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# Lack of antagonism between the biocontrol agent *Gliocladium virens** and vesicular arbuscular mycorrhizal fungi

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SUMMARY

The effect of the fungal biocontrol agent *Gliocladium virens* Miller, Giddens & Foster on the colonization of cucumber by the VA mycorrhizal fungi *Glomus etunicatum* Becker & Gerdemann and *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe was investigated. Inoculum of *G. virens*, grown on wheat bran or peatmoss-Czapek substrate, was added to soil artificially infested with *Pythium ultimum* Trow. Both damping-off of cucumber and pathogen population density were reduced in treatments with *G. virens*. Inoculum of *G. virens* was also added to soils containing increasing propagule densities of *Glomus etunicatum* or *Glomus mosseae*. After one week of incubation, the soils were planted with cucumber seeds, and the percent mycorrhizal colonization of roots was determined after 12 d. When grown on wheat bran, *G. virens* was phytotoxic and increased mycorrhizal colonization by *Glomus etunicatum*. However, *G. virens* had no effect on *Glomus etunicatum* when added as peatmoss-Czapek inoculum. Amendment of soil with peatmoss-Czapek inoculum of *G. virens* reduced colonization by *Glomus mosseae* when compared to a non-amended control. Inoculum colonized by other fungal biocontrol agents and sterilized with propylene oxide gave a similar reduction, suggesting that the substrate, not *G. virens*, was responsible for the reduction in VA mycorrhizal colonization. This evidence suggests that the fungal biocontrol agent *G. virens* does not have a detrimental impact on these VA mycorrhizal fungi, and would be compatible if applied as a dual-inoculum.

Key words: Vesicular-arbuscular mycorrhizal fungi, *Gliocladium virens*, cucumber, *Glomus etunicatum*, *Glomus mosseae*.

## INTRODUCTION

The control of soil-borne diseases with fungal biocontrol agents has elicited considerable recent research interest. Increased concern about the environmental impacts of agrochemicals in soil and ground water and the lack of effective chemical controls for many soil-borne diseases has stimulated this trend. One of the most widely studied genera of fungal biocontrol agents is *Gliocladium* (Papavizas, 1985), which has experimentally controlled Pythium damping-off diseases (Howell, 1982), Rhizoctonia damping-off (Lewis & Papavizas, 1985) and damping-off by *Sclerotium rolfsii* (Papavizas & Lewis, 1989). *Gliocladium virens* antagonizes fungal pathogens by mycoparasitism (Papavizas & Collins, 1990) and by production of anti-fungal compounds (Howell & Stipanovic, 1983).

Despite this increasing interest in the development and commercialization of biological control strategies, very few studies have investigated the possible interactions between fungal biocontrol agents and vesicular-arbuscular (VA) mycorrhizal fungi. These mycorrhizal fungi form a symbiotic association with the roots of most crop plants, resulting in increased phosphorus nutrition (Stribley, 1987), drought tolerance (Nelson, 1987), and disease resistance (Schenck, 1987). If biocontrol agents are antagonistic to the VA mycorrhizal symbiosis, then these benefits would be lost. However, fungal biocontrol agents might also enhance mycorrhizal colonization, by making the root more susceptible to colonization, or by controlling mycoparasites and other antagonists of the VA mycorrhizal fungi. Meyer & Linderman (1986) and Von Alten, Lindeman & Schönbeck, (1989) have demonstrated a stimulation of mycorrhizal infection with bacterial biocontrol agents.

Mycorrhizal fungi may also influence the fungal biocontrol agents. These interactions may be direct between organisms, or indirect as mediated by the

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host plant. Mycorrhizal fungi significantly alter the physiology of the root, especially root exudation, thus altering the composition of microorganisms in the rhizosphere. The influence of mycorrhizal fungi on the microbial composition of the rhizosphere is called the 'mycorrhizosphere effect' (Linderman, 1988) and is reviewed by Paulitz & Linderman (1991) and Linderman & Paulitz (1990). For example, mycorrhizal fungi might compete with the biocontrol agents for carbon, thus reducing the population of the latter in the rhizosphere (Paulitz & Linderman, 1989). Reduction of root damage by biocontrol agents might enhance the efficiency of the VA mycorrhizal symbiosis. Afek, Menge & Johnson (1990) demonstrated significantly enhanced VA mycorrhizal colonization of pepper and cotton when *Pythium* damage was reduced with the fungicide metalaxyl.

