a systemic increase in proteinase inhibitors (Ryan and Green 1972) and other compounds (Tallamy 1985) that may deter further insect feeding, and there is even some evidence that plants can respond to distress signals emitted by their neighbours (Baldwin and Schultz 1983; Farmer and Ryan 1990). In this context, it does not seem unreasonable that plants respond to parasite infection by strengthening their defense mechanisms against further invasion.

For the phenomenon of induced resistance to be directly selected for during plant evolution, there must be situations in which this attribute increases the fitness of the plant. The induction of systemic resistance beyond the tissue given the induction treatment seems to require limited, but significant, tissue necrosis (Jenns and Kuć 1979), but necrosis that is different from that caused by wounding (Hammerschmidt *et al.* 1982). This means that resistance is likely to be induced only by compatible plant-parasite interactions that result in limited, necrotic, lesions, or by incompatible interactions that result in necrotic flecks. Inoculation with parasites for which the plant is a nonhost, resulting in the expression of basic resistance, may not induce systemic resistance (Jenns and Kuć 1979), presumably because of the more restricted response by the plant. Therefore, the induction of systemic resistance would seem to be limited to rather specific types of plant-parasite interactions and we do not know whether such interactions occur with high frequency in natural ecosystems. If the plant is already resistant to the invading parasite, is there a selective advantage of inducing systemic resistance to a compatible race of the same, or other, pathogen when it is possible that such organisms may not be present? The most predictable advantage of induced resistance would be in the case of compatible interactions that form limited lesions, since induced systemic resistance would reduce the incidence of autoinfection, and in some cases, reduce the rate of development of an epidemic. It is not clear, however, how common such infections are in natural ecosystems, and whether they outnumber infections by, for example, organisms such as rust and powdery mildew fungi and systemic viruses that cause little necrosis and do not appear to elicit induced resistance (indeed, they may induce localized susceptibility towards microbes for which the plant is normally resistant). An interesting idea is that the prevalence of necrotic-fleck types of resistance to biotrophic organisms is the result of selective pressure for types of parasite-specific resistance that induce systemic resistance in the plant to other pathogens. However, it is possible that in natural ecosystems, localized, rather than systemic, induced resistance may be more important. Localized resistance can be elicited by fungi that cause wall modifications rather than cell death (Gregersen and Smedegaard 1989) and, therefore,

may be elicited by the hundreds of microbes that try to infect nonhost plants. Such a phenomenon would explain why aseptically-grown barley is more susceptible to powdery mildew than plants grown under normal conditions (Sahashi *et al.* 1989).

Rather than induced resistance being selected for because of its role in disease protection, it is possible that it is a secondary, and perhaps even fortuitous, phenomenon associated with the changes that occur in plants in response to certain types of stress. Certainly there are many similarities in the changes that occur in induced plants to those elicited by other environmental or biotic stresses, and many of these changes appear to be irrelevant to the subsequent resistance of the plant to the inducing pathogen (Linthorst *et al.* 1989; Ye *et al.* 1990). Not only will induced resistance elicited by one microbe protect against a different organism, but there are many other examples of plants responding to one stress becoming more resistant to another. For example, cotton seedlings exposed to spider mites become more resistant to the fungus, *Verticillium dahliae*, and *vice versa* (Karban *et al.* 1987), although, as described in chapters by Deverall and Dann and by Hammerschmidt and Yang-Cashman, successful induction of disease resistance by pathogens may not always result in expression of resistance to arthropod herbivore attack (Ajlan and Potter 1991, 1992). Low temperature hardening of the grass, *Poa pratensis,* increases resistance to the rust fungus, *Puccinia poae-nemoralis* (Tronsmo 1984). Perhaps, induced resistance, like basic resistance (Heath 1991), may be part of a multipurpose stress defense system selected for by factors other than, or in addition to, parasite pressure.

If induced resistance can protect a plant against a significant proportion of potential pathogens, it seems reasonable to ask why, in crop plants, this resistance is not expressed all the time. The most obvious explanation is that the changes induced in protected plants have a fitness cost. This possibility is supported by studies of "super-induction" in cucumber with *Pseudonomas syringae pv. syringae* in which the plants were visibly stunted and exhibited veinal chlorosis in developing leaves (Rasmussen *et al.* 1991) and by the deleterious effects of constitutively increased peroxidase levels in transgenic tobacco plants (Lagrimini *et al.* 1990). However, no deleterious effects on fitness were observed in tomato following the induction of high levels of proteinase inhibitors, apparently because they represented only a small part of the nitrogen budget of the plant (Brown 1988). Therefore, it is still unclear whether the expression of induced resistance represents a significant energy drain on the plant. The fact that induced resistance may be elicited by surface microbes (Gregersen and Smedegaard 1989; Sahashi *et al.* 1989) suggests that in nature, plants may well exist in a perpetual induced state.

## 4. Can Induced Resistance be Overcome?

If induced resistance occurs in natural ecosystems, it is pertinent to ask whether parasites can evolve to overcome it in the same way that they can evolve to become successful parasites of what were previously nonhost plants or plants with parasite-specific

resistance. This question also is of practical importance if induced resistance becomes commercially important as a means of protecting crop plants against disease. Although induced resistance will protect plants against a wide variety of pathogens, it often does not protect against them all (Kuć 1982; Bashan and Cohen 1983)). We do not know if this is because the defenses activated in protected plants just happen not to be effective against all parasites, or because the unaffected pathogens have specifically evolved means to circumvent this additional form of resistance in their hosts. Ultimately, the durability of any form of resistance depends on how easily random mutation in the parasite can produce some means of negating it (Heath 1985). Pathogens can become resistant to site-specific pesticides (Dekker 1976) or overcome parasite-specific resistance more readily that they can overcome the effects of multisite pesticides or multicomponent basic resistance, illustrating the principle that the more specific the molecular recognition events that govern an interaction, the higher the chance that small genetic changes in the parasite may prevent this recognition (Heath 1985). Therefore, the general nonspecificity of induced resistance bodes well for it being an exploitable form of durable resistance. Such a conclusion is supported by the apparently continued effectiveness of induced resistance in protecting tobacco against *Peronospora tabacina* over a four year field study in Mexico, despite high levels of pathogen inoculum (Tuzun *et al.* 1992).

## 5. Summary and Conclusions

Induced systemic resistance in crop plants appears to be relative nonspecific, and multicomponent in nature. In these features, and in some of its apparent mechanisms, it resembles basic resistance and age-related resistance more than parasite-specific resistance. It seems likely that, for fungi at least, some of the changes that occur in protected plants may directly inhibit fungal growth, while others may "prime" the plant so that it responds faster and more vigorously to any damage caused by attempted cell ingress. Whether induced resistance occurs in wild plants is unknown, and the number of cultivated species in which is has been documented is still relatively small. Nevertheless, this form of resistance fits the emerging concept that plants respond to various stresses in their environment in ways that make them less susceptible to further damage.

The nature and significance of induced resistance in natural ecosystems is completely unknown, as is whether it evolved in response to pathogen pressure or as a fortuitous result of the evolution of responses to other forms of stress. The indications that this form of resistance may be elicited by surface microbes and saprophytes is an area worth investigating further and it raises the possibility that wild plants may be in a state of perpetual induction. Whether there is an energy cost of induced resistance, and whether its expression significantly reduces the fitness of the plant, are all topics that need to be investigated before we can fully understand the role of this resistance relative to parasite-specific resistance in wild plants.

The similarity of induced resistance to basic resistance suggests that it may be similarly durable with respect to the ease with which resistance may be overcome by a particular pathogen. This possibility, together with the fact that induced resistance can be triggered in otherwise susceptible plants by various "environmentally friendly" abiotic treatments such as ultraviolet radiation (Stevens *et al.* 1990), bodes well for the successful commercial application of this type of disease protection.

## References


Ajlan, A.M. and Potter, D.A. (1991) 'Does immunization of cucumber against anthracnose by *Colletotrichum lagenarium* affect host suitability for arthropods?' Entomologia Experimentalis Applicata 58, 83-91.

Ajlan, A.M. and Potter, D.A. (1992) 'Lack of effect of tobacco mosaic virus-induced systemic acquired resistance on arthropod herbivores in tobacco' Phytopathology 82, 647-651.

Arribas, A., Revilla, G., Zarra, I., and Lorences, E.P. (1991) 'Changes in cell wall polysaccharides during the growth of *Phaseolus vulgaris* leaves', Journal of Experimental Botany 42, 1181-1187.

Baldwin, I.T. and Schultz, J.C. (1983) 'Rapid changes in tree leaf chemistry induced by damage: evidence for communication between plants', Science 221, 277-279.Bashan, B. and Cohen, Y. (1983) 'Tobacco necrosis virus induce systemic resistance in cucumbers against *Sphaerotheca fuliginea*', Physiological Plant Pathology 23, 137-144.

Bhattacharyya, M.K. and Ward, E.W.B. (1986) 'Expression of gene-specific and age-related resistance and the accumulation of glyceollin in soybean leaves infected with *Phytophthora megasperma* f.sp. *glycinea*', Physiological and Molecular Plant Pathology 29, 105-113.

Biro, R.L., Hunt Jr., E.R., Erner, Y., and Jaffe, M.J. (1980) 'Thigmomorphogenesis: changes in cell division and elongation in the internodes of mechanically-perturbed or ethrel-treated bean plants', Annals of Botany 45, 655-664.

Braber, J.M. (1980) 'Catalase and peroxidase in primary bean leaves during development and senescence', Zeitschrift fur Pflanzenphysiolgie 97, 135-144.

Brown, D.G. (1988) 'The cost of plant defense: an experimental analysis with inducible proteinase inhibitors in tomato', Oecologia 76, 467-470.

Browning, J.A. (1981) 'The agro-ecosystem - natural ecosystem dichotomy and its impact on phytopathological concepts', in J.M. Thresh (ed.), Pests, Pathogens and Vegetation, Pitman, Boston, pp. 159-172.

Cloud, A.M.E. and Deverall, B.J. (1987) 'Induction and expression of systemic resistance to the anthracnose disease in bean', Plant Pathology 36, 551-557.

Conti, G.G., Bassi, M., Carminucci, D., Gatti, L., and Bocci, A.M. (1990) 'Preinoculation with Tobacco Necrosis Virus enhances peroxidase activity and lignification in cucumber as a resistance response to *Sphaerotheca fuliginea*', Journal of Phytopathology 128, 191-202.

Dean, R.A. and Kuć, J. (1987) 'Rapid lignification in response to wounding and infection as a mechanism for induced systemic protection in cucumber', Physiological and Molecular Plant Pathology 31, 69-81.

Dekker, J. (1976) 'Acquired resistance to fungicides', Annual Review of Phytopathology 14, 405-428.

Elliston, J., Kuć, J., and Williams, E.B. (1977a) 'Effect of heat treatment on the resistance of *Phaseolus vulgaris* to *Colletotrichum lindemuthianum* and *Colletotrichum lagenarium*', Phytopathologische Zeitschrift 88, 43-52.

