Systemic Resistance Induced by *Trichoderma hamatum* 382 in Cucumber Against Phytophthora Crown Rot and Leaf Blight

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ABSTRACT

Khan, J., Ooka, J. J., Miller, S. A., Madden, L. V., and Hoitink, H. A. J. 2004. Systemic resistance induced by *Trichoderma hamatum* 382 in cucumber against Phytophthora crown rot and leaf blight. Plant Dis. 88:280-286.

Phytophthora root rot, crown rot, leaf and stem blight, and fruit rot of cucumber can cause serious losses, and are difficult to control. Although composts can be used successfully for control of Phytophthora root rots, little is known about their effects on *Phytophthora* diseases of aboveground plant parts. This research shows that the severity of Phytophthora root and crown rot of cucumber caused by *Phytophthora capsici* was suppressed significantly in cucumber transplants produced in a composted cow manure–amended mix compared with those in a dark sphagnum peat mix. In split root bioassays, *Trichoderma hamatum* 382 (T_{382}) inoculated into the compost-amended potting mix significantly reduced the severity of Phytophthora root and crown rot on paired roots in the peat mix. This effect did not differ significantly from that provided by a drench with benzothiadiazole (BTH) or mefenoxam (Subdue MAXX). Based on area under disease progress curves, T_{382} also significantly reduced the severity of Phytophthora leaf blight in transplants produced in the compost mix compared with controls not inoculated with T_{382}. Efficacy of T_{382} did not differ significantly from that provided by a drench with BTH. T_{382} remained spatially separated from the pathogen in plants in both the split root and leaf blight bioassays, suggesting that these effects were systemic in nature.

Additional keywords: biological control, induced systemic resistance, ISR, systemic acquired resistance, SAR, transplant mixes

Phytophthora capsici Leonian is one of the most destructive pathogens of cucumber in the United States and several other parts of the world (17,38,39,44). This pathogen has a wide host range that includes other cucurbits (38,43), pepper (29,43,49), tomato (46), cocoa (26), and macadamia (32). On cucumber, *P. capsici* causes damping-off, crown and root rot, stem lesions, foliar blight, and fruit rot (31,38,44). In temperate climates such as in the midwestern United States, *P. capsici* infects susceptible hosts throughout the growing season and survives as dormant oospores during the winter (36). The disease is very difficult to control (44). Cucumber varieties resistant to *P. capsici* are not available to our knowledge.

The principal procedures for control of Phytophthora blight on cucumber include cultural practices and the use of fungicides (44). In Michigan, resistance to the systemic fungicide mefenoxam developed rapidly in *P. capsici* after its application in the field (35,36). Recommended cultural practices include crop rotation, planting on well drained soils or on appropriately constructed raised beds, and the use of disease-free transplants (37). Subsoiling and chisel plowing have been recommended to improve drainage (18). Flooding and puddle formation around the crown of seedlings in beds must be avoided to reduce the formation, release, and dissemination of zoospores (5,15).

Organic amendments such as composts can have beneficial effects on the physical properties of soils related to water infiltration and retention (14). Compost amendments have been shown to affect the severity of diseases caused by several soilborne plant pathogens (22), and they have been tested for their ability to suppress Phytophthora blight of pepper. Unfortunately, their impacts on this disease were variable or not significant (28,29). Ristaino and Johnston (44) concluded, therefore, that inadequate information is available on the effects of compost amendments on *Phytophthora* diseases of these vegetable crops to warrant control recommendations.

Composts have been used successfully for some time for suppression of Phytophthora crown and root rots of nursery and fruit crops produced in plug mixes, container media (2,20,24,34,40,51,53), or field soils (3,16,54,58,61). *Pseudomonas* spp. (2), *Pantoea* spp. (formerly *Enterobacter* spp.; 30,33,57), *Bacillus* spp. (4,19,56), actinomycetes (2,19), and fungi including *Trichoderma* spp. (45,49,52) have been identified from compost-amended substrates as potential biocontrol agents of Phytophthora root and crown rots. The composition of the organic matter in the compost-amended substrate is critical to sustained biological control (55).

Composts may also induce systemic resistance (ISR) in plants to several diseases, including foliar diseases, although the results can be variable (30,41,62,63). Krause et al. (30) demonstrated that less than 2% of 80 different batches of composts tested induced systemic resistance in radish against bacterial leaf spot. The effect was due to the activity of specific biocontrol agents in the batches of composts that suppressed bacterial leaf spot. They identified *Trichoderma hamatum* 382 (Bonord.) Bainier (T_{382}) as the most active inducer of ISR in radish. *Trichoderma harzianum* Rifai T-203 has been shown to induce systemic resistance to *P. capsici* in pepper seedlings raised from seed treated with this biocontrol agent (50). ISR-active biocontrol agents could possibly be inoculated into composts after peak heating but before substantial colonization of the substrate with mesophyllic microorganisms. This may reduce the severity of this disease on cucumber transplants produced in potting mixes amended with such composts. The objectives of this research were to determine: (i) whether compost-amended transplant mixes inoculated with T_{382} can reduce the severity of the root as well as the foliar blight phase of this *Phytophthora* disease of cucumber; and (ii) whether any observed effect of compost or T_{382} on disease suppression was systemic in nature in cucumber seedlings produced in transplant mixes. A preliminary report on this work has been published (27).

