CHAPTER 6

Potential Application of Plant Growth-Promoting Rhizobacteria to Induce Systemic Disease Resistance

Sadik Tuzun and Joseph W. Kloepper

CONTENTS

I. INTRODUCTION

Our planet faces new challenges with its ever increasing population, the limited amount of agricultural land available to supply food demand, and the pollution problems associated with the extensive use of agri-chemicals. The need for an increased food supply and the use of intensive farming and mass monoculture have led to the development of new chemicals to control diseases and insects and these have disrupted the balance of agricultural systems. These disruptions may have eliminated some of the biological agents which have helped plants to remain healthy in their environment. Today, we know that

plants have quite sophisticated defense mechanisms that can be activated prior to disease development by environmental factors and microorganisms. Chapter 5 of this book describes such a phenomenon, called induced systemic resistance or plant immunization, that has coevolved with plants and may have contributed to plant survival. Our research has been concerned about the induction of systemic resistance to diseases in plants by manipulation of microbial populations naturally present in the plants' environment. In this chapter, we briefly describe key events in the evolution of the concept of induced systemic resistance, briefly review research on plant growth-promoting rhizobacteria (PGPR) and their role in disease control, describe the research on PGPR-mediated induced systemic resistance, describe current research on the range of diseases controlled by PGPR-mediated induced systemic resistance, and, finally, briefly review the ongoing research to establish the mechanism of PGPR-mediated systemic resistance and to develop their field applications.

II. INDUCTION OF DISEASE RESISTANCE IN PLANTS

Effective mechanisms for resistance to infectious agents have evolved in plants. Such mechanisms, although adequate for the survival of a species, may not satisfy the demands for high crop yield and quality imposed by modern agriculture. As early as 1933, Chester[1] reported development of resistance to diseases in plants following infection. Induced disease resistance can be defined as the process of active resistance dependent on the host plant's physical or chemical barriers, activated by biotic or abiotic agents.[2] Research in laboratories worldwide has demonstrated induction of disease resistance in susceptible varieties of over 25 crops including cereals, cucurbits, legumes, solanaceous plants, and tree and small fruits[3] against a broad spectrum of leaf[4–8] and root pathogens.[9] Today we know that resistance can be induced by prior inoculation with pathogens,[3,10,11] nonpathogens,[12,13] seed treatment with specific PGPR strains,[14] and microbial metabolites and other chemicals.[15–19] Plant immunization has also been demonstrated to be induced by previous treatment with plant-derived materials[20–26] including extracts of compost.[27] An important aspect of induced systemic resistance is that the phenomenon is nonspecific; that is, a single inducer can immunize plants against diverse pathogens. In cucumber, treatment of the first true leaf with a necrosis-forming organism protects the plant against subsequent infection by at least 13 pathogens, including fungi, bacteria, and viruses.[4,6]

III. PLANT GROWTH-PROMOTING RHIZOBACTERIA (PGPR)

Free-living root and soil bacteria have been studied for the past century as possible inoculants for enhancing crop productively. While early work

indicated that some bacteria, such as *Azotobacter* spp., could periodically increase plant growth, the population density of many inoculated bacteria declined upon introduction to agricultural soils. With the advent of bacterial marking systems, it was demonstrated that select rhizosphere bacteria could colonize plant roots in the presence of an indigenous soil microflora.[28] Such bacteria which exhibit root colonization have been termed "rhizobacteria",[29] and issues related to the root colonization process have recently been reviewed.[30] Effects of rhizobacteria on host plants may be deleterious, neutral, or beneficial, and beneficial rhizobacteria have been termed "plant growth-promoting rhizobacteria (PGPR)".[31]

Throughout the 1980s, many descriptive studies documented that PGPR strains may exert beneficial effects through plant growth promotion[32–37] or biological disease control.[32,34–41] The PGPR reports summarized in these reviews include the effects of many different bacterial groups on many host plants. Therefore, it is difficult to make comprehensive summary statements of common principles. However, some broad conclusions may be drawn from these reports. Most PGPR strains appear to have beneficial effects on plants by more than one mechanism. Growth promotion and biological control are frequently, but not always, expressed by the same PGPR strain. Several bacterial metabolites have been associated with biological control, including siderophores, HCN, volatile and nonvolatile antibiotics, cell-wall degrading enzymes, and antifungal factors. Induced systemic resistance was not included in the list of possible modes of action in most PGPR studies.