The purpose of this study was to examine the effect of *G. virens* on colonization of cucumber roots by VA mycorrhizal fungi, under conditions where the fungal biocontrol agent could antagonize a plant pathogen.

## MATERIALS AND METHODS

### *Soil*

For greenhouse experiments, a 1:1 mixture of Willamette sandy loam soil and river sand was used (Paulitz & Linderman, 1989). To eliminate the native species of VA mycorrhizal fungi, the soil-sand mix was pasteurized at 70 °C for 45 min. A water filtrate from non-pasteurized soil was added back to pasteurized soil to reestablish the native microflora. This filtrate was prepared by mixing 200 g of non-pasteurized soil with 2 l of water, filtering the soil-water mix through a 38 $\mu$m sieve to remove spores of VA mycorrhizal fungi, and mixing the filtrate with 40 l of pasteurized soil. The filtrate-amended pasteurized soil mix was incubated in open containers in the greenhouse for 1 month, and was periodically moistened.

### *Biological materials*

The fungal biocontrol agent was *Gliocladium virens* Miller, Giddens & Foster strain GV-P (GV) (Howell, 1982). This fungus was initially cultured on potato-dextrose agar, and was stored in sterile soil. The plant pathogen was *Pythium ultimum* Trow isolate N1 (Lifshitz, Sneh & Baker, 1984), which was maintained on water agar.

Two species of VA mycorrhizal fungi were used: *Glomus etunicatum* Becker & Gerdemann and *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe. The inoculum of *Glomus etunicatum*, a mixture of sand, roots, and spores, was obtained from Native Plants Incorporated (NPI), Salt Lake City, Utah. Pot culture inoculum of *G. mosseae* was obtained from mycorrhizal greenhouse-grown pigeon pea.

The plant host used in all experiments was cucumber (*Cucumis sativus* L., 'Straight Eight').

### *Fungal inoculum*

Inoculum of *G. virens* was grown on two substrates, wheat bran and peatmoss-Czapek. Wheat bran substrate was prepared by passing the bran (commercial-grade chicken feed) through a 2-mm sieve. Equal volumes of water and bran were mixed together by hand, and 0.94 l Mason jars were filled halfway with the substrate. Each jar was sealed with a canning lid in which two 6-mm diameter holes were punched. A 70-mm diameter filter disc (Fungi Imperfecti, P.O. Box 7634, Olympia, WA 98507) was placed on the inside of the lid before sealing. This permitted air exchange while maintaining sterility. The substrate was autoclaved for 1 h on two consecutive days. *Gliocladium virens* was grown on PDA under fluorescent lights (20 h light, 4 h dark) at room temperature. Ten ml of sterile water was added to two-week-old fungal cultures, and conidia were dislodged with a sterile glass rod. The substrate was inoculated with 5 ml of the conidial suspension. The cultures were shaken and incubated at 26 °C for 3 d, and placed in a cold-room at 4 °C, before the fungus had sporulated.

Peatmoss-Czapek inoculum was prepared by sieving Canadian sphagnum peatmoss moss through a 2-mm sieve. Mason jars (0·94 l) were filled with 50 ml of peatmoss and 100 ml of Czapek broth (Difco). The jars were sealed and autoclaved, and the substrate was inoculated as described above. The inocula were incubated at 26 °C for three weeks, air-dried, and ground in a Wiley mill with a 1-mm sieve. Another control treatment (PO) was prepared by treating 50 g of a 1:1:1:1 (w/w/w/w) mixture of the inocula of *G. virens* and three other fungi with propylene oxide. The three other fungi were *Talaromyces flavus* (Klocker) Stolk & Samson strain Tf-1 (Marois, Fravel & Papivazas, 1984), *Trichoderma harzianum* Rifai WT-6-6 (Papavizas, Lewis & Abd-El Moity, 1982), and *T. harzianum* T-95 (Chang, Chang & Baker, 1986). The inoculum was moistened with 10 ml of distilled water and placed in a 0·94 l Mason jar with a cotton ball saturated with 4 ml of propylene oxide. The jar was sealed for 2 d and opened under a fume hood for 1 d. The sterilized inoculum was spread on a greenhouse bench and air dried for two more days to remove all traces of propylene oxide. A sample of the sterilized inoculum was sprinkled on PDA to verify sterility.

