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Potential of *Trichoderma virens* for Biocontrol of Root Rot and Vine Decline in *Cucumis melo* L. Caused by *Monosporascus cannonballus*

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ABSTRACT

Root rot and vine decline disease of muskmelon, incited by *Monosporascus cannonballus*, causes severe economic losses in many hot semi-arid production areas. No effective control measures for Monosporascus root rot and vine decline are presently available. Genetic resistance to this disease is not satisfactory in commercial melon cultivars. We have attempted to determine the potential for biocontrol of root rot and vine decline disease on muskmelon using *Trichoderma virens*. *T. virens* exhibited *in vitro* antibiotic activity by inhibiting mycelial growth of *M. cannonballus* and other vine decline pathogens such as *Didymella bryoniae*, *Macrophomina phaseolina* and *Phomopsis cucurbitae*. A gliotoxin producing strain of *T. virens* (TV-6) demonstrated stronger inhibition of the fungal pathogens when compared with *T. virens* strain TV-4 which does not produce gliotoxin. In addition to antibiotic activity, *T. virens* demonstrated parasitism of *M. cannonballus* mycelium. Under greenhouse conditions, *T. virens* colonized the root systems of muskmelon plants, significantly reduced colonization by *M. cannonballus* of muskmelon seedling roots, and suppressed severity of seedling disease using a seed treatment. *T. virens* strains TV-4 and TV-6 performed best in suppression of *M. cannonballus* root rot among the *T. virens* strains and isolates tested. Preliminary data suggest that strains of the biocontrol agent *T. virens* may have potential as an additional control strategy for the integrated management of root rot and vine decline diseases of muskmelon.

RESUMEN

La pudrición de la raíz y de la enredadera del melón causada por *Monosporascus cannonballus*, ocasiona pérdidas económicas severas en muchas áreas productoras semiáridas de clima caliente. No existen actualmente medidas de control eficaces disponibles contra las pudriciones causadas por Monosporascus. Los cultivares comerciales de melón no presentan resistencia genética satisfactoria a esta enfermedad. Nosotros hemos intentado determinar el potencial de biocontrol de las pudriciones de la raíz y de la enredadera del melón usando *Trichoderma virens*. *T. virens* mostró actividad antibiótica *in vitro* al inhibir el crecimiento micelial de *M. cannonballus* y de otros patógenos que causan pudrición de la enredadera tales como *Didymella bryoniae*, *Macrophomina phaseolina* y *Phomopsis cucurbitae*. La cepa de *T. virens* (TV-6) productora de una gliotoxina mostró mayor inhibición de los hongos patógenos en comparación con la cepa de *T. virens* TV-4 que no produce gliotoxina. Además de la actividad antibiótica, *T. virens* también mostró parasitismo del micelio de *M. cannonballus*. Bajo condiciones de invernadero, *T. virens* colonizó el sistema radical de las plantas del melón, redujo significativamente la colonización por *M. cannonballus* en las raíces de la plántula y suprimió la severidad de la enfermedad en las plántulas cuando se usó como tratamiento a la semilla. Entre las cepas y aislamientos de *T. virens* estudiados, las cepas TV-4 y TV-6 mostraron la mejor supresión de la pudrición de la raíz por *M. cannonballus*. Los resultados preliminares, sugieren que las cepas del agente de biocontrol *T. virens* pueden tener potencial como estrategia de control adicional para el manejo integrado de la pudrición de la raíz y la enredadera del melón.

Additional index words: Cucumis melo, Cucurbitaceae, cucurbits, antibiosis, parasitism, tissue colonization

Biocontrol of plant diseases is a promising strategy with increased emphasis in both research and plant protection industries. Biocontrol has attracted great interest because of ineffective chemical controls and the increased regulation of chemical registration and use. The use of some commonly applied and environmentally harmful pesticides such as methyl bromide will be prohibited in the near future. Biocontrol is especially attractive for soilborne diseases since these pathogens are difficult to reach in the growing medium with specific pesticides. Excessive use of broad spectrum or persistent chemicals may result in soil contamination, fungicide resistance, or other harmful effects. The concept of biocontrol is based on microbial interactions that directly protect the host or reduce the inoculum of the pathogen in a

particular environment (Cook and Baker, 1983). The mechanisms of biocontrol may be antibiosis, parasitism, competition, predation, and/or induction of plant systemic resistance. Some biocontrol agents have reached commercial development and utilization. For example, *Agrobacterium radiobacter* strain K84 was used to control crown gall of several plant species; non-pathogenic *Fusarium oxysporum* strains were developed to control Fusarium wilt of tomato, flax and other crops; *Pseudomonas fluorescens* was used to reduce bacterial and fungal diseases of cereals and other crops; and plant damping-off was suppressed by *Trichoderma viride* or *Pythium oligandrum* (Maloy, 1993).

