European Journal of Plant Pathology **106:** 817–824, 2000.

Evaluation of induction of systemic resistance in pepper plants (*Capsicum annuum*) to *Phytophthora capsici* using *Trichoderma harzianum* and its relation with capsidiol accumulation

Ahmed Sid Ahmed, Consuelo Pérez Sánchez and Maria Emilia Candela*
*Department of Plant Biology, Faculty of Biology, Campus de Espinardo, University of Murcia, 30100 Espinardo (Murcia), Spain; *Author for correspondence (Fax: +34 968 363963; E-mail: mcandela@um.es)*

Accepted 29 August 2000

Key words: induced systemic resistance, biocontrol

Abstract

The effect of pepper seed and root treatments with *Trichoderma harzianum* spores on necrosis caused in stems by *Phytophthora capsici* inoculation and on the course of capsidiol accumulation in the inoculated sites were studied. The results indicate that seed treatments significantly reduced stem necrosis, which fell by nearly a half compared with the values observed in plants grown from non-treated seeds. Necrosis was also reduced in plants whose roots were drenched with various doses of *T. harzianum* spores, although the extent of necrosis was not correlated with the dose used. Attempted isolation of *P. capsici* and *T. harzianum* from the zones immediately contiguous with the necrotic zones revealed the presence of the former but not of the latter, suggesting that there was no direct contact between them in the zones of isolation, which means that there was no competition for space. The percentage of *P. capsici* isolated 9 days after inoculation was greater in non-treated inoculated plants than in treated inoculated plants. These results suggest that *T. harzianum*, introduced into the subterranean part of the plant, induces a systemic defense response against *P. capsici* in the upper part of the plant. Analysis of capsidiol in the stems of treated inoculated plants by the end of the sixth day after inoculation, revealed that its concentration was more than seven-fold greater than in non-treated and inoculated plants, while after 9 days, the concentration of capsidiol decreased in the treated inoculated plants and increased in the non-treated inoculated plants. The high concentration of capsidiol detected in treated and inoculated stems after 6 days might be one of the contributing factors, but not necessarily the main factor, in delaying lesion development in the stems of pepper plants.

Introduction

Trichoderma harzianum is a biocontrol agent of several pathogens (Papavizas, 1985; Elad et al., 1993; Lo et al., 1998), among them *Phytophthora* spp. (Smith et al., 1990; Sid Ahmed et al., 1999). In a recent study (Sid Ahmed et al., 1999), it was observed that the *T. harzianum* reduced the root rot in pepper caused by *Phytophthora capsici*, this reduction being related to a reduction of the *P. capsici* population density. Its principal antagonistic action was attributed to its direct interaction with the pathogens involved through antibiosis (Ghisalberti and Sivasithamparam, 1991) and/or mycoparasitism (Lorito et al., 1994) associated with competition for nutrients and space.

In a recent study (Yedidia et al., 1999), it was suggested that *T. harzianum* T-203 may induce a defensive response in cucumber plants by increasing peroxidase and chitinase activities. Research into the resistance of pepper to *P. capsici* points to increasing evidence that such resistance is related to metabolic changes in the plant, among them the production of the phytoalexin capsidiol (Candela et al., 1995; Egea et al., 1996a,b; García-Pérez et al., 1998). Egea et al. (1996a) observed

a correlation between capsidiol accumulation and the degree of resistance in different varieties of pepper plants.

Capsidiol is the principal phytoalexin synthesized by pepper plants exposed to infection or tissue damage (Stoessl et al., 1973). Its accumulation in tissues may inhibit the development of *P. capsici* infection (Hwang, 1994; Egea et al., 1996a). However, the accumulation of this phytoalexin in inoculated tissue and its relation with the resistance induced by a fungal biocontrol agent such as *T. harzianum* has not been investigated.

The objectives of this study were: (i) to evaluate the effect of pepper seed treatment or root drenching with *T. harzianum* on necrosis in stems inoculated with *P. capsici* and (ii) to analyse the accumulation of capsidiol in the inoculated sites and its relation with the *T. harzianum* treatment.

Material and methods

Seeds of Pepper cr. Yolo Wonder, a variety susceptible to *P. capsici*, were used. *P. capsici* isolate 17 (Candela et al., 1995) was cultivated and maintained in potato dextrose agar (PDA, Difco, Detroit) at 25 °C. *T. harzianum* isolate 2413 (provided by Colección Española de Cultivos Tipos, Valencia, Spain), a biocontrol agent (Sid Ahmed et al., 1999), was cultivated in PDA at 25 °C and maintained at 4 °C. The spore suspension was prepared by covering a 7-day-old culture with a 3% (w/v) sucrose solution and gently scraping with a sterile glass rod. The suspension was collected in sterile tubes and its concentration adjusted to the required concentration.