Soil inoculum of *Pythium ultimum* was prepared according to the methods of Paulitz & Baker (1987). For infested treatments, inoculum of *P. ultimum* was mixed in the soil to give a final population density of 54 cfu $g^{-1}$.

Inoculum of *G. virens* was hand-mixed with the greenhouse soil at 1 % (w/w). Population densities of

*G. virens* in the soil were determined by dilution-plating on TME selective medium (Papavizas & Lumsden, 1982). Populations of *P. ultimum* were determined by dilution plating on Pythium selective medium (Mircetich & Kraft, 1973).

### *Biological control of* Pythium *damping-off of cucumber by* Gliocladium virens

In the biological control experiment, four treatments were used: non-infested control (no *P. ultimum* or *G. virens*), infested control (*P. ultimum* only), *G. virens* (wheat bran) + *P. ultimum*, and *G. virens* (peatmoss-Czapek) + *P. ultimum*. Each treatment was placed in ten 6·5-cm plastic pots (150 g per pot). Each pot was planted with ten cucumber seeds, and placed in a growth chamber (12 h light-dark cycle) where the soil temperature was maintained at 23 °C. Pots were watered daily with distilled water. After 7 d, the emergence of seedlings was recorded for each treatment. Soil samples from five pots in each treatment were taken at the beginning and end of the experiment. Serial dilutions of a soil suspension from each sample were plated on TME and Pythium-selective media.

### *Effect of* Gliocladium virens *on VA mycorrhizal fungi*

To determine the effect of *G. virens* on mycorrhizal colonization, the propagule density-root colonization relationships were compared in treatments with and without *G. virens*. Six propagule densities of *Glomus etunicatum* or *Glomus mosseae* were prepared by diluting pot culture-sand inoculum with filtrate-amended pasteurized soil. The propagule densities in the soil were adjusted to 18·60, 9·30, 4·65, 2·33 and 1·16 spores of *Glomus etunicatum* $g^{-1}$, and 4·68, 2·34, 1·7, 0·58, 0·29, 0·15 spores of *Glomus mosseae* $g^{-1}$. Inoculum of *G. virens* was applied to the soil, as described in *Fungal inoculum*. Soil from each treatment was placed in ten 1·5-cm diameter X 16-cm plastic tubes (Cone-Tainer Co., Canby, OR) (70 g $tube^{-1}$). The tubes were placed in the greenhouse in controlled 'air bath' temperature boxes, which maintained root zone temperatures at 26 °C. Lighting was supplemented with high-pressure sodium-vapor lamps (350–480 $\mu E\ m^{-2}\ s^{-1}$). The soil was watered daily, and after one week of incubation, one cucumber seed was planted in each tube.

After 12 d, the plants were removed from the tubes, the roots were washed, and placed in water. The roots were cleared and stained according to the methods of Phillips & Hayman (1970). The percent mycorrhizal colonization was determined with the line intersect method, and mean colonization was calculated for each fungal treatment- VA mycorrhiza inoculum density. Linear regressions were performed on the root colonization-inoculum density data.

### *Statistical analyses*

All experiments were performed twice. Data from the biological control experiment were analyzed by one-way analysis of variance and Duncan's mean separation test. Linear regression models were constructed from the inoculum density-root colonization data. All correlations were determined to be significant, based on the coefficient of correlation and the degrees of freedom. Statistical differences in the regression coefficients were determined from 95% confidence limits calculated from standard errors. Confidence limits around the predicted Y (ˆY) values from the regression were also calculated, and the ˆY values of measured X values for each fungal biocontrol agent treatment were compared to the ˆY values of the control treatment.