Biocontrol research has been conducted on numerous diseases of different crops (Cook and Baker, 1983; Chet, 1987). Some work on biocontrol of diseases of cucurbit crops has also been done. Fusarium wilt of muskmelon caused by *F. oxysporum* f. sp. *melonis* was suppressed by seed or soil treatment with *Trichoderma harzianum* (Sivan and Chet, 1986) or *Aspergillus niger* (Mukherjee and Sen, 1998). Soft rot disease of muskmelon seedlings caused by *Erwinia carotovora* subs. *carotovora* was reduced using the biocontrol agent *Pseudomonas fluorescens* (El-Hendawy et al., 1998). An isolate of *A. niger* was found to inhibit the growth of a range of pathogenic *Fusarium* isolates causing wilt of watermelon *in vitro* (Naik and Sen, 1993). Larkin et al. (1993) reported that soil suppressiveness was associated with a watermelon cultivar 'Crimson Sweet' with moderate resistance to Fusarium wilt (caused by *F. oxysporum* f. sp. *niveum*) but not with a susceptible cultivar 'Florida Giant'. Induction of systemic resistance in cucumber against several diseases, including Fusarium wilt, by treatment with rhizobacteria (Liu et al., 1995a, 1995b, 1995c; Wei, et al., 1996), non-pathogenic *Rhizoctonia* isolates (Sneh and Ichielevich, 1998), or composts (Zhang, et al., 1996a; Zhang et al., 1998), has been demonstrated. Hassouna et al. (1998) reported that biocontrol of soilborne plant pathogens (*F. oxysporum* f. sp. *lycopersici*, *R. solani*, and *Pythium* sp.) attacking cucumber by N_2-fixing rhizobacteria (*Azospirillum brasilense*, *Azotobacter chrococcum*, and *Klebsiella pneumoniae*) in a semi-arid environment might be due to induction of systemic resistance, antagonism, and/or plant growth promotion. Fusarium wilt of cucumber also was reduced by chitinolytic bacteria (Singh et al., 1999). Transformants of *Trichoderma longibrachiatum* over-expressing the ß-1,4-endoglucanase gene egl1 showed enhanced biocontrol of Pythium damping-off of cucumber caused by *P. ultimum* (Migheli et al., 1998). *Trichoderma virens* (J. H. Miller, Giddens & A. A. Foster) Arx (formerly *Gliocladium virens*, J. H. Miller, Giddens & A. A. Foster) has been reported to be a mycoparasite or antibiotic-producing antagonist of fungal plant pathogens (Howell et al. 1993; Howell, 1981, 1998; Lumsden et al., 1992, Tu, 1980). The potential of *T. virens* as an effective biocontrol agent for several soilborne root or seedling diseases was demonstrated (Elad et al., 1986; Howell, 1981; Lewis and Papavizas, 1985; Smith et al., 1990; Zhang et al., 1996b).

Muskmelon (*Cucumis melo* L.) is an important horticultural crop in the U. S. Due to continuous and intensive cultivation of these crops in the same areas, soilborne diseases have increased in number and severity. In recent years, a group of soilborne diseases, known as vine declines, have become prevalent, causing serious economic losses to muskmelon. As many as ten fungi, including *Acremonium cucurbitacearum* A. Alfaro-Garcia, W. Gams, et J. Garcia-Jimenez, *Didymella bryoniae* (Auersw.) Rehm, *Monosporascus cannonballus* Pollack & Uecker, *Macrophomina phaseolina* (Tassi) Goidanich, and *Phomopsis cucurbitae* McKeen, have been reported to cause vine decline diseases in cucurbits (Bruton, 1998; Bruton et al., 1998a; Miller et al., 1995). Vine declines of melons generally occur at 10 to 20 days before harvesting

Table 1. Antibiosis of *Trichoderma* virens against selected fungal pathogens causing muskmelon vine declines.

	Fungal growth reduction (%)	
Fungus	*T. virens* TV-4 metabolites[z]	*T. virens* TV-6 metabolites[y]
Acremonium cucurbitacearum	70.5±0.80[x]	82.0±1.80[x]
Didymella bryoniae	45.4±0.95	100
Monosporascus cannonballus	73.4±4.17	100
Macrophomina phaseolina	99.6±0.27	100
Phomopsis cucurbitae	81.8±0.71	100

[z]Metabolites of *T. virens* TV-4 or TV-6 were leached into the assay medium when the strains were grown on cellophone sheets which overlaid the assay medium.
[x]Standard errors were calculated from ten replicates of two similar experiments.

Table 2. Effect of seed treatment with preparations of *Trichoderma virens* on root rot of muskmelon seedlings caused by *Monosporascus cannonballus*.

Treatment (*T. virens* strain or isolate)	Seedling emergence (%)	Mean disease severity index (DSI)
Experiment 1		
TV-980026	28±4.4[z]	2.15 a[y]
TV-980034	88±3.4	1.86 ab
Control	82±4.4	1.82 ab
TV-980035	82±3.4	1.81 ab
TV-980033	82±10.0	1.72 abc
TV-980030	80±4.9	1.56 abc
TV-980024	75±6.2	1.51 bc
TV-4	73±7.8	1.41 c
TV-6	66±6.9	1.32 c
Experiment 2		
Control	83±5.5	3.71 a
TV-4	87±2.7	2.18 b
TV-6	75±3.1	2.14 b

[z]Standard errors were calculated from ten replicates.
[y]Means with the same letter within a column in each experiment are not significantly different ($P \leq 0.05$) based on the Ryan -Elinot-Gabriel-Welsch multiple range test.

(Bruton et al., 1998a). Monosporascus root rot and vine decline particular environment (Cook and Baker, 1983). The mechanisms of biocontrol may be antibiosis, parasitism, competition, predation, and/or induction of plant systemic resistance. Some biocontrol agents have reached commercial development and utilization. For example, *Agrobacterium radiobacter* strain K84 was used to control crown gall of several plant species; non-pathogenic *Fusarium oxysporum* strains were developed to control Fusarium wilt of tomato, flax and other crops; *Pseudomonas fluorescens* was used to reduce bacterial and fungal diseases of cereals and other crops; and the potential of *T. virens* as an agent for biocontrol of Monosporascus root rot and vine decline of muskmelon.

Objectives of this study were to 1) test the *in vitro* activity of *T. virens* against selected vine decline pathogens of muskmelon; 2) determine the ability of *T. virens* to parasitize *M. cannonballus*; 3) evaluate effects of muskmelon seed treatment with *T. virens* for control of root rot caused by *M. cannonballus* under greenhouse conditions, and 4) determine the colonization of muskmelon plant root systems by *T. virens* after seed treatment. A preliminary report of this work has been published in the form of an abstract (Bruton et al., 1998b).

