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Prior inoculation with non-pathogenic fungi induces systemic resistance to powdery mildew on cucumber plants*

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Abstract

A spray inoculation of the first leaf of 2-leaf stage cucumber plants with a non-pathogenic isolate of *Alternaria cucumarina* or *Cladosporium fulvum* before a challenge inoculation with the pathogen *Sphaerotheca fuliginea* induced systemic resistance to powdery mildew on leaves 2–5. Systemic resistance was expressed by a significant ($p < 0.05$) reduction in the number of powdery mildew colonies produced on each leaf of the induced plants, as compared with water-sprayed plants. Systemic resistance was evident when a prior inoculation with each of the inducing fungi was administered 1, 3 or 6 days before the challenge inoculation with *S. fuliginea*. Increasing the inoculum concentration of *A. cucumarina* or *C. fulvum* enhanced the systemic protection and provided up to 71.6% or 80.0% reduction, respectively, in the number of colonies produced on upper leaves, relative to controls. Increasing the inoculum concentration of *S. fuliginea* used for challenge inoculation, increased the number of powdery mildew colonies produced on both induced and non-induced plants. Pre-treated plants, however, were still better protected than controls, indicating that the level of systemic protection was related to the *S. fuliginea* inoculum concentration. The induction of systemic resistance against powdery mildew by biotic agents, facilitates the development of a wide range of disease management tools.

Introduction

Cucumber powdery mildew caused by *Sphaerotheca fuliginea* is a major disease, attacking field- and greenhouse-grown cucumber plants. Disease control is generally based on the use of fungicides, but the need for reduced pesticide levels on agricultural products, together with the lack of commercially acceptable resistant cucumber cultivars, dictate the need for alternative methods of disease control. One of the potential methods of reducing the severity of foliar diseases is the induction of systemic resistance, achieved through the use of chemicals or biotic inducing agents (Sticher et al., 1997; Ye et al., 1995). Elicitors enhance the level of translocatable signal molecules which results in coordinated induction of genes controlling various defence mechanisms. Salicylic acid, jasmonic acid and ethylene have been shown to be involved in induced resistance, but the actual systemically transported signal has yet to be identified (Durner et al., 1997; Thomma et al., 1998). Two signal transduction pathways involved in induced disease resistance have been distinguished so far: a salicylic acid-dependent pathway (Durner et al., 1997) and a salicylic acid-independent pathway (Pieterse et al., 1996; Schaffrath et al., 1997) that involves jasmonic acid and ethylene (Penninckx et al., 1996; Pieterse et al., 1996). The salicylic acid-independent pathway and the jasmonic acid and ethylene-dependent pathway which is triggered by non-pathogenic, induced systemic resistance-inducing rhizobacteria, have been described (Pieterse et al., 1996; 1998). It has recently been stated that induced systemic resistance, mediated by non-pathogenic soil

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bacteria follows a salicylic acid-independent pathway that requires jasmonic acid and ethylene and does not involve the synthesis of pathogenesis-related proteins, while systemic acquired resistance (SAR) is salicylic acid-dependent and can be initiated by various pathogenic and chemical agents (van Loon et al., 1998).

Systemic resistance against various diseases can be induced by spraying the lower leaves of cucumber plants with solutions of oxalate or phosphate salts (Mucharromah and Kuć, 1991). In previous studies we demonstrated that a foliar spray of phosphate salts (Reuveni et al., 1993) and application of phosphate through a hydroponic system (Reuveni et al., 2000), induced systemic resistance against powdery mildew on cucumber plants, and against common rust and northern leaf blight on maize (Reuveni et al., 1996). This treatment has recently become a practical means for control of powdery mildew diseases in a wide range of crops, such as grape, apple, mango and nectarine (Reuveni and Reuveni 1998).

Prior inoculation of the lower leaves with biotic agents such as avirulent or hypovirulent pathogens, which cause limited local lesions, or with non-pathogens, induces systemic resistance against a wide range of fungal, bacterial and viral pathogens (Hammerschmidt and Smith Becker, 1997; Sticher et al., 1997; Ye et al., 1995). Such inoculation activates resistance mechanisms in the non-inoculated leaves and provides long-lasting protection against a broad spectrum of diseases. Induction of resistance against *Alternaria brassica* was observed in mustard following prior inoculation with an avirulent *A. brassica* isolate (Vishwanath et al., 1999) and in barley against *Erysiphe graminis* f.sp. *hordei* after pre-inoculation with the saprophytic fungus *Cladosporium macrocarpum* (Gregersen and Smedegaard, 1989).