Elliston, J., Kuć, J., Williams, E.B., and Rahe, J.E. (1977b) 'Relationship of phytoalexin accumulation to local and systemic protection of bean against anthracnose', Phytopathologische Zeitschrift 88, 114-130.

Esquerré-Tugayé, M.-T., Lafitte, C., Mazau, D., Toppan, A., and Touzé, A. (1979) 'Cell surfaces in plant-microorganism interactions. II. Evidence for the accumulation of hydroxyproline-rich glycoproteins in the cell wall of diseased plants as a defense mechanism', Plant Physiology 64, 320-326.

Farmer, E.E. and Ryan, C.A. (1990) 'Interplant communication: airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves', Proceedings of the National Academy of Sciences, USA 87, 7713-7716.

Fluhr, R., Sessa, G., Sharon, A., Ori, N., and Lotan, T. (1991) 'Pathogenesis-related proteins exhibit both pathogen-induced and developmental regulation', in H. Hennecke and D.P.S. Verma (eds.), Advances in Molecular Genetics of Plant-Microbe Interactions Vol. I, Kluwer Academic Publishers, The Netherlands, pp. 387-394.

Gregersen, P.L. and Smedegaard, V. (1989) 'Induction of resistance in barley against *Erysiphe graminis* f. sp. *hordei* after preinoculation with the saprophytic fungus, *Cladosporium macrocarpum*', Journal of Phytopathology 124, 128- 136.

Hammerschmidt, R., Nuckles, E.M., and Kuć, J. (1982) 'Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*', Physiological Plant Pathology 20, 73-82.

Heath, M.C. (1974) 'Light and electron microscope studies of the interactions of host and nonhost plants with cowpea rust - *Uromyces phaseoli* var. *vignae*', Physiological Plant Pathology 4, 403-414.

Heath, M.C. (1981) 'The suppression of the development of silicon-containing deposits in French bean leaves by exudates of the bean rust fungus and extracts from bean rust-infected tissue', Physiological Plant Pathology 18, 149-155.

Heath, M.C. (1985) 'Implications of nonhost resistance for understanding host-parasite interactions', in J.V. Groth and W.R. Bushnell (eds.), Genetic Basis of Biochemical Mechanisms of Plant Disease, APS Press, St. Paul, pp. 25-42.

Heath, M. C. (1991) 'Evolution of resistance to fungal parasitism in natural ecosystems', (Tansley Review No. 33), New Phytologist 119, 331-343.

Heilbronn, J. and Harrison, J.G. (1989) 'Effects of bean leaf age on pathogenicity by *Botrytis fabae*', Journal of Phytopathology 126, 272-278.

Jenns, A.E. and Kuć, J. (1979) 'Graft transmission of systemic resistance of cucumber to anthracnose induced by *Colletotrichum lagenarium* and Tobacco Necrosis Virus', Phytopathology 69, 753-756.

Karban, R., Adamchak, R., and Schnathorst, W.C. (1987) 'Induced resistance and interspecific competition between spider mites and a vascular wilt fungus', Science 235, 678-680.

Kovats, K., Binder, A., and Hohl, H.R. (1991) 'Cytology of induced systemic resistance of cucumber to *Colletotrichum lagenarium*', Planta 183, 484-490.

Kuć, J. (1982) 'Induced immunity to plant disease', BioScience 32, 854-860.

Lagrimini, M., Bradford, S., and Rothstein, S. (1990) 'Peroxidase-induced wilting in transgenic tobacco plants', The Plant Cell 2, 7-18.

Lazarovits, G, Stössel, and Ward, E.W.B. (1981) 'Age-related changes in specificity and glyceollin production in the hypocotyl reaction of soybeans to *Phytophthora megasperma* var. *sojae*', Phytopathology 71, 94-97.

Linthorst, H.J.M., Meuwissen, R.L.J., Kauffmann, S., and Bol, J.F. (1989) 'Constitutive expression of pathogenesis-related proteins PR-1, GRP, and PR-S in tobacco has no effect on virus infection', The Plant Cell 1, 285-291.

Oerke, E.-C., Steiner, U., and Schönbeck. (1989) 'The efficiency of induced resistance under practical culture conditions V. Powdery mildew and grain yield of winter and spring barley in relation to nitrogen fertilization', Journal of Plant Diseases and Protection 96, 140-153.

Rasmussen, J.B., Hammerschmidt, R., and Zook, M.N. (1991) 'Systemic induction of salicylic acid accumulation in cucumber after inoculation with *Pseudomonas syringae* pv. *syringae*', Plant Physiology 97, 1342-1347.

Reuveni, M., Tuzun, S., Cole, J.S., Siegel, M.R., and Kuć, J. (1986) 'The effects of plant age and leaf position on the susceptibility of tobacco to blue mold caused by *Peronospora tabacina*', Phytopathology 76, 455-458.

Ryan, C.A. and Green, T.R. (1974) 'Proteinase inhibitors in natural plant protection', Recent Advances in Phytochemistry 8, 123 -140.

Sahashi, N., Tsuji, H., and Shishiyama, J. (1989) 'Barley plants grown under germ-free conditions have increased susceptibility to two powdery mildew fungi', Physiological and Molecular Plant Pathology 34, 163-170.

Schmele, I., and Kauss, H. (1990) 'Enhanced activity of the plasma membrane localized callose synthase in cucumber leaves with induced resistance', Physiological and Molecular Plant Pathology 37, 221-228.

Stein, B.D., Klomparens, K.L., and Hammerschmidt, R. (1993) 'Histochemistry and ultrastructure of the induced resistance response of cucumber plants to *Colletotrichum lagenarium*', Journal of Phytopathology 137, 177-188.

Stermer, B.A. and Hammerschmidt, R. (1987) 'Association of heat shock induced resistance to disease with increased accumulation of insoluble extensin and ethylene synthesis', Physiological and Molecular Plant Pathology 31, 453-461.

Stevens, C., Khan, V.A., Tang, A.Y., and Lu, J.Y. (1990) 'The effect of ultraviolet radiation on mold rots and nutrients of stored sweet potatoes', Journal of Food Protection 53, 223- 226.

Stumm, D., and Gessler, C. (1986) 'Role of papillae in the induced systemic resistance of cucumbers against *Colletotrichum lagenarium*', Physiological and Molecular Plant Pathology 29, 405-410.

Tallamy, D.W. (1985) 'Squash beetle feeding behaviour: an adaptation against induced cucurbit defenses', Ecology 66, 1574-1579.

Tronsmo, A.M. (1984) 'Resistance to the rust fungus *Puccinia poae-nemoralis* in *Poa pratensis* induced by low-temperature hardening', Canadian Journal of Botany 62, 2891-2892.

Tuzun, S., Juarez, J., Nesmith, W.C., and Kuć, J. (1992) 'Induction of systemic resistance in tobacco against metalaxyl-tolerant strains of *Peronospora tabacina* and the natural occurrence of the phenomenon in Mexico', Phytopathology 82, 425-429.

Ward, E.W.B. (1989) 'Susceptibility of immature soybean leaves to *Phytophthora* species', Physiological and Molecular Plant Pathology 34, 393-402.

Woloshuk, C.P., Meulenhoff, J.S., Sela-Buurlage, M., van den Elzen, P.J.M., and Cornelissen, B.J.C. (1991) 'Pathogen-induced proteins with inhibitory activity toward *Phytophthora infestans*', The Plant Cell 3, 619-628.

Wyatt, S.E., Pan, S.Q., and Kuć, J. (1991) 'ß-1,3-Glucanase, chitinase, and peroxidase in tobacco tissue resistant and susceptible to blue mold as related to flowering, age and sucker development', Physiological and Molecular Plant Pathology 39, 433-440.

Ye, X.S., Pan, S.Q. and Kuć, J. (1989) 'Pathogenesis-related proteins and systemic resistance to blue mould and tobacco mosaic virus induced by tobacco mosaic virus, *Peronospora tabacina* and aspirin', Physiological and Molecular Plant Pathology 35, 161-175.

Ye, X.S., Pan, S.Q., and Kuć, J. (1990) 'Association of pathogenesis-related proteins and activities of peroxidase, ß-1,3-glucanase and chitinase with systemic induced resistance to blue mould of tobacco but not to systemic tobacco mosaic virus', Physiological and Molecular Plant Pathology 36, 523-531.

Ye, X.S., Jarlfors, U., Tuzun, S., Pan, S.Q., and Kuć, J. (1992) 'Biochemical changes in cell walls and cellular responses of tobacco leaves related to systemic resistance to blue mold (*Peronospora tabacina*) induced by tobacco mosaic virus', Canadian Journal of Botany 70, 49-57.

# PRACTICAL APPLICATION AND IMPLEMENTATION OF INDUCED RESISTANCE

SADIK TUZUN and JOSEPH KLOEPPER
*Department of Plant Pathology*
*Alabama Agricultural Experiment Station*
*Biological Control Institute*
*Auburn University*
*Auburn, AL 36849*
*USA*

## 1. Introduction

The use of intensive farming and mass monoculture resulted in the extensive use of chemical pesticides to control plant pests and diseases. The use of chemical pesticides, however, will become increasingly restricted due to concerns for the environment and health. The need for extensive toxicological and field testing prior to release of new chemicals and development of pest or pathogen resistance to some current pesticides may also contribute to a reduction in pesticide utilization. We are faced with the challenge of finding more effective, safer and economical ways to protect plants against pests to help feed an ever increasing world population. Utilization of plants' own defense mechanisms by application of technologies to induce resistance may contribute toward a solution to this problem. Many theoretical and experimental aspects of induced systemic resistance (immunization) are described in detail in the previous chapters. Because there are few examples of field tests with induced resistance, the wide scale application of the phenomenon is not yet established. This chapter will discuss some of the approaches that can be utilized in progressing toward the practical application of induced systemic resistance.



*R. Hammerschmidt and J. Kuć (eds.), Induced Resistance to Disease in Plants,* 152–168.

## 2. Induction of Resistance in Plants

Research in laboratories world-wide has demonstrated immunization in over 25 crops, including cereals, cucurbits, legumes, solanaceous plants, trees and small fruits (Tuzun and Kuć 1991), against a broad spectrum of leaf (Dean and Kuć 1985; Kuć 1983, 1985, 1987a,b; Tuzun and Kuć 1989, 1991) and root pathogens (Gessler and Kuć 1982). The extent of protection may vary among plant species. The agents involved induce a host response which in turn protects either treated parts of the plant (local immunization) (Dehne *et al.* 1984; Giri and Sinha 1979; Weltzien 1989) or parts of the plant distant from the treated area (systemic immunization) (Dean and Kuć 1985; Gottstein and Kuć 1989; Tuzun and Kuć 1985a,b). Generally, protection persists for a long time, and in the case of tobacco for the life of the plant (Tuzun and Kuć 1985a). A lag period is needed for development of resistance (Tuzun and Kuć 1985a, 1989), and the signal for induction of systemic resistance in cucumbers and tobacco is graft transmissible (Jenns and Kuć 1979; Tuzun and Kuć 1985b). Translocation of the signal can be prevented by girdling (Guedes *et al.* 1980; Tuzun and Kuć 1985b), and in tobacco protection is transmitted to regenerants via tissue culture (Tuzun and Kuć 1987). The signal (Kuć 1987b) is of plant origin, and it affects developed and unopened leaves in the bud. A low molecular-weight chemical with signal activity has been isolated in cucumbers (L. Matthews-Faught, personal communications) which may have practical application.