MATERIALS AND METHODS

Biocontrol agents and vine decline pathogens. Two *T. virens* strains, TV-4 ('P'-group) and TV-6 ('Q'-group), were obtained from C. R. Howell at the Southern Crops Research Lab, USDA-ARS, College Station, TX. Six other *T. virens* isolates (TV-980024, TV-980026, TV-980030, TV-980033, TV-980034, and TV-980035) were isolated from muskmelon plant roots collected in Costa Rica. The vine decline pathogens included *A. cucurbitacearum* (SP 934941), *D. bryoniae* (OK 963095), *M. cannonballus* (TX 912035), *M. phaseolina* (TX 922049), and *P. cucurbitae* (OK 951062). These respective fungi were previously isolated from muskmelon plant roots exhibiting symptoms of vine decline. All *T. virens* strains and isolates, and the fungal pathogens were hyphal-tipped, and stored in glass vials containing sterilized moist Redi-earth soil mix (Scotts-Sierra Hort. Products Co., Marysville, OH) in the laboratory at 23°C. Fungal cultures for subsequent experiments were obtained by transferring a small amount of the fungal colonized soil from the storage to potato dextrose agar (PDA), and incubating the fungi at 25°C for 4 days.

Antibiotic activity of T. virens to vine decline pathogens in vitro. The technique developed by Zhang et al. (1996b), using PDA covered with cellophane to grow biocontrol agents, was used in this study. Two *T. virens* strains, TV-4 and TV-6, were grown on cellophane covered PDA at 25°C for three days. The cellophane and biocontrol agent were subsequently removed from the PDA. Any secondary metabolites, produced by the biocontrol agents, which leached into the agar remained. Five fungal pathogens, *A. cucurbitacearum*, *D. bryoniae*, *M. cannonballus*, *M. phaseolina*, and *P. cucurbitae*, were grown on PDA for 3 days at 25°C prior to transfer to the PDA containing the secondary metabolites of the biocontrol agents. A 0.5-cm diameter fungal colonized PDA disc was then transferred to the center of the PDA plates containing the secondary metabolites of the biocontrol agents. Fungal pathogens were grown on PDA without the secondary metabolites of the biocontrol agents to serve as controls. Radial growth was measured after 3 days incubation at 25°C. The experiment was conducted twice with 5 replicates.

Parasitism of M. cannonballus by biocontrol agents. To observe parasitism of *M. cannonballus* by *T. virens* on plates containing soil extract agar (Howell, 1987), a 0.5-cm diameter

Table 3. Colonization of muskmelon seedling hypocotyls and roots by *Trichoderma virens* 28 days after planting of *T. virens* treated seeds in the greenhouse.

Treatment (*T. virens* strain)	*T. virens* colonized tissue samples (%)[z]					
	Non-surface sterilized			Surface sterilized		
	Hypocotyl	Taproot	2nd root	Hypocotyl	Taproot	2nd root
TV-4	100 a[y]	72.5 b	100 a	80.0 b	70.0 b	2.5 c
TV-6	100 a	77.5 ab	90 ab	90.0 ab	67.5 b	2.5 c
Control	0	0	0	0	0	0

[z]The tissue samples were from the experiment 1.

[y]Means with the same letter within a row are not significantly different ($P \leq 0.05$) based on the Ryan-Einot-Gabriel-Welsch multiple range test.

Table 4. Colonization of muskmelon seedling hypocotyls and roots by *Monosporascus cannonballus* 28 days after planting of *T. virens* treated seeds in the greenhouse.

Treatment (*T. virens* strain)	*M. cannonballus* colonized tissue samples (%)[z]		
	Hypocotyl	Taproot	Secondary root
Control	22.5 a[y]	40.0 a	0 a
TV-6	0 b	0 b	0 a
TV-4	2.5 b	0 b	2.5 a

[z] The tissue samples collected from experiment 1 and were surface-sterilized.

[y] Means with the same letter in the same column are not significantly different ($P \leq 0.05$) based on the Ryan-Einot-Gabriel-Welsch multiple range test.

PDA disc colonized by *T. virens* was placed on one side of the plate, and a 0.5-cm diameter PDA disc bearing *M. cannonballus* mycelia was placed on the other. The distance between discs was 4 cm. After the biocontrol agents and *M. cannonballus* grew together (4 days at 25°C), parasitism of *M. cannonballus* by the biocontrol agents was observed and photographed under a light microscope.

To observe parasitism of *M. cannonballus* by the biocontrol agents using a scanning electron microscope (SEM), a 0.5-cm diameter disc bearing *T. virens* mycelia and a 0.5-cm PDA disc bearing *M. cannonballus* were placed 2 cm apart on a piece of muskmelon root tissue in a plate containing moist filter paper. The fungi were incubated at 25°C for 4 days. The colonized root tissues were then fixed, and processed according to a procedure described by Bruton et al. (1998c). The parasitism of *M. cannonballus* by *T. virens* was examined and photographed using a JEOL 6400 SEM system.

Effect of T. virens seed treatment on Monosporascus root rot of muskmelon under greenhouse conditions. Preparations of *T. virens* strains (TV-4 and TV-6) and isolates (TV-980024, TV-980026, TV-980030, TV-980033, TV-980034, and TV-980035) were prepared as follows: *T. virens* was grown in a liquid culture (100 ml) consisting of 4% ground wheat bran, 1% ground peat moss and 95% water. The pH of the culture was adjusted to 4.0. After the culture was shake incubated (150 rpm) for one week at 25°C, the contents were centrifuged at 16,000 x g for 10 min, and the supernatants decanted. The pellets were spread in large petri dishes, and air-dried under a hood. The dry material was ground to pass through a 0.43-mm sieve.