In the present study, systemic resistance against *S. fuliginea* in cucumber by means of a limited, prior inoculation with the non-pathogenic fungi *Alternaria cucumarina* and *Cladosporium fulvum* was studied. In addition, the effects of the concentration of the inoculum of *S. fuliginea* used for challenge inoculation, and of *A. cucumarina* or *C. fulvum*, the agents used for inducing systemic resistance, were evaluated.

Materials and methods

Plants

Cucumber plants (*Cucumis sativum* L. 'Delilla') were grown in a greenhouse in 10 cm-diameter plastic pots containing peat, soil and vermiculite (1 : 1 : 1, v/v/v). Twice weekly, the plants were watered to saturation with a 0.1% 20–20–20 (N–P–K) fertilizer solution.

Cultures

Isolates of *A. cucumarina* and *C. fulvum* were obtained from greenhouse-grown cucumber and tomato plants, respectively, maintained on Petri dishes containing Potato Dextrose Agar (PDA, Difco) medium and incubated at 25 °C in darkness. Cultures were transferred periodically to fresh medium and used for prior inoculation (induction) of plants.

Induction of systemic resistance by prior inoculation with A. cucumarina *or* C. fulvum

Plants with the first true leaf expanded and the second true leaf approximately two-thirds expanded were used (unless stated otherwise) in all experiments. Conidia of each inducing fungus were brushed separately from Petri dishes into 30 ml of distilled water containing two drops of Tween-20. Conidial suspensions at known inoculum concentrations of each fungus were used for prior inoculation (induction) of the first true leaf. The upper surface of leaf 1 was sprayed with 1 ml of the conidial suspension or water, while to avoid contamination, other parts of the plant and the pot were covered. After inoculation, the plants were incubated in a dew chamber at 20 °C for 24 h in darkness. They were then kept in a growth chamber (24 °C, 120 $\mu E\, m^{-2}\, s^{-1}$, 16 h of light per day) until challenged with a conidial suspension of *S. fuliginea*. The non-induced control plants were treated with water and kept under similar conditions in a separate growth chamber, in order to avoid contamination. In experiments to determine the 'duration' and the speed of induced systemic resistance, freshly prepared conidial suspensions of *A. cucumarina* or *C. fulvum* were sprayed onto the first true leaf 1, 3 or 6 days prior to challenge inoculation of the upper leaves. In this case all the plants were challenged on the same day (6 days after the first induction).

Pathogen inoculation, and effect of inoculum concentration

An isolate of *S. fuliginea* obtained from plants in a field, was maintained on cucumber plants grown in a growth chamber. Inoculum was collected from freshly sporulating infected leaves 9–12 days after inoculation.

Conidia were gently brushed into 30 ml of distilled water containing two drops of Tween-20 and counted with the aid of a haemocytometer to give a suspension of 3×10^4 conidia ml^{-1} (unless stated otherwise). In experiments to determine the effect of inoculum concentration on induced systemic resistance, various concentrations of conidial suspensions were prepared and used to inoculate the induced and non-induced plants. For inoculation, the upper surface of each non-induced leaf (leaf number 2–4) of each plant was uniformly sprayed with a conidial suspension delivered from a hand glass sprayer, while the first leaf was protected from inoculation. After inoculation, the plants were incubated in a dew chamber at 20 °C for 24 h in darkness, and were then kept in a growth chamber (24 °C, 120 μ E m^{-2} s^{-1}, 16 h of light per day) for disease development. Non-inoculated control plants were kept under similar conditions in a separate growth chamber in order to avoid contamination.

Assessment of induced resistance

Induced systemic resistance was determined by counting the number of colonies of powdery mildew produced on each leaf of each treated plant at the time points indicated.

Statistical analysis

Analysis of variance (ANOVA) was applied using the SAS GLM procedure (SAS Institute, Cary, NC, 1992), and Duncan's Multiple Range Test was applied to determine whether differences between treatments were statistically significant. Each experiment was repeated twice and data presented are of one typical experiment. At least six to eight plants were used in each treatment in complete randomized design.