MATERIALS AND METHODS

Preparation of transplant mixes. A sphagnum peat transplant mix, referred to hereafter as the "peat mix," was prepared by blending coarse horticultural grade perlite (Ball Seed Company, West Chicago, IL) with sphagnum peat classified as H_4 on the von Post decomposition scale

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Accepted for publication 21 October 2003.

Publication no. D-2003-1222-02R

(42) obtained from Premier Peat Moss Ltd., Quebec, Canada, at a volumetric ratio of 3:7. This mix is low in microbial carrying capacity (8) and conducive to Pythium and Phytophthora root rots (7,24). A composted cow manure–amended transplant mix, referred to hereafter as the "composted cow manure mix," was prepared by blending H_4 sphagnum peat with coarse horticultural grade perlite and composted cow manure at a volumetric ratio of 4.5:4.0:1.5. The composted cow manure had been prepared from sawdust-bedded dairy manure at the Ohio State University windrow composting facility until it reached a stability level of 0.5 mg CO_2-C g^{-1} dry weight day^{-1} (11). This type of composted cow manure incorporated into mixes at 15% (vol/vol) supports natural suppression of Pythium root rot of cucumber for approximately 200 days after planting (55). The peat mix was amended with 5.4 g of dolomitic lime, 3.6 g of calcium carbonate (<0.15 mm), 1.1 g of super phosphate, 1.1 g of potassium nitrate, and 1.1 g of gypsum per liter of mix. The composted cow manure mix was amended with 5.4 g of dolomitic lime and 3.6 g of calcium carbonate only. The pH of the mixes at the time of planting and throughout the growth period ranged from 5.5 to 6.2. Water was added during preparation (3 min) of the mixes in a concrete mixer to adjust the moisture content of the mixes to 50% (wt/wt).

All transplant mixes referred to hereafter as "natural mixes" were used directly after blending. "Heated transplant mixes" were incubated for 5 days at 60°C to reduce populations of biocontrol agents effective against Phytophthora root rots (3,21,24,54). The air capacity of each of the mixes in 10-cm-tall pots was at least 15% (vol/vol), and percolation rates exceeded 2 cm min^{-1}. Slow release fertilizer, Osmocote 17-6-12 plus minor nutrients (Scotts Company, Marysville, OH) was incorporated into the transplant mixes just before planting at a rate of 17.5 g per liter of mix.

Inoculation of transplant mixes with *Trichoderma hamatum* 382. Inoculum of T_{382} was grown for 7 days in petri plates on a *Trichoderma* selective medium (13), transferred to potato dextrose agar (DFCO PDA), and incubated for 14 days in continuous light at 24°C to produce abundant conidia. The plate culture was diced into a 250-ml Erlenmeyer flask containing 100 ml of sterilized distilled water. A drop of Tergitol NP_{10} (J. T. Baker Chemical Co., Phillipsburg, NJ) was added, and the flask was then shaken to dislodge conidia. This conidial suspension was filtered through two layers of cheesecloth into a sterilized 250-ml flask. The concentration of conidia was then determined with the aid of a hemocytometer. This suspension was blended into transplant mixes during their preparation to establish an initial inoculum density of 1.0×10^5 CFU T_{382} g^{-1} dry weight mix. The inoculated transplant mixes were then incubated for 7 days at 25°C. Control transplant mixes were not inoculated with T_{382}. The population of T_{382} in the transplant mixes was determined by dilution plating on the *Trichoderma* selective medium. Duplicate 10-g (wet weight) samples of a mix were suspended in 90 ml of 0.1% sterilized water agar, diluted, and then plated in triplicate on the selective *Trichoderma* medium. Colonies of T_{382} were counted after 10 days of incubation at 24°C. The identity of three presumed T_{382} colonies per sample was verified by light microscopy. Phialides produced by T_{382} examined under a microscope served to verify the identity of *T. hamatum* according to Bissett (6). The identity of these colonies was confirmed by polymerase chain reaction (PCR) using T_{382}-specific primers SCE16 and SCH19 (1) for each of three colonies per potting mix sample.