## 3. Plant Immunization In Nature

Researchers working on induced systemic resistance are often asked why are plants in nature not universally protected against pathogens if induced resistance can occur in the field. There may be many answers to this question, one of which may be the lack of the required lag time for the activation of plant defense responses or inoculum density sufficient enough to induce resistance. Success of induced resistance is also dependent on environmental conditions (Falkhof *et al.* 1988; Karban 1987) and nutrient level in the soil (Oerke *et al.* 1989). Many of us involved in field research, however, have periodically observed some "unexplained" results. During the 1984-1985 growing season in Puerto-Rico, we observed that unsprayed control tobacco plants had significantly less blue mold symptoms than those sprayed with a contact fungicide (mancozeb). One might immediately assume that the mancozeb treatment increased susceptibility to blue mold. Closer examination, however, revealed that the control plants survived blue mold early in the crop season and somehow became more resistant to disease development in late season. In contrast to that, the mancozeb treatment effectively protected plants early in the season; however, later in the season heavy rain frequently washed off the fungicide, leaving susceptible leaf tissue without any protection. Indeed, surviving nontreated plants were protected against blue mold the rest of the season. In Mexico, cooperators talked about selecting so-called "resistant individuals" from the field and how they were subsequently disappointmented when none of the progeny was resistant (Tuzun *et al.*,

1992). This examples supports the contention that induced systemic resistance exists under field conditions.

Although most of the research on plant immunization has been conducted in the laboratory and greenhouse, there are a number of reports indicating its effectiveness in protecting crop plants under field conditions (Halasz 1965; Cox *et al.* 1976; Caruso and Kuć 1977; Metlitski *et al.* 1978; Giri and Sinha 1979; Dehne *et al.* 1984; Tuzun et al. 1986, 1991, 1992; Steiner *et al.* 1988). In the following sections we will give detailed examples of some of these studies which describe the feasibility of manipulating host defensive mechanisms toward practical disease control.

## 4. Pathogenic Organisms as Inducers of Resistance

Development of resistance to diseases in plants following infection has been known for over 60 years (Chester, 1933). In order to achieve successful protection, plants must recover from initial infection or must be infected at a site that does not normally become diseased. For example, immunization against blue mold in tobacco can be achieved by stem injection with live sporangiospores of the causal organism of blue mold, *Peronospora tabacina* (Cruickshank and Mandryk 1960; Tuzun and Kuć 1985a). The site of injection appears to be important since only delivery of spores into the tissue external to the xylem induces systemic resistance without stunting. In a similar way, protection can be achieved in tobacco varieties with the N gene for resistance to tobacco mosaic virus (TMV), in which the virus does not become systemic, by inoculation of two or three bottom leaves with TMV (Ye *et al.* 1989). More interestingly, both of these pathogens induce resistance against either pathogen equally well, which may indicate activation of multiple mechanisms once the state of resistance is achieved.

The systemic protection of tobacco against blue mold is somehow unique since it was first reported on systemically stem-infected and stunted field-grown tobacco in Australia (Pont 1959). Extensive field tests conducted over a 3-year period in Kentucky and Puerto Rico utilizing a modified technique (which employs injection of a sporangiospore suspensions of *P. tabacina* into stem tissue external to the xylem) further indicated that tobacco plants can be protected against blue mold under various field conditions (Tuzun *et al.* 1986). These tests indicated that immunized plants had reduced lesion number and size compared to the nontreated controls, and the level of protection obtained by stem injections with *P. tabacina* was comparable to that obtained by treatment with metalaxyl. Furthermore, even in the absence of disease, immunized plants grew more vigorously and yields were increased up to 20%, compared to controls (Tuzun *et al.* 1986). Further field experiments were conducted in Mexico for three growing season to test the effectiveness of immunization against-metalaxyl-tolerant strains of *P. tabacina* (Tuzun *et al.* 1992). Highly significant reductions in the number and size of blue mold lesions were observed on plants injected with *P. tabacina*, compared to noninjected controls, regardless of metalaxyl applications. Some vigorously growing plants with necrotic stem lesions but markedly reduced blue mold on the foliage were

observed in heavily infected commercial fields indicating the natural occurrence of immunization against blue mold in the gulf coast of Mexico (Tuzun *et al.* 1992). Tolerance to immunization was not evident, although conditions for its development were very favorable. In field trials, plants derived via tissue culture from immunized parents were also protected against blue mold compared to plants derived from control parents (Tuzun and Kuć 1987).

Cucurbits have been utilized as a model system to establish many aspects of plant immunization, including applicability of the phenomenon to the real world. Cucumbers can be protected in the field by application of biological as well as chemical inducers (Caruso and Kuć 1977; Doubrava *et al.* 1989; Gottstein and Kuć 1989; Mucharromah and Kuć 1989).

These studies provide evidence that immunization of plants with biotic or abiotic inducers effectively controls disease in the field. Utilization of pathogenic organisms in the field, however, may create problems if handled carelessly. Culture filtrates of pathogenic organisms may provide a safer alternative, and several studies have indicated induction of resistance in many crop species by microbial metabolites (Trivedi and Sinha 1976; Dehne *et al.* 1984; Doke *et al.* 1987; Kopp *et al.* 1989; Maiss 1987; Ozeretskovskaya *et al.* 1988). Avirulent, hypovirulent and/or attenuated strains of pathogenic organisms (Kroon *et al.* 1991; Martyn *et al.* 1991; Sneh *et al.* 1989; van Asch *et al.* 1992) as well as L-forms of pathogenic bacteria (Amijee *et al.* 1992) may provide additional alternatives. Cabbage plants were successfully protected against the black rot pathogen, *Xanthomonas campestris* pv. *campestris* (XCC), under field conditions by prior application of attenuated strains of XCC or an isolate of *Xanthomonas campestris* pv. *malvacearum*, nonpathogenic on cabbage (Jetiyonan *et al.* 1993). In these experiments, conducted at several locations over two years, inducing bacteria were introduced into natural openings of leaves utilizing an organosilicone surfactant (Silwet) prior to transplanting and three weeks prior to pathogen challenge. Utilization of silwet to introduce organisms into leaf tissue may have a large scale practical application by eliminating the need for injection.

## 5. Nonpathogenic Organisms as Inducers of Resistance

Non-pathogenic organisms that have been utilized by several research groups to induce resistance include suspensions and/or culture filtrates of saprophytic bacteria (Schmidt 1988; Schönbeck *et al.* 1980) such as *Bacillus subtilis* (Mais 1987; Oerke *et al.* 1989), *B. thuringensis* (Roveratti *et al.* 1989), *B. pumulis* and *Erwinia herbicola* (Reiss *et al.* 1988), and saprophytic fungi (Gregersen and Smedegaard-Peterson 1989). There are also several reports that suggest induction of resistance by mycorrhiza against fungi (Rosendahl 1985), bacteria (Garcia-Garrido and Ocampo 1988) and nematodes (Carling *et al.* 1989). Although most of these studies are conducted in controlled environments, spraying plants with *B. subtilis* effectively controlled powdery mildew on barley under field conditions (Oerke *et al.* 1989).

Application of inducing agents to leaf surfaces makes the inducing agents more susceptible to stress conditions such as UV irradiation, rain washing, and temperature fluctuations. Application of good root colonizers to seed or soil may protect inducing agents from some of these negative effects. Recently, studies in our group and others indicated a subgroup of rhizosphere bacteria, and fungi can effectively induce resistance in plants while providing other beneficial effects to plants (Kloepper *et al.* 1993, Meera *et al.* 1993; Tuzun and Kloepper 1994). Free-living root and soil bacteria have been studied for the past century as possible inoculants for enhancing crop productivity. Utilization of natural microbial strains as inducers of plant defensive responses may increase the chance of their applicability and offer a practical way to deliver plant immunization. Since plant growth-promoting rhizobacteria (PGPR) are beneficial to plants in many ways, and they may provide a mean for practical application of induced resistance by seed or soil application, we will review the studies in this area in detail.

## 6. Plant Growth-Promoting Rhizobacteria (PGPR) as Inducers of Disease Resistance

Beneficial effects of PGPR that have been documented by many research groups can be summarized as: (1) direct plant growth promotion (Kapulnik 1991; Schroth and Becker 1990), (2) biological disease control (Bakker *et al.*, 1991; Kloepper 1991, 1993; Schippers *et al.* 1991; Schroth and Becker, 1990), and (3) inducing host resistance (Kloepper *et al.* 1993; Tuzun and Kloepper 1994). The reports summarized in these reviews include the effects of many different bacterial groups on many host plants. Plant growth-promoting fungi (PGPF), however, have been described more recently from the rhizosphere of turf grass (Hyakumachii *et al.* 1992), and such fungi also appear to induce systemic resistance against antrachnose disease caused by *Colletotrichum orbiculare* in cucumber plants (Meera *et al.* 1993).

Although it was suggested previously (Scheffer 1983; Voisard *et al.* 1989), direct pathological evidence for the induction of systemic resistance by PGPR was first published for three plant-pathogen systems in 1991. In a carnation system applications of *Pseudomonas* sp. strain WCS417 to rockwool cubes resulted in protection from wilt caused by *Fusarium oxysporum* f. sp. *dianthi* (van Peer *et al.* 1991). The pathogen was spatially separated from the PGPR strain by inoculating into stems one week after PGPR application, and separation was confirmed by the failure to isolate WCS417 from stems. In a bean system , seed treatment with a *P. fluorescens* PGPR strain led to reductions in the numbers of foliar lesions caused by subsequent inoculations of *P. syringae* pv. *phaseolicola* (Alstrom 1991). In another report with cucumber, 94 known PGPR strains were examined for ability to control *C. orbiculare* (Wei et al. 1991). Six PGPR strains applied as seed treatments consistently resulted in significant reductions in anthracnose lesion diameters and lesion numbers when the pathogen was applied 21 days after planting. In a subsequent study with the 6 inducing PGPR (Kloepper *et al.* 1993), none of the inducing strains was recovered from leaf petioles, confirming the spatial separation of pathogen and PGPR. These reports with carnation, bean, and cucumber demonstrate

that saprophytic root-associated bacteria may act as agents of induced systemic resistance, and hence, they serve to expand the potential mechanisms by which PGPR may exert biological disease control.