Seed treatment and root drenching with *T. harzianum* spores

Pepper seeds were disinfected with 2% sodium hypochlorite for 5 min and rinsed three times in sterile distilled water before being introduced into the *T. harzianum* suspension adjusted to 5×10^6 spores/ml. This dose was optimized on basis of unpublished data. After 10 min, the seeds were withdrawn and dried in a Petri dish with sterile filter paper. As a control, seeds were treated with a sterile sucrose solution. To determine the population density adhering to each seed, 4–5 treated seeds were ground in a sterile mortar with 9 ml of peptone–water at 0.1% (w/v). The suspension was serially diluted and aliquots (0.1 ml) of appropriate dilutions were plated on PDA. The dishes were incubated in darkness at 25 °C and colonies counted after 36 h. The density of *T. harzianum* was 2.5×10^4 CFU/seed.

The treated seeds were sown (one seed per plug) in trays with 48 square plugs ($4.5 \times 4.5 \times 13$ cm) containing sterile mixture of peat (Humus King, Floraska, Germany) and sand (3 : 1, v/v; pH 6.5 ± 0.2), which had been autoclaved at 121 °C for 1 h on each one of two consecutive days. The trays were arranged in a randomised complete block in a culture chamber with a 16 h photoperiod at 25 °C and 75–85% relative humidity. They were watered every 2 or 3 days and received a nutritive solution (Plantavit, Reckitt & Colman. S.A., Bilbao, Spain) every 2 weeks until five leaves were formed. In another treatment, the roots were drenched with four concentrations of spore suspension at 2×10^3, 2×10^5, 3×10^7 and 5×10^8 spores/ml, 10 ml of each suspension being added to the substrate surface around the stem base of each plant. After 7 days, the plants were inoculated with *P. capsici*.

Evaluation of *T. harzianum* treatments on necrosis caused by *P. capsici*

Pepper plants with 5–7 true leaves grown from treated seeds were wounded (5 mm long and less than 1 mm deep) in the stem by mean of a sterile scalpel approximately 7 cm above the substrate surface. Five mm diameter discs of *P. capsici* cut from the edge of a growing mycelium colony were placed on the wounded sites and covered with aluminium foil containing moist cotton wool as indicated in Figure 1A. These plants were designated treated and inoculated (T & I). Non-treated and inoculated plants (NT & I), treated plants inoculated with PDA (T & NI) and non-treated and non-inoculated plants (NT & NI) were used as control. Forty plants per treatment were used and the experiment was repeated twice, the plants being kept in a culture chamber at 25 °C with a 16 h photoperiod. The extent of necrosis was measured after 3, 6 and 9 days in the plants grown from treated seeds and at 3, 6, 9 and 15 days in the plants treated by root drenching. To detect the presence of *T. harzianum* and the extent of *P. capsici* within the stem, two sections 2.5 cm long were cut, as indicated in Figure 1B, from either side of the necrotic zone on days 3, 6 and 9. Each of these sections was cut into 5 subsections of 0.5 cm, which were designated zones 1

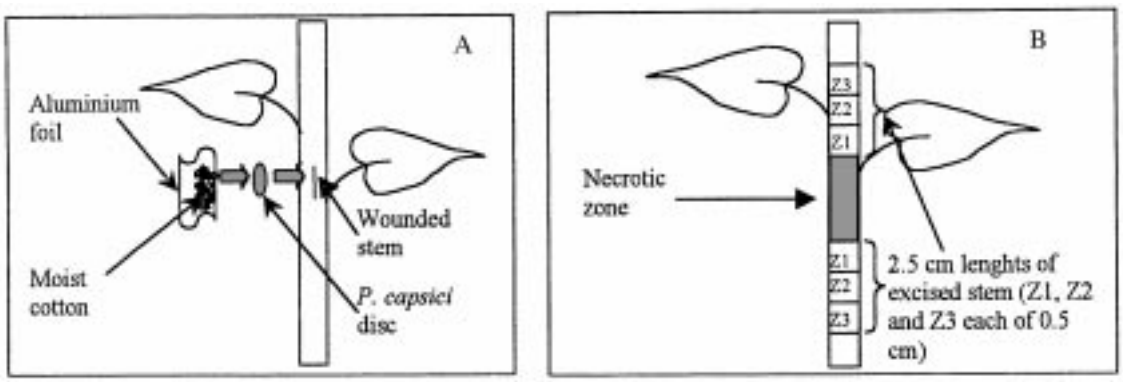

Figure 1. Diagram of stem inoculation (A) and 2.5 cm stem zones excised for *P. capsici* isolation (B).