Inoculum of *P. capsici*. A culture of *P. capsici* (OP97) received from M. K. Hausbeck, Department Plant Pathology, Michigan State University, East Lansing, was transferred onto a *Phytophthora* selective medium (PBNC medium; 47). It contained 1.0 g of sucrose, 0.2 g of yeast extract, 0.027 g of pentachloronitrobenzene (75% Terraclor, WP), 0.02 g of benomyl (50% Benlate, WP), 0.1 g of neomycin, 0.01 g of chlorphenicol, 0.1 g of cholestrol, 200 ml of V8 juice filtered through Whatman no. 1 filter paper, and 20 g of agar in 1 liter of distilled water. The culture was incubated 3 days at 25°C, transferred to V8 juice agar (47), and stored at 4°C till use. Millet seed inoculum (20) and a zoospore suspension prepared according to Chen and Zentmyer (12) were used as inoculum sources of *P. capsici*. Millet seed inoculum was prepared by adding 25 g of millet seed and 0.02 g of asparagine to 18 ml of water in a 250-ml Erlenmeyer flask. It was capped with aluminum foil and then autoclaved for 20 min at 121°C on three successive days (24). The flask was shaken immediately after each autoclaving to avoid the formation of a clumped mass. It was inoculated with a mycelial plug from a 7-day-old culture of *P. capsici*, incubated 3 weeks at 24°C, and shaken daily to break up particles. The millet seed inoculum was then air-dried in a transfer hood for 24 h and ground in a Wiley mill to a particle size <1 mm diameter. This inoculum was blended with the transplant mixes at a rate of 0.5 g of dry weight per liter of mix. Zoospore inoculum of *P. capsici* was produced from mycelial mats grown in Difco lima bean broth. Three 5-mm-diameter plugs removed from the edge of a 7-day-old culture of *P. capsici* were placed in petri plates containing 15 ml of lima bean broth and incubated 3 days at 24°C. They were then washed four times at 1-h intervals with a salt solution (12). The washed mycelial plugs were next incubated 48 h under fluorescent light to stimulate production of sporangia. Sporangial cultures were incubated 30 min at 4°C, returned to 24°C, and zoospores were discharged after 1 h. The concentration of zoospores in the salt solution was determined with a hemocytometer and adjusted to inoculum concentrations ranging from 5 to 5,000 zoospores per ml through dilution with autoclaved water.

Phytophthora damping-off bioassay. The ability of T_{382} to suppress Phytophthora damping-off of cucumber in the two transplant mixes was examined on *Cucumis sativus* L. 'Straight Eight'. In a preliminary experiment, it was determined that 0.5-g of millet seed inoculum of *P. capsici* incorporated per liter of transplant mix just before planting caused enough disease to differentiate the severity of damping-off caused in the two transplant mixes. Transplant mix treatments included the natural and the heated peat mix, the natural and the heated composted cow manure mix, and finally, both the natural and the heated mixes inoculated with T_{382} as described above. Transplant potting mixes were placed in 400-ml Styrofoam pots with a perforated base (400 ml of potting mix per pot) and then seeded (eight seeds per pot) using five pots per treatment. Slow release fertilizer was used as described above. Pots were arranged in a randomized complete block design and incubated in a greenhouse for 7 days at 22 to 27°C under a combination of daylight and supplemental lighting (225 $\mu E\ m^{-2}\ s^{-1}$; 16 h per day). Plants were watered as needed. Seven days after seeding, the seedlings were rated for damping-off severity utilizing a scale in which 1 = symptomless, 2 = small lesion, 3 = postemergence damping-off, and 4 = preemergence damping-off. The identity of the pathogen was verified by plating of infected root or stem tissues on PBNC medium followed by verification with light microscopy. The population of T_{382} in the transplant mixes and the heated control transplant mixes was determined by dilution plating on the *Trichoderma* selective medium and verified with the PCR procedure as described above. The experiment was performed three times.

Analysis of variance (ANOVA) was used to determine the effects of transplant potting mix treatments on Phytophthora damping-off severity. The mix treatment factors included: transplant potting mix type (peat mix and composted cow manure mix), mix infestation (mix infested or not infested with *P. capsici*), heat treatment (heated or not), and inoculation (mix inoculated or not inoculated with T_{382}). The response variable was the percentage of seedlings in each replication with a damping-off severity rating ≥3 (Y). This was transformed to $Y^* = \text{arcsine}(\sqrt{Y})$ to obtain a constant variance. A four-way analysis of

variance was performed with the MIXED procedure of SAS (Statistical Analysis System, Cary, NC). The least significant difference (LSD) at $P = 0.05$ was calculated to compare differences among the means for pairs of interaction means.

Split root bioassay. A split root cucumber bioassay, described previously for detection of systemic effects in cucumber roots by composts against Pythium damping-off and root rot (62), was used. Cucumber seeds (*Cucumis sativus* 'Straight Eight') surface sterilized 30 s in 1% sodium hypochlorite were rinsed three times with sterilized distilled water and then planted in the heated peat mix using one seed per 200-ml Styrofoam pot. Seeded pots were incubated in a greenhouse at 22 to 27°C, as described above. After 12 days, roots of the seedlings were removed carefully from the mix, washed with running tap water as described previously (62), divided into approximately equal split root portions, and transplanted as split root plants into 600-ml pots. The mixes in the paired pots were separated by a distance of 5 mm, which avoided cross contamination of mix ingredients during careful irrigation of plants. The pot on one side of all split root seedlings was filled with the heated peat mix, which was infested 6 days later with a 5-ml zoospore suspension (5 zoospores per ml) of the pathogen, *P. capsici*. The other side of each pot contained the "inducer" treatments, which included: (i) the heated composted cow manure mix as a control; (ii) the heated composted cow manure mix fortified with T_{382}; (iii) the heated composted cow manure mix drenched 3 days after transplanting with benzothiadiazole (BTH; Syngenta Crop Protection Inc., Greensboro, NC) using 16 ml of a 10 µg BTH per ml solution per split root seedling to induce systemic resistance (SAR) in cucumber (25,59); and finally (iv) the heated composted cow manure mix drenched 3 days after transplanting with the fungicide mefenoxam (*R*-2-[(2,6-dimethylphenyl)-methoxy-acetylamino]-propionic acid methyl ester; 22% a.i.) at a dose of 0.08 ml per liter of solution using 25 ml per seedling (Syngenta Crop Protection).