IV. PGPR AS INDUCERS OF DISEASE RESISTANCE

Initial suggestions that some beneficial bacteria may act as agents to induce systemic resistance came from two reports in the 1980s. Scheffer[42] reported that prior inoculation of elm trees with four fluorescent pseudomonad strains led to significant reductions in systemic foliar symptoms of *Ophiostoma ulmi,* the Dutch elm disease pathogen. Some of the bacterial strains exhibited only weak antibiosis *in vitro* to the fungal pathogen, and Scheffer suggested that these strains might cause plant protection by enhancing host resistance. In another system, Voisard et al.[43] investigated the mechanisms for biological control of *Thielaviopsis basicola* by PGPR strain CHAO, a strain of *Pseudomonas fluorescens.* Production of HCN was found by molecular analysis to be associated with biological control activity and with promotion of root hair growth. It was suggested that HCN produced by PGPR strain CHAO might induce plant defense mechanisms.

Direct pathological evidence for the induction of systemic resistance by PGPR was published for three systems in 1991. In a carnation system,[44] applications of *Pseudomonas* sp. strain WCS417 to rockwool cubes resulted in protection from wilt of *Fusarium oxysporum* f. sp. *dianthi.* The pathogen was spatially separated from the PGPR strain by inoculating into stems 1 week after

PGPR application, and separation was confirmed by a failure to isolate WCS417 from stems. In a bean system,[45] 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.* In another report with cucumber,[46] 94 known PGPR strains were examined for their ability to control anthracnose caused by *Colletotrichum orbiculare.* Six PGPR strains applied as seed treatments consistently resulted in significant reductions in anthracnose lesion diameter and lesion numbers when the pathogen was applied 21 days after planting. In a subsequent study with the six inducing PGPR,[47] none of the inducing strains was recovered from leaf petioles, confirming the spatial separation of pathogen and PGPR. Together 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.

V. SPECTRUM OF PGPR-MEDIATED INDUCED SYSTEMIC RESISTANCE

Current work in our laboratories to determine the spectrum of protection was achieved with two of the inducing PGPR strains used by Wei et al.[46] on cucumber. Both strains, applied as seed treatments, significantly reduce mean diameter of lesions induced by foliar-applied *C. orbiculare.* Protection against cucumber mosaic virus (CMV) is affected by the viral inoculation site. One PGPR strain significantly protected against CMV inoculated onto cotyledons.[48] Protection was evident as complete blockage of symptom development on PGPR-induced plants, which differs from protection reported against CMV for classical induced systemic resistance.[49] With classical induced resistance, CMV symptoms were delayed but not completely blocked upon induction. 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 induce 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.[50] Preliminary studies with Fusarium wilt demonstrate that one PGPR strain reduces the rate of symptom development and plant death.[50] 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 of PGPR.

VI. BIOCHEMICAL MECHANISMS

While pathological effects may be considered as direct evidence for induced resistance, some biochemical data are required to conclude that the observed decrease in disease severity resulted from host defense reactions rather than from PGPR-produced antifungal metabolites. Resistance in classically immunized plants is expressed as a reduction both in size and number of lesions and in sporulation.[6] In cucurbits, appressoria of *C. lagenarium* penetrate much less into immunized cucumber than into control plants.[51] Immunization of tobacco, however, appears to restrict fungal development within leaves after penetration.[52] In general, immunization sensitizes plants so that they respond rapidly to infectious agents. Treatments with immunizing agents rapidly activate multiple mechanisms of disease resistance which, in susceptible plants, are latent or are expressed too late to control disease. These mechanisms include the accumulation of antimicrobial low molecular weight chemicals (phytoalexins and leaf surface diterpenes)[53–56] and protective biopolymers (lignin, callose, and hydroxyproline-rich glycoproteins).[57–59] Increase in activity of enzymes in the pathways leading to production of such products, and increases in the amount of other primary gene products such as chitinases, β-1,3-glucanases, peroxidases, and other pathogenesis related (PR-) proteins, has also been detected.[60] The induction of systemic resistance to *P. tabacina* in tobacco coincided with the accumulation of β-1,3-glucanases, chitinases, and other PR-proteins,[61] and an anionic isozyme of peroxidase.[62] Enhanced peroxidase activity,[57,63] induction of chitinases,[64,65] and β-1,3-glucanases[66] have been found in systemically protected cucumber plants. Synthesis of these proteins appears to be regulated at the level of mRNA accumulation.[67,68] Chitinases and β-1,3-glucanases, which are biologically active against fungi by hydrolyzing cell wall polymers, and peroxidases, which generate H_2O_2 and oxidize phenols, are important in lignin biosynthesis. These enzymes are also likely to be a part of the multicomponent mechanisms effective in the immunization of plants against disease. The multicomponent nature of defense mechanisms associated with immunization result in a very stable resistance.