(immediately next to the necrosis) to 5 (further away from the necrosis). The sections were disinfected with 1% sodium hypochlorite for 5 min, rinsed three times with sterile distilled water and placed in TSM medium (Askew and Laing, 1993) to detect *T. harzianum* and P5VPP-BH (Papavizas et al., 1981) to detect *P. capsici*. The plates were incubated at 25 °C for 3–5 days.

Capsidiol extraction from plants

To detect capsidiol, 13–14 stem sections of pepper plants per treatment were taken from the inoculated sites at 3, 6 and 9 days. The experiment was repeated a minimum of three times. Capsidiol was extracted following the method described by Egea et al. (1996b), which, consisted of cutting out the necrotic zones, and a comparable area from the healthy part, weighing and triturating in a mortar with liquid nitrogen. Capsidiol was extracted from the resulting powder by adding a mixture of chloroform : methanol (2 : 1, v/v) and separated by thin layer chromatography (Silica gel, Merck, Germany). Quantification was made by a gas chromatograph (Carlos Erba Instrument Vega Series 6130, Italy) equipped with FID 40 flame ionisation detector and WCOSTE-40 capillary column. This column was programmed as follows: 190 °C, rising to 220 °C in 5 min, kept for 10 min and then decreasing to 190 °C, 1 min waiting and 1 min cooling. Injector and detector were kept at 240 and 300 °C, respectively. The carrier gases were helium and nitrogen at a 2 and 30 ml/min flow rate, with hydrogen and air at a 300 and 30 ml/min.

Dynamics of *T. harzianum* in rhizosphere and on roots

To detect *T. harzianum* initially introduced on the seed and to determine its population density in the rhizosphere and on the root, the subterranean parts of 20 treated plants were collected and the substrate adhering to the root was removed. One gram of substrate from each plant and two 1 cm sections of the root were used, one from the sections cut from both primary and secondary roots near the seed and another from sections cut from the root tips. The root tissues from each part were macerated in a sterilised mortar with 9 ml of peptone–water and decimal dilutions from macerated roots and substrate were plated on TSM medium. The population density was determined as propagules per cm (ppcm) of single root section and as propagules per gram (ppg) of substrate in the rhizosphere 6, 9, 15, 20 and 30 days. Counts were made in the dishes containing between 30 and 150 colony forming units (CFU). The experiment was repeated three times.

Statistical analysis

The effects of *T. harzianum* treatments on *P. capsici* necrosis were analyzed by one-way ANOVA using Statgraphics plus software for Windows, V.2.1 (Statistical Graphics Corp., Maryland, USA) and the means were separated using Fisher's Least Significant Difference (LSD) test at $P = 0.05$. The data for each experiment were analyzed separately and then pooled because the treatment $\times$ experiment interaction was not significantly different at $P = 0.05$.

Results

Effect of T. harzianum *treatments on necrosis caused by* P. capsici

Generally, necrosis was slow to develop in pepper plants treated with *T. harzianum* and inoculated with *P. capsici* (Figure 2). Three days after inoculation with *P. capsici*, the NT & I and T & I plants showed a small dark brown stretch of necrosis, measuring an average of 5.3 and 2.4 mm, respectively. Small points of necrosis could be seen in the T & NI and NT & NI plants. After 6 days, the mean length of necrosis in the NT & I plants had reached 22.9 mm, almost twice the length of the necrosis observed in T & I plants. After 9 days, the length of necrosis had reached 48.6 mm in NT & I plants, which usually resulted in stem withering and plant death. The progress of necrosis was significantly ($P = 0.05$) lower in T & I plants (27.7 mm), although it did not cease. In T & NI and NT & NI plants, some lesions developed were significantly ($P = 0.05$) smaller than those observed in T & I plants.

Drenching the roots with suspensions of *T. harzianum* spores had a variable protective effect, which did not depend on the spore concentration used (Figure 2B). An analysis of variance at 3, 6, 9 and 15 days confirmed that there was no statistically significant relation between the doses of spores added and the length of necrotic tissue recorded. However, there was a significant ($P = 0.05$) difference between the infection which developed in T & I plants and that developed in NT & I plants: this began to be noticed 3 days after inoculation and continued to progress. After 6 days, necrosis had reached 14.8–17.2 mm in T & I plants compared with a mean 21.5 mm in NT & I plants. By day 9, these values had increased to 35.3–36.5 mm in T & I plants and 41 mm in NT & I plants. Although there was still a difference between the two responses, the necrosis in T & I plants had extended 40–44 mm by day 15. The reduction in length of necrosis obtained by drenching roots with *T. harzianum* spores was less pronounced than that obtained by treating the seeds (Figure 2A and B). The isolations from T & I and NT & I plants are shown in Figure 3. No significant difference was observed between mycelium growth