The experiment testing the effects of *T. virens* strains and/or isolates on Monosporascus root rot of muskmelon in the greenhouse was conducted twice. Two *T. virens* strains (TV-4 and TV-6) and six isolates (TV-980024, TV-980026, TV-980030, TV-980033, TV-980034, and TV-980035) were used in experiment 1. *T. virens* strains (TV-4 and TV-6) were tested in experiment 2. The experiments were conducted as follows: The preparations of *T. virens* strains or isolates were coated onto muskmelon seeds (cv. 'Hy-Mark') at the rate of 0.1g of preparation per g of seed, using a latex sticker (Rhoplex B-15J, Rohm and Haas, Philadelphia, PA). Biocontrol agent treated seeds were planted in methyl bromide fumigated soil infested with *M. cannonballus* (TX 912035) at the rate of 10 colony forming units per g of soil in pots (14.5 x 11.0 cm), while non-treated seeds planted in *M. cannonballus* infested soil served as controls. Ten seeds were planted in each pot containing 0.71 kg of pathogen infested soil, and ten pots were used for each treatment. The pots were transferred to the greenhouse, plant emergence was recorded, and plants were thinned to 5 per pot. Plants were fertilized weekly with 0.1% Peters 20:20:20 (Grace-Sierra Hort. Products, Milpitas, CA). The experiments were conducted in the greenhouse with mean soil temperatures of 24 and 31°C for night and day, respectively. After 28 days, plant height was recorded, and then the soil was gently washed away to expose the roots for subsequent disease rating. The disease rating scale was as follows: (hypocotyl = RH) 1 = healthy with no lesions or discoloration; 2 = slight discoloration; 3 = moderate discoloration and/or with lesions; 4 = moderate maceration; and 5 = severe maceration; (stem/root junction = RSR) 1 = healthy with no lesions or discoloration; 2 = slight discoloration; 3 = moderate discoloration but firm: 4 = moderate discoloration with loss of firmness; 5 = severe discoloration and mushy; (primary root= R1R) 1 = healthy with no lesions; 2 = up to 25% with slight discoloration; 3 = > 25% slightly discolored or with lesions; 4 = moderate discoloration and/or slight maceration; 5 = severe discoloration and/or macerated; (secondary roots = R2R) 1= healthy with no lesions or discoloration; 2 = slight discoloration; 3 = slight discoloration with up to 25% root mass reduction; 4 = moderate discoloration with up to 50% root mass reduction; 5 = severe discoloration with >50% root mass reduction. A disease severity index (DSI) was derived by: DSI=(RH+RSR+R1R+R2R)/4, where RH, RSR, R1R and R2R, respectively, are the average disease rating for hypocotyl, stem/root junction, primary root, and secondary root; 4 represents the number of different ratings used to evaluate disease reaction. Isolations were made from plants taken from representative treatments to verify the presence of the inoculated fungus.

Colonization of muskmelon plant roots by the biocontrol agent T. virens under greenhouse conditions. Muskmelon root systems were recovered from the experiments described above by washing the roots with tap water. The roots from *T. virens* treated or control plants were separated into hypocotyl, tap roots and secondary roots. The hypocotyl, tap and secondary roots were then cut into 1-cm pieces. Half of the samples were washed with sterile distilled water, and the other half of the samples were surface-sterilized with 0.5% NaOCl for 30 seconds. The surface sterilized and non-surface sterilized tissue samples were incubated in plates containing PDA amended with rifampicin (100 µg/ml) and metalaxyl (200 µg/ml) and benlate (1 µg/ml). Each plate contained 10 pieces of roots or hypocotyls, and 4 plates were used for each treatment. The plates were incubated for 7 days at 25°C. The *T. virens* colonies and *M. cannonballus* colonies were recognized on the medium, based on their colony morphology. Percentage of hypocotyl and root samples bearing *T. virens* or *M. cannonballus* or both was recorded separately.

Data analysis. Analysis of variance of the data was performed by the general linear model procedure of SAS (SAS Institute, Cary, NC). Treatment means were compared using the Ryan-Einot-Gabriel-Welsch multiple range test ($P \leq 0.05$).

RESULTS

Antibiotic effect of T. virens on different vine decline pathogens in vitro. Both *T. virens* strains (TV-4 and TV-6) exhibited antibiotic effects against each of the vine decline pathogens (Table 1). *T. virens* TV-6 completely suppressed the growth of *D. bryoniae*, *M. cannonballus*, *M. phaseolina*, and *P. cucurbitae*, and the growth of *A. cucurbitacearum* was reduced 82%. *T. virens* TV-4 showed the strongest antibiotic activity to *M. phaseolina*, and the least antibiotic activity against *D. bryoniae*.

Parasitism of M. cannonballus by T. virens. Light microscopy demonstrated that *T. virens* TV-4 was parasitic to