Four studies have indicated that specific PGPR may stimulate the production of biochemical compounds associated with host defense. Van Peer and his colleagues[44] observed increased accumulation of phytoalexins in carnation plants treated with PGPR (isolate WCS417) following pathogen inoculation. In a bean system, Hynes and Lazarovits[69] found that levels of a PR-protein increased in leaves following seed treatment with PGPR strains. Plant root colonization by PGPR was associated with enhanced lignification of stems or leaves in bean[70] and wheat.[71] Inoculation of bean roots with a *P. putida* PGPR

strain led to an increased abundance of mRNA encoding PR1a protein in leaves.[72] 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 classically induced controls.[14] Some, but not all, inducing PGPR strains were associated with enhanced mRNA encoding acidic chitinases.[73] It will be necessary to conduct further biochemical investigations of how PGPR-mediated and classical induced resistance affect host defense-related compounds.

VII. FIELD APPLICATIONS

Plant immunization can be a natural, safe, effective, persistent, and durable alternative to the use of pesticides in controlling plant diseases. However, the problems associated with utilization of pathogens as inducers and limitation in the application technologies may affect practical application. Chemical inducers, on the other hand, may provide a better means of induction. Some chemicals are rather inexpensive and easily obtainable, such as phosphates used for the induction of resistance in cucumbers,[26] while others such as β-ionone derivatives[21] may not be produced as economically and easily. The method of application, e.g., root treatment with the inducers[74] or seed treatment, may reduce the cost of application. It is important to note, however, the efficacy of induced resistance in plants, at least in some cases, depends on the environmental conditions.[75]

Most of the research on plant immunization has been conducted in the laboratory and greenhouse; however, there are a number of reports indicating its effectiveness in protecting crop plants under field conditions.[16,25,76–83] Immunization of tobacco against blue mold was first reported on systemically stem-infected and stunted field-grown tobacco in Australia.[84] Extensive field tests with a modified technique which employs injection of sporangiosporal suspensions of *P. tabacina* into stem tissue external to the xylem were conducted over a 3-year period in Kentucky and Puerto Rico with a metalaxyl-sensitive strain of the fungus.[80] These tests indicated that immunized plants were protected as well as those treated with metalaxyl. Furthermore, even in the absence of disease, immunized plants grew more vigorously and yields were up to 20% greater than those of the controls.[80] Further field experiments were conducted in Mexico during 1986 to 1989 to test the effectiveness of immunization against metalaxyl-tolerant strains of *P. tabacina*.[81,82] Highly significant reductions in the numbers and size of blue mold-induced lesions were observed on plants injected with *P. tabacina* as compared to nonstem-injected controls,

regardless of metalaxyl applications. Some vigorously growing plants with necrotic stem lesions but markedly reduced blue mold were observed in heavily infected commercial fields indicating the natural occurrence of immunization against blue mold in the Gulf Coast of Mexico.[82] Tolerance to immunization was not evident, although conditions for its development were very favorable. Treatments with chemical inducers such as β-ionone and 3-*n*-butroyl-β-ionone protected tobacco against metalaxyl-sensitive and tolerant strains of the fungus in greenhouse and field trials.[21,85] In field trials, plants derived by tissue culture from immunized parents were also less affected by blue mold than were plants derived from control parents.[86] These studies provide evidence that immunization of plants with biotic or abiotic inducers results in effective and stable disease control in the field.