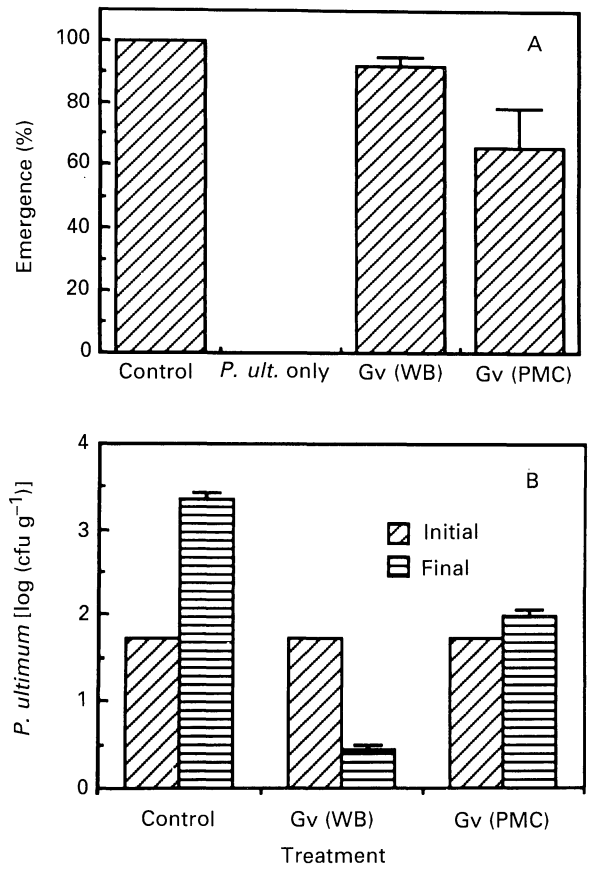


**Fig. 1.** Biological control of *Pythium ultimum* by *Gliocladium virens*. A. Effect of *G. virens* on emergence of cucumber in *Pythium*-infested soil. Emergence expressed as a percent of non-infested control. P. ult. only = *P. ultimum* only, added at 54 cfu $g^{-1}$. Gv (WB) = *G. virens*-wheat bran inoculum. Gv (PMC) = *G. virens*-peatmoss-Czapek inoculum. B. Effect of *G. virens* on population densities of *Pythium ultimum*. Bars represent standard errors. Initial = population density of *P. ultimum* at start of experiment; final = population density 7 d later.

## RESULTS

### *Biological control of* Pythium *damping-off of cucumber by* Gliocladium virens

Treatment of soil with inocula of *G. virens* grown on two different substrates gave significant control of Pythium damping-off (Fig. 1A). Both inocula also reduced increases in the population density of *P. ultimum* (Fig. 1B). In the unamended control, the pathogen populations increased 1·5 log units, but in the *G. virens*-wheat bran treatment, the populations were reduced by 1·3 log units. In the wheat bran treatment, the population density of *G. virens* increased from 4·5 to 6·7 log cfu $g^{-1}$ during the course of the experiment. In the peatmoss-Czapek treatment, *G. virens* was added at 6·5 log cfu $g^{-1}$, but did not increase over the 7 d of the experiment.

