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Induced systemic resistance in *Trichoderma harzianum* T39 biocontrol of *Botrytis cinerea*

Geert De Meyer[1], Joseph Bigirimana[1], Yigal Elad[2] and Monica Höfte[1,*]
[1] *Laboratory of Phytopathology, Faculty of Agricultural and Applied Biological Sciences, University Gent, Coupure Links, 653, B-9000 Gent, Belgium (Fax: 32-9-2646238);* [2] *Department of Plant Pathology, The Volcani Center, ARO, Bet Dagan 50250, Israel; Author for correspondence*

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Abstract

Biocontrol of *Botrytis cinerea* with *Trichoderma* spp. is generally believed to result from direct interaction of the biocontrol agent with the pathogen or from a *Trichoderma*-induced change in environmental conditions that affects *B. cinerea* development. In this work we provide arguments for the participation of induced plant defence in *T. harzianum* T39 control of *B. cinerea*. In tomato, lettuce, pepper, bean and tobacco, *T. harzianum* T39 application at sites spatially separated from the *B. cinerea* inoculation resulted in a 25–100% reduction of grey mould symptoms, caused by a delay or suppression of spreading lesion formation. Given the spatial separation of both micro-organisms, this effect was attributed to the induction of systemic resistance by *T. harzianum* T39. The observation that in bean the effect of *T. harzianum* T39 was similar to that of the rhizobacterium *Pseudomonas aeruginosa* KMPCH, a reference strain for the induction of systemic resistance, confirmed this hypothesis. Since *B. cinerea* control on tobacco leaves sprayed with *T. harzianum* T39 was similar to the control on leaves from *T. harzianum* T39 soil-treated plants, induction of plant defence might also participate in biocontrol on treated leaves.

Introduction

Biocontrol of the necrotrophic fungus *Botrytis cinerea* Pers. ex Fr. (teleomorph *Botryotinia fuckeliana* (de Bary) Whetz.) by *Trichoderma* spp. can be mediated by mechanisms that either directly or indirectly affect *B. cinerea* development. Direct modes of action include mycoparasitism (Labudova and Gogorova, 1988) and production of inhibitory compounds (Tronsmo and Raa, 1977; Tronsmo and Dennis, 1977). Examples of indirect mechanisms are competition for nutrients (Zimand et al., 1995) and space (Dubos et al., 1982) because the presence of *Trichoderma* changes the environment for *B. cinerea* development. These different strategies are not mutually exclusive since *T. harzianum* T39 combines competition for nutrients (Zimand et al., 1995) and interference with pathogenicity enzymes (Zimand et al., 1996) to control *B. cinerea*.

Some studies have demonstrated that *Trichoderma* spp. can also affect the host plant. Addition of *T. viride* cellulase to grapevine cell cultures induced plant defence reactions such as the hypersensitive response and phytoalexin production (Calderon et al., 1993). Recently, a similar induction of plant defence reactions by *T. longibrachiatum* in tobacco plants was linked to an increased resistance to *Phytophthora parasitica* var. *nicotianae* (Chang et al., 1997). This suggests an indirect biocontrol effect of *Trichoderma* through the induction of plant resistance.

In the case of pathogen infections, plant defence mechanisms are not only activated at the infection site, but also, to a lesser extent, in distant plant parts that were not infected. Consequently pathogen infections lead to a systemic increase in resistance throughout the plant, termed systemic acquired resistance (SAR) (Ryals et al., 1996). Root colonisation by specific non-pathogenic micro-organisms such as plant growth-promoting rhizobacteria (Tuzun and Kloepper, 1994) and fungi (Meera et al., 1994) can also induce a systemic increase in resistance. The latter phenom-

enon is similar to SAR, but is called induced systemic resistance (ISR) because different signalling pathways might be involved.

This work deals with induced plant defence in *T. harzianum* T39 biocontrol of *B. cinerea*. We checked induction of systemic resistance by *T. harzianum* T39 by studying *B. cinerea* control after a prior, spatially separated application of *T. harzianum* T39, that excluded the possibility of other mechanisms in biocontrol. In addition, *T. harzianum* T39 was compared to the rhizobacterium *Pseudomonas aeruginosa* KMPCH (De Meyer and Höfte, 1997) for induction of systemic resistance to *B. cinerea.* Finally ISR by *T. harzianum* T39 was compared to the total biocontrol effect that is obtained when the biocontrol agent is applied at the site of *B. cinerea* inoculation. Preliminary results on this topic have already been reported by Bigirimana et al. (1997).