Earlier research indicated that cucumbers can be protected in the field by the application of biological as well as chemical inducers.[22,26,78] Two field trials were conducted with PGPR on cucumber in 1992 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.[87] 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 *Erwinia tracheiphila* were observed. 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 more superior plant protection than classical induced systemic resistance.

Many questions remain to be addressed concerning the use of PGPR as agents of induced systemic resistance. The length of protection period obtained by PGPR-mediated induced resistance is unknown. The bacterial components which trigger induced resistance must be elucidated. In addition, a comparison should be made of the translocatable plant signal between PGPR-mediated and classical induced resistance. Answers to these questions will help assessment of the potential agricultural usefulness of PGPR as agents of induced systemic resistance.

REFERENCES

1. **Chester, K. S.,** The problem of acquired physiological immunity in plants *Q. Rev. Biol.*, 8, 128 and 275, 1933.

2. **Kloepper, J., Tuzun, S., and Kuć, J.,** Proposed definitions related to induced disease resistance, *Biocontrol Sci. Tech.*, 2, 349, 1992.
3. **Tuzun, S. and Kuć, J.,** Plant immunization: An alternative to pesticides for control of plant diseases in the greenhouse and field. In *Biological Control of Plant Diseases,* Bay-Peteren, J., Ed., FFTC Book Series No. 42, Taiwan, 30, 1991.
4. **Dean, R. A. and Kuć, J.,** Induced systemic protection in plants, *Trends Biotechnol.*, 3, 125, 1985.
5. **Kuć, J.,** Induced systemic resistance in plants to diseases caused by fungi and bacteria. In *The Dynamics of Host Defense,* Bailey, J. A. and Deverall, B. J., Eds., Academic Press, Sydney, 191, 1983.
6. **Kuć, J.,** Expression of latent genetic information for disease resistance in plants. In *Cellular and Molecular Biology of Plant Stress,* Key, J. L. and Kosuge, T., Eds., Alan R. Liss, New York, 303, 1985.
7. **Kuć, J.,** Plant immunization and its applicability for disease control. In *Innovative Approaches to Plant Disease Control,* Chet, I., Ed., John Wiley & Sons, New York, 255, 1987.
8. **Tuzun, S. and Kuć, J.,** Induced systemic resistance to blue mold. In *Blue Mold of Tobacco,* McKeen, W. E., Ed., American Phytopathology Society Press, St. Louis, MO, 177, 1989.
9. **Gessler, C. and Kuć, J.,** Induction of resistance to *Fusarium* wilt in cucumber by root and foliar pathogens, *Phytopathology,* 72, 1439, 1982.
10. **Tuzun, S. and Kuć, J.,** A modified technique for inducing systemic resistance to blue mold and increasing growth of tobacco, *Phytopathology,* 75, 1127, 1985.
11. **Cloud, A. M. E. and Deverall, B. J.,** Induction and expression of systemic resistance to the anthracnose disease in bean, *Plant Pathol.*, 36, 551, 1987.
12. **Schonbeck, F., Dehne, H. W., and Beicht, W.,** Activation of unspecific resistance mechanisms in plants, *J. Plant Dis. Prot.*, 87, 654, 1980.
13. **Roveratti, D. S., Teixeira, A. R. R., and Morai, W. B. C.,** *Bacillus thuringiensis* — A new perspective for an induced protection to coffee leaf rust. *J. Phytopathol.*, 126, 149, 1989.
14. **Wei, G., Tuzun, S., and Kloepper, J. W.,** Comparison of biochemical responses in cucumber systemically protected against *Colletotrichum orbiculare* by prior leaf inoculation with the pathogen or seed treatment with rhizobacteria, *Phytopathology,* 82, 1109, 1992.
15. **Trivedi, N. and Sinha, A. K.,** Resistance induced in rice plants against *Helminthosporium* infection by treatment with various fungal fluids, *J. Phytopathol.*, 86, 335, 1976.
16. **Dehne, H. W., Stenzel, K., and Schonbeck, F.,** The efficiency of induced resistance under practical culture conditions III. Reproduction of powdery mildew on induced resistant plants, *J. Plant Dis. Prot.*, 91, 258, 1984.
17. **Chai, H. B. and Doke, N.,** Systemic activation of O_2^- generating reaction, superoxide dismutase, and peroxidase in potato plants in relation to induction of systemic resistance to *Phytophthora infestans, Ann. Phytopathol. Soc. Jpn.*, 53, 585, 1987.