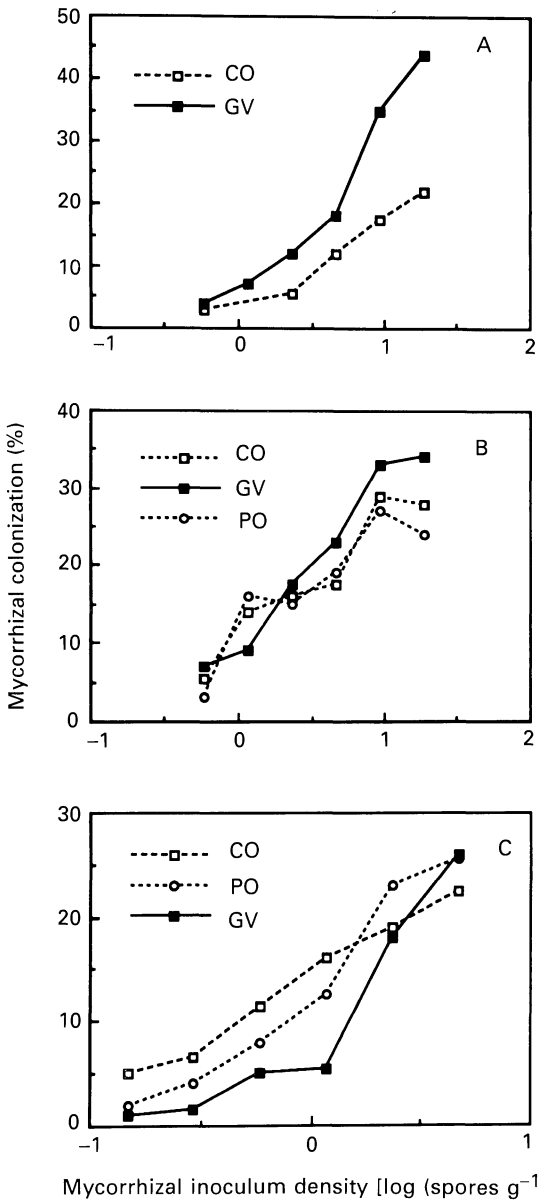


**Fig. 2.** Effect of *Gliocladium virens* on root colonization of cucumber by *Glomus etunicatum* and *Glomus mosseae*. A. Colonization by *Glomus etunicatum* in soil amended with *Gliocladium virens* (GV) inoculum grown on wheat bran, compared to non-amended control treatment (CO). B. Colonization by *Glomus etunicatum* in soil amended with *Gliocladium virens* (GV) inoculum grown on peatmoss-Czapek, compared to unamended treatment (CO) or treatment amended with inoculum sterilized by propylene oxide (PO). C. Colonization by *Glomus mosseae* in soil amended with *Gliocladium virens*-inoculum grown on peatmoss-Czapek, compared to CO or PO controls. Treatments were planted with cucumber one week after fungal biocontrol agents were added, and cucumber roots were harvested 12 d after planting.

### *Effect of* Gliocladium virens *on VA mycorrhizal fungi*

When *G. virens* was added to the soil as wheat bran inoculum (GV treatment), colonization by *Glomus etunicatum* was stimulated, when compared to the non-amended control (Fig. 2A). The ^Y values of this treatment were significantly higher than the ^Y values of the control treatment at all inoculum densities except the lowest. The regression slope of the GV treatment was also significantly higher than the control. However, GV-treated plants were severely stunted, and showed phytotoxic effects, possibly from fungal toxins in the inoculum. When *G. virens* was added as peatmoss-Czapek inoculum, no significant effect on colonization by *G. etunicatum* was observed, when compared to the treatment amended with propylene-oxide killed inoculum (PO) (Fig. 2B). Also, no differences were detected between the non-amended (control) and PO treatments.

Colonization of cucumber by *Glomus mosseae* was significantly reduced by all treatments with peatmoss-Czapek inoculum (Fig. 2C). The addition of the peatmoss substrate reduced the pH of the soil from 5·9 to 5·5, which may have inhibited mycorrhizal colonization. However, no significant differences were detected between the *G. virens* treatment and the PO treatment.

## DISCUSSION

Despite the well-documented deleterious effects of *G. virens* on plant pathogenic fungi, no consistent detrimental impacts on VA mycorrhizal fungi were observed. Kohl & Schlosser (1989) reported similar results, finding that *Trichoderma hamatum* and *T. harzianum* did not inhibit the colonization of maize by *Glomus etunicatum*. Similarly, Calvet, Pera & Barea (1988) observed that *Trichoderma* spp. did not inhibit the spore germination of *Glomus mosseae*. In our experiments, the biocontrol agent was added to the soil in high populations with a food base, and was in contact with the mycorrhizal fungi 7 d before the host root was present. In the experiments with wheat-bran inoculum, the population densities of *G. virens* increased 20 times, although this may have been due to sporulation. These conditions should

have been optimal for the expression of any negative interaction. However, no negative effects were detected. In one case, (Fig. 2A), *G. virens* actually increased colonization when expressed as percent of root colonized. However, this may have been an artifact resulting from a slower-growing root system, since this treatment was phytotoxic. Subsequent work suggests that a phytotoxic substance is produced when *G. virens* is grown on wheat bran, since *G. virens*-wheat bran inoculum that was sterilized with propylene oxide showed the same effect (unpublished results). This phytotoxin may be viridiol, a compound with herbicidal activity that is produced by *G. virens* (Howell & Stipanovic 1984; Jones, Lanini & Hancock, 1988). This fungal toxin may have made the root more susceptible to colonization by the mycorrhizal fungus.