Current work in our laboratories is underway to determine the spectrum of protection achieved with two of the inducing PGPR strains used by Wei *et al.* (1991) on cucumber. Both strains, applied as seed treatments, significantly reduced mean diameter of lesions induced by foliar-applied *C. orbiculare*. PGPR-mediated protection against cucumber mosaic virus (CMV) is affected at the viral inoculation site. One PGPR strain significantly protected against CMV that was mechanically inoculated onto cotyledons (Liu *et al.* 1992). Protection was evident as prevention of symptom development on PGPR-induced plants, which differs from protection reported against CMV for classical induced systemic resistance (Bergstrom *et al.* 1982). With classical induced resistance, CMV symptoms were delayed but not prevented. With the PGPR system, both strains delayed, but did not stop, symptom development when CMV was inoculated onto the first, second, or third leaves, which is equivalent to results with classical induced resistance. The same two PGPR strains induced protection of cucumber against *Pseudomonas syringae* pv. *lachrymans* as seen by a significant reduction in mean lesion number and lesion size compared to noninduced controls (Liu *et al.* 1993a).

Preliminary studies with Fusarium wilt demonstrate that one PGPR strain reduced the rate of symptom development and plant death (Liu *et al.* 1993b). With *Fusarium*, a split root system was used to ensure spatial separation of PGPR and the pathogen. PGPR were applied to one-half of the roots after splitting, and *Fusarium* was incorporated into the soil in which the other half of the roots were growing. While this system demonstrates the potential to use PGPR-mediated induced resistance for protection from a soilborne vascular wilt pathogen, it is fundamentally different from the other PGPR systems which use seed treatments. PGPR applied as seed treatments may induce systemic resistance earlier in the plant's life, and hence, more work is needed to compare the biochemical responses of plants induced by seed and root treatments with PGPR.

Several studies have indicated that specific PGPR may stimulate the production of biochemical compounds associated with host defense. Van Peer and his colleagues (1991) observed increased accumulation of phytoalexins in carnation plants treated with PGPR (isolate WCS417) following pathogen inoculation. In a bean system, Hynes & Lazarovits (1989) found that levels of a PR-protein increased in leaves following seed treatment with PGPR strains. Plant root colonization by PGPR was associated with increased peroxidase activity (Albert and Anderson 1987) and enhanced lignification of stems or leaves in bean (Anderson and Guerra, 1985) and wheat (Frommel *et al.* 1991). Inoculation of bean roots with a *P. putida* PGPR strain led to an increased abundance of mRNA encoding PR1a protein in leaves (Zdor 1992). These reports clearly demonstrate that particular bacteria inoculated onto seeds or roots may elicit systemic physiological changes in plants.

Early investigations with PGPR-mediated induced resistance in cucumbers suggested that the biochemical response of the plant may depend on the inducing PGPR strain. Some inducing PGPR were associated with enhanced peroxidase activity similar to that

observed with classical induced controls (Wei *et al.* 1992). Some, but not all, inducing PGPR strains were associated with enhanced mRNA encoding acidic chitinases (Wei 1993). It will be necessary to conduct further biochemical investigations of how PGPR-mediated and classical induced resistance affect host defense-related compounds.

Two field trials were conducted with PGPR on cucumber in 1992 and two in 1993 to compare disease protection levels of PGPR-mediated and classical induced resistance. PGPR were applied as seed treatments, and classical induced resistance was achieved by inoculation of the first leaf with *C. orbiculare*. In the first trial, plants were challenge-inoculated with *P. syringae* pv. *lachrymans*. Compared to the noninduced control plants, all those treated with PGPR showed significant reductions in mean lesion diameter, whereas those treated to obtain classical induced resistance showed significant increase in lesion diameter. Growth promotion, measured as the number of leaves per plant and the weight of fruit per plant, resulted from use of 2 of 3 PGPR strains. In the second field trial, plants were not challenge-inoculated because natural infections of *E. tracheiphila* were observed (Fig. 1).

**Figure 1.** Protection of cucumbers against *E. tracheiphila* by PGPR seed treatment (left) compared to controls (right) under field conditions.

All 3 PGPR strains, but not the classical induced resistance treatment, resulted in significant reductions in symptom expression. Hence PGPR-mediated induced resistance can be observed under field conditions and, at least in some cases, may lead to superior plant protection from classical induced systemic resistance.

## 7. Plant-Derived Materials as Inducers of Resistance

There are several reports indicating the resistance- inducing activity of plant extracts as well as components of plant extracts (Doke and Chai 1985; Doubrava *et al.* 1988; Giri and Sinha 1979; Kopp *et al.* 1989; Salt *et al.* 1986; Yamada *et al.* 1990). Doubrava

*et al.* (1988) found that treatment of first leaves with spinach extracts induced resistance against *C. orbiculare* in cucumber. Further studies determined that oxalic acid in spinach extracts was the active entity (Doubrava *et al.* 1988). Weltzien (1989) reported that compost extracts can also induce resistance against diseases. Decomposed leaves or leaf extracts of *Aegle marmelos* induced resistance against *Sclerotinia sclerotium* in chickpea (Singh *et al.* 1990). Aqueous extracts of healthy barley leaves induced oversized papillae formation and in turn resistance to powdery mildew in barley seedlings (Yokoyama *et al.* 1991). These studies, although limited, indicate the biological diversity of inducers which can have practical applications.

## 8. Chemicals as Inducers of Resistance

In addition to treatment with oxalates (Doubrava *et al.* 1988), dipotassium/sodium or tripotassium/sodium phosphates (Gottstein and Kuć 1989; Mucharromah and Kuć 1989; Walters and Murray 1992), treatments with other chemicals such as unsaturated fatty acids (Cohen *et al.* 1991); dinitro aniline herbicides (Cohen *et al.* 1992), salicylic acid, 7-methanocarbonyl benzol-1,2,3-thiadiazol, ethaphon (Okuno *et al.* 1991), chitosan


**Figure 2.** Protection of tobacco against metalaxyl-tolerant strains of *P. tabacina* by application of 3-n-butyryoyl-ß-ionone (left) compared to controls (right). Mexican cigar tobacco was protected against natural stem infection of plants with the fungus as well as leaf infections by weekly application of the inducer. Over 90% of the untreated controls were stunted due to high incidence of stem infections, although, they were naturally protected from leaf infections due to systemic protection achieved via stem infections.

(Pospieszny *et al.* 1991), DL-alanine dodecylester HCL (Arimoto *et al.* 1991), N-cyanomethyl-2-chloroisonicotinamide (Seguchi *et al.* 1992), ß-ionone and 3-n-butryoyl-ß-ionone (Salt *et al.* 1986), and 2,6-dichloro-isonicotic acid (INA, Metraux *et al.* 1991) can induce resistance. Beta-ionone derivatives protected tobacco against metalaxyl sensitive

and tolerant strains of the fungus in greenhouse and field trials (Salt *et al.* 1986; Figure 2). Extensive field studies utilizing ß-ionone derivatives (Figure 2) and INA (R. Hammerschmidt, personal communications) suggest the possiblity of utilizing chemicals, which do not have direct activity against pathogens, effectively to induce systemic resistance in the field.

## 9. UV-C and $CO_2$ as Inducers of Resistance

Exposure to low quantities of ultraviolet light (254 nm UV-C) has been shown to reduce post harvest diseases (Wilson and Wisniewski 1989). This effect appears to be more than just germicidal and indeed associated with changes in host defensive responses, such as increases in phenylalanine ammonia lyase activity (Stevens *et al.* 1991) and phytoalexin accumulation (Creasy and Coffee 1988; Hadwiger and Schwochau 1973; Reilly and Klarman 1980). UV light increases changes in transcriptional activity of plant defense genes similar to changes detected upon tratment with fungal elicitors in cell suspension cultures (Chapell and Hahlbrock 1984). Treatment of avocado fruits with $CO_2$ increased the levels of antifungal dienes and in turn decreased the decay by *C. gloeosporioides* (Prusky *et al.* 1991). This technology could be developed for safe and effective immunization of fruits and vegetables to control post harvest diseases.

## 10. Conclusions

Plant immunization can be a natural, safe, effective, persistent and durable alternative to pesticides in controlling plant diseases. Although it has not yet been utilized on a large scale, there are reports of the practical application of plant immunization. For many years, hypovirulent strains of TMV have been used in the greenhouse (Rast 1975) and field (Japan-personal observation) to protect tomato against virulent strains of the virus. In addition to viruses, bacteria and fungi can also be used as inducers for the control of diseases. However, this may not be as economical in some cases, especially in areas with highly developed agriculture, and using pathogenic organisms may cause additional problems.

Several reports reviewed in this article clearly indicate that nonpathogenic organisms can be used successfully to induce resistance in plants. Development of application technologies to utilize some of these organisms to provide the best protection is essential. Utilization of organosilicone surfactants, such as Silwet, that deliver microorganisms into stomates and hydathodes (Jetiyonan *et al.* 1993) may overcome some of the difficulties associated with practical application of biological inducers. Seed treatment with select

PGPR strains (Wei *et al.* 1991), or root treatment with some biologicals (Molot and Mas 1985) may reduce the cost of application and therefore may have an immediate applicability. Treatment of crops with plant-derived materials and compost extracts, as well as UV-C and $CO_2$ treatment of fruits and vegetables may have niches for practical application to control certain high-value crops and vegetables.

Chemical inducers may provide better means of application possibilities for induction of resistance, providing that they are easily accessible and not harmful. Some natural chemicals are rather inexpensive and easily obtainable, such as phosphates used for the induction of resistance in cucumbers (Gottstein and Kuć 1989), while others such as beta-ionone derivatives (Salt *et al.* 1986) may not be produced as economically and easily. It is important to note, however, the efficacy of induced resistance in plants, at least in some cases, depends on the environmental conditions (Falkoff *et al.* 1988).

In tobacco, resistance to blue mold is transferred to progeny from immunized parents via tissue culture (Tuzun and Kuć 1987), and such regenerants were protected in the field. The use of tissue culture regenerants may find practical field application with plants such as tobacco which are transplanted to the field. Plant immunization may also provide means to add additional resistance to commercially available varieties bred by conventional techniques or to improve their resistance.

Plant immunization may find its niche in sustainable agriculture as one of a variety of practices that promote plant health and reduce plant disease. Reduction of the use of synthetic pesticides for disease control through plant immunization may be most practical in Integrated Pest Management programs where the level of defense reactions in the plants could be detected by assaying indicator enzyme activities prior to a booster inducer application or treatment with pesticides.