Materials and methods

Plants

Tobacco (*Nicotiana tabacum* L.) cv. Xanthi, tomato (*Lycopersicon esculentum* Mill.) cv. 144, lettuce (*Lactuca sativa* L.) cv. Iceberg and bell pepper (*Capsicum annuum* L.) cv. Mazurka were grown in the greenhouse at 15–26 °C in pots (diameter 17 cm) with a peat-vermiculite-volcanic gravel mixture (2:1:2). Plants were fed weekly with appropriate fertiliser and never treated with fungicides. Pepper and tobacco plants were used for experiments when 10 weeks old, while for tomato and lettuce plant age was 12 and 8 weeks, respectively. Bean plants (*Phaseolus vulgaris* L.) cv. Prelude were grown in pots (diameter 7 cm) with potting compost soil (Klassmann-Deilmann, Germany) in the greenhouse at 18–28 °C and used for experiments when 3 weeks old.

Micro-organisms and culture conditions

On bean, *B. cinerea* infections were performed with isolate R16, that resulted from the cross SAS56 x SAS405 (Faretra and Pollastro, 1991), while other plants were infected with isolate B16, obtained from a naturally infected cucumber flower (Elad, 1988). The fungus was grown and maintained on potato dextrose agar (Difco). Conidia from 10–14 day old cultures were collected in water and cleared from mycelial debris by filtration through cheesecloth. Conidial concentration was determined with a haemacytometer and adjusted when necessary. The biocontrol agent *T. harzianum* T39, that controls grey mould in greenhouse vegetable crops and vine plants after foliar application (Elad et al., 1993; O'Neill et al., 1996), was used in its commercial formulation TRICHODEX 25P (Makhteshim Chemical Works Ltd, Be'er Sheva, Israel). Preliminary experiments had demonstrated that the ingredients of the formulation did not induce resistance to *B. cinerea* and that, in this respect, non-formulated conidia of *T. harzianum* T39 had the same effect as formulated ones. Inoculum of the rhizobacterium *P. aeruginosa* KMPCH, a salicylic acid-producing mutant of *P. aeruginosa* 7NSK2 (Buysens et al., 1996), was prepared from overnight cultures on King's medium B (King et al., 1954) agar plates at 37 °C. Bacteria were scraped from the plates, washed twice in sterile demineralized water and resuspended to a concentration of approximately 10^9 CFU ml^{-1} (De Meyer and Höfte, 1997).

Application of biocontrol agents

In tobacco, tomato, lettuce and pepper, *T. harzianum* T39 was applied in a 10^6 conidial ml^{-1} suspension. For soil treatment, 2 ml of the conidial suspension was injected into the substrate of every plant using a syringe without needle. Tobacco leaves were sprayed with 2 ml of the conidial suspension before the *B. cinerea* challenge. Bean plants were inoculated with the same technique, but using a 4×10^7 conidia ml^{-1} suspension. While spraying the first pair of bean leaves, targeting of other plant parts was avoided to ensure that *T. harzianum* T39 would not be applied to the first trifoliate leaf that was later challenged with *B. cinerea*. For comparison of ISR induction by *P. aeruginosa* KMPCH and *T. harzianum* T39, both micro-organisms were applied to bean roots by a combined seed and soil treatment. Bean seeds were soaked for five minutes in a 10^8 conidia and 10^8 CFU ml^{-1} suspension and subsequently planted in a soil amended with 10^8 conidia and 5×10^7 CFU g^{-1}, for *T. harzianum* T39 and *P. aeruginosa* KMPCH, respectively. Controls were soaked in water and grown in untreated soil.