Six days after transplanting, zoospore inoculum of *P. capsici* was applied to the surface of the heated peat mix at a 1-cm distance from where the split root system penetrated the surface of the potting mix using inoculum densities of 0 and 25 zoospores per seedling as 5-ml suspensions. The inducer portion of the split root seedlings was not infested with *P. capsici*. Ten replicate split root seedlings (one plant per paired pot system) were used per treatment using a randomized complete block design. The severity of crown and root rot in the seedlings on the infested side in the heated peat mix was determined 21 days after infestation, using a scale in which: 1 = symptomless; 2 = mild root rot (one to three small lesions per root system); 3 = moderate root rot (one to three large lesions per root system); 4 = severe root rot and crown rot; 5 = severe root and crown rot and large stem lesion; and 6 = dead plant. Infected crown and stem sections were plated onto the PBNC medium to verify the identity of the pathogen. The population of T_{382} in the inducer transplant potting mix and the heated control mixes was determined by dilution plating on the *Trichoderma* selective medium and verified with the PCR procedure as described above. The population of T_{382} in the stem of cucumber plants grown in the transplant mix inoculated with T_{382} and in control plants was determined by harvesting a 1-cm stem section at a 5-cm distance above the crown of the plant after the final disease severity ratings had been made (five stem sections per treatment). Samples were macerated in a sterilized Ziploc freezer pouch containing a 1:4 ratio (wt/wt) of plant tissue to sterilized dilution buffer. This suspension was serially diluted and then plated in triplicate onto the *Trichoderma* selective medium. The experiment was performed three times.

ANOVA was used to determine the effects of infestation with *P. capsici* and inducer mix on the (transformed) percentage of plants with Phytophthora crown and root rot. Percent diseased plants for each replication (Y) was transformed to $Y^* = \text{arcsine}(\sqrt{Y})$ to obtain a constant variance prior to analysis. The marginal effects nonparametric analysis of Brunner and Puri (9,48) was used to determine the effects of infestation with *P. capsici* and inducer mix on severity rating. The marginal effects analysis, which uses a rank transformation of the data (R^*) and derived relative treatment effects, was recently developed as a nonparametric method for properly testing main-effect and interaction hypotheses regarding the distribution of ordinal (and other) data that do not have a normal distribution (9,10,48). The least significant difference (LSD; $P = 0.05$) for Y^* (based on ANOVA) and R^* (based on marginal effects) was determined when factors or interactions were significant.

Phytophthora leaf blight bioassay. The ability of T_{382} to induce systemic resistance in the foliage of cucumber plants (*C. sativus* 'Straight Eight') against Phytophthora blight as an inoculant in transplant potting mixes was determined in the heated composted cow manure mix inoculated with this biocontrol agent. In this experiment, a drench with BTH served as a positive SAR control. Cucumber seeds, surface sterilized as described above, were germinated in 400-ml Styrofoam pots (two seeds per pot) containing the heated composted cow manure mix or the same but fortified with T_{382} as described above. Plants were grown at 22 to 25°C in the greenhouse for 12 days with a 16-h photoperiod (light intensity 225 µE m^{-2} s^{-1}). Ten-day-old cucumber plants were drenched carefully with 16 ml per plant of a 10 µg BTH per ml solution so that the BTH penetrated the mix at a 2-cm distance from the stem. After 12 days, the plants were incubated overnight in a moisture chamber (24 to 25°C; relative humidity >95%). Within 1 h after having been positioned in the chamber, plants were inoculated with *P. capsici* by placing a 10-µl drop containing 0, 25, or 50 zoospores near the midrib on the surface of the first true leaf. The following morning, plants were returned to a greenhouse (22 to 25°C and 225 µE m^{-2} s^{-1}; 16-h photoperiod) equipped with a fogger to maintain 60 to 80% relative humidity (RH) and enhance symptom development.

The severity of Phytophthora blight was determined through 11 days after inoculation on the basis of a disease severity rating scale in which: 1 = symptomless, 2 = small lesion, 3 = large lesion, 4 = leaf blight, 5 = leaf blight and stem lesion, and 6 = dead plant. A randomized complete block design was used. Each treatment was replicated 16 times (two plants per pot = one replication). Blighted leaf sections were plated onto PBNC medium to verify the identity of the pathogen. The population of T_{382} in the fortified mix and the heated control mix was determined by dilution plating on the *Trichoderma* selective medium and verified with the PCR procedure as described above. The population of T_{382} in leaves of cucumber plants grown in the various treatments was determined according to the procedure as described above. This experiment was performed three times.

Areas under disease progress curves were determined for percentage of plants with symptoms (YA) and disease severity rating (RA) from day 0 through day 11 after inoculation. ANOVA was used to determine the effects of inducer treatment mix and inoculum density of *P. capsici* on the (transformed) percentage of plants with symptoms of blight (disease incidence) and YA. Percent diseased plants (Y) was transformed to $Y^* = \text{arcsine}(\sqrt{Y})$ to obtain a constant variance prior to analysis. The marginal effects nonparametric analysis (9,48) was used to determine the effects of inducer treatment mix and inoculum density on blight severity rating and area under the severity rating curve. This analysis is based on ranks of severity rating (R^*) and area (RA^*). The least significant difference (LSD; $P = 0.05$) for Y^* and YA (based on ANOVA), and R^* and RA^* (based on marginal effects [48]), was determined when factors or interactions were significant.