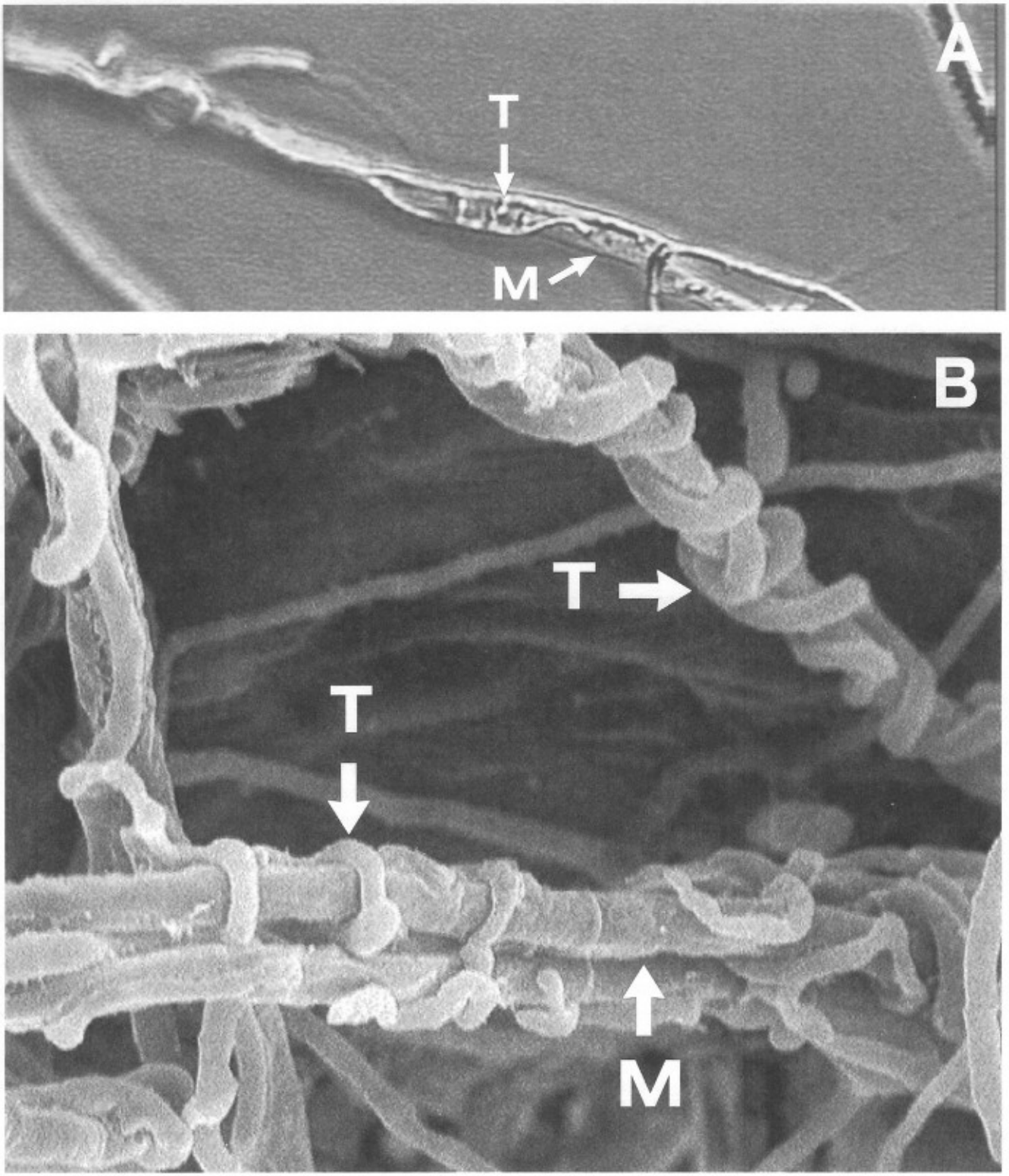

Fig. 1. Parasitism of *Monosporascus cannonballus* by *Trichoderma virens*. (A) light microscopy image illustrating the penetration and peg development of *T. virens* within the host mycelium of *M. cannonballus* (X 400); and (B) scanning electron microscope image showing *T. virens* (TV-4) [T] coiled around the mycelium of *M. cannonballus* (TX 912035) [M] (X 1000).

M. cannonballus when the two fungi were grown together on the soil extract agar (Fig. 1A). *T. virens* attached and penetrated the hyphae of *M. cannonballus* forming mycelial pegs within the host fungus. Parasitism of *M. cannonballus* by *T. virens* TV-4 was also observed using SEM when TV-4 and *M. cannonballus* were introduced on muskmelon root tissue (Fig. 1B). Parasitism of *M. cannonballus* by *T. virens* strain TV-6 was similar to that observed with the strain TV-4 (data not shown).

Effect of seed treatment with T. virens on Monosporascus root rot of muskmelon plants under greenhouse conditions. Eight *T. virens* strains and isolates were tested in experiment 1 to evaluate their effects on Monosporascus root rot of muskmelon seedlings. The results showed that different *T. virens* strains and isolates performed differently in suppressing Monosporascus root rot of muskmelon (Table 2). *T. virens* strains TV-4 and TV-6 significantly ($P \leq 0.05$) reduced disease severity compared with control treatment. However, the other *T. virens* isolates (TV-980024, TV-980026, TV-980030, TV-980033, TV-980035, and TV-980034) did not significantly ($P \leq 0.05$) suppress the disease. TV-4 and TV-6 were the only *T. virens* strains tested in experiment 2. The results were similar to those of experiment 1. Significant disease reduction ($P \leq 0.05$) on muskmelon seedlings was achieved by seed treatment with both TV-4 and TV-6 when compared to the disease on control plants (Table 2).

Colonization of muskmelon seedling roots by T. virens and M. cannonballus under greenhouse conditions. When muskmelon seeds were coated with *T. virens* and planted in pots containing soil infested with *M. cannonballus*, hypocotyls, tap roots, and secondary roots were colonized by *T. virens* (TV-4 and TV-6) during the 28-day experimental period (Table 3). Surface sterilization of tissue only reduced *T. virens* colonization by 10 to 20% on hypocotyls, and 3.4 to 13.5% on taproots. Surface sterilization of tissue greatly reduced isolation of *T. virens* on the secondary roots. *T. virens* isolates TV-4 and TV-6 were similar in their ability to colonize the root systems of muskmelon seedlings. Colonization of muskmelon seedling hypocotyls and taproots by *M. cannonballus* was significantly ($P \leq 0.05$) reduced by seed treatment with *T. virens* TV-4 or TV-6 (Table 4). Colonization of secondary roots of both control and *T. virens* treated seedlings by *M. cannonballus* was very low (0 to 2.5%).