B. cinerea *inoculation and scoring of disease symptoms*

Tomato, lettuce and pepper plants were inoculated with *B. cinerea* by spraying the leaves with a 5×10^5 conidia ml^{-1} suspension amended with 0.5 mg ml^{-1} glucose

and 0.5 mg ml^{-1} KH_2PO_4. Disease severity on whole plants was evaluated according to the percentage coverage of rot. For pepper, the percentage of leaves that detached from the stem was also recorded. In tobacco four leaves per plant were each inoculated with ten 30 μl drops of the same inoculum further supplemented with 5 mg ml^{-1} potato dextrose broth (Sigma). In this case, disease severity was evaluated according to the 0–7 index described by Zimand et al. (1996), where 0 = symptomless leaf tissue; 1 = 1 to 12%, 2 = 13 to 25%, 3 = 26 to 50%, and 4 = 51 to 100% necrotic area under the drop; and 5 = expansion zone around the drop $<$ 2mm, 6 = expansion zone 2 to 5 mm, and 7 = expansion zone $>$ 5 mm. In bean, *B. cinerea* was applied to leaves at a rate of ten 10 μl drops per plant of a 10^6 conidia ml^{-1} suspension amended with 20 μg ml^{-1} glucose. Subsequently, diameters of spreading lesions were recorded and summed per plant to make the total lesion diameter, which served as disease severity parameter. After challenge with *B. cinerea*, plants were incubated at 20 °C and 95% relative humidity.

Spatial separation of T. harzianum *T39 and* B. cinerea

In treatments where *T. harzianum* T39 was not applied to the leaf challenged with *B. cinerea*, a possible *T. harzianum* T39 colonisation of the challenged leaf was routinely checked on some plants. Leaves were macerated in sterile demineralized water and plated on half strength potato dextrose agar (Difco) amended with 50 mg l^{-1} rose bengale and 250 mg l^{-1} chloramphenicol. Plates were incubated at 24 °C for two to four days and colonies were compared with *T. harzianum* T39 on morphological criteria.

Experimental design and statistical analysis

Experiments with tobacco, tomato, lettuce and pepper consisted of 6 replicates per treatment and were laid out in a complete randomised design. Since conclusions based on three different trials were similar, findings of one experiment are presented in the results. For statistical analysis, arcsin transformed data were subjected to analysis of variance and treatments were compared with Fisher's protected least significant difference (LSD) test. Bean experiments consisted of at least 8 plants per treatment and were performed at least 4 times. The total lesion diameter (TLD) was analysed by a general linearized model, in which experiments were pooled because interaction between treatment and experiment was not significant. Fisher's LSD test was used to compare means. Contrast analysis to determine the additive effect of leaf and soil treatment was performed with the coefficients -1, 1, 1 and -1 for control, leaf only, soil only and leaf and soil treatments, respectively. For all techniques the $P = 0.05$ threshold was used.

Results

T. harzianum T39 soil applicaton 7 days before *B. cinerea* challenge significantly reduced grey mould severity in tomato, lettuce and pepper (Figure 1), although the biocontrol agent was not detected on the leaves of these plants. For pepper, grey mould coverage in the canopy did not give a complete picture of *B. cinerea* development because leaves colonised by the pathogen tended to detach from the stem. Figure 2A clearly indicates that *T. harzianum* T39 soil treatment also reduced the deleafing of pepper plants. As a result, the reduction of grey mould coverage on the leaves (Figure 1) should be combined with the reduction in deleafing to estimate the real reduction in *B. cinerea* development. In pepper, the *B. cinerea* spray inoculation also caused a considerable amount of stem infections (Figure 2B), that were not related to the deleafing since the stem infections did not develop from petiole wounds. *T. harzianum* T39 soil treatment was particularly effective against these stem infections, since treated plants did not show stem infections at all, while control plants had a coverage of about 20%.

In bean, *T. harzianum* T39 was applied to soil and/or primary leaves 7 days before the *B. cinerea* challenge on the first trifoliate leaf. This ensured spatial separation of both micro-organisms since in none of the treatments could *T. harzianum* T39 be isolated from the first trifoliate leaf at the time of the *B. cinerea* challenge. Figure 3 shows that not only *T. harzianum* T39 soil treatment but also leaf treatment significantly reduced the TLD. Combination of leaf and soil treatment was even more effective and reduced the TLD by about 35%, compared to the control. Contrast analysis showed that better *B. cinerea* control in the combined treatment could be explained by a model including leaf and soil treatment as additive factors. To compare the effect of *T. harzianum* T39 soil treatment to ISR by *P. aeruginosa* KMPCH, both strains were applied in a combined seed and soil treatment that did not result in *T. harzianum* T39 colonisation of the challenged leaf. It appeared (Figure 4) that both *T. harzianum* T39 and