18. **Maiss, E.,** 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, *Arch. Phytopathol. Pflanzenschutz,* Berlin, 4, 275, 1987.
19. **Ozeretskovskaya, O. L., Chaleva, L. I., Avduishko, S. A., Chalenko, G. I., and Karavaeva, K. A.,** Eicosanoids as inducers of enhanced resistance of potato to late blight, *Sov. Plant Physiol.*, 35, 141, 1988.
20. **Doke, N. and Chai, H. B.,** Activation of superoxide generation and enhancement of resistance against compatible races of *Phytophthora infestans* in potato plants treated with digitonin, *Physiol. Plant Pathol.*, 27, 323, 1985.
21. **Salt, S. D., Tuzun, S., and Kuć, J.,** Effects of β-ionone and abscisic acid on the growth of tobacco and resistance to blue mold. Mimicry of effects of stem infection by *Peronospora tabacina* Adam, *Physiol. Mol. Plant Pathol.*, 28, 287, 1986.
22. **Doubrava, N. S., Dean, R. A., and Kuć, J.,** Induction of systemic induced resistance to anthracnose caused by *Colletotrichum lagenarium* in cucumber by oxalate and extracts from spinach and rhubarb leaves, *Physiol. Mol. Plant Pathol.*, 33, 69, 1988.
23. **Kopp, M., Rouster, J., Fritig, B., Darvill, A., and Albersheim, P.,** Host-pathogen interactions XXXII. A fungal glucan preparation protects *Nicotianae* against infection by viruses, *Plant Physiol.*, 90, 208, 1989.
24. **Yamada, T., Hiramoto, T., Tobimatsu, R., Shiraishi, T., and Oku, H.,** Elicitor-like substances present in barley and wheat seeds, *J. Phytopathol.*, 128, 89, 1990.
25. **Giri, D. N. and Sinha, A. K.,** Effect of nontoxic chemical on brown spot disease in rice seedlings, *Intl. Rice Res. Newslett.*, 4, 10, 1979.
26. **Gottstein, H. D. and Kuć, J.,** Induction of systemic resistance in cucumber by phosphates, *Phytopathology,* 79, 176, 1989.
27. **Weltzien, H. C.,** Some effects of composted organic materials on plant health, *Agric. Ecosystems Environ.*, 27, 439, 1989.
28. **Kloepper, J. W., Schroth, M. N., and Miller, T. D.,** Effects of rhizosphere colonization by plant growth-promoting rhizobacteria on potato plant development and yield, *Phytopathology,* 70, 1078, 1980.
29. **Schroth, M. N. and Hancock, J. G.,** Disease suppressive soil and root-colonizing bacteria, *Science,* 216, 1376, 1982.
30. **Kloepper, J. W. and Beauchamp, C. J.,** A review of issues related to measuring colonization of plant roots by bacteria, *Can. J. Microbiol.*, 38, 1219, 1992.
31. **Kloepper, J. W. and Schroth, M. N.,** Plant growth promoting rhizobacteria on radishes. In *Proceeding of the 4th Int. Conf. on Plant Pathogenic Bacteria,* Vol. 2, Gibert-Clarey, Tours, France, 879, 1978.
32. **Jagnow, G., Höflich, G., and Hoffman, K. H.,** Inoculation of non-symbiotic rhizosphere bacteria: possibilities of increasing and stabilizing yields, *Angew. Botanik,* 65, 97, 1991.
33. **Kapulnik, Y.,** Plant growth-promoting rhizobacteria. In *Plant Roots: The Hidden Half,* Waisel, Y., Eshel, A., and Kafkafi, U., Eds., Marcel Dekker, New York, 717, 1991.