DISCUSSION

Our results demonstrated that *T. virens* produced secondary metabolites *in vitro* that exhibited antibiotic activity against all five vine-decline fungi tested including *M. cannonballus*. *T. virens* is known to produce such antibiotics as gliotoxin, dimethylgliotoxin, gliovirin, viridin, viridiol, and heptelidic acid (Howell et al. 1993; Howell, 1998; Lumsden et al., 1992). Howell et al. (1993) further reported that *T. virens* strains could be separated into 'P'- or 'Q'-groups based on their antibiotic production profiles. 'P'-group strains of *T. virens* produce gliovirin, and heptelidic acid, whereas, 'Q'-group strains produce gliotoxin and dimethylgliotoxin. Both 'P'- and 'Q'-group strains produce viridin and viridiol. In the present study, TV-4 belongs to the 'P'-group, and TV-6 belongs to the 'Q'-group of *T. virens*. The antibiotic activity of *T. virens* TV-6 was greater than that of TV-4 against all fungal pathogens tested. Different antibiotic effects between TV-4 and TV-6 may be due to gliotoxin production since TV-6 produces the antibiotic and TV-4 does not. Gliotoxin appears to be one of the most important antibiotics involved in antibiosis by *T. virens*. Therefore, production of antibiotics by *T. virens* may be an important mechanism in the biocontrol of *M. cannonballus* and other fungal pathogens that cause vine decline diseases in cucurbits. On the other hand, the *in vitro* production of antimicrobial compounds by *T. virens* does not ensure that biocontrol agents will produce them *in vivo* and play a role in the biocontrol of plant diseases. Howell (1998) stated that research to date on the role of antibiotics in the biocontrol process has shown them to be important in some systems butless so in others.

Mycoparasitism by biocontrol agents of target plant pathogens is one of mechanisms of biocontrol of plant diseases. A number of fungal species are capable of parasitizing other fungal species, including many plant pathogens. For instance, *Pythium oligandrum* has been reported as an aggressive mycoparasite of many plant pathogens such as *Pythium ultimum* (Deacon, 1976). *T. virens* is parasitic to *R. solani* (Howell, 1981, 1987), and *Sclerotinia sclerotiorum* (Tu, 1980). *T. harzianum* has been demonstrated to parasitize *R. solani*, *Pythium aphanidermatum*, *Sclerotium rolfsii*, and *M. phaseolina* (Howell, 1990). The current study demonstrated that *T. virens* is parasitic to *M. cannonballus* which can cause severe root rot and vine decline disease of muskmelon. To our knowledge, this is the first report of parasitism of *M. cannonballus* by *T. virens*. The mycoparasitism of *M. cannonballus* by *T. virens* may be one of the mechanisms operating in biocontrol of disease caused by *M. cannonballus* on muskmelon. However, the role of mycoparasitism in biocontrol of the vine-decline pathogen, *M. cannonballus*, requires additional investigation.

The ability of an introduced biocontrol organism to colonize the host plant root is a major requirement for effective biocontrol of root diseases. A biocontrol agent applied by seed treatment must establish itself in the spermosphere of the germinating seed and then become established on the root system. In the current study, we demonstrated that *T. virens* colonized the muskmelon seedling hypocotyls, taproot and secondary roots during the experimental period (up to 28 days). By root tissue surface sterilization, we were able to distinguish between root surface colonization and root epidermis penetration by *T. virens*. Surface sterilization did not greatly reduce hypocotyl and taproot colonization by *T. virens*. This suggests that a significant amount of *T. virens* hyphae penetrated the epidermis of muskmelon seedling root system and colonized the cortex tissue of the hypocotyls and taproots. However, surface sterilization greatly reduced secondary root colonization by *T. virens* on muskmelon seedlings. There may be two possible reasons for this: 1) *T. virens* may not colonize the epidermis of the secondary roots; or 2) surface sterilization might kill both surface and internal *T. virens* hyphae, since the

secondary roots are much finer compared to the hypocotyls or taproots. Vine decline diseases of melons usually occur at 10 to 20 days prior to harvest, but infection of melon plant roots by vine decline pathogens generally occurs in the early stages of plant development (Bruton et al., 1998a, Miller et al., 1995). Therefore, an ideal biocontrol agent for controlling the vine decline diseases of melons should be a good root colonizer. It should also competitively proliferate, and establish its population on the rhizoplane and in the rhizosphere during the entire melon growing season. However, colonization of muskmelon plant roots by *T. virens* in the greenhouse may be much different from that in the fields.

In the present study, we also demonstrated that *T. virens* greatly reduced the colonization of muskmelon seedling hypocotyl and taproot by *M. cannonballus* after introduction of *T. virens* through seed treatment. The actual colonization by *M. cannonballus* of muskmelon hypocotyl and taproot which developed from *T. virens* treated seeds might be higher than what we detected using a single medium to isolate both fungi, since this medium is more suitable for growth of *T. virens*. *T. virens*, which grows faster than *M. cannonballus* on this assay medium, might restrict the growth of *M. cannonballus* from the hypocotyl and root tissues. Mechanisms regarding the reduction of *M. cannonballus* on the muskmelon seedling root system by *T. virens* need additional studies.

T. virens has been reported as a potential biocontrol agent for a number of plant root diseases (Elad et al., 1986; Howell, 1981, 1991; Lewis and Papavizas, 1985; Smith et al., 1990; Zhang et al., 1996b). The biocontrol by this fungus of root rot of muskmelon plants caused by *M. cannonballus* was examined twice in the greenhouse. Although disease severity in each of the tests was at a different level, the results from both experiments indicated that the disease was significantly reduced by both *T. virens* strains (TV-4 and TV-6). This is the first time that *T. virens* has been demonstrated to have potential in the biocontrol of diseases caused by *M. cannonballus* on muskmelon, although extensive research has been done with *T. virens* in the biocontrol of plant diseases caused by other fungal pathogens (Howell, 1981; Howell, 1991; Tu and Vaartaja, 1981; Lumsden and Locke, 1989). The data from experiment 1 demonstrates that differences exist between *T. virens* strains or isolates in the biocontrol of Monosporascus root rot of muskmelon. This suggests that superior *T. virens* strains might be found through testing a large number of isolates collected from different sources.