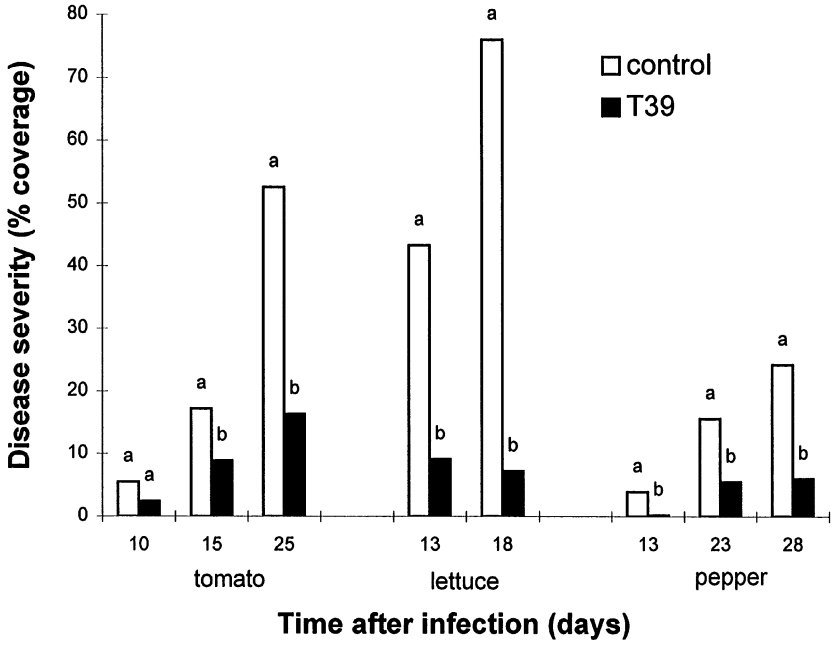

Figure 1. Effect of *Trichoderma harzianum* T39 soil treatment on *Botrytis cinerea* disease severity in the canopy of tomato, lettuce and pepper. *T. harzianum* T39 was applied 7 days before plants were inoculated with *B. cinerea*. Each bar is the mean of 6 replications. Bars with the same letter do not differ significantly at $P \geq 0.05$ by Fisher's protected LSD test.

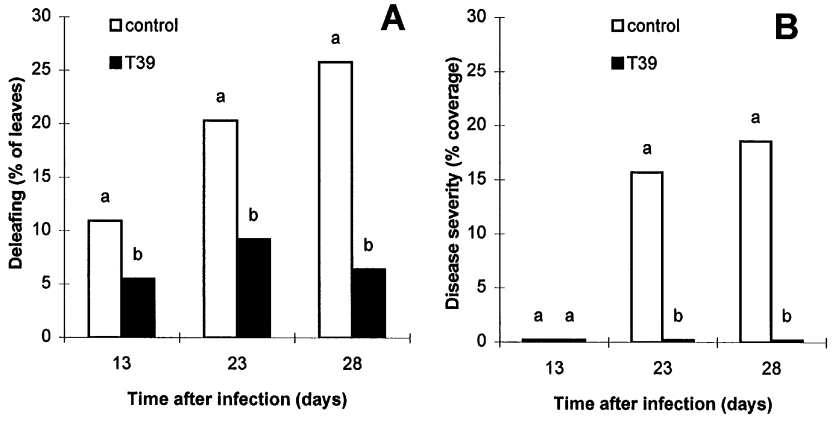

Figure 2. Effect of *Trichoderma harzianum* T39 soil treatment on deleafing (A) and stem infections (B) caused by *Botrytis cinerea* in bell pepper. *T. harzianum* T39 was applied 7 days before *B. cinerea* inoculation and symptoms were evaluated at various time points after inoculation. Each bar is the mean of 6 replications. Bars with the same letter do not differ significantly at $P \geq 0.05$ by Fisher's protected LSD test.