34. **Kloepper, J. W.,** Plant growth-promoting rhizobacteria as biological control agents of soilborne diseases. In *Biological Control of Plant Diseases,* Bay-Petersen, J., Ed., FFTC Book Series No. 42, Taipei, Taiwan, 142, 1991.
35. **Lugtenberg, B. J. J., de Weger, L. A., and Bennett, J. W.,** Microbial stimulation of plant growth and protection from disease, *Curr. Opin. Biotechnol.,* 2, 457, 1991.
36. **Schippers, B., Bakker, A. W., Bakker, P. A. H. M., and van Peer, R.,** Beneficial and deleterious effects of HCN-producing pseudomonads on rhizosphere interactions. In *The Rhizosphere and Plant Growth,* Keister, D. L. and Cregan, P. B., Eds., Kluwer Academic Publishers, The Netherlands, 211, 1991.
37. **Schroth, M. N. and Becker, J. O.,** Concepts of ecological and physiological activities rhizobacteria related to biological control and plant growth promotion. In *Biological Control of Soil-Borne Plant Pathogens,* Homby, D., Ed., CAB International, Wallingford, Oxon, U.K., 389, 1990.
38. **Bakker, P. A. H. M., van Peer, R., and Schippers, B.,** Suppression of soilborne plant pathogens by fluorescent pseudomonads: mechanisms and prospects. In *Biotic Interactions and Soil-Borne Diseases,* Beemster, A. B. R., Bollen, G. J., Gerlagh, M., Ruissen, M. A., Schippers, B., and Tempel, A., Eds., Elsevier, Amsterdam, 1991, 217.
39. **Défago, B., Berling, C. H., Burger, U., Haas, D., Kahr, G., Keel, C., Voisard, C., Wirthner, P., and Wüthrich, B.,** Suppression of black root rot of tobacco and other root diseases by strains of *Pseudomonas fluorescens:* potential applications and mechanisms. In *Biological Control of Soil-Borne Plant Pathogens,* Hornby, D., Ed., CAB International, Oxon, U.K., 93, 1990.
40. **Kloepper, J. W.,** Plant growth-promoting rhizobacteria as biological control agents. In *Soil Microbial Technologies,* Metting, B., Ed., Marcel Dekker, New York, 255, 1993.
41. **Thomashow, L. S. and Weller, D. M.,** Application of fluorescent pseudomonads to control root diseases of wheat and some mechanisms of disease suppression. In *Biological Control of Soil-Borne Plant Pathogens,* Hornby, D., Ed., CAB International, Oxon, U.K., 109, 1990.
42. **Scheffer, R. J.,** Biological control of Dutch elm disease by *Pseudomonas* species, *Ann. Appl. Biol.,* 103, 21, 1983.
43. **Voisard, C., Keel, C., Haas, D., and Défago, G.,** Cyanide production by *Pseudomonas fluorescens* helps suppress black root rot of tobacco under gnotobiotic conditions, *EMBO J.,* 8, 351, 1989.
44. **van Peer, R., Niemann, G. J., and Schippers, B.,** Induced resistance and phytoalexin accumulation in biological control of Fusarium wilt of carnation by *Pseudomonas* sp. strain WCS417r, *Phytopathology,* 81, 728, 1991.
45. **Alström, S.,** Induction of disease resistance in common bean susceptible to halo blight bacterial pathogen after seed bacterization with rhizosphere pseudomonads, *J. Gen. Appl. Microbiol.,* 37, 495, 1991.
46. **Wei, G., Kloepper, J. W., and Tuzun, S.,** Induction of systemic resistance of cucumber to *Colletotrichum orbiculare* by select strains of plant growth-promoting rhizobacteria, *Phytopathology,* 81, 1508, 1991.
47. **Kloepper, J. W. and Beauchamp, C. J.,** A review of issues related to measuring colonization of plant roots by bacteria, *Can. J. Microbiol.,* 38, 1219, 1992.