Results

Effect of inoculum concentration of A. cucumarina *and* C. fulvum *on induced systemic resistance*

To study the effect on inoculum density of non-pathogenic fungi on the level of induced resistance, cucumber plants were pre-inoculated with different concentrations of conidia of *A. cucumarina* and *C. fulvum* and 24 h later were challenge inoculated with *S. fuliginea*. The data in Table 1 show that increasing the concentration of *A. cucumarina* for prior inoculation on the first leaf from 1×10^4 to 20×10^4 conidia ml^{-1} significantly ($p < 0.05$) improved the systemic resistance and provided 71.6% protection against powdery mildew on the upper leaves, relative to the controls. Similar results were obtained when *C. fulvum* was used as an inducer for prior inoculation. Increasing the concentration from 3×10^4 to 150×10^4 conidia ml^{-1}, improved systemic protection by 80.0% in terms of the number of colonies produced on upper leaves (Table 1). None of the concentrations applied in these experiments damaged the plant tissue.

Table 1. Effect of inoculating leaf 1 of cucumber with increasing inoculum concentrations of *A. cucumarina* or *C. fulvum* on induced systemic resistance against powdery mildew[a]

A. cucumarina			*C. fulvum*		
Inoculum concentration ($\times 10^4$/ml)	Colonies/ plant	% Protection vs. control	Inoculum concentration ($\times 10^4$/ml)	Colonies/ plant	% Protection vs. control
0	39.5 a[b]	—	0	17.0 a[b]	—
1	23.8 b	39.7	3	11.4 ab	32.9
4	19.0 bc	51.9	15	10.8 ab	36.5
20	11.2 c	71.6	75	8.2 b	51.8
			150	3.4 c	80.0

[a]The first leaf of 2-leaf-old plants was inoculated (induced) with various concentrations of *C. fulvum* or *A. cucumarina* as described in Materials and methods. After 24 h plants were challenge inoculated with a suspension of 3×10^4 or 1.5×10^4 conidia ml^{-1} of *S. fuliginea*, for *A. cucumarina* and *C. fulvum*, respectively. Data represent mean numbers of colonies produced on leaves 2 and 3, 15 days after challenge. The results are from one typical experiment performed three times with six replications.

[b]Mean numbers within columns followed by different letters are significantly ($p < 0.05$) different according to Duncan's Multiple Range Test.

Effect of increasing the time interval between induction of resistance and challenge inoculation

Nine different experiments were conducted in order to determine the effectiveness of each of the fungal isolates in inducing systemic resistance against powdery mildew. Relative to the number of powdery mildew colonies produced on plants treated with water, the fungal isolates of both *A. cucumarina* and *C. fulvum* were found to be effective for induction of systemic resistance (Table 2). This was not influenced by the number of days (1, 3 or 6) elapsing between induction and the challenge with *S. fuliginea*. Analysis of variance performed for each leaf indicated significant ($p < 0.05$) effects of *A. cucumarina* and *C. fulvum* (Table 2) on the numbers of colonies produced on leaves 2, 3, 4 and 5. In all cases, the number of powdery mildew colonies was greater on the leaves of non-induced control plants than on those of induced plants (Table 2).

Table 2. Induced systemic protection to powdery mildew on each upper leaf of cucumber plants by prior inoculation with *A. cucumarina* or *C. fulvum* on leaf 1

Induction: number of days before challenge[a]	Mean numbers of powdery mildew colonies on each of leaves 2–5, 19 days after challenge with *S. fuliginea*			
	2	3	4	5
Induction with *A. cucumarina*				
Control	11.3 a[b]	16.8 a	20.7 a	19.5 a
1	3.9 b	5.8 bc	7.5 b	4.5 b
3	5.3 b	5.5 bc	8.0 b	2.7 b
6	0.8 b	3.5 c	2.7 b	4.3 b
Induction with *C. fulvum*				
Control	11.3 a[b]	16.8 a	20.7 a	19.5 a
1	3.0 b	4.8 b	8.2 b	5.8 b
3	3.8 b	11.0 ab	6.0 b	3.0 b
6	2.8 b	4.2 b	4.8 b	4.3 b

[a]Two-leaf-old plants were induced by spray inoculation of the upper surface of leaf 1 with a suspension of 4×10^4 conidia ml^{-1} of *A. cucumarina* or 100×10^4 conidia ml^{-1} of *C. fulvum*. Control plants were sprayed with water. At 1, 3 and 6 days after induction, plants were challenge inoculated with a suspension of 3×10^4 conidia ml^{-1} of *S. fuliginea*. The results are from one typical experiment for each inducing fungus, performed three times with six replications.