RESULTS

Suppression of Phytophthora damping-off induced by *T. hamatum* 382. Based on ANOVA, there were significant ($P < 0.05$) effects of heat treatment, of

inoculation with T_{382}, and of infestation with *P. capsici* on (transformed) percentage of plants with damping-off severity ratings ≥3 (*Y**; Table 1). There also were significant two-way interactions of T_{382} and *P. capsici* infestation (because T_{382} had no effect if the pathogen was not present), potting mix and heat treatment (because heating resulted in less of an increase in disease with the peat mix than with the compost), T_{382} and heat treatment (because heating the mix had a much smaller effect when T_{382} was present than when it was not), and heat treatment and *P. capsici* infestation (because heat treatment had no effect on disease if the pathogen was not present). There were also some marginal ($P \leq 0.10$) three-way interactions involving mix, heat treatment, and infestation, and also mix, heat treatment, and inoculation with T_{382}. Comparisons were made based on the mean values for combinations of the four factors because of several significant interactions.

Significance of the effects of T_{382} on the severity of Phytophthora damping-off is illustrated by the differences in the mean *Y** values (Table 1). T_{382} significantly reduced the severity of Phytophthora damping-off of cucumber in the heated composted cow manure mix (0.8 versus 1.5) and in the heated peat mix (0.8 versus 1.1). However, it did not significantly affect disease severity in the natural mixes. The mean *Y** values for the natural peat and the composted cow manure (without T_{382}) mixes were significantly lower than values in the heated mixes (0.8 versus 1.1 for peat; 0.5 versus 1.5 for compost). Finally, effects of mix, heat treatment, and T_{382} on severity of disease were not observed in the control treatments that were not infested with *P. capsici*. These results were consistent among the three experiments.

The population of T_{382} recovered at planting on the selective *Trichoderma* medium from the inoculated composted cow manure mix was 4.3×10^5 CFU g^{-1} dry weight transplant mix. Seven days after planting when the seedlings were rated for damping-off severity, the population of T_{382} in the composted cow manure mix had increased to 3.5×10^6 CFU g^{-1} dry weight mix. The population of T_{382} in the inoculated peat mix was 4.9×10^5 CFU g^{-1} dry weight mix at planting, and it reached 6.4×10^5 CFU g^{-1} dry weight mix 7 days later when seedlings were rated for disease severity. T_{382} was not recovered from any of the heated mixes not fortified with T_{382}. *Trichoderma* isolates recovered on the selective medium were verified as T_{382} using PCR. Similar population trends of T_{382} were observed in two additional experiments in this study.

Systemic effects in split root plants. Heated peat mix infested with *P. capsici* (25 zoospores per seedling) was paired with four different inducer (and pathogen-free) treatment mixes in a split-root system. There were significant ($P < 0.05$) effects of pathogen infestation, inducer treatment, and their interaction on ranks of crown and root rot severity (*R**) on the infested side of the pots, based on the nonparametric marginal-effects analysis (9,10,48), and transformed plant disease incidence (*Y**), based on ANOVA. The interaction was significant because the inducer treatment had no effect if the pathogen was not present (Table 2).

There was no significant difference in mean *Y** for plants exposed to heated compost inoculated with T_{382} and those exposed to heated compost without T_{382} (0.94 versus 0.79). Both BTH and mefenoxam inducer treatments significantly decreased the mean *Y** compared with the heated compost control mix. The drench with mefenoxam was significantly more effective than T_{382} in reducing the (transformed) incidence of disease.

Even though inoculation of the heated compost mix with T_{382} did not reduce disease incidence, mean rank of disease severity rating (on the side that was exposed to inoculum but not exposed to the inducer) was reduced significantly ($P = 0.05$) by this inducer mix treatment (Table 2). In particular, the mean rank of crown and root rot rating for the compost mix inoculated with T_{382} (*R** = 48) was significantly lower than the mean rank (*R** = 62) for the compost that was not inoculated with T_{382}. Moreover, mean ranks for the mixes with BTH and mefenoxam were significantly less than for the heated compost control mix. There was no significant difference in mean *R** for the mix with T_{382} and the mix with BTH drench (Table 2); however, the drench with mefenoxam resulted in a significantly lower mean *R** than found for the compost mix inoculated with T_{382}. These results were consistent among the three experiments.

At planting, the population of T_{382} in the heated composted cow manure mix portion of the split root plants was 4.9×10^5 CFU g^{-1} dry weight transplant mix. When plants were rated for disease severity 28 days after transplanting, the population had increased to 6.7×10^6 CFU g^{-1} dry weight transplant mix. T_{382} was not recovered from the stem of the plants grown in control mixes, indicating that the pathogen and the biocontrol agent remained spatially separated. Its identity was verified with PCR.