Although *T. virens* TV-6 (gliotoxin producer) showed stronger inhibition of the growth of *M. cannonballus in vitro* as compared with TV-4 which does not produce gliotoxin, the reduction of disease by TV-4 and TV-6 was the same in both the greenhouse experiments. This suggests that gliotoxin may not play a significant role in biocontrol of the disease caused by *M. cannonballus* on muskmelon. Consequently, other mechanisms such as mycoparasitism, competition and induction of plant systemic resistance may be important in biocontrol of *M. cannonballus* by *T. virens*. This needs further study.

An adverse effect of *T. virens* treatments on seed germination and initial seedling development of muskmelon was observed in one of the two greenhouse experiments. The seeds treated with preparations of *T. virens* TV-4 and TV-6 showed 9 to 16% less seedling emergence compared with control treatment in experiment 1, and slow initial development of seedlings. One of the metabolites produced by *T. virens* is viridiol, which is phytotoxic to the roots of crop plants such as cotton (Howell and Stipanovic, 1984). This negative effect of *T. virens* on muskmelon seedlings is likely due to the presence of viridiol in the *T. virens* preparations. The problem might be solved by the following approaches: smaller amounts of *T. virens* preparations may be used to treat muskmelon seeds; viridiol deficient mutants of *T. virens* may be developed and used for making inoculum preparations; and viridiol inhibitors may be applied in cultures of *T. virens* to reduce or eliminate the viridiol during the inoculum preparation process (Howell and Stipanovic, 1994).

Although positive test results on the biocontrol of *M. cannoballus* by *T. virens* under greenhouse conditions was observed, results may be different under field conditions. Field tests on the biocontrol of vine decline diseases on muskmelon are currently being conducted. While we are still in the early stages of evaluating *T. virens* for biocontrol of *M. cannonballus* root rot and vine decline disease on muskmelon, our results suggest that biocontrol may provide a new strategy for the integrated management of vine decline diseases of cucubits.

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LITERATURE CITED

Bruton, B. D. 1998. Soilborne diseases in Cucurbitaceae: pathogen virulence and host resistance, p. 143-146. In: McCreight, J. (ed.). Cucurbitaceae '98, Amer. Soc. Hort. Sci. Press, Alexandria, Va.

Bruton, B. D., V. M. Russo, J. Garcia-Jimenez, and M. E. Miller. 1998a. Carbohydrate partitioning, cultural practices, and vine decline diseases of cucurbits, p.189-200. In: McCreight, J. (ed.). Cucurbitaceae '98. Amer. Soc. Hort. Sci. Press, Alexandria, VA.

Bruton, B. D., Zhang, J. X., Howell, C. R., and Miller, M. E. 1998b. Evaluation of *Trichoderma virens* as a potential biocontrol agent of *Monosporascus cannonballus* in muskmelon. Phytopathology 88:S12. (Abstr)

Bruton, B. D., J. Fletcher, S. D. Pair, M. Shaw, and H. Sittertz-Bhathar. 1998c. Association of a phloem-limited bacterium with yellow vine disease in cucurbits. Phytopathology 82:512-520.

Chet, I. 1987. Innovative approaches to plant disease control. John Wiley & Sons, New York.

Cook, R. J., and K. F. Baker. 1983. The nature and practice of biological control of plant pathogens. The American Phytopathological Society, St. Paul, MN.

Deacon, J. W. 1976. Studies of *Pythium oligandrum*, an aggressive parasite of other fungi. Trans. British Mycol.

Society 66:383-391.

Elad, Y., Y. Zvieli, and I. Chet. 1986. Biological control of *Macrophomina phaseolina* (Tassi) Goid by *Trichoderma harzianum*. Crop Prot. 5:288-292.

El-Hendawy, H. H., I. M. Zeid, and Z. K. Mohamed. 1998. The biological control of soft rot disease in melon caused by *Erwinia carotovora* subs. *carotovora* using *Pseudomononas fluorescens*. Microbiol. Res. 153:55-60.

Hassouna, M. G., M. A. M. El-Saedy, and H. M. A Saleh. 1998. Biocontrol of soil-borne plant pathogens attacking cucumber (*Cucumis sativus*) by rhizobacteria in a semiarid environment. Arid Soil Res. Rehabilitation 12:345-357.

Howell, C. R. 1981. Effect of *Gliocladium virens* on *Pythium ultimum*, *Rhizoctonia solani*, and damping-off of cotton seedlings. Phytopathology 72:496-498.

Howell, C. R. 1987. Relevance of mycoparasitism in the biological control of *Rhizoctonia solani* by *Gliocladium virens*. Phytopathology 77:992-994.

Howell, C. R. 1990. Fungi as biological control agents, p. 257-286. In: J. P. Nakas, and C. Hagedorn, (eds.). Biotechnology of Plant -Microbe Interactions. McGraw-Hill Publishing Company, New York.

Howell, C. R. 1991. Biological control of Pythium damping-off of cotton with seed-coated with preparation of *Gliocladium virens*. Phytopathology 81:738-741.

Howell, C. R. 1998. The role of antibiosis in biocontrol, p.173-184. In: G. E. Harman and C. P. Kubicek, (eds.). *Trichoderma* and *Gliocladium* Volume 2, Taylor and Francis, London.

Howell, C. R., and R. D. Stipanovic. 1984. Phytotoxicity to crop plants and herbicidal effects on weeds of viridiol produced by *Gliocladium virens*. Phytopathology 74:1346-1349.