In order to take advantage of plant immunization, a plant's natural means of protection against disease, we need more applied research to stimulate the interest of industry in commercialization of current methodologies and participating in the development of new technologies. We also need to change our current thinking about plant protection. Plants can be immunized and perhaps we are harming ourselves by the application of pesticides to stop fungal activity in the early stages of plant development when the induction process is most effective. If we wish to reduce pollution and strive for a healthful environment for our children, we must consider alternatives to pesticides for the control of plant pests and diseases. It appears logical to take advantage of mechanisms which helped plants withstand the test of time and contributed to the survival of plants throughout evolution. Hopefully, we will someday utilize plant immunization in an integrated program for plant protection.

## References

Albert, F. and Anderson, A.J. (1987) 'The effect of *Pseudomonas putida* colonization on root surface peroxidase', Plant Physiology 85, 535-541.

Alström, S. (1991) 'Induction of disease resistance in common bean susceptible to halo blight bacterial pathogen after seed bacterization with rhizosphere pseudomonads', Journal of General and Applied Microbiology 37, 495-501.

Amijee, F., Allan, E.J., Waterhouse, R.H., Glover, L.A., and Paton, A.M. (1992) 'Non-pathogenic association of L-form bacteria (*Pseudomonas syringae* pv. *phaseolica*) with bean plants (*Phaseolus vulgaris*) and its potential for biocontrol of halo blight disease', Biocontrol Science and Technology 2, 203-214.

Anderson, A.J., Guerra, D. (1985) 'Responses of bean to root colonization with *Pseudomonas putida* in a hydroponic system' Phytopathology 75, 992-995.

Arimoto, Y., Homma, Y., Yoshino, R. and Saito, S. (1991) 'Generational succession of DL-alanine dodecylester HCL-induced resisitance to blast disease in rice plants', Annals Phytopathological Society of Japan 57, 522-525.

Bakker, P.A.H.M., van Peer, R., Schippers, B. (1991) 'Suppression of soil-borne plant pathogens by fluorescent pseudomonads: mechanisms and prospects' in A.B.R. Beemster, G.J. Bollen, M. Gerlagh, M.A. Ruissen, B. Schippers, and A. Tempel, (eds.), Biotic Interactions and Soil-Borne Diseases, Elsevier, Amsterdam, The Netherlands, pp. 217-223.

Bergstrom, G.C., Johnson, M.C. and Kuć, J. (1982) 'Effects of local infection of cucumber by *Colletotrichum lagenarium*, *Pseudomonas lachrymans* and tobacco necrosis virus on systemic resistance to cucumber mosaic virus', Phytopathology 72, 922-925.

Carling, D.E., Roncadori, R.W., and Hussey, R.S. (1989) 'Interactions of vesicular-abuscular mycorrhizal fungi, root-knot nematode, and phosphorus fertilization in soybean', Plant Disease 73, 730-733.

Caruso, F.L. and Kuć, J. (1977) 'Field protection of cucumber, watermelon, and muskmelon against *Colletotrichum lagenarium* by *Colletotrichum lagenarium*', Phytopathology 67, 1290-1292.

Chappell, J. and Hahlbrock, K. (1984) 'Transcription of plant defence genes in response to UV light or fungal elicitor', Nature 311, 76-78.

Chester, K.S. (1933) 'The problem of acquired physiological immunity in plants', Quarterly Review of Biolology 8, 128-154, 275-324.

Cohen, R., Cuppels, D.A., Brammal, R.A. and Lazoravitts, G. (1991) 'Induction of resistance towards bacterial pathogens of tomato by exposure of the host to dinitroaniline herbicides', Phytopathology 82, 110-114.

Cohen, Y., Gisi, U. and Mossinger, E. (1991). 'Systemic resistance of potato plants against *Phytophthora infestans* induced by unsaturated fatty acids', Physiological and Molecular Plant Pathology 38, 255-263.

Cox, J.E., Fraser, L.R. and Broadbend, P. (1976) 'Stem pitting of grape fruit. Field protection by the use of mild strains: An evaluation of trials in two climatic districts', in E. C. Calvan (ed.), Proc. 7th. Conf. Intern. Organ. Citrus. Virol., Gainesville, Univ. Florida Press, pp. 68-70.

Creasy, L.L. and Coffee, M. (1988) 'Phytoalexin production potential of grape berries', Journal of the American Society for Horticultural Science 113, 230-234.

Cruickshank, I.A.M and Mandryk, M. (1960) 'The effect of stem infection of tobacco with *Peronospora tabacina* on foliage reaction to blue mold', Journal of the Australian Institute of Agricultural Research 26, 369-372.

Dean, R.A. and Kuć, J. (1985) 'Induced systemic protection in plants', Trends in Biotechnology 3, 125-129.

Dehne, H.W., Stenzel, K. and Schönbeck, F. (1984) 'The efficiency of induced resistance under practical culture conditions III. Reproduction of powdery mildew on induced resistant plants', Journal of Plant Diseases and Protection 91,-258-265.

Doke, N. and Chai, H.B. (1985) 'Activation of superoxide generation and enhancement of resistance against compatible races of *Phytophthora infestans* in potato plants treated with digitonin', Physiological Plant Pathology 27, 323-334.

Doke, N., Ramirez, A.V., and Tomiyama, K. (1987) 'Systemic induction of resistance in potato plants against *Phytophthora infestans* by local treatments with hyphal wall components of the fungus', Journal of Phytopathology 119, 232-239.

Doubrava, N.S., Dean, R.A. and Kuć, J. (1988) 'Induction of systemic induced resistance to anthracnose caused by *Colletotrichum lagenarium* in cucumber by oxalate and extracts from spinach and rhubarb leaves', Physiological and Molecular Plant Pathology 33, 69-79.

Falkhof, A.G., Dehne, H.W., and Schönbeck, F. (1988) 'Dependence of the effectiveness of induced resistance on environmental factors', Journal of Phytopathology 123, 311-321.

Frommel, M.I., Nowak, J., and Lazarovits, G. (1991) 'Growth enhancement and developmental modifications of *in vitro* grown potato (*Solanum tuberosum* spp. *tuberosum*) as affected by a nonfluorescent *Pseudomonas* sp.', Plant Physiology 96, 928-936.

Garcia-Garrido, J.M., and Ocampo, J.A. (1988) 'Interaction between *Glomus mossae* and *Erwinia carotovora* and its effects on the growth of tomato plants', New Phytologist 110, 551-555.

Gessler, C. and Kuć, J. (1982) 'Induction of resistance to *Fusarium* wilt in cucumber by root and foliar pathogens', Phytopathology 72, 1439-1441.

Giri, D.N. and Sinha, A.K. (1979) 'Effect of nontoxic chemical on brown spot disease in rice seedlings' International Rice Research Newsletter 4, 10-11.

Gottstein, H.D. and Kuć, J. (1989) 'Induction of systemic resistance in cucumber by phosphates', Phytopathology 79, 176-179.

Gregersen, P.L., and Smedegaard, V. (1989) 'Induction of resistance in barley against *Erisiphe graminis* f. sp. *hordei* after preinoculation with saprophytic fungus, *Cladosporium macrocarpum*', Journal of Phytopathology 124, 128-136.

Guedes, M.E., Richmond, S. and Kuć, J. (1980) 'Induced systemic resistance to anthracnose in cucumber as influenced by the location of the inducer inoculation with *Colletotrichum lagenarium* and onset of flowering and fruiting', Physiological Plant Pathology 17, 229-233.

Hadwiger, L.A. and Scwochau, M.E. (1971) 'Ultraviolet light-induced formation of pisatin and phenylalanine ammonia lyase', Plant Physiology 47, 588-590.

Halasz, E. (1965) 'The possibility of preimmunity in maize smut (*Ustilago maydis*)', Acta Agronomica 14, 27-35.

Hynes, R.K., and Lazarovits, G. (1989) 'Effect of seed treatment with plant growth promoting rhizobacteria on the protein profiles of intercellular fluids from bean and tomato leaves', Canadian Journal of Plant Pathology 11, 191.

Jenns, A. and Kuć, J. (1979) 'Graft transmission of systemic resistance of cucumber to anthracnose induced by *Colletotrichum lagenarium* and tobacco necrosis virus', Phytopathology 69, 753-756.

Jetiyonan, K., Backman, P.A. and Tuzun, S. (1993) 'Use of polysilicone surfactant for delivery of resistance-inducing strains of *Xanthomonas campestris* pvs. for control of black rot in cabbage', Proceedings 6th. International Congress of Plant Pathology, p 65.

Kapulnik, Y. (1991) 'Plant growth-promoting rhizobacteria', in Y. Waisel, A. Eshel, and U. Kafkafi, (eds.), Plant Roots: The Hidden Half, Marcel Dekker, Inc., New York, NY, pp. 717-729.

Karban, R. (1987) 'Environmental conditions affecting the strength of induced resistance to mites in cotton', Oecologia 73, 414-419.

Kloepper, J.W. (1991) 'Plant growth-promoting rhizobacteria as biological control agents of soilborne diseases', in J. Bay-Petersen, (ed.), Biological Control of Plant Diseases. FFTC Book Series No. 42. Food and Fertilizer Technology Center, Taipei, Taiwan pp. 142-156.

Kloepper, J.W. (1993) 'Plant growth-promoting rhizobacteria as biological control agents', in B. Metting, (ed.), Soil Microbial Technologies, Marcel Dekker, Inc., New York, NY, pp. 255-274.

Kloepper, J.W., Tuzun, S., Liu, L. and Wei, G. (1993) 'Plant growth-promoting rhizobacteria as inducers of sytemic disease resistance', in R.D. Lumsden and J.L. Waughn (eds.), Pest Management: Biologically Based Technologies', ACS Conference Proceedings Series, American Chemical Soc. Press. pp. 156-165.

Kopp, M., Rouster, J., Fritig, B., Darvill, A. and Albersheim, P. (1989) 'Host-pathogen interactions XXXII. A fungal glucan preparation protects *Nicotianae* against infection by viruses', Plant Physiology 90, 208-216.

Kroon, B.A.M., Scheffer, R.J. and Elgersma, D.M. (1991) 'Induced resistance in tomato plants against fusarium wilt invoked by *Fusarium oxysporum* f.sp. *dianthi*', Netherlands Journal of Plant Pathology 97, 401-408.

Kuć, J. (1983) 'Induced systemic resistance in plants to diseases caused by fungi and bacteria', in J.A. Bailey and B.J. Deveral (eds.), The Dynamics of Host Defense, Academic Press, Sydney, pp. 191-221.

Kuć, J. (1985) 'Expression of latent genetic information for disease resistance in plants', in J.L. Key and T. Kosuge (eds.), Cellular and Molecular Biology of Plant Stress, Alan R. Liss, NY, pp. 303-318.

Kuć, J. (1987a) 'Plant immunization and its applicability for disease control', in I. Chet, (ed.), Innovative Approaches to Plant Disease Control, John Wiley, NY, pp. 255-274.

Kuć, J. (1987b) 'Translocated signals for plant immunization', Annals of the New York Academy of Sciences 494, 213-223.

Liu, L., Kloepper, J. W., and Tuzun, S. (1992) 'Induction of systemic resistance against cucumber mosaic virus by seed inoculation with select rhizobacteria strains', Phytopathology 82, 1108.