P. aeruginosa KMPCH reduced the TLD by about 35% compared to the control.

In tobacco, where a *T. harzianum* T39 soil treatment 7 days before *B. cinerea* inoculation reduced *B. cinerea* disease severity (Figure 5A), *T. harzianum* T39 was also applied at earlier time points. Two weeks after *B. cinerea* inoculation, a significant *B. cinerea* control was still obtained after *T. harzianum* T39 application 1 and 3 days before challenge. This was not the case after simultaneous application because the significant reduction in disease severity 7 days after inoculation was only a delay in *B. cinerea* development. Two weeks after inoculation, the disease severity for this treatment was only marginally lower than for control plants. In the same set-up *T. harzianum* T39 was also applied to the challenged leaf 0, 1, 3 and 7 days before *B. cinerea*

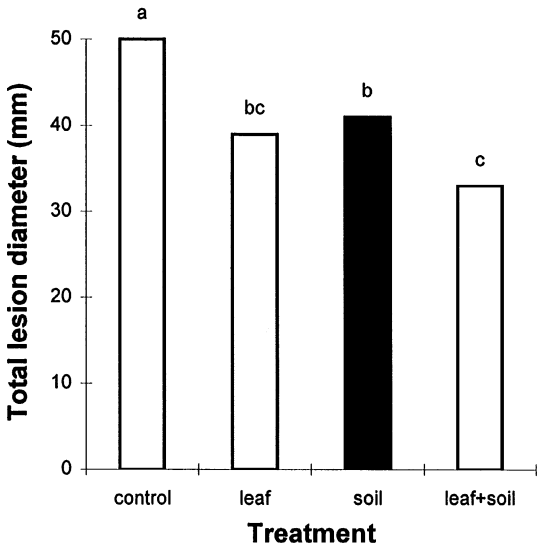

Figure 3. Influence of *Trichoderma harzianum* T39 application site on *Botrytis cinerea* infections in bean. *T. harzianum* T39 was applied to soil and/or first leaves of 3 weeks old plants. One week later, the first trifoliate leaves were inoculated with *B. cinerea*. Infections were evaluated 5 days after inoculation. Statistical analysis was performed with a general linearized model on pooled data from four separate experiments and treatment means were compared with Fisher's LSD test. Bars with the same letter do not differ significantly at $P \geq 0.05$.

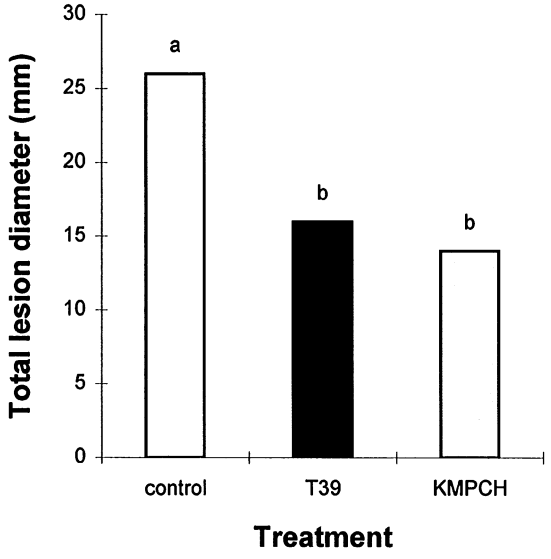

Figure 4. Influence of a combined seed and soil treatment with *Trichoderma harzianum* T39 or *Pseudomonas aeruginosa* KMPCH on *Botrytis cinerea* infections on the first leaves of 3 weeks old bean plants, evaluated 5 days after inoculation. Statistical analysis was performed with a general linearized model on pooled data from five separate experiments and treatment means were compared with Fisher's LSD test. Bars with the same letter do not differ significantly at $P \geq 0.05$.

inoculation. Figure 5 shows that leaf and soil treatment resulted in a similar reduction in disease severity, when the biocontrol agent was applied at the same time point before *B. cinerea* challenge. This indicates that the site of *T. harzianum* T39 application did not influence the developmental stage of the *B. cinerea* infection. However, the spreading of established *B. cinerea* infections (disease index 5–7) in *T. harzianum* T39 leaf-treated plants was 25–50% slower than in control and *T. harzianum* T39 soil-treated plants (results not shown).