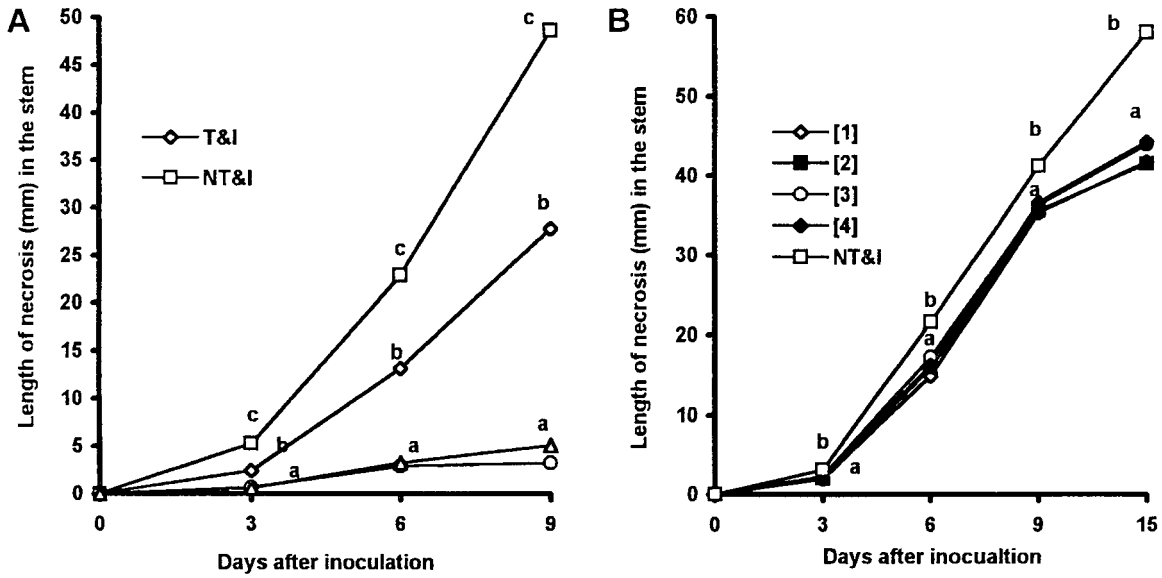

Figure 2. Progress of necrosis in stems of pepper (c.v. Yolo Wonder) plants grown from seeds treated with *T. harzianum* (A) or treated by drenching the roots with four concentrations ([1] $= 5 \times 10^8$, [2] $= 3 \times 10^7$, [3] $= 2 \times 10^5$ or [4] $= 2 \times 10^3$ spores/ml) of *T. harzianum* (B) and inoculated with *P. capsici*. The values representing necrosis are the means per plant and treatment from two experiments (data from two experiments were pooled because the experiment × treatment interaction was not significant). The means with the same letter at 3, 6 or 9 days are not significantly different according to Fisher's LSD test at $P = 0.05$. T & I, plants treated with *T. harzianum* and inoculated with *P. capsici*; NT & I, plants no treated and inoculated with *P. capsici*; NT & NI, plants not treated and not inoculated; T & NI, plants treated with *T. harzianum* and not inoculated.