[b]Mean numbers within columns followed by different letters are significantly ($p < 0.05$) different according to Duncan's Multiple Range Test.

Effect of S. fuliginea *inoculum concentration on the level of inducion of systemic resistance*

To study the effect of inoculum density of the challenge pathogen on the level of induced resistance by *A. cucumarina* and *C. fulvum*, cucumber plants were challenged inoculated with different concentrations of conidia of *S. fuliginea*. Increasing the concentrations of *S. fuliginea* inoculum significantly increased the number of colonies produced on both induced and non-induced plants (Tables 3 and 4). Systemic protection induced by the inducing fungi was related to inoculum concentration and resistance was significant only under the lower concentrations of the challenge inoculum. Increasing the inoculum concentration of *S. fuliginea* up to 20 or 24×10^4 conidia ml^{-1} decreased the systemic protection (Tables 3 and 4).

Table 3. Effect of inoculum concentration of *S. fuliginea* on induction of systemic protection to cucumber powdery mildew by prior inoculation with *A. cucumarina*[a]

S. fuliginea concentration	13 days		16 days		18 days	
	Control	Induced	Control	Induced	Control	Induced
1×10^4	21.0 b[b]A[c]	6.4 bB	28.7 bA	12.2 bB	34.5 bA	18.0 bB
4.5×10^4	44.3 abA	12.7 abB	57.0 abA	24.8 abB	65.5 abA	33.5 abB
20×10^4	61.8 aA	26.5 aA	77.5 aA	40.7 aA	86.7 aA	53.7 aA

[a]The first leaf of 2-leaf-old plants was sprayed with a suspension of 5×10^4 conidia ml^{-1} of *A. cucumarina* (induced) or with water (control), and 24 h later plants were challenge inoculated with suspensions of 1, 4.5 or 20×10^4 conidia ml^{-1} of *S. fuliginea*.

[b]Numbers within a column (inoculum concentration) followed by different lower case letters are significantly ($p < 0.05$) different according to Duncan's Multiple Range Test.

[c]Numbers in rows (control or induced) within each rating point followed by different upper case letters are significantly ($p < 0.05$) different according to the same test.

Table 4. Effect of inoculum concentration of *S. fuliginea* on induction of systemic protection to cucumber powdery mildew by prior inoculation with *C. fulvum*[a]

S. fuliginea concentration	14 days		17 days		21 days	
	Control	Induced	Control	Induced	Control	Induced
0.8×10^4	13.8 b[b]A[c]	0.8 bB	19.8 bA	2.6 bB	47.0 bA	11.6 bB
4.5×10^4	54.4 abA	24.4 abB	65.2 abA	38.4 abB	81.4 abA	50.6 abA
24×10^4	84.8 aA	41.6 aA	105.8 aA	53.4 aA	141.4 aA	69.0 aA

[a]The first leaf of 2-leaf-old plants was sprayed with a suspension of 150×10^4 conidia ml^{-1} of *C. fulvum* (induced) or with water (control), and 24 h later plants were challenge inoculated with suspensions of 0.8, 4.5 or 24×10^4 conidia ml^{-1} of *S. fuliginea*.
[b]Numbers within a column (inoculum concentration) followed by different lower case letters are significantly ($p < 0.05$) different according to Duncan's Multiple Range Test.
[c]Numbers in rows (control or induced) within each rating point followed by different upper case letters are significantly ($p < 0.05$) different according to the same test.