48. **Liu, L., Kloepper, J. W., and Tuzun, S.,** Induction of systemic resistance against cucumber mosaic virus by seed inoculation with select rhizobacteria strains, *Phytopathology,* 82, 1108, 1992.
49. **Bergstrom, G. C., Johnson, M. C., and Kuć, J.,** 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, 1982.
50. **Liu,** 1992, unpublished.
51. **Richmond, S., Kuć, J., and Elliston, J.,** Penetration of cucumber leaves by *Colletotrichum lagenarium* is reduced in plants systemically protected by previous infection by the pathogen, *Physiol. Plant Pathol.,* 14, 329, 1979.
52. **Stolle, K., Zook, M., Shain, L., Hebard, F., and Kuć, J.,** Restricted colonization by *Peronospora tabacina* and phytoalexin accumulation in immunized tobacco leaves, *Phytopathology,* 78, 1193, 1988.
53. **Kuć, J. and Preisig, C.,** Fungal regulation of disease resistance mechanisms in plants, *Mycologia,* 76, 5, 767, 1984.
54. **Kuć, J. and Rush, J. S.,** Phytoalexins, *Arch. Biochem. Biophys.,* 236, 455, 1985.
55. **Salt, S. D. and Kuć, J.,** Elicitation of disease resistance in plants by the expression of latent genetic information. In *Bioregulators for Pest Control* Hedin, P. A., Ed., American Chemical Society, Washington, D.C., 47, 1985.
56. **Tuzun, S., Reuveni, M., Siegel, M. R., and Kuć, J.,** The effect of removing leaf surface compounds with acetone from immunized and nonimmunized resistant tobacco plants on their susceptibility to blue mold, *Phytopathology* 79, 1024, 1989.
57. **Hammerschmidt, R. and Kuć, J.,** Lignification as a mechanism for induced systemic resistance in cucumber, *Physiolol. Plant Pathol.,* 20, 61, 1982.
58. **Hammerschmidt, R., Lamport, D. T. A., and Muldoon, E. P.,** Cell wall hydroxyproline enhancement and lignin deposition as an early event in the resistance of cucumber to *Cladosporium cucumerinum, Physiol. Plant Pathol.* 24, 43, 1984.
59. **Hammerschmidt, R., Nuckles, E., and Kuć, J.,** Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium, Physiol. Plant Pathol.,* 20, 73, 1982.
60. **Lawton, M. A. and Lamb, C. J.,** Transcriptional activation of plant defense genes by fungal elicitor, wounding and infection, *Mol. Cell. Biol.,* 7, 335, 1987.
61. **Tuzun, S., Rao, M. N., Vogeli, U., Schardl, C. L., and Kuć, J.,** Induced systemic resistance to blue mold: Early induction and accumulation of β-1,3 glucanases, chitinases, and other pathogenesis related proteins (b-proteins) in immunized tobacco, *Phytopathology,* 79, 979, 1988.
62. **Ye, X. S., Pan, S. Q., and Kuć, J.,** Activity, isozyme pattern and cellular localization of peroxidase as related to induced systemic resistance to blue mold of tobacco, *Phytopathology,* 80, 1295, 1990.
63. **Smith, J. and Hammerschmidt, R.,** Comparative study of acidic peroxidases associated with induced resistance in cucumber, muskmelon and water melon *Physiol. Mol. Plant Pathol.,* 33, 255, 1988.
64. **Toppan, A. and Roby, D.,** Chitinase activity of melon plants infected by *Colletotrichum lagenarium* or treated with ethylene, *Agronomie,* 2, 829, 1982.