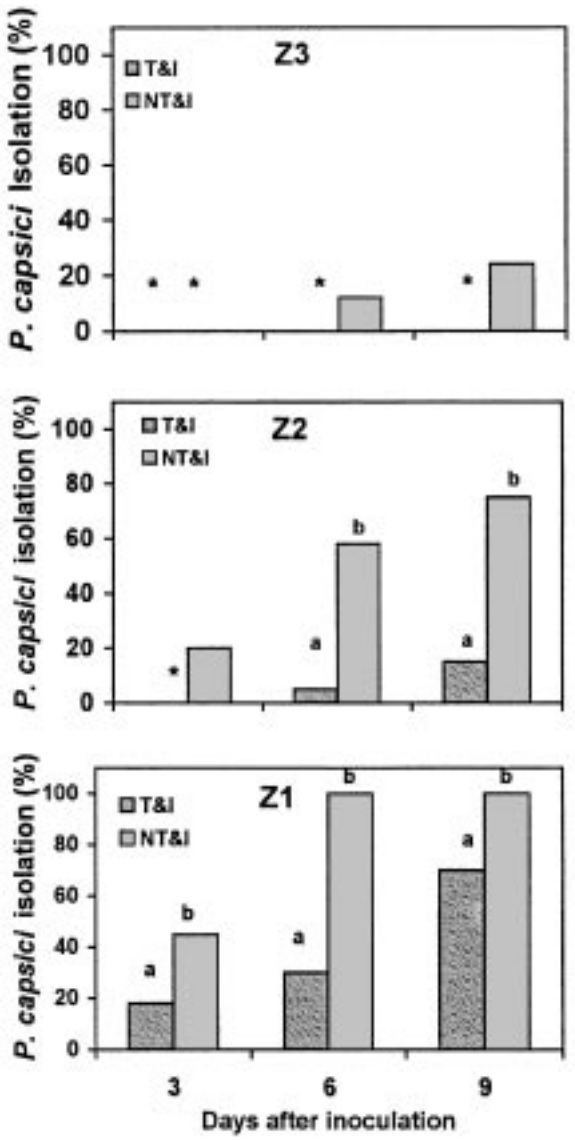

Figure 3. Percentage of stem zone sections in which *P. capsici* was recovered from pepper plants grown from seeds treated (T & I) or not treated (NT & I) with 2.5×10^4 CFU/seed *T. harzianum*. Values represent the mean percentages of corresponding zones (Z1, Z2, Z3) above and below necrotic areas in which *P. capsici* was recovered. **P. capsici* was not recovered. The percentages are the means from two experiments (data from two experiments were pooled because there was no significant interaction between treatment and experiment). Bar values at 3, 6 or 9 days with the same letter are not significantly different according to Fisher's LSD test at $P = 0.05$.

towards the top or towards the stem base, and so the mean of both values was taken.

Isolations showed that no stem contained *T. harzianum* while *P. capsici* was isolated at day 3 in 45% and 21% of zones 1 and 2, respectively, of NT & I plants and 18% and 0% in the same zones of T & I plants. At day 6, *P. capsici* was present in 70%, 58% and 12% of zones 1, 2 and 3, respectively of NT & I plants, while in T & I plants it was limited to 30 and 5% of zones 1 and 2, respectively. After 9 days, *P. capsici* colonized 100, 70 and 24% of zones 1, 2 and 3, while in T & I plants it colonised 70% and 15% of zones 1 and 2 but was not present in zone 3. *P. capsici* was not isolated from zones 4 or 5.

Capsidiol accumulation

The concentration of capsidiol detected in the stems of pepper plants of the different treatments 3, 6 and 9 days after inoculation with *P. capsici* is shown in Table 1. Three days after inoculation the amount of capsidiol detected in the necrotic zones of the NT & I plants was statistically similar to that detected in T & I plants, but significantly above that detected in T & NI plants. On day 6, the amount of capsidiol accumulated in T & I plants was 7.4 times greater than in NT & I plants. The

Table 1. Accumulation of the phytoalexin, capsidiol, in the necrotic zones of the stems of pepper plants grown from seeds treated with *T. harzianum* and inoculated with *P. capsici*

	Concentration of capsidiol in μg/g of fresh tissue[1]		
	Day3	Day6	Day9
T & I	46.72 c[2]	241.97 d	33.03 b
NT & I	44.93 c	32.68 b	121.76 c
T & NI	35.15 b	63 c	32 b
NT & NI	15.89 a	19.9 a	13.25 a

[1] The accumulation of capsidiol was measured 3, 6 and 9 days after inoculating the stems with *P. capsici*.

[2] The concentration of capsidiol are the means of two extractions per treatment and experiment (data from three experiments were pooled because treatment × experiment interaction was not significant). Each extract was analysed three or four times by gas chromatography. The means with the same letter in each column are not significantly different according to Fisher's LSD test at $P = 0.05$.

amount accumulated in T & NI plants was less than in either of the above. On day 9, levels of capsidiol were significantly greater in NT & I than T & I plants. Levels in T & NI plants were similar to those detected in T & I plants.

Dynamic of *T. harzianum* in rhizosphere and on roots

An analysis of the *T. harzianum* populations on the roots and in the rhizosphere showed that the *T. harzianum* applied to seeds had spread along the plant roots and into the rhizosphere. Six days after inoculation with *P. capsici*, the population density of *T. harzianum* was 2.43×10^2 ppcm (propagules per centimetre of root) on roots near the seed, 0.26×10^2 ppcm on the root tips and 14.8×10^4 ppg of substrate in the rhizosphere. At day 9, these values had fallen to 2.13×10^2, 0.25×10^2 ppcm and 14×10^4 ppg, respectively, further falling to 2×10^2, 0.20×10^2 ppcm and 12×10^4 ppg. On day 20, the densities were 2.1×10^2, 0.22×10^2 ppcm and 15×10^4 ppg and on day 30, 1.7×10^2, 0.13×10^2 ppcm and 9×10^4 ppg, respectively, on roots near the seed, on root tips and in rhizosphere.