Liu, L., Kloepper, J.W. and Tuzun, S. (1993a) 'Induction of systemic resistance against cucumber bacterial angular leaf spot caused by *Pseudomonas syringae* pv. *lachrymans* with two plant-growth promoting rhizobacteria', Phytopathology 83, 1340.

Liu, L., Kloepper, J.W. and Tuzun, S. (1993b) 'Induction of systemic resistance to *Fusarium oxysporum* by PGPR strains', Proceedings of the 6th International Congress of Plant Pathology, p. 214.

Maiss, E. (1987) 'Use of resistance induction through culture filtrates of *Stachybotrys chartarum* (Ehrenb. ex Link) Hughes and *Bacillus subtilis* (Ehrenburg) Cohn against virus diseases under commercial conditions of crop production', Archiv für Phytopathologie und Pflanzenschutz 4, 275-283.

Martyn, R.D., Biles, C.L. and Dillard, E.A. (1991) 'Induced resistance to *Fusarium* wilt of watermelon under simulated field conditions', Plant Disease 75, 874-877.

Meera, M.S., Shivanna, M.B., Kageyama, K., and Hyakumachi, M. (1993) 'Induction of systemic resistance in cucumber plants using turfgrass rhizosphere fungi', Annals of the Phytopathological Society of Japan 59, 279.

Metlitskii, L.V., Ozeretskovskaya, O.L., Dorozhkin, N.A., Ivanyuk, V.G., Chalova, L.I., Yurganova, L.A. and Baraidze, V.G. (1978) 'Induction of resistance to parasitic fungi in potatoes', Applied Biochemistry and Microbiology 14, 208-215.

Métraux, J.P., Goy, P.A., Staub, T., Speich, J., Steinman, A., Ryals, J. and Ward, E. (1991) 'Induced sytemic resistance in cucumber in response to 2,6-dichloro-isonicotinic acid and pathogens', in H. Hennecke, D.P.S. Verma (Eds), Advances in Molecular Genetics of Plant-Microbe Interactions Vol 1, Kluver Academic Publishers, Netherlands, pp. 432-439.

Molot, P.M. and Mas, P. (1985) 'Resistance induction on pepper and tomato after dipping roots into water-soluble elicitor', Journal of Phytopathology 112, 315-321.

Mucharromah, E. and Kuć, J. (1989) 'Oxalic acid, $K_2HPO_4$ and $K_3HPO_4$ as inducers of systemic resistance against diseases caused by fungi, bacteria and viruses in cucumber', Phytopathology 79, 1144.

Oerke, E.C., Steiner, U. and Schönbeck, F. (1989) 'The efficiency of induced resistance under practical culture conditions V. Powdery mildew and grain yield of winter and spring barley in relation to nitrogen fertilization', Zeitschrift für Pflanzenkrankheiten and Pflanzenschutz 96, 140-153.

Okuno, T., Nakayama, M., Okajima, N. and Furasawa, I. (1991) 'Systemic resistance to downy mildew and appearance of acid soluble proteins in cucumber leaves treated with biotic and abiotic inducers', Annals of the Phytopathological Society of Japan 57, 203-211.

Ozeretskovskaya, O.L., Chaleva, L.I., Avduishko, S.A, Chalenko, G.I. and Karavaeva, K.A. (1988) 'Eicosanoids as inducers of enhanced resistance of potato to late blight', Soviet Plant Physiology 35, 141-148.

Pont, W. (1959) 'Blue mold (*Peronospora tabacina* Adam) of tobacco in north Queensland. Some aspects of chemical control', Queensland Journal of Agricultural Science 16, 299-327.

Pospieszny, H., Chirkov, S. and Atabekov, J. (1991). 'Induction of antiviral resistance in plants by chitosan', Plant Science 79, 63-68.

Prusky, D., Plumbley, R.A. and Kobiler, I. (1991) 'Induction of resistance of avacado fruits to *Colletotrichum gloeosporioides* attack using $CO_2$ treatment', in C.L. Wilson and E. Chalutz Eds., Biologiical Control of Postharvest Diseases of Fruits and Vegetables, Workshop Proceedings, USDA/ARS 92, pp 243-255.

Rast, A.T.B. (1975) 'Variability of tobacco mosaic virus in relation to control of tobacco mosaic in glasshouse tomato crops by resistant breeding and cross protection', Agric. Res. Report 834, Institute of Phytopathologic Research, Wageningen, the Netherlands.

Reiss, E., Schubert, J. and Naumann, K. (1988) 'Determination of microbial resistance inducers and their preliminary charecterization', Archiv für Phytopathologie und Pflanzenschutz 24, 335-340.

Reilly, J.J. and Klarman, W.L. (1980) 'Thymine dimer and glyceollin accumulation in UV-irradiated soybean suspension cultures', Environmental and Experimental Botany 20, 131-134.

Rosendahl. S. (1985) 'Interactions between vesicular-arbuscular mycorrhizal fungus *Glomus fasciculatum* and *Aphonomyces euteiche* root rot of peas', Phytopathologische Zeitschrift 114, 31-40.

Roveratti, D.S., Teixeira, A.R.R. and Morai, W.B.C. (1989) '*Bacillus thuringiensis*-A new perspective for an induced protection to coffee leaf rust', Journal of Phytopathology 126, 149-159.

Salt, S.D., Tuzun, S. and Kuć, J. (1986) 'Effects of beta-ionone and abscisic acid on the growth of tobacco and resistance to blue mold. Mimicry of effects of stem infection by *Peronospora tabacina* Adam', Physiological and Molecular Plant Pathology 28, 287-297.

Scheffer, R.J. (1983) 'Biological control of Dutch elm disease by *Pseudomonas* species', Annals of Applied Biology 103, 21-30.

Schmidt, D. (1988) 'Prevention of bacterial wilt of grasses by phylloplane bacteria', Journal of Phytopathology 122, 253-260.

Schönbeck, F., Dehne, H.W. and Beicht, W. (1980) 'Activation of unspecific resistance mechanisms in plants', Journal of Plant Diseases and Protection 87, 654-666.

Schroth, M.N., and Becker, J.O. (1990) 'Concepts of ecological and physiological activities rhizobacteria related to biological control and plant growth promotion', In D. Hornby, (ed.), Biological Control of Soil-Borne Plant Pathogens, CAB International, Wallingford, Oxon, U.K., pp. 389-414.

Seguchi, K., Kurotaki, M., Sediko, S. and Yamaguchi, I. (1992) 'Action mechanism of N-cyanomethyl-2-chloroisonicotinamide in controlling rice blast disease', Journal of Pesticide Science 17, 107-113.

Singh, U.P., Ram, D. and Tewari, V.P. (1990) 'Induction of resistance in chickpea (*Cicer arietianum*) by *Aegle marmelos* leaves against *Sclerotinia sclerotiorum*', Zeitschrift für Pflanzenkrankheiten Pflanzenschutz 97, 439-443.

Sneh, B., Ichielevich-Auster, M. and Plaut, Z. (1989) 'Mechanism of seedling protection induced by hypovirulent isolate of *Rhizoctonia solani*', Canadian Journal of Botany 67, 2135-2141.

Steiner, U., Oerke, E.C., and Schönbeck, F. (1988) 'The efficiency of induced resistance under practical culture conditions IV. Powdery mildew grain yield of winter barley cultivars with induced resistance and fungicide treatment', Journal of Plant Diseases and Protection 95, 506-517.

Stevens, C., Lu, J.Y., Khan, V.A., Wilson, C.L., Chalutz, E. and Droby, S. (1991) 'Ultraviolet light induced resistance against postharvest diseases in vegetables and fruits', in C.L. Wilson and E. Chaultz, Eds. Biological Control of Postharvest Diseases Fruits and Vegetables, Workshop Proceedings, USDA/ARS 92, pp 268-290.

Trivedi, N. and Sinha, A.K. (1976) 'Resistance induced in rice plants against *Helminthosporium* infection by treatment with various fungal fluids', Journal of Phytopathology 86, 335-344.

Tuzun, S., Juarez, J., Nesmith, W.C. and Kuć, J. (1992) 'Induction of sytemic resistance in tobacco against metalaxyl-tolerant strains of Peronospora *tabacina* and the natural occurrence of the phenomenon in Mexico', Phytopathology 82, 425-429.

Tuzun, S. and Kloepper, J.W. 1994. 'Induced systemic resistance by plant growth-promoting rhizobacteria', in M.H. Ryder, P.M. Stephens and G.D. Bowen (eds.), Improving Plant Productuvity with Rhizosphere Bacteria, Proc. 3rd. International Workshop on Plant Growth-Promoting Rhizobacteria, CSIRO, Australia, pp. 104-109.

Tuzun, S. and Kuć, J. (1985a) 'A modified technique for inducing systemic resistance to blue mold and increasing growth of tobacco', Phytopathology 75, 1127-1129.

Tuzun, S. and Kuć, J. (1985b) 'Movement of a factor in tobacco infected with *Peronospora tabacina* Adam which systemically protects against blue mold', Physiological Plant Patholology 26, 321-330.

Tuzun, S. and Kuć, J. (1987) 'Persistence of induced systemic resistance to blue mold in tobacco plants derived via tissue culture', Phytopathology 77, 1032-1035.

Tuzun, S. and Kuć, J. (1989) 'Induced systemic resistance to blue mold', in W.E. McKeen (ed.), Blue Mold of Tobacco, American Phytopathological Society Press, pp. 177-200.

Tuzun, S. and Kuć, J. (1991) 'Plant immunization: An alternative to pesticides for control of plant diseases in the greenhouse and field', in J.-Bay-Petersen (ed.), Biological Control of Plant Diseases, FFTC Book Series No. 42, Taiwan, pp. 30-40.

Tuzun, S., Nesmith, W., Ferriss, R.S. and Kuć, J. (1986) 'Effects of stem injections with *Peronospora tabacina* on growth of tobacco and protection against blue mold in the field', Phytopathology 76, 938-941.

van Asch, M.A.J., Rijkenberg, F.H.J., and Coutinho T.A. (1992) 'Resistance induced in wheat by an avirulent race of *Puccinia recondita* f. sp. *tritici*', Plant Disease 76, 412-415.

van Peer, R., Niemann, G.J. and Schippers, B. (1991) 'Induced resistance and phytoalexin accumulation in biological control of Fusarium wilt of carnation by *Pseudomonas* sp. strain WCS417r', Phytopathololgy 81, 728-734.

Voisard, C., Keel, C., Haas, D. and Défago, G. (1989) 'Cyanide production by *Pseudomonas fluorescens* helps suppress black root rot of tobacco under gnotobiotic conditions', The EMBO Journal 8, 351-358.

Walters, D.R. and Murray, D.C. (1992) Induction of sytemic resistance to rust in Vicia faba by phosphate and EDTA: effects on calcium', Plant Pathology 41, 444-448.