Howell, C. R. and R. D. Stipanovic. 1994. Effect of sterol biosynthesis inhibitors on phytotoxin (viridiol) production by *Gliocladium virens* in culture. Phytopathology 84:969-972.

Howell, C. R., R. D. Stipanovic, and R. D. Lumsden. 1993. Antibiotic production by strains of *Gliocladium virens* and its relation to the biocontrol of cotton seedling diseases. Biocontrol Sci. Technol. 3:435-441.

Larkin, R. P., D. L. Hopkins, and F. N. Martin. 1993. Effect of successive watermelon plantings on *Fusarium oxysporum* and other microorganisms in soils suppressive and conducive to Fusarium wilt of watermelon. Phytopathology 83:1097-1105.

Lewis, J. A., and G. C. Papavizas. 1985. Effect of mycelial preparations of *Trichoderma* and *Gliocladium* on populations of *Rhizoctonia solani* and incidence of damping-off. Phytopathology 75:812-817.

Liu, L., J. W. Kloepper, and S. Tuzun. 1995a. Induction of systemic resistance in cucumber by plant growth-promoting rhizobacteria: Duration of protection and effect of host resistance on protection and root colonization. Phytopathology 85:1064-1068.

Liu, L., J. W. Kloepper, and S. Tuzun. 1995b. Induction of systemic resistance in cucumber against bacteria angular leaf spot by plant growth-promoting rhizobacteria. Phytopathology 85:843-847.

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

Lumsden, R. D., and J. C. Locke. 1989. Biological control of damping-off caused by *Pythium ultimum* and *Rhizoctonia solani* with *Gliocladium virens* in soilless mix. Phytopathology 79:361-366.

Lumsden, R. D., C. J. Ridout, W. E. Vendemia, R. M. Harrison, and J. F. Waters. 1992. Characterization of major secondary metabolites produced in soilless mix by a formulated strain of the biocontrol fungus *Gliocladium virens*. Can. J. Microbiol. 38:1274-1280.

Maloy, O. C. 1993. Biological control, p.135-249. In: O. C. Maloy, (ed.). Plant Disease Control: Principles and Practice, John Wiley & Sons, Inc., New York.

Martyn, D. R., B. R. Lovic, and M. E. Miller. 1995. Monsporascus root rot/vine decline of melons - A case study, p.36-43. In: G. Lester and J. Dunlap, (eds.). Cucurbitaceae'94, Gateway Printing, TX.

Martyn, R, D., and M. E. Miller. 1996. Monosporascus root rot and vine decline, an emerging disease of melons worldwide. Plant Dis. 80:716-725.

Migheli, Q., L. Gonzalez-Candelas, L. Dealessi, A. Camponogara, and D. Ramon-Vidal. 1998. Transformants of *Trichoderma longibrachiatum* overexpressing the ß-1,4-endoglucanase gene egl1 show enhanced biocontrol of *Pythium ultimum* on cucumber. Phytopathology 88: 673-677.

Miller, M. E., R. D. Martyn, B. R. Lovic, and B. D. Bruton. 1995. An overview of vine decline diseases of melons, p.31-35. In: G. Lester and J. Dunlap, (eds.). Cucurbitaceae'94, Gateway Printing, TX.

Mukherjee, K., and B. Sen. 1998. Biological control of Fusarium wilt of muskmelon by formulations of *Aspergillus niger*. Israel J. Plant Sci. 46:67-72.

Naik, M. K., and B. Sen. 1993. Effectiveness of biocontrol agents against a spectrum of *Fusarium* isolates causing wilt of watermelon. Indian J. Plant Prof. 1:19-22.

Singh, P. P., Y. C. Shin, C. S. Park, and Y. R. Chung. 1999. Biological control of Fusarium wilt of cucumber by chitinolytic bacteria. Phytopathology 89:92-99.

Sivan, A., and I. Chet. 1986. Biological control of *Fusarium* spp. in cotton, wheat and muskmelon by *Trichoderma harzianum*. J. Phytopathology 116:39-47.

Smith, V. L., W. F. Wilcox, and G. E. Harman. 1990. Potential for Biological control of Phytophthora root and crown rots of apple by *Trichoderma* and *Gliocladium* spp. Phytopathology 80:880-885.

Sneh., B., and M. Ichielevich-Auster. 1998. Induced resistance of cucumber seedlings caused by some non-pathogenic *Rhizoctonia* (np-R) isolates. Phytoparasitica 26:27-38.

Tu, J. C. 1980. *Gliocladium virens*, a destructive mycoparasite of *Sclerotinia sclerotiorum*. Phytopathology 70:670-674.

Tu, J. C., and V. Vaartaja. 1981. The effect of the hyperparasite *Gliocladium virens* on *Rhizoctonia* solani and rhizoctonia rot of white beans. Can. J. Bot. 59:22-27.

Wei, G., J. W. Kloepper, and S. Tuzun. 1996. Induced systemic resistance to cucumber diseases and induced plant growth by plant growth-promoting rhizobacteria under field conditions. Phytopathology 86:221-224.

Zhang, W., W. A. Dick, and H. A. J. Hoitink. 1996a. Compost-induced systemic acquired resistance in cucumber to Pythium root rot and anthracnose. Phytopathology 86:1066-1070.

Zhang, W., D. Y. Han, W. A. Dick, K. R. Davis, and H. A. J. Hoitink. 1998. Compost and compost water extract-induced systemic acquired resistance in cucumber and arabidopsis. Phytopathology 88:450-455.

Zhang, J. X., C. R. Howell, and J. L. Starr. 1996b. Suppression of *Fusarium* colonization of cotton roots and Fusarium wilt by seed treatments with *Gliocladium virens* and *Bacillus subtilis*. Biocontrol Sci. Technol. 6:175-187.