Systemic effects against Phytophthora leaf blight. The inducer treatments and inoculum concentration of *P. capsici* significantly ($P < 0.05$) affected (transformed) percentage of plants with Phy-

Table 1. Suppression of Phytophthora damping-off of cucumber (*Cucumis sativus* 'Straight Eight') induced by *Trichoderma hamatum* 382 (T_{382}) in a composted cow manure-amended compared with a peat transplant mix

			Phytophthora damping-off severity					
			Mean disease severity[v]		Mean percent severity ≥3 (*Y*)[w]		Mean transformed percent severity ≥3 (*Y**)[x]	
Mix treatment	Heat treatment[t]	*Trichoderma* inoculum[u]	Control[y]	Infested[y]	Control	Infested	Control	Infested
Peat mix	Natural	–	1.2	2.2	5.0	52.5	0.1	0.8
Peat mix	Natural	+	1.2	2.0	5.0	45.0	0.1	0.7
Compost mix	Natural	–	1.0	1.6	0.0	22.5	0.0	0.5
Compost mix	Natural	+	1.1	1.7	2.5	25.0	0.1	0.5
Peat mix	Heated	–	1.1	2.8	2.5	77.5	0.1	1.1
Peat mix	Heated	+	1.0	2.3	0.0	55.0	0.0	0.8
Compost mix	Heated	–	1.2	3.2	5.0	95.0	0.1	1.5
Compost mix	Heated	+	1.0	2.2	0.0	52.5	0.0	0.8
$LSD_{0.05}$[z]							0.27	

[t] Natural represents transplant mixes stored at 25°C. Heated mixes were incubated at 60°C for 5 days.

[u] "+" = Inoculated with *T. hamatum* 382 during formulation of transplant mixes to an initial inoculum density of 1.0×10^5 CFU g^{-1} dry weight mix; "–" = not inoculated.

[v] Mean damping-off severity, determined 7 days after planting on five pots with eight plants each using a disease severity scale in which 1 = symptomless, 2 = small lesion, 3 = postemergence damping-off, and 4 = preemergence damping-off.

[w] Mean percentage of plants with a damping-off severity rating ≥3.

[x] Mean transformed percentage of plants with a damping-off severity rating ≥3, expressed as "$Y^* = \arcsin(\sqrt{Y})$", calculated to obtain a constant variance.

[y] Infested with 0.5 g millet seed inoculum of *P. capsici* per liter transplant mix; control mixes were not infested.

[z] Analysis based only on transformed data. Differences in means within columns larger than the least significant difference (LSD) value are significant ($P = 0.05$).

tophthora leaf blight symptoms (*Y**), mean rank of severity (*R**), area under the disease incidence progress curve (*YA*), and mean rank of area under the severity rating curve (*RA**). Although there were large differences in measures of disease between plants inoculated with 0 and 25 zoospores per 10 µl, there was only a slight or no increase in disease measures when inoculation concentration increased from 25 to 50 spores per 10-µl droplet (Table 3). There also was a significant ($P < 0.05$) interaction of inoculum concentration and inducer treatment for *YA*, *R**, and *RA**. This could be attributed to lack of differences in disease measures among the inducer treatments at the 0 level of inoculum concentration (control) versus differences that were observed on plants inoculated with zoospore concentrations of 25 or 50 per 10-µl droplet (Table 3).

Symptoms were first observed 72 h after inoculation on the inoculated foliage, and *P. capsici* was recovered on PBNC medium from lesions. Slightly more than half (52.5%) of the plants grown in the heated composted cow manure mix that were also inoculated with 50 zoospores per leaf developed symptoms of Phytophthora blight within 11 days after inoculation (Table 3). Comparisons of *Y**, *YA*, *R**, or *RA** generally led to the same conclusions regarding the inducer treatment and inoculum-concentration effects. Infestation of the potting mix with T_{382} significantly reduced the measures of disease compared with the uninfested control. For instance, use of T_{382} reduced *Y** from 0.71 (or 45% diseased plants) down to 0.39 (or 25%) when

Table 2. Systemic protective effects induced by *Trichoderma hamatum* 382 (T_{382}), benzothiadiazole (BTH), or the fungicide mefenoxam in cucumber (*Cucumis sativus* 'Straight Eight') against crown and root caused by *Phytophthora capsici* using split root plants

		Phytophthora crown and root rot severity in split cucumber roots[v]							
Paired split root heated mix treatments[v]		Mean % diseased plants (*Y*)		Mean transformed % diseased plants (*Y**)[w]		Mean disease severity (*R*)[x]		Mean severity rank (*R**)[y]	
Infested mix	Inducer mix	Control	Infested	Control	Infested	Control	Infested	Control	Infested
Peat mix	Compost mix (control)	0.0	60.0	0.0	0.94	1.0	2.7	32	62
Peat mix	Compost mix + T_{382}	0.0	50.0	0.0	0.79	1.0	1.6	32	48
Peat mix	Compost mix + BTH	0.0	30.0	0.0	0.47	1.0	1.7	32	44
Peat mix	Compost mix + mefenoxam	0.0	10.0	0.0	0.16	1.0	1.1	32	36
$LSD_{0.05}$[z]				0.45				12	

[v] A heated (60°C; 5 days) peat mix was infested 12 days after transplanting of seedlings as split root plants with a 5-ml zoospore suspension (25 zoospores per plant) of *P. capsici* on one side of the split root system. It was paired with uninfested inducer mix treatments that included the heated compost mix (control), the same mix but fortified with T_{382} (1.0×10^6 CFU g^{-1} dry weight mix), or drenched with BTH (10 µg ml^{-1} solution; 16 ml per pot), or drenched with mefenoxam (0.08 ml per liter solution; 25 ml per pot).