Discussion

This report demonstrates that limited prior inoculation of the lower leaf with non-pathogenic fungi induced systemic resistance against powdery mildew in cucumber. Evidently, this systemic resistance was not influenced by the number of days which elapsed between the induction by either inducing agent, *A. cucumarina* or *C. fulvum*, and the challenge inoculation with *S. fuliginea*, nor by leaf age and position on the plant. These findings are different from those of our previous studies, when systemic resistance to powdery mildew was obtained by a single spray of the first leaf with phosphates (Reuveni et al., 1993). In that case, the best protection was observed on leaf 2 when the first true leaf was induced, and it progressively declined on leaves 3 and 4. It was also evident that the systemic protection induced by phosphate was greater when the first true leaf was induced 2 h (0 day) before inoculation than when it was induced 2 or 4 days before (Reuveni et al., 1993). Although the mechanism(s) by which these fungi act to restrict disease development is still unknown, the present results suggest that the plant's general response to infection is rapidly triggered by a possible rapid translocation of the 'immunity signal' from the lower, induced leaf to the upper, protected leaves. It is unclear whether these inducers have similar modes of action to that of phosphates, although this phenomenon is certainly not specific (Fought and Kuć, 1996). This interpretation was further supported by the fact that a similar level of systemic resistance was obtained when both phosphates and the inducing fungi were used for induction treatment (Reuveni et al., unpublished data).

The present data extend earlier reports that systemic resistance to powdery mildew in cucumber can be induced by biotic agents such as *C. lagenarium* and TNV. Moreover, the present data indicate that increasing the inoculum concentration of either *A. cucumarina* or *C. fulvum* increased the level of systemic protection, as has been previously reported in *C. lagenarium*–cucumber interaction (Dean and Kuć, 1986). Increasing the inoculum concentration of *S. fuliginea* used in the challenge inoculation, increased the number of powdery mildew colonies produced on both induced and non-induced plants. The induced plants were significantly more protected than controls under the lower concentrations of the challenge inoculum of the pathogen, indicating that the level of systemic protection was related to inoculum concentration of the challenge pathogen (Tables 3 and 4). In practical terms, it means that under field conditions protection against powdery mildew can be induced at the early stages of the epidemic development and may keep the inoculum level of the pathogen low enough to avoid further development of the disease.

The present report provides further evidence for the presence of resistance mechanisms in susceptible plants, as hypothesized by Kuć (1987) and that these mechanisms can be activated by restricted infection by various non-pathogens. It is possible that non-pathogenic fungi are able to disturb the epidermal cells releasing elicitors from the pathogen or from the epidermal cell walls as a response to their presence, without causing visible lesions and thereby induce defence reactions. In spite of the fact that none of our studies showed any damage, subsequent to the induction treatment by phosphate (Reuveni and Reuveni, 1998), or by

A. cucumarina or *C. fulvum*, it is nevertheless, possible that these biotic or abiotic inducers elicit the release of a signal. This signal might affect the expression of the defence genes, which then make the plants more responsive to subsequent infection. This hypothesis might be supported by the evidence of the absence of specificity of the biotic and abiotic inducers, when protection was effectively induced against fungi, bacteria and viruses (Hammerschmidt and Smith Becker, 1997; Sticher et al., 1997; Ye et al., 1995). Studies with strains of plant growth-promoting rhizobacteria (PGPR) support this evidence: two strains of PGPR induced systemic resistance against various cucumber pathogens including *Colletotrichum orbiculare*, cucumber mosaic virus and *Fusarium oxysporum* (Tuzun and Kloepper, 1995; Wei et al., 1991).

As natural occurrence of the fungi *A. cucumarina* and *C. fulvum* might be sporadic and therefore limited in the fields and the greenhouses, the feasibility of this strategy will require artificial application of inoculum to commercial productions to ensure its practical implementation. The induction of systemic resistance in cucumber by non-pathogens can provide a practical supplement to conventional methods, since it extends the selection of tools available for environmentally-friendly disease management, and furthers our aim of establishing effective integrated pest management in cucumber production.