Discussion

Treatment of pepper plant seeds with *T. harzianum* significantly reduced the extent of necrosis caused by *P. capsici* in stems. Necrosis developed significantly more slowly in T & I than NT & I plants. Isolation from zones near the inoculation sites after 9 days showed that *P. capsici* mycelium was present in 100% of zone 1, 75% of zone 2 and 24% of zone 3 in NT & I plants, but only 70% of zone 1 and 15% of zone 2 in T & I plants. This behaviour seems to be related with slower pathogen growth in the tissue of T & I plants. However, necrosis continued to develop slowly in T & I plants, so that any resistance must be considered as quantitative rather than qualitative.

T. harzianum has been described as a biocontrol agent for several pathogens (Papavizas, 1985; Ahmad and Baker, 1987; Claydon and Allan, 1987; Elad et al., 1993; Harman et al., 1993; Lorito et al., 1993; Kapat et al., 1998; Sid Ahmed et al., 1999), among them *P. capsici* (Sid Ahmed et al., 1999). Its antagonistic activity is partly related with the production of antifungal metabolites such as enzymes (Lorito et al., 1994) and toxins (Claydon and Allan, 1987) and with competition for space and nutrients (Papavizas, 1985). The results described here indicate that the response achieved in the aerial parts of T & I plants was probably mediated at a distance by the presence of the biocontrol agent on the root surface. The absence of *T. harzianum* from the stems of treated plants indicated that there was no physical contact with *P. capsici*, which rules out competition. However, the absence of *T. harzianum* in the stems does not rule out the possibility that its metabolites might have been transferred to the inoculation site, where they could have reacted with the pathogen to inhibit its growth or induce other defense mechanisms in the affected tissues.

Several biocontrol agents such as *Pseudomonas* spp. (Rasmussen et al., 1991; van Peer et al., 1991; Maurhofer et al., 1994; Liu et al., 1995; Wei et al., 1996) have been used to induce resistance in plants. These authors indicated that the presence of such bacteria on plants, although spatially separated from the pathogen, increases their defense capacity. The presence of *T. harzianum* on the seed and its subsequent spreading along the emerged root and into the rhizosphere, as demonstrated by the counts made during this experiment, was related with a reduction in necrosis in the aerial part of the growing plants. This result suggests that *T. harzianum* can produce a systemic protection effect in inoculated tissues.

The use of a biocontrol agent like *T. harzianum* might be more advantageous than the classical induced systemic resistance induced by pathogenic or attenuated microorganisms. For example, there would be fewer associated risks than with using a pathogen, which itself might become a source of infection during cultivation of crops. Additionally, a biocontrol agent can be used to treat seeds or soil, both of which must be considered more rational than using pathogens in a controlled inoculation to induce systemic resistance.

The accumulation of capsidiol in T & I plants was statistically similar to that detected in NT & I plants on day 3, but had increased by day 6, before falling by the ninth day after inoculation. The increase in capsidiol concentration on day 6, was related to the treatment of the seeds by *T. harzianum*. This high concentration in the tissues of T & I plants might be one of the factors responsible for the decrease in necrosis. Previous studies have shown a relation between capsidiol accumulation in the stems of pepper plants and their degree of resistance against *P. capsici* (Hwang, 1994; Egea et al., 1996a). Hwang (1994) inoculated the stems of two pepper varieties (one resistant and the other susceptible)

and after 7 days detected a significantly higher concentration of capsidiol in the resistant plants. In one aspect of the present study, treatment of the seeds of the susceptible variety (Yolo Wonder) with *T. harzianum* and subsequent inoculation with *P. capsici* stimulated the production of capsidiol, which reached its highest concentration at 6 days, when it was seven times higher than in NT & I plants. Such an accumulation of capsidiol in the necrotic zone might contribute to the slowing down of the vegetative growth of *P. capsici* and to a decrease in infection (Egea et al., 1996a). Previous studies have pointed to the fungistatic proprieties of capsidiol at a mean concentration of 3.75 mM and its fungitoxic proprieties at 5 mM (Egea et al., 1996a). However, high phytoalexin concentrations may be toxic to the same plant and so its seems reasonable to expect that plants may have a mechanism for regulating the production of such compounds (Vidhyasekaran, 1997). The fall in capsidiol levels 9 days may have been caused by the plant itself (Vidhyasekaran, 1997) and/or *P. capsici* (Ward and Stoessl, 1973), and several studies have pointed to such a fall after maximum levels have been reached (Hwang, 1994; Egea et al., 1996a). However, in light of these finding it may be wondered why, if the concentration of this phytoalexin falls after 9 days, does necrosis not reach the same rate of progress as in NT & I plants. Although the amount of capsidiol accumulation in treated plants was related with the length of necrosis, its intervention as the principal agent responsible for resistance can probably be discarded. It seems very likely that the reduction in the progression of necrosis caused by *P. capsici* in the stems of treated plants was the result of more than one defense mechanism being activated by the presence of *T. harzianum* in the subterranean parts of the plants.