[w] Mean transformed percent diseased plants, expressed as "$Y^* = \arcsin(\sqrt{Y})$", calculated to obtain a constant variance.

[x] Mean crown and root rot severity rating in the heated peat mix determined 21 days after infestation, based on 10 replicates per treatment using a disease severity scale in which 1 = symptomless, 2 = mild root rot, 3 = moderate root rot, 4 = severe root rot and/or crown rot, 5 = severe root and crown rot and large stem lesion, and 6 = dead plant.

[y] Mean rank of disease severity rating across replications (*R**).

[z] Analyses based only on transformed data. An analysis of variance (ANOVA) was performed on *Y**, and a marginal relative-effects nonparametric analysis (9,48) was performed on *R**. Then, least significant differences (LSD) were calculated for both *Y** and *R** based on the standard error of the differences. For *R**, the LSD was based on the average standard error of the difference between pairs of rank means. Differences in means larger than the LSD value are significant ($P = 0.05$).

Table 3. Systemic resistance induced by treatment of transplant mixes with *Trichoderma hamatum* 382 (T_{382}) or benzothiadiazole (BTH) in cucumber (*Cucumis sativus* 'Straight Eight') against Phytophthora leaf blight[r]

		Phytophthora blight severity				Area under disease progress curves[y]		
Inducer treatment[s]	*Phytophthora* density[t]	Mean % diseased plants (*Y*)[u]	Transformed mean % diseased plants (*Y**)[v]	Mean severity rating (*R*)[w]	Mean severity rank (*R**)[x]	% diseased plants (*YA*)	Severity rating	Severity rank (*RA**)
Control	0	0.0	0.00	1.0	58	0.0	1.0	58
Control	25	45.0	0.71	2.0	122	36.8	1.6	122
Control	50	52.5	0.82	2.3	135	42.2	1.9	136
T_{382}	0	0.0	0.00	1.0	58	0.0	1.0	58
T_{382}	25	25.0	0.39	1.5	93	20.5	1.3	93
T_{382}	50	30.0	0.47	1.6	99	24.6	1.4	100
BTH	0	0.0	0.00	1.0	58	0.0	1.0	58
BTH	25	32.5	0.51	1.5	100	25.8	1.3	99
BTH	50	30.0	0.47	1.4	92	20.7	1.2	91
$LSD_{0.05}$[z]			0.29		11	14.7		12

[r] Plants were grown in a heated (60°C; 5 days) composted cow manure-amended transplant mix.

[s] Transplant mix fortified during its formulation with T_{382} to an initial density of 1.0×10^5 CFU g^{-1} dry weight mix or drenched 9 days after seeding with 16 ml of a 10 µg BTH ml^{-1} solution per plant, or with water (control).

[t] First true cucumber leaf was inoculated near the midrib 11 days after seeding with a 10-µl inoculum droplet containing 0, 25, or 50 zoospores of *P. capsici*.

[u] Mean percent blight on infected leaves (disease incidence) was determined 11 days after inoculation, based on 16 replicates of two plants each.

[v] Mean transformed percent diseased plants, expressed as "$Y^* = \arcsin(\sqrt{Y})$", calculated to obtain a constant variance.

[w] Mean blight severity rating determined 11 days after inoculation, based on 16 replicates of two plants each, using a disease severity rating scale, in which: 1 = symptomless, 2 = small lesion, 3 = large lesion, 4 = leaf blight, 5 = leaf and stem blight, 6 = dead plant.

[x] Mean rank of disease severity rating across replications (*R**).

[y] Area under disease progress curves through 11 days after inoculation, based on disease incidence (*YA*) or severity rating (*RA*). For areas based on ratings, the mean rank of the areas across the replications (*RA**) was determined.

[z] Except for area under the disease incidence curve, analyses based on transformed data. An analysis of variance (ANOVA) was performed for *Y** and *YA*, and a marginal relative effects nonparametric analysis (9,48) was performed on *R** and *RA**. Then, least significant differences (LSD) were calculated for *Y**, *YA**, *R**, and *RA** based on the standard error of the differences. For *R** and *RA**, the LSD was based on the average standard error of the difference between pairs of rank means. Differences in the means larger than the LSD value are significant ($P = 0.05$).

plants were inoculated with the 25 zoospore per 10 ml suspension of *P. capsici* (Table 3). The mean rank for area under the severity curve (*RA**) was reduced from 122 down to 93 with the use of T_{382} at this inoculum concentration. Use of a BTH drench for the inducer treatment also reduced the measures of disease compared with the control. Furthermore, there were no significant differences in disease measurements between BTH drench treatment and T_{382}-inoculation treatment (at the same inoculum concentration of the pathogen). These results were consistent between two Phytophthora leaf blight bioassays.