In one experiment, the edaphic conditions had a greater influence on the colonization of cucumber than the fungal biocontrol agent. Colonization by *Glomus mosseae* was inhibited by the addition of the peatmoss-Czapek inoculum, even when the inoculum was sterilized (Fig. 2C). This inhibition may be due to the reduction in pH caused by the addition of the peatmoss inoculum at 1 % (w/w). *Glomus mosseae* was shown to be inhibited in lower pH soils (Davis, Young & Linderman, 1983). This points out the difficulty of interpreting experiments where fungi are grown on inoculum with natural substrates used as a food base or carrier. When added at high levels, these substrates may also influence mycorrhizal colonization. The use of propylene oxide-sterilized inoculum as a control would overcome this problem, unless the inoculum had already accumulated large amounts of fungitoxic compounds.

There are several possible reasons why no detrimental effects were seen. VA mycorrhizal fungi might not be sensitive to antagonism by *G. virens*. VA mycorrhizal fungi may be tolerant of anti-fungal compounds produced by *G. virens* and may not be susceptible to mycoparasitism. Most of the literature on mycoparasitism of VA mycorrhizal fungi have dealt with spore parasites, and very few hyphal parasites have been observed. Spores of VA mycorrhizas contain large food reserves, and germ tubes can grow long distances before colonizing a host root. Since VA mycorrhizal fungi are incapable of saprophytic growth during this stage, they may not be affected by microbial competition. Once the VA mycorrhizal fungus has established a symbiosis with the host plant, it derives its nutrition from the host cells via hyphae and arbuscles, and might be immune to antagonism in the rhizosphere. In this experiment, we only measured the effects of *G. virens* on primary colonization, but *G. virens* might still influence secondary colonization and growth of external hyphae, both of which occur in the rhizosphere.

Very little is known about the interactions between VA mycorrhizal fungi and biological control agents. To our knowledge, this is the first study of interactions between *G. virens* and VA mycorrhizal fungi. In order for microbial pesticides to be registered by government regulatory agencies, the effect of biocontrol agents on non-target beneficial microorganisms such as mycorrhizal fungi must be ascertained. Our results suggest that the fungal biocontrol agent *G. virens* might be compatible with VA mycorrhizal fungi, and could be applied in the same inoculum to further enhance plant growth. However, additional research is still needed, using combinations of these and other VA mycorrhizal fungi, fungal biocontrol agents, and pathogens. This future research should focus on experimental plant systems which respond to all three of the microbial components. Only then can possible interactions between these microorganisms and with the plant be observed. Compatible combinations identified from studies such as those described in this paper could be introduced into this more complex system, and could be screened for further enhancement of biocontrol.

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## REFERENCES

Afek, U., Menge, J. A. & Johnson, E. L. V. (1990). Effect of *Pythium ultimum* and metalaxyl treatments on root length and mycorrhizal colonization of cotton, onion, and pepper. *Plant Disease* **74**, 117–120.

Calvet, C., Pera, J. & Barea, J. M. (1988). Interactions of *Trichoderma* spp. with *Glomus mosseae* and two wilt pathogenic fungi. *2nd European Symposium on Mycorrhizae*, August 14–20, Prague.

Chang, Y. C., Chang, Y. C. & Baker, R. (1986). Increased growth of plants in the presence of the biological control agent *Trichoderma harzianum*. *Plant Disease* **70**, 145–148.

Davis, E. A., Young, J. L. & Linderman, R. G. (1983). Soil lime level (pH) and VA-mycorrhiza effects on growth responses of sweetgum seedlings. *Soil Science Society of America Journal* **47**, 251–256.

Howell, C. R. (1982). Effect of *Gliocladium virens* on *Pythium ultimum*, *Rhizoctonia solani*, and damping-off of cotton seedlings. *Phytopathology* **72**, 496–498.

Howell, C. R. & Stipanovic, R. D. (1983). Gliovirin, a new antibiotic from *Gliocladium virens*, and its role in the biological control of *Pythium ultimum*. *Canadian Journal of Microbiology* **29**, 321–324.

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

Jones, R. W., Lanini, W. T. & Hancock, J. G. (1988). Plant growth response to the phytotoxin viridiol produced by the fungus *Gliocladium virens*. *Weed Science* **36**, 683–687.

Kohl, J. & Schlosser, E. (1989). Effect of two *Trichoderma* spp. on the infection of maize roots by vesicular-arbuscular mycorrhiza. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* **96**, 439–443.

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

LIFSHITZ, R., SNEH, B. & BAKER, R. (1984). Soil suppressiveness to a plant pathogenic *Pythium* species. *Phytopathology* **74**, 1054–1061.