Various growth-promoting bacteria such as *Pseudomonas fluorescens* WCS417r elicit an ISR in carnation (van Peer et al., 1991), radish (Hoffland et al., 1996), cucumber (Liu et al., 1995) and *Arabidopsis* spp. (Pieterse et al., 1996). To elucidate the molecular mechanism responsible for this type of defense reaction, Van-Wees et al. (1997) using heat killed WCS417r or purified bacterial outer membrane lipopolysaccharides (LPS) to elicit systemic resistance in carnation, observed that they were as effective as were live bacteria. LPS of the outer membrane of WCS417r seem to be the main ISR inducing determinant in radish and carnation, and LPS containing cell walls also elicit ISR in Arabidopsis (Van-Wees et al., 1997). The same authors using bacterial mutants lacking the O-antigenic side chain of the LPS (OA^-), observed that the induced protection was similar to that induced by wild type WCS417r and suggested that the bacteria might use more than a single factor to elicit systemic resistance. Calderón et al. (1993, 1994), using cellulase from *T. viride* to elicit vine cell suspensions, observed a hypersensitivity reaction accompanied by physiological changes, localized cell death and an increase in benzoic acid and resveratrol levels.

T. harzianum, when used to treat pepper seeds, was able to induce resistance against *P. capsici* in relatively distant parts of plant. Previous experiments (Sid Ahmed et al., 1999) have shown that *T. harzianum* can be added directly to the substrate in contact with *P. capsici* to reduce root rot, suggesting that part of the mechanism responsible for such biocontrol activity is the production of inhibitory metabolites and competition for the space and nutrients available in the medium. The findings of the present study suggest that *T. harzianum* may have one or more mechanism responsible for antagonistic activity, including the induction of resistance. Capsidiol, although seeming to participate in protection, more probably combines with other factors responsible for the defense. Further studies are necessary to elucidate the resistance induction mechanism at molecular level.

In conclusion, *T. harzianum* probably uses a combination of biocontrol mechanisms against *P. capsici*, among which the induction of resistance in plants plays an important role. This, combined with other mechanisms, may contribute to the biocontrol of plant rot in pepper caused by *P. capsici*.

Acknowledgements

This work was supported in part by the FEDER project IFD97-2302.

References

Ahmad JS and Baker R (1987) Rhizosphere competence of *Trichoderma harzianum*. Phytopathology 77: 182–189

Askew DJ and Laing MD (1993) An adapted selective medium for the quantitative isolation of *Trichoderma* species. Plant Pathol 42: 686–690

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

Calderón AA, Zapata JM and Ros Barceló A (1994) Peroxidase-mediated formation of resveratrol oxidation products during the hypersensitive-like reaction of grapevine to an elicitor from *Trichoderma viride.* Physiol Mol Plant Pathol 44: 289–299

Candela ME, Alcazár MD, Espin A, Egea C and Almela L (1995) Soluble phenolic acids in *Capsicum annuum* stems infected with *Phytophthora capsici*. Plant Pathol 44: 116–123

Claydon N and Allan M (1987) Antifungal alkyl pyrones of *Trichoderma harzianum*. Trans Brit Mycol Soc 88: 503–513

Egea C, Alcázar MD and Candela ME (1996a) Capsidiol: Its role in the resistence of *Capsicum annuum* to *Phytophthora capsici*. Physiol Plant 98: 737–742

Egea C, García-Pérez MD and Candela ME (1996b) Capsidiol accumulation in *Capsicum annuum* stems during the hypersensitive reaction to *Phytophthora capsici.* J Plant Physiol 149: 762–764

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

García-Pérez MD, Egea C and Candela ME (1998) Defense response of pepper (*Capsicum annuum*) suspension cells to *Phytophthora capsici*. Physiol Plant 103: 527–533

Ghisalberti EL and Sivasithamparam K (1991) Anti fungal antibiotics produced by *Trichoderma* spp. Soil Biol Biochem 23: 1011–1020

Harman GE, Hayes CK, Lorito M, Broadway RM, Di pietro A, Peterbauer C and Tronsmo A (1993) Chitinolytic enzymes of *Trichoderma harzianum*: Purification of chitobiosidase and endochitinase. Phytopathology 83: 313–318