The population of *Trichoderma* recovered on the selective medium from the fortified composted cow manure mix at planting was 5.2×10^5 CFU g^{-1} dry weight transplant mix. When final disease severity ratings were made (23 days after planting), the population of *Trichoderma* recovered on the selective medium was 5.9×10^6 CFU g^{-1} dry weight mix. T_{382} was the only *Trichoderma* isolate obtained on the selective medium from these mix samples, based on the production of phialides on the medium and verification by PCR. T_{382} was not recovered at planting nor at 23 days after planting from the control-heated mix. T_{382} also was not isolated from the leaves of plants grown in the transplant potting mixes fortified with T_{382}. These results were consistent in the two experiments.

DISCUSSION

The biocontrol agent T_{382} significantly reduced the severity of Phytophthora damping-off of cucumber in the heated composted cow manure mix. Even so, T_{382} was not as effective against this phase of the disease as the microbial community that naturally colonized the composted cow manure mix (Table 1). Thus, T_{382} only partially restored the suppressive effect (lost from heating) that was naturally present in the composted cow manure mix. This agrees with previous reports on the role of specific biocontrol agents in suppression of Phytophthora damping-off and root rots which show that control of these diseases is due to the interactions of many biocontrol agents naturally present in composts (20,21,61). The general suppression phenomenon is considered to best explain biological suppression of these damping-off and root rot diseases (8).

The effects of T_{382} observed in this work against foliar Phytophthora blight of cucumber suggest that it had a specific role in suppression of this phase of the disease, and data in Table 3 provided direct evidence for a systemic effect. T_{382} was as effective as the SAR inducer BTH in reducing both the severity of Phytophthora blight and the percentage of blighted plants. It provided this degree of control while the pathogen, *P. capsici*, remained spatially separated on the plant from the biocontrol agent, which verified that the effect was systemic in nature. The split root experiments provided additional evidence for the induction of a systemic response in cucumber by T_{382} against *P. capsici*. T_{382} consistently reduced the severity of root rot in plants harvested from the infested heated peat mix that were paired as split root plants with the heated composted cow manure mix colonized by T_{382}. In this system, the pathogen also remained spatially separated in the plant from the biocontrol agent. The systemic effect induced by T_{382} supports that reported for another *Trichoderma* isolate, *Trichoderma harzianum* T_{203} (60). *Pythium oligandrum* Drechsler seems to induce a similar response in tomato, an effect that is enhanced by compost amendments (41). The significance of the role of ISR provided by T_{382} and other ISR-active biocontrol agents in control of Phytophthora root rot relative to that provided by microorganisms, which colonized the compost-amended potting mix naturally, needs to be explored further.

A question that could be asked is whether the degree of disease control provided through ISR by T_{382} on cucumber in compost-amended transplant mixes would be of interest to farmers. Under the controlled conditions and with high uniform inoculum doses used in this work, the reduction of disease obtained through inoculation of the compost-amended transplant mix with T_{382} was less than 50%. This may prove valuable to growers because the disease is so difficult to control with fungicides (36,44). Although we do not have data on field performance of T_{382} against Phytophthora blight of cucumber, it has been effective in nurseries on *Pieris japonica* Thunb. against leaf blight, stem blight, and dieback caused by *Phytophthora parasitica* Dastur. The percentage of *Pieris* plants killed during this naturally occurring Phytophthora leaf blight and stem dieback epidemic was reduced from 26% in the control compost-amended container medium to 4% in the same medium that had been inoculated during potting with T_{382} (23). This suggests that T_{382} may also prove useful for the production of vegetable crops in ground beds where transplants increasingly are planted in compost-amended soils, a production system that is similar to containerized production of plants.

An advantage of ISR over the use of *Phytophthora*-specific fungicides, for example, is the spectrum of diseases that can be suppressed by systemic induced resistance. In a stem dieback epidemic caused by *Botryosphaeria dothidea* (Moug.:Fr.) Ces. & de Not. on *Myrica pennsylvanica* Loisel., the percentage of plants killed was reduced from 21% in the control to 6% in the compost-amended container medium that had been inoculated with T_{382} (23). This disease is very difficult to control with fungicides, and its incidence in nurseries on container-produced crops is also difficult to predict. It would seem, therefore, that the degree of control provided by T_{382} can be significant in container production systems, particularly for difficult-to-control diseases such as Botryosphaeria dieback. For Phytophthora dieback and leaf blight diseases, T_{382} may be able to reduce fungicide use by the nursery industry, a feat that was accomplished several decades ago for control of Phytophthora root rots through the use of naturally suppressive compost-amended media (20,21,53,61). ISR provided by *Trichoderma* may prove most useful for organic transplant production systems where the choice of pesticides is even more limited and composts often are used to improve soil fertility and quality.

ACKNOWLEDGMENTS

This research was supported by Research Grant Award No. US-3108-99 from BARD, The United States-Israel Binational Agricultural Research and Development Fund, by a grant from the Ohio Water Development Authority, Columbus, OH, and by a scholarship (15003631) from the Fullbright/Near East Asia Program. Salaries and research support were provided by state and federal funds to the Ohio Agricultural Research and Development Center, Ohio State University. We thank C. A. Musselman and T. L. Moore for providing technical assistance.

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