References

Dean RA and Kuć J (1986) Induced systemic protection in cucumbers: effects of inoculum density on symptom development caused by *Colletotrichum lagenarium* in previously infected and uninfected plants. Phytopathology 67: 186–189

Durner J, Shah J and Klessig DF (1997) Salicylic acid and disease in plants. Trend Plant Sci 2: 266–274

Fought L and Kuć J (1996) Lack of specifiin pextracts and chemicals as inducers of systemic resistance in cucumber plants to anthracnose. J Phytopathol 144: 1–6

Gregersen Per L and Smedegaard V (1989) Induction of resistance in barley against *Erysiphe graminis* f.sp. *hordei* after preinoculation with the saprophytic fungus, *Cladosporium macrocarpum*. J Phytopathol 124: 128–136

Hammerschmidt R and Smith Becker J (1997) Acquired resistance to disease in plants. Hort Rev 19: 248–289

Kuć J (1987) Plant immunization and its applicability for disease control. In: Chet I (ed) Innovative Approaches to Plant Disease Control (pp 255–274) J Wiley & Sons, Inc., New York

Mucharromah E and Kuć J (1991) Oxalates and phosphates induce systemic resistance against diseases caused by fungi, bacteria and viruses in cucumber. Crop Prot 10: 265–270

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

Pieterse CMJ, van Wees SCM, Hoffland E, van Pelt JA and van Loon LC (1996) Systemic resistance in *Arabidopsis* induced by biocontrol bacteria is independent of salicylic acid accumulation and pathogenesis-related gene expression. Plant Cell 8: 1225–1237

Pieterse CMJ, van Wees SCM, van Pelt JA, Knoester M, Laan R, Gerrits H, Weisbeek PJ and van Loon LC (1998) A novel signaling pathway controlling induced systemic resistance in *Arabidopsis*. Plant Cell 10: 1571–1580

Reuveni R and Reuveni M (1998) Foliar fertilizer therapy – a concept in integrated pest management. Crop Prot 17: 111–118

Reuveni M, Agapov V and Reuveni R (1993) Induction of systemic resistance to powdery mildew and growth increase in cucumber by phosphates. Biol Agric Hort 9: 305–315

Reuveni R, Reuveni M and Agapov V (1996) Foliar sprays of NPK fertilizers induce systemic protection against *Puccinia sorghi* and *Exserohilum turcicum* and growth response in maize. Eur J Plant Pathol 102: 339–348

Reuveni R, Dor G, Raviv M, Reuveni M and Tuzun S (2000) Systemic resistance against *Sphaerotheca fuliginea* in cucumber plants exposed to phosphate in hydroponics system, and its control by foliar spray of mono-potassium phosphate. Crop Protect 19: 355–361

Schaffrath U, Freydle E and Dudler R (1997) Evidence for different signalling pathways activated by inducers of acquired systemic resistance in wheat. Mol Plant–Microbe Interact 10: 779–783

Sticher L, Mauch-Mani B and Metraux J-P (1997) Systemic acquired resistance. Annu Rev Phytopathol 35: 235–270

Thomma BPH, Eggermont K, Penninckx IAMA, Mauch-Mani B, Vogelsang T, Bammue PBA and Broekaert WF (1998) Separate jasmonate-dependent and salicylate-independent defence response pathways in *Arabidopsis* are essential for resistance to distinct microbial pathogens. Proc Nat Acad Sci USA 95: 1507–1511

Tuzun S and Kloepper J (1995) Potential application of plant growth-promoting rhizobacteria to induce systemic disease resistance. In: Reuveni R (ed) Novel Approaches to Integrated Pest Management (pp 115–128) CRC Press, Boca Raton, Florida

Van Loon LC, Bakker PAHM and Pieterse CMJ (1998) Systemic resistance induced by rhizosphere bacteria. Annu Rev Phtopathol 36: 453–483

Vishwanath, Kolte SJ, Singh MP and Awasthi RP (1999) Induction of resistance in mustard (*Brassica juncea*) against *Alternaria* black spot with an avirulent *Alternaria brassica* isolate-D. Eur J Plant Pathol 105: 217–220

Wei G, Kloepper JW and Tuzun S (1991) Induction of systemic resistance of cucumber to *Colletotricum orbiculare* by select strains of plant growth-promoting rhizobacteria. Phytopathology 81: 1598–1512

Ye XS, Strobel N and Kuć J (1995) Induced systemic resistance (ISR): activation of natural defense mechanisms for plant disease control as part of integrated pest management (IPM). In: Reuveni R (ed) Novel Approaches to Integrated Pest Management (pp 95–113) CRC Press, Boca Raton, Florida, USA