Hoffland E, Hakulinen J and Van Pelt JA (1996) Comparison of systemic resistence induced by avirulent and nonpathogenic *Pseudomonas* species. Phytopathology 86: 757–762

Hwang BK (1994) Effects of age-related resistance and metalaxyl on capsidiol production in pepper plants infected with *Phytophthora capsici*. In: Daniel M and Purkayastha RP (eds) Handbook of Phytoalexin Metabolism and Action (pp 503–523) Marcel Dekker, Inc, New York

Kapat A, Zimand G and Elad Y (1998) Effect of two isolates of *Trichoderma harzianum* on the activity of hydrolytic enzymes produced by Botrytis cinerea. Physiol Mol Plant Pathol 52: 127–137

Liu L, Kloepper JW and Tuzun S (1995) Induction of systemic resistance in cucumber against Fusarium wilt by plant growth-promoting rhizobacteria. Phytopathology 85: 695–698

Lo CT, Nelson EB, Hayes CK and Harman GE (1998) Ecological studies of transformed *Trichoderma harzianum* strains 1295-22 in the rhizosphere and on the phylloplane of creeping bentgrass. Phytopathology 88: 129–136

Lorito M, Harman GE, Hayes CK, Broadway RM, Tronsmo A, Woo SL and Di Pietro A (1993) Chitinolytic enzymes produced by *Trichoderma harzianum*: Antifungal activity of purified endochitinase and chitobiosidase. Phytopathology 83: 302–307

Lorito M, Hayes CK, Di Pietro A, Woo SL and Harman GE (1994) Purification, characterization, and synergistic activity of glucan 1,3-B-glucosidase and an N-acetyl-B-glucosaminidase from *Trichoderma harzianum*. Phytopathology 84: 398–405

Maurhofer M, Hase C, Meuwly P, Métraux and Défago G (1994) Induction of systemic resistance of tobacco to tobacco necrosis virus by the root-colonizing *Pseudomonas fluorescens* strains CHA0: Influence of the *gac*A gene and pyoveridine production. Phytopathology 84: 139–146

Papavizas GC (1985) *Trichoderma* and *Gliocladium*: Biology, ecology, and potential for biocontrol. Annu Rev Phytopathol 23: 23–54

Papavizas GC, Bowers JH and Johnston SA (1981) Selective isolation of *Phytophthora capsici* from soils. Phytopathology 71: 129–133

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

Rasmussen JB, Hammerschmidt R and Zook MN (1991) Systemic induction of salicylic acid accumulation in cucumber after inoculation with *Pseudomonas syringae* pv *syringae*. Plant Physiol 97: 1342–1347

Sid Ahmed A, Pérez SC, Egea GC and Candela CME (1999) Evaluation of the capacity of *Trichoderma harzianum* in controlling rot caused by *Phytophthora capsici* in pepper plants. Plant Pathol 48: 58–65

Smith VL, Wilcox WF and Harman GE (1990) Potential for biological control of Phytophthora root and crown rots of apple by *Trichoderma* and *Gliocladium* spp. Phytopathology 80: 880–885

Stoessl A, Unwin CH and Ward EWB (1973) Postinfectional inhibitors from plants: fungal oxidation of capsidiol in pepper fruit. Phytopathology 63: 1225–1231

van Peer R, Niemann GJ and Schippers B (1991) Induced resistance and phytoalexin accumulation in biological control of Fusarium wilt of carnation by *Pseudomonas* sp. strain WCS417r. Phytopathology 81: 728–734

Van-Wees SC, Pieterse CMJ, Trijssenaar A, Van-t-Westende YA, Hartog F and Van Loon LC (1997) Differential induction of systemic resistance in *Arabidopsis* by biocontrol bacteria. Mol Plant–Microbe Interact 10: 716–724

Vidhyasekaran P (1997) Phytoalexins. In: Vidhyasekaran P (ed) Fungal Pathogenesis in Plants and Crops: Molecular Biology and Host Defense Mechanisms (pp 380–455) Marcel Dekker, Inc, New York

Ward EWB and Stoessl A (1973) Postinfectional inhibitors from plants. III. Detoxification of capsidiol, an antifungal compound from peppers. Phytopathology 62: 1186–1187

Wei G, Kloepper JW and Tuzun S (1996) Induced systemic resistance to cucumber diseases and increased plant growth by plant growth-promoting rhizobacteria under field conditions. Phytopathology 86: 221–224

Yedidia I, Benhamou N and Chet I (1999) Induction of defense responses in cucumber plants (*Cucumis sativus* L.) by the biocontrol agent *Trichoderma harzianum*. Appl Environ Microbiol 65: 1061–1070