The effect of *T. harzianum* T39 application at sites spatially separated from the *B. cinerea* inoculation became particularly significant when, in control plants, established *B. cinerea* infections developed into rapidly spreading lesions causing grey mould coverage levels of more than 15% (Figure 1). *T. harzianum* T39-treated plants showed less extensive lesions which indicates that *B. cinerea* is restricted in the early stages of development. This hypothesis is confirmed by the observation that spreading *B. cinerea* lesions on *T. harzianum* T39 soil-treated bean and tobacco had the same growth rate as lesions on control plants (results not shown). The restriction of *B. cinerea* development was expressed in a delay or suppression of spreading lesion formation depending on host plant and *T. harzianum* T39 application time. In lettuce and pepper (Figure 1), for instance, grey mould coverage on *T. harzianum* T39 soil-treated plants did not increase with time indicating that *B. cinerea* lesions were completely restricted. The same applies for tobacco plants where *B. cinerea* infections were restricted within the inoculum drop when *T. harzianum* T39 was applied at least one day before pathogen challenge (Figure 5A). In the case of simultaneous application, however, spreading lesions appeared on *T. harzianum* T39 soil-treated plants, but were smaller than on control plants (Figure 5A). Given the generally constant growth rate of spreading *B. cinerea* lesions, this indicates that lesion formation was delayed. In other crops such as tomato (Figure 1) and bean (Figures 3 and 4), delay and suppression of spreading lesion formation occurred in a combined fashion. In bean the relative importance of both parameters varied between the different experiments. Therefore the LTD was the most consistent indicator of disease severity because it represents both parameters when a constant growth rate is assumed for spreading lesion development.

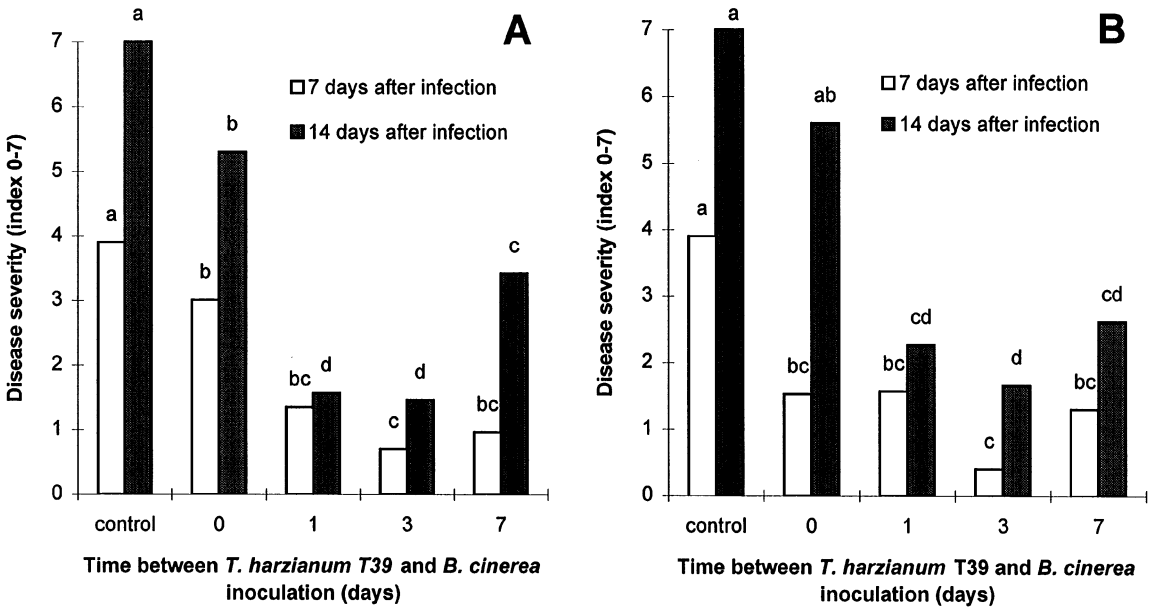

Figure 5. Influence of *Trichoderma harzianum* T39 application site and time on *Botrytis cinerea* infections in tobacco. *T. harzianum* T39 was either applied to the soil (A) or to leaves later challenged with *B. cinerea* (B). Disease severity was evaluated 7 and 14 days after inoculation according to a 0–7 disease index. Each bar is the mean of 6 replicates. For each time point after infection, bars with the same letter do not differ significantly at $P \geq 0.05$ by Fisher's protected LSD test.