Wei, G. (1993) 'Investigations on induced sytemic resistance with plant growth-promoting rhizobacteria', Ph.D. Dissertation, submitted to Auburn University Graduate School, 134 pp.

Wei, G., Kloepper, J.W. and Tuzun, S. (1991) 'Induction of systemic resistance of cucumber to *Colletotrichum orbiculare* by select strains of plant growth-promoting rhizobacteria. Phytopathololgy 81, 1508-1512.

Wei, G., Tuzun, S. and Kloepper, J.W. (1992) 'Comparison of biochemical responses in cucumber systemically protected against *Colletotrichum orbiculare* by select strains of plant growth-promoting rhizobacteria', Phytopathology 82, 1109.

Weltzien, H.C. (1989) 'Some effects of composted organic materials on plant health', Agriculture, Ecosystems and Environment 27, 439-446.

Wilson, C.L. ans Wisnievski, M.E. (1989) 'Biological control of postharvest diseases of fruits and vegetables: an emerging technology', Annual Review of Phytopathology 27, 425-441.

Yamada, T., Hiramoto, T., Tobimatsu, R., Shiraishi, T. and Oku, H. (1990) 'Elicitor-like substances present in barley and wheat seeds', Journal of Phytopathology 128, 89-98.

Ye, X.S., Pan, S.Q. and Kuć, J. (1989) 'Pathogenesis-related proteins and systemic resistance to blue mold and tobacco mosaic virus induced by tobacco mosaic virus, *Peronospora tabacina* and asprin', Physiological and Molecular Plant Pathology 35, 161-175.

Yokoyama, K., Aist, J.R. and Bayles, C.J. (1991) 'A paplilla-regulating extract that induced resistance to barley powdery mildew', Physiological and Molecular Plant Pathology 39, 71-78.

Zdor, R.E. and Anderson, A.J. (1992) 'Influence of root colonizing bacteria on defense responses of bean', Plant and Soil 140, 99-107.

# INDUCED SYSTEMIC RESISTANCE - AN OVERVIEW

JOSEPH KUĆ
*Department of Plant Pathology*
*University of Kentucky*
*Lexington, Kentucky 40546*
*USA*

It is difficult to understand why it has taken so long for the scientific community and agrichemical industry to recognize the hazards to human health and the environment of the increased dependence on pesticides and to acknowledge the potential of induced systemic resistance in plants and appreciate its significance to fundamental science and as a technology for plant disease control. The early reviews by Chester (Chester, 1933) brought together many descriptions of the phenomenon. This was followed by the well documented experiments by Ross and his colleagues (Ross, 1966), primarily with N-gene tobacco and tobacco mosaic virus, and by reports from my group with green bean, pear, apple, potato, cucumber, muskmelon, watermelon and tobacco and a broad spectrum of pathogens including fungi, bacteria and viruses (Kuć, 1982, 1985, 1987, 1993). What became clear from these reports was that susceptible plants had the genetic information for effective disease resistance mechanisms and that these mechanisms could be expressed systemically for extended periods of time by prior restricted inoculation with pathogens, avirulent forms of pathogens, cultivar-nonpathogenic races, microbial components and chemicals which themselves were not antimicrobial (Kuć, 1982, 1985, 1987, 1991-1993). The reports eroded the concept that a gene for resistance caused the production of a compound which directly inhibited development of a pathogen and susceptible plants, lacking such a gene, could not produce the compound. It was evident that defense compounds were common to resistant and induced resistant plants as well as susceptible plants. This does not preclude mechanisms for resistance in noninduced resistant plants which may be in addition to those induced by biotic or abiotic agents. Examples of such mechanisms include the presence of a preformed antimicrobial compound at the plant surface, e.g. duvatriendiols in tobacco leaves, steroid glycoalkaloids in potato tuber peel; leaf surface features which are not conducive to penetration of a fungal pathogen through



*R. Hammerschmidt and J. Kuć (eds.), Induced Resistance to Disease in Plants,* 169–175.

stomata; presence of compartmentalized antifungal agents which are released after injury caused by infection, e.g. glycosides of phenolic compounds and their hydrolytic and oxidative products. Underlying such mechanisms, however, are the general response mechanisms for resistance common to resistant and susceptible plants.

The early pioneer research with phytoalexins established many basic principles valid for induced resistance. Plants respond to pathogens and nonpathogens by synthesizing low molecular weight, generally lipophilic compounds which inhibit the growth of fungi and bacteria *in vitro* and accumulate at the sites of infection to levels which inhibit the development of some pathogens. Phytoalexins have a broad antifungal spectrum of activity, and specific phytoalexins are not produced in response to infection by specific pathogens. This lack of specificity with regard to production and action evident for phytoalexins is also evident for other defense compounds, e.g. lignin, chitinases, ß-1,3-glucanases, hydroxyproline-rich glycoproteins, anti-fungal pathogenesis-related proteins and callose. Structural similarities, however, are evident for phytoalexins within plant families. Thus, isoflavonoid and pterocarpan phytoalexins are produced in the Leguminosae but not the Solanceae, and sesquiterpenoid phytoalexins are produced in the Solanceae but not the Leguminosae. Further specificity for phytoalexins is evident within families, e.g. phaseollin is produced by green bean and not pea, pisatin is produced by pea and not green bean, and capsidiol is produced by tobacco and pepper but not potato. Phytoalexins have most often been reported as defense compounds against fungi, less often against bacteria, and it is difficult for this author to see a role for phytoalexins in resistance to viruses.

It is clear that phytoalexin structures and activity do not explain the often high specificity in plant-pathogen interaction. Biotic and abiotic agents cause phytoalexin synthesis and accumulation. The genes for the synthesis of phytoalexins are present in resistant and susceptible plants, even those reported to lack genes for resistance to a pathogen (Kuć, 1991, 1992). Specificity with phytoalexins probably resides in the regulation of the rapidity and magnitude of their synthesis and accumulation and this is under genetic control of host and pathogen. As with phytoalexins, all other suggested defense compounds produced by a given plant (lignin, phenolic cross-linked cell wall polymers, hydroxyproline-rich glycoproteins, callose, thionins, chitinases, ß-1,3-glucanases, peroxidases, phenoloxidases and non hydrolytic antifungal pathogenesis-related (PR) proteins) can be produced equally well by susceptible and resistant cultivars given the proper conditions for elicitation. This general observation is the basis for induced systemic resistance. Before challenge infection, induced systemic resistance elevates the levels of some defense compounds and sensitizes the plant to rapidly produce the same or other compounds after infection.

What initiates the cascade of events that leads to the profound metabolic changes associated with induced systemic resistance? All the evidence reported is in agreement with the proposal that a signal(s) is produced in the inoculated or chemically-treated leaf, stem or root, and this signal or a second signal(s) is translocated throughout the plant where it conditions a resistant state (Dean and Kuć, 1986, Guedes *et al.*, 1980; Jenns and Kuć, 1979; Tuzun and Kuć 1985). Tissue receiving the signal has not been reported to

generate more signal (Dean and Kuć, 1986). Clearly, several signals may be involved with induced systemic resistance. The initial signal(s) would be the first produced in response to the infection or stress and need not be the translocatable signal(s). The translocatable signal(s) may result directly or indirectly in the elicitation of defense compounds. Different defense compounds may be elicited by different translocatable signals or the translocatable signals can give rise to secondary signals which elicit different defense compounds or groups of compounds. Some compounds applied to plants to induce resistance may function as translocatable signals and lead directly or indirectly to the cascade of metabolic events leading to induced systemic resistance.

It is unlikely that an organic chemical, synthesized in the plant as a result of infection or treatment with a chemical, is the initial signal. Such an organic chemical would require a signal to initiate its synthesis. However, a compartmentalized compound(s), released because of injury or metabolic perturbation, could serve as the initial signal(s). Compartmentalization would prevent the compound from influencing metabolism and its slow and persistent release could result in the cascade of signals and metabolic events leading to induced systemic resistance. Such a compartmentalized signal(s) might function as an alarm signal conditioning an umbrella response to protect the plant against infectious disease as well as other stresses.

A recent report presents strong evidence that an electrical signal may be the initial signal for the synthesis of proteinase inhibitors in plant tissue distant from that receiving an injury (Wildon *et al.*, 1992). It is unlikely that salicylic acid is the initial signal or translocated signal. Excision of inducer leaf one in cucumber inoculated with Pseudomonas syringae, before export of salicylic acid into leaf two was detected, resulted in induced resistance in leaf two and subsequent accumulation of salicylic acid (Rasmussen *et al.* 1991). Ethephon increases PR proteins in tobacco, with the exception of acidic ß-1,3-glucanase, and increases N-gene resistance to TMV but not resistance to *Peronospora tabacina* (Ye *et al.* 1992). The increased levels of PR-proteins and resistance to TMV are not accompanied by increased free or conjugated salicylic acid in the leaf treated with ethephon or the leaves above (Kuć, 1993). Resistance could not be induced in transgenic tobacco plants expressing a hydroxylase which converts salicylic acid to catechol (Gaffney *et al.* 1993); however, grafted scions of tobacco not containing the hydroxylase gene were induced by inoculating the hydroxylase-containing rootstocks (Vernooij *et al.* 1994) That salicylic acid is not essential for induced resistance is further supported by the report that 2,6-dichloro-isonicotinic acid (INA) induced systemic resistance in tobacco plants containing the hydroxylase gene (S. Uknes, personal communication, 15th International Botanical Congress). Salicylic acid and many other compounds, including the phytoalexins, accumulate in some infected plant tissues in response to a signal(s). Together with ethylene, jasmonate, cytokinins and a multitude of other compounds which can induce resistance, salicylic acid elicits some of the putative defense compounds.

Why do I refer to the defense compounds as putative? It is not evident that any one of the individual defense compounds is the cause of fungal or bacterial containment in resistant plants. The role of the reported defense compounds in protecting plants against

viruses is even less certain. Collectively and in the proper chronological order of appearance and proper location within plant tissue relative to the location and development of a fungal or bacterial pathogen, some of the defense compounds may be effective in restricting pathogen development. Not all of the defense compounds need be active against all pathogens for resistance. Thus, plant chitinase would not hydrolyze the walls of fungi not containing chitin, but could be effective in hydrolyzing the walls of some fungi containing chitin. Anyone of the defense compounds also need not be 100% effective in containing the development of a pathogen, but collectively and in the proper order of appearance, several compounds may be highly effective.

In another perspective, the putative defense compounds listed earlier may be considered as failed defense compounds to a successful pathogen. It could be argued that the successful pathogen has coped with the failed mechanisms by one or several strategies. These strategies include avoiding recognition by the host as non-self and thereby avoiding elicitation of host defense compounds during critical stages of pathogen development, suppression of the synthesis or activity of host defense compounds, detoxication of defense compounds, restriction of uptake of such compounds, ability to pump out absorbed compounds, and development of cell walls resistant to plant hydrolases.