LINDERMAN, R. G. (1988). Mycorrhizal interactions with the rhizosphere microflora: the mycorrhizosphere effect. *Phytopathology* **78**, 366–371.

LINDERMAN, R. G. & PAULITZ, T. C. (1990). Mycorrhizal-rhizobacterial interactions. In: *Biological Control of Soil-Borne Plant Pathogens*, (Ed. by D. Hornby). CAB International, Wallingford, UK.

MAROIS, J. J., FRAVEL, D. R. & PAPAVIZAS, G. C. (1984). Ability of *Talaromyces flavus* to occupy the rhizosphere and its interaction with *Verticillium dahliae*. *Soil Biology and Biochemistry* **16**, 387–390.

MEYER, J. R. & LINDERMAN, R. G. (1986). Response of subterranean clover to dual inoculation with vesicular-arbuscular mycorrhizal fungi and a plant growth-promoting bacterium, *Pseudomonas putida*. *Soil Biology and Biochemistry* **18**, 185–190.

MIRCETICH, S. M. & KRAFT, J. M. (1973). Efficiency of various selective media in determining *Pythium* populations in soil. *Mycopathologia et Mycologia Applicata* **50**, 151–161.

NELSON, C. E. (1987). The water relations of vesicular-arbuscular mycorrhizal systems. In: *Ecophysiology of VA Mycorrhizal Plants*, (Ed. by G. R. Safir), pp. 71–92. CRC Press, Boca Raton, Florida, USA.

PAPAVIZAS, G. C. & COLLINS, D. J. (1990). Influence of *Gliocladium virens* on germination and infectivity of sclerotia of *Sclerotium rolfsii*. *Phytopathology* **80**, 627–630.

PAPAVIZAS, G. C. & LEWIS, J. A. (1989). Effect of *Gliocladium* and *Trichoderma* on damping-off and blight of snapbean caused by *Sclerotium rolfsii* in the greenhouse. *Plant Pathology* **38**, 277–286.

PAPAVIZAS, G. C., LEWIS, J. A. & ABD-EL MOITY, T. H. (1982). Evaluation of new biotypes of *Trichoderma harzianum* for tolerance to benomyl and enhanced biocontrol capabilities. *Phytopathology* **72**, 126–132.

PAPAVIZAS, G. C. & LUMSDEN, R. D. (1982). Improved medium for isolation of *Trichoderma* spp. from soil. *Plant Disease* **66**, 1019–1020.

PAPAVIZAS, G. C. (1985). *Trichoderma* and *Gliocladium*: biology, ecology and potential for biocontrol. *Annual Review of Phytopathology* **23**, 23–54.

PAULITZ, T. C. & BAKER, R. (1987). Biological control of Pythium damping-off of cucumbers with *Pythium nunn*: Population dynamics and disease suppression. *Phytopathology* **77**, 335–340.

PAULITZ, T. C. & LINDERMAN, R. G. (1989). Interactions between fluorescent pseudomonads and VA mycorrhizal fungi. *New Phytologist* **113**, 37–45.

PAULITZ, T. C. & LINDERMAN, R. G. (1991). Mycorrhizal interactions with soil organisms. In: *Handbook of Applied Mycology. Vol. 1. Soil and Plants*. (Ed. by D. K. Arora, Rai, K. G. Mukerji & G. Knudsen) Marcel Dekker, New York (in the press).

PHILLIPS, J. M. & HAYMAN, D. S. (1970). Improved procedure for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* **55**, 158–161.

SCHENCK, N. C. (1987). Vesicular-arbuscular mycorrhizal fungi and the control of fungal root diseases. In: *Innovative Approaches to Plant Disease Control*. (Ed. by I. Chet), pp. 179–191. John Wiley & Sons, New York.

STRIBLEY, D. P. (1987). Mineral nutrition. In: *Ecophysiology of VA Mycorrhizal Plants*, (Ed. by G. R. Safir), pp. 59–70. CRC Press, Boca Raton, Florida, USA.

VON ALTEN, H., LINDEMANN, A. & SCHÖNBECK, F. (1989). Increasing VA-mycorrhization with applications of rhizosphere bacteria. *Beltsville Symposium XIV. The Rhizosphere and Plant Growth*. (abstract)