Discussion

Development of *B. cinerea* infections was consistently reduced after a prior *T. harzianum* T39 treatment on plant parts spatially separated from the site of *B. cinerea* inoculation. *T. harzianum* T39 soil treatment reduced *B. cinerea* stem infections in pepper (Figure 2B) and *B. cinerea* leaf infections in tomato, lettuce, pepper, tobacco and bean (Figures 1–5). In bean, the same control of *B. cinerea* leaf infections was obtained after a prior *T. harzianum* T39 spray on spatially separated leaves (Figure 3). Because in these experiments *T. harzianum* T39 could not directly interact with *B. cinerea* or affect the environment for *B. cinerea* development for reasons of spatial separation, induction of systemic resistance is, by exclusion of alternatives, the most likely explanation of the *B. cinerea* control. Moreover, the observed phenomenon shares other characteristics with ISR. First of all, *T. harzianum* T39 ISR was not only effective against *B. cinerea*, but worked, just like ISR and SAR (Kloepper et al., 1997; Ryals et al., 1996) against more than one pathogen. *T. harzianum* T39 soil treatment reduced anthracnose symptoms of *Colletotrichum lindemuthianum* in bean (Bigirimana et al., 1997) and also reduced white mould in lettuce (Y. Elad, unpubl.). A second argument for ISR involvement is that the resistance to both *B. cinerea* (Figure 4) and mild *C. lindemuthianum* infections (Bigrimana et al., 1997) in *T. harzianum* T39 soil-treated bean plants, was of the same level as in plants treated with *P. aeruginosa* KMPCH, a reference strain for ISR (De Meyer and Höfte, 1997). Finally, the effect of *T. harzianum* T39 soil treatment needed, just like ISR, some time to develop in the host plant. In tobacco, *B. cinerea* was only efficiently controlled when inoculated 1 or more days after *T. harzianum* T39 treatment (Figure 5). Compared with pathogen-induced systemic resistance this lag period of one day is rather short but nonetheless possible, since Smith et al. (1991) detected systemic resistance 1 day after induction with *Pseudomonas syringae* pv. *syringae*. However, the real lag period for induction of systemic resistance by *T. harzianum* T39 could be more than 1 day because *B. cinerea* infections might still be affected by induced resistance during their slow initial development. The same hypothesis would also explain that, in tobacco, *T. harzianum* T39 soil treatment at the moment of *B. cinerea* challenge was still partially effective (Figure 5). In this case the induced resistance that developed after the *B. cinerea* inoculation probably came too late to suppress spreading lesion formation, but managed to delay the infection.

In tobacco, the strikingly similar *B. cinerea* control after local *T. harzianum* T39 application and spatially separated soil application (Figure 5) seems to suggest that induction of resistance is also involved in the containment of the pathogen when *T. harzianum* T39 is applied at the infection site. This could explain why dead *T. harzianum* T39 cells partially controlled *B. cinerea* in bean (Elad, 1996) but is no proof of induced resistance at the site of *T. harzianum* T39 application. On *T. harzianum* T39-treated leaves other modes of action, like competition for nutrients (Zimand et al., 1995) and suppression of *B. cinerea* pathogenicity enzymes (Zimand et al., 1996), can be involved in *B. cinerea* control. The observation that established *B. cinerea* lesions spread more slowly on *T. harzianum* T39-treated tobacco leaves, than on leaves of control or *T. harzianum* T39 soil-treated plants (results not shown) is a clear indication that induced resistance is not the only mode of action involved.

Further work is needed to evaluate the importance of induced resistance in *B. cinerea* control on *T. harzianum* T39-treated leaves. The possible relation between the suppression of *B. cinerea* pathogenicity enzymes by *T. harzianum* T39 and induced resistance is also intriguing because the suppression of pathogenicity enzymes might lead to an accumulation of oligogalacturonide elicitors that in turn activate plant defence (Zimand et al., 1996).

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References

Bigirimana J, De Meyer G, Poppe J, Elad Y and Höfte M (1997) Induction of systemic resistance on bean (*Phaseolus vulgaris*) by *Trichoderma harzianum*. Med Fac Landbouww Univ Gent 62: 1001–1007

Buysens S, Heungens K, Poppe J and Höfte M (1996) Involvement of pyochelin and pyoverdin in suppression of *Pythium*-induced damping-off of tomato by *Pseudomonas aeruginosa* 7NSK2. Appl Environ Microbiol 62: 865–871