Having mentioned the possibility of failed defense mechanisms, I also believe it is important to introduce a second philosophical consideration. Do a few exceptions negate a generalization? High and rapid phytoalexin accumulation associated with susceptibility in a given plant-pathogen interaction need not mean that phytoalexins do not have a role in plant defense. Not all the defense compounds need be effective against all pathogens under all environmental conditions. The question of how the successful pathogen copes with the multiple defense mechanisms is as important to answer as is the elucidation of the mechanisms.

Aside from providing information relevant to mechanisms for disease resistance and their regulation, induced systemic resistance provides a technology for disease control which has the potential for reducing our dependence on chemical pesticides. Induced systemic resistance is likely to be as safe for health and the environment as disease resistant plants since the reported mechanisms activated in both are the same. Foreign genes are not introduced. Induced systemic resistance is systemic, persistent and multicomponent with different sites responsible for activity of the different components. Therefore, it is likely that induced systemic resistance will be stable compared to single metabolic site-directed chemical pesticides. It is not necessary to establish the nature of the translocated signal, mechanism for signal transduction or even to identify all components of the multiple response before utilizing induced systemic resistance technology for practical disease control. What is necessary is to refine the technology and improve formulation of the active agents to maximize the effectiveness, persistence and reliability of induced systemic resistance. Since compounds as simple and safe as di- and tripotassium phosphate and isonicotinic acid can be used to induce systemic resistance, the technology is not limited to the use of microorganisms as inducers (Mucharromah and Kuć, 1991; Gottstein and Kuć, 1989; Staub *et al.* 1993). We are fortunate that people

did not wait for the elucidation of the metabolic mode of action of aspirin before using it as a pain-killer. There are numerous examples where induced systemic resistance has successfully been applied for disease control in the field. They will be considered in subsequent chapters. In my research group, we have demonstrated the effectiveness of induced systemic resistance in the field for cucumber, muskmelon, watermelon and tobacco (Kuć, 1987; Kuć and Strobel, 1992; Caruso and Kuć, 1977; Gottstein and Kuć, 1989; Tuzun *et al.* 1986; Tuzun *et al.* 1992).

What are the problems to be faced in the application of induced systemic resistance for practical disease control? Induced systemic resistance has recently received a great deal of attention because of the increased concern for health and the environment, increased restrictions on the use of chemical pesticides, the withdrawal of many effective pesticides from use and the increased costs of developing, testing and marketing new products. The use of chemicals as inducers of systemic resistance offers an exciting possibility for disease control since their use would be compatible with existing foliar spray and soil application equipment. Compounds used as inducers, however, must be safe for animal health and the environment, otherwise we have merely substituted one chemical problem for another. Many of the compounds my research group developed for induced systemic resistance are on the U.S.A. Environmental Protection Agency's list of compounds exempt from tolerances. Thus, development of safe inducers is not a major hurdle to be overcome before utilizing induced systemic resistance. Commercialization of safe, inexpensive, effective and easily obtained chemicals for plant disease control may, however, be difficult. Pesticide research and commercialization have largely been driven by the ease of obtaining a patent monopoly and a highly profitable product. Though costs for development and production of compounds for induced systemic resistance would likely be far less than for existing organic pesticides, it is doubtful that major agrichemical companies would be anxious to rush into the commercialization of a product that could be purchased at the local pharmacy or supermarket. A smaller, environmentally-oriented company might be a more likely candidate. Such a company might recognize that proper formulation of a mix of active ingredients could become a major money maker, while at the same time serving the best interests of agriculture, the environment and the consumer. Companies make profits selling products ranging from yogurt to fertilizer without the need of patents.

Problems of the variability of effectiveness of induced systemic resistance under varying climatic and agronomic conditions, encountered with biological control in general, must be addressed. Induced systemic resistance depends upon a physiological response by the plant and therefore can, under certain conditions, be less consistent than chemical pesticides which kill the infectious agent. Induced systemic resistance utilizes the plant's own defense mechanisms to restrict development of pathogens. It has the advantage of controlling a broad spectrum of plant diseases including those caused by viruses and bacteria for which effective and economically-sound chemical pesticides are not available. Continued research directed at understanding the induction and regulation of induced systemic resistance and the defense compounds would help in maximizing reliability. Induced systemic resistance need not be an "either or" technology. Induced

systemic resistance readily integrates into a program utilizing disease resistant plants, biocontrol, genetic engineering and appropriate pesticides. Induced systemic resistance could not only decrease the quantity of pesticide needed for disease control but could also increase the effectiveness and useful life of those on the market.

Development of new technology to solve a problem often creates a new problem for which we need a new technology. Induced systemic resistance is not a final solution to all plant disease problems. It does deserve to be extensively and vigorously explored for its potential in providing an effective, inexpensive, natural, consumer- friendly and environmentally-friendly technology for plant disease control.

## References


Caruso, F. and Kuć, J. (1977) 'Field protection of cucumber, watermelon and muskmelon against *Colletotrichum lagenarium* by *Colletotrichum lagenarium*', Phytopathology 67,1285-1289.

Chester, K. (1933) 'The problem of acquired physiological immunity in plants', Quarterly Review of Biology 8,129-154, 275-324.

Dean, R. and Kuć, J. (1986) 'Induced systemic protection in cucumber: The source of the "signal"', Physiological and Molecular Plant Pathology 28,227-233.

Gaffney, T., L. Friedrich, B. Vernooij, D. Negrotto, G. Nye, S. Uknes, E. Ward, H Kessman and Ryals, J. (1993) 'Requirement of salicylic acid for the induction of systemic acquired resistance', Science 261, 754-756.

Gottstein, H. and Kuć, J. (1989) 'Induction of systemic resistance to anthracnose in cucumber by phosphates', Phytopathology 79,176-179.

Guedes, M., S. Richmond and Kuć, J. (1980) 'Induced systemic resistance to anthracnose in cucumber as influenced by the location of the inducer inoculation with *Colletotrichum lagenarium* and onset of flowering and fruiting', Physiological Plant Pathology 17,29-233.

Jenns, A. and Kuć, J. (1979) 'Graft transmission of systemic resistance of cucumber to anthracnose induced by *Colletotrichum lagenarium* and tobacco necrosis virus', Phytopathology 69,753-756.

Kuć, J. (1982) 'Induced immunity to plant disease', Bioscience 32,854-860.

Kuć, J. (1985) 'Increasing crop productivity and value by increasing disease resistance through non-genetic techniques', In: Forest Potentials, Productivity and Value, Weyerhauser Science Symp. 4 (Eds. R. Ballard *et al.* ) Weyerhauser Co. Press pp 147-190.

Kuć, J. (1987) 'Plant immunization and its applicability for disease control', In: Innovative Approaches to Plant Disease Control (Ed. I. Chet) John Wiley, N.Y. pp 255-274.

Kuć, J. 1991. Phytoalexins: perspectives and prospects. In: Mycotoxins and Phytoalexins (Eds. R. Sharma, D. Salunkhe). CRC Press, Boca Raton pp. 595603.

Kuć, J. (1992) 'Antifungal compounds from plants', In: Phytochemical Resources for Medicine and Agriculture. (Eds. H. Nigg, D. Seigler) Plenum, N.Y., pp 159-184.

Kuć, J. (1993) 'Non pesticide control of plant disease', In: Proc. 10th International Symp. on Systemic fungicides and Antifungal Compounds. (Eds. H. Lyr, C. Polter) Eugen Ulmer, Stuttgart, pp 225-237.

Kuć, J. and Strobel, N (1992) 'Induced resistance using pathogens and nonpathogens', In: biological control of Plant diseases. NATO ASI Series (Eds. E. Tjamos, G. Papavizas, R. Cook), Plenum, N.Y. pp 295-303.

Mucharromah, E. and Kuć, J. (1991) 'Oxalate and phosphates induce systemic resistance against diseases caused by fungi, bacteria and viruses in cucumber', Crop Protection 10, 265-270.

Rasmussen, J., R. Hammerschmidt, and Zook, M. (1991) 'Systemic induction of salicylic acid and its accumulation in cucumber after inoculation with *Pseudomonas syringae* pv. *syringae*', Plant Physiology 97, 1342-1347.

Ross, A. (1966) 'Systemic effects of local lesion formation', In: Viruses of plants (Eds, A. Beemster and S. Dykstra) North Holland Publ. Amsterdam pp. 127-150.

Staub, T., P. Ahl Goy and Kessman, H. (1993) 'Chemically induced disease resistance in plants', In: Proceedings of the 10th International Symposium on Systemic Fungicides and Antifungal Compounds (Eds H. Lyr and C. Polter) Eugen Ulmer, Stuttgart, pp 239-249.

Tuzun, S. and Kuć, J. (1985) 'Movement of a factor in tobacco infected with *Peronospora tabacina* which systemically protects against blue mold', Physiological Plant Pathology 26,321-330.

Tuzun, S., W. Nesmith, R. Ferriss and Kuć, J. (1986) 'Effects of stem injections with *Peronospora tabacina* on growth of tobacco and protection against blue mold in the field', Phytopathology 76,938-941.

Tuzun, S., J. Juarez, W. Nesmith and Kuć, J. (1992) 'Induction of systemic resistance in tobacco against metalaxyl-tolerant strains of *Peronospora tabacina* and the natural occurrence of the phenomenon in Mexico', Phytopathology 82,425-429.

Wildon, D., J. Thain, P. Minchin, I. Gubb, A. Riley, A. Skipper, Y. Doherty, P. O'Donnell and Bowles, D. (1992) 'Electrical signalling and systemic proteinase inhibitor induction in the wounded plant', Nature 360,62-65.

Vernooij, B., Friedrich, L., Morse, A., Reist, R., Kolditz-Jawhar, R., Ward, E., Uknes, S., Kessmann, H., Ryals, J. (1994) 'Salicylic acid is not the translocated signal responsible for inducing systemic acquired resistance but is required for signal transduction', The Plant Cell 6, 959-965.

Ye, X., S. Pan and Kuć, J. (1992) 'Specificity of induced systemic resistance as elicited by ethephon and tobacco mosaic virus in tobacco', Plant Science 84,1-9.

# Index

## Developments in Plant Pathology

1. R. Johnson and G.J. Jellis (eds.): *Breeding for Disease Resistance.* 1993 ISBN 0-7923-1607-X
2. B. Fritig and M. Legrand (eds.): *Mechanisms of Plant Defense Responses.* 1993 ISBN 0-7923-2154-5
3. C.I. Kado and J.H. Crosa (eds.): *Molecular Mechanisms of Bacterial Virulence.* 1994 ISBN 0-7923-1901-X
4. R. Hammerschmidt and J. Kuć (eds.), *Induced Resistance to Disease in Plants.* 1995 ISBN 0-7923-3215-6

KLUWER ACADEMIC PUBLISHERS – DORDRECHT / BOSTON / LONDON