65. **Métraux, J. P. and Boller, T.,** Local and systemic induction of chitinase in cucumber plants in response to viral, bacterial and fungal infections, *Physiol. Mol. Plant Pathol.,* 28, 161, 1986.
66. **Rabenantoandro, Y., Auriol, P., and Tozue, A.,** Implication of β-glucanase in melon anthracnose, *Physiol. Plant Pathol.,* 8, 313, 1976.
67. **Carr, J. P., Dixon, D. C., and Klessig, D. F.,** Synthesis of pathogenesis-related proteins in tobacco is regulated at the level of mRNA accumulation and occurs on membrane bound polysomes, *Proc. Natl. Acad. Sci. U.S.A.,* 82, 7999, 1985.
68. **Dixon, R. A., Bolwell, G. P., Hamdan, M. A. M. S., and Robbins, M. P.,** Molecular biology of induced resistance. In *Genetics and Plant Pathogenesis,* Day, P. R. and Jellis, G. J., Eds., Blackwell, Boston, 245, 1987.
69. **Hynes, R. K. and Lazarovits, G.,** Effect of seed treatment with plant growth promoting rhizobacteria on the protein profiles of intercellular fluids from bean and tomato leaves, *Can. J. Plant Pathol.,* 11, 191, 1989.
70. **Anderson, A. J. and Guerra, D.,** Responses of bean to root colonization with *Pseudomonas putida* in a hydroponic system, *Phytopathology,* 75, 992, 1985.
71. **Frommel, M. I., Nowak, J., and Lazarovits, G.,** Growth enhancement and developmental modifications of *in vitro* grown potato (*Solanum tuberosum* spp. *tuberosum*) as affected by a nonfluorescent *Pseudomonas* sp. *Plant Physiol.,* 96, 928, 1991.
72. **Zdor, R. E. and Anderson, A. J.,** Influence of root colonizing bacteria on defense responses of bean, *Plant Soil,* 140, 99, 1992.
73. **Wei,** 1992, unpublished.
74. **Molot, P. M. and Mas, P.,** Resistance induction on pepper and tomato after dipping roots into water-soluble elicitor, *J. Phytopathol.,* 112, 315, 1985.
75. **Falkhof, A. G., Dehne, H. W., and Schoenbeck, F.,** Dependence of the effectiveness of induced resistance on environmental factors, *J. Phytopathol.,* 123, 311, 1988.
76. **Halász, E.,** The possibility of preimmunity in maize smut (*Ustilago maydis). Acta Agron.,* 14, 27, 1965.
77. **Cox, J. E., Fraser, L. R., and Broadbend, P.,** Stem pitting of grapefruit. Field protection by the use of mild strains: An evaluation of trials in two climatic districts. In *Proc. 7th Conf. Intern. Organ. Citrus. Virol.,* Calvan, E. C., Ed., University of Florida Press, Gainesville, 68, 1976.
78. **Caruso, F. L. and Kuć, J.,** Field protection of cucumber, watermelon, and muskmelon against *Colletotrichum lagenarium* by *Colletotrichum lagenarium, Phytopathology,* 67, 1290, 1977.
79. **Metlitskii, L. V., Ozeretskovskaya, O. L., Dorozhkin, N. A., Ivanyuk, V. G., Chalova, L. I., Yurganova, L. A., and Baraidze, V. G.,** Induction of resistance to parasitic fungi in potatoes, *Appl. Biochem. Microbiol.,* 14, 208, 1978.
80. **Tuzun, S., Nesmith, W., Ferriss, R. S., and Kuć, J.,** Effects of stem injections with *Peronospora tabacina* on growth of tobacco and protection against blue mold in the field, *Phytopathology,* 76, 938, 1986.
81. **Tuzun, S., Juarez, J., Nesmith, W. C., and Kuć, J.,** Immunization of tobacco against metalaxyl-tolerant strains of *Peronospora tabacina.* In *Blue Mold Disease of Tobacco,* Main, C. E. and Spurr, H. W., Jr., Eds., Tobacco Literature Service, N.C. State Univ., Raleigh, 157, 1990.

82. **Tuzun, S., Juarez, J., Nesmith, W. C., and Kuć, J.,** 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, 1992.
83. **Steiner, U., Oerke, E. C., and Schonbeck, F.,** The efficiency of induced resistance under practical culture conditions IV. Powdery mildew grain yield of winter barley cultivars with induced resistance and fungicide treatment, *J. Plant Dis. Prot.,* 95, 506, 1988.
84. **Pont, W.,** Blue mold (*Peronospora tabacina* Adam) of tobacco in north Queensland. Some aspects of chemical control, *Queensl. J. Agric. Sci.,* 16, 299, 1959.
85. **Tuzun, S.,** 1990, unpublished.
86. **Tuzun, S. and Kuć, J.,** Persistence of induced systemic resistance to blue mold in tobacco plants derived via tissue culture, *Phytopathology,* 77, 1032, 1987.
87. **Wei, G.,** 1992, unpublished.

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Edited by

Reuven Reuveni, Ph.D.
Division of Plant Pathology
Agricultural Research Organization
Newe Ya'ar Research Center
Haifa, Israel

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