Calderon AA, Zapata JM, Munoz R, Pedreno MA and Ros-Barcelo A (1993) Resveratrol production as a part of the hypersensitive response of grapevine cells to an elicitor from *Trichoderma viride*. New Phytol 124: 455–463

Chang P-FL, Xu Y, Narasimhan ML, Cheah KT, D'Urzo MP, Damsz B, Kononowicz AK, Abad L, Hasegawa PM and Bressan RA (1997) Induction of pathogen resistance and pathogenesis-related genes in tobacco by a heat-stable *Trichoderma* mycelial extract and plant signal messengers. Physiol Plant 100: 341–352

De Meyer G and Höfte M (1997) Salicylic acid produced by the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 induces resistance to leaf infection by *Botrytis cinerea* on bean. Phytopathology 87: 588–593

Dubos B, Jailloux F and Bulit J (1982) Protection du vignoble contre la pourriture grise: les propriétés antagonistes du *Trichoderma* a l'égard du *Botrytis cinerea*. Les Colloques de l'INRA 11: 205–219

Elad Y (1988) Ultrastructural scanning electron microscopy study of parasitism of *Botrytis cinerea* Pers. on flowers and fruit of cucumber. Trans Br Mycol Soc 91: 185–190

Elad Y (1996) Mechanisms involved in the biological control *of Botrytis cinerea* incited diseases. Eur J Plant Pathol 102: 719–732

Elad Y, Zimand G, Zaqs Y, Zuriel S and Chet I (1993) Use of *Trichoderma harzianum* in combination or alteration with fungicides to control cucumber grey mould (*Botrytis cinerea*) under commercial greenhouse conditions. Plant Pathol 42: 324–332

Faretra F and Pollastro S (1991) Genetic bases of resistance to benzimidazole and dicarboximide fungicides in *Botryotinia fuckeliana* (*Botrytis cinerea*). Mycol Res 95: 943–951

King EO, Ward M and Raney DE (1954) Two simple media for demonstration of pyocyanin and fluorescin. J Lab Clin Med 44: 301–307

Kloepper JW, Tuzun S, Zehnder GW and Wei G (1997) Multiple disease protection by rhizobacteria that induce systemic resistance – historical precedence. Phytopathology 87: 136–137

Labudova I and Gogorova I (1988) Biological control of phytopathogenic fungi through lytic action of *Trichoderma* species. FEMS Microbiol Lett 52: 193–198

Meera MS, Shivanna MB, Kageyama K and Hyakumachi M (1994) Plant growth promoting fungi from zoysiagrass rhizosphere as potential inducers of systemic resistance in cucumbers. Phytopathology 84: 1399–1406

O'Neill TM, Niv A, Elad Y and Shtienberg D (1996) Biological control of *Botrytis cinerea* on tomato stem wounds with *Trichoderma harzianum*. Eur J Plant Pathol 102: 635–643

Ryals JA, Neuenschwander UH, Willits MG, Molina A, Steiner H and Hunt MD (1996) Systemic acquired resistance. Plant Cell 8: 1809–1819

Smith JA, Hammerschmidt R and Fulbright DW (1991) Rapid induction of systemic resistance in cucumber by *Pseudomonas syringae* pv. *syringae*. Physiol Mol Plant Pathol 38: 223–235

Tronsmo A and Dennis C (1977) The use of *Trichoderma* species to control strawberry fruit rots. Neth J Plant Pathol 83 (Suppl 1): 449–455

Tronsmo A and Raa J (1977) Antagonistic action of *Trichoderma pseudokoningii* against the apple pathogen *Botrytis cinerea*. Phytopathol Z 89: 216–220

Tuzun S and Kloepper JW (1994) Induced systemic resistance by plant growth-promoting rhizobacteria. In: Ryder MH, Stephens

PM and Bowen GD (eds.) Improving plant productivity with rhizosphere bacteria, Proc. 3rd. International Workshop on Plant Growth-Promoting Rhizobacteria (pp. 104–109) CSIRO, Australia

Zimand G, Elad Y, Gagulashvily N and Chet I (1995) Effect of the biocontrol agent *Trichoderma harzianum* T-39 on the pathogenicity of *Botrytis cinerea*. Phytoparasitica 23: 241–242

Zimand G, Elad Y and Chet I (1996) Effect of *Trichoderma harzianum* on *Botrytis cinerea* pathogenicity. Phytopathology 86: 1255–1260