Potential for Biological Control of Phytophthora Root and Crown Rots of Apple by *Trichoderma* and *Gliocladium* spp.

V. L. Smith, W. F. Wilcox, and G. E. Harman

Postdoctoral associate, associate professor, and professor, Departments of Plant Pathology and Horticultural Science, Cornell University, New York State Agricultural Experiment Station, Geneva 14456.

Present address of first author: Department of Plant Pathology and Ecology, Connecticut Agricultural Experiment Station, P. O. Box 1106, New Haven 06504.

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ABSTRACT

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A system was developed to identify isolates of *Trichoderma* and *Gliocladium* with potential for biocontrol of *P. cactorum*. Seedlings from open-pollinated McIntosh apples were grown in potting mix infested with both *Phytophthora cactorum* and candidate biocontrol fungi (*Trichoderma* and *Gliocladium* spp.). After 14 days of growth and a 72-hr flooding interval, significant reductions in root damage and increases in plant weight, compared with seedlings exposed to *P. cactorum* alone, were observed with some isolates of biocontrol candidates. Presence of both the pathogen and biocontrol fungi in the potting mix significantly influenced plant weight and the incidence of hypocotyl infection, and chi-square analysis indicated a lack of independence between qualitative root damage ratings and the presence of biocontrol agents. In the absence of *P. cactorum*, growth of apple seedlings was significantly increased by the presence of some isolates of biocontrol fungi. The system developed will aid in the identification of isolates of *Trichoderma* and *Gliocladium* spp. with potential for biocontrol of *P. cactorum*.

Phytophthora root and crown rots are primary causes of decline and death of deciduous fruit trees in North America. *Phytophthora cactorum* (Leb. & Cohn) Schroet., *P. cambivora* (Petri) Buisman, *P. cryptogea* Pathybridge & Lafferty, *P. megasperma* Dreschler, *P. drechsleri* Tucker, *P. parasitica* Dastur, and a number of unidentified *Phytophthora* spp. have been documented as causal agents of these diseases on apple trees (14,19,20). Of these species, *P. cactorum* has the widest geographical range of occurrence on apple, the longest history of association with this host, and has been among the most virulent in comparative pathogenicity tests (14,19). Current control recommendations (e.g., 31) consist of an integrated approach, including the selection and/or modification of planting site and adoption of subsequent cultural practices to minimize episodes of soil saturation near the tree crown, avoidance of rootstocks highly susceptible to these pathogens, and the use of systemic fungicides in high-risk situations. Nevertheless, disease outbreaks are common where planting site availability, selection of susceptible rootstocks to maximize other desirable horticultural traits or reduction of economic inputs lead to the omission of one or more of these components. It would therefore be desirable to identify an additional component that could be incorporated into the present management system to increase the degree of control potentially available.

Species of *Trichoderma* have provided varied levels of biological control of a number of important soilborne plant pathogens, including *Sclerotium cepivorum* Berk. (1), *Rhizoctonia solani* Kühn (6,25), *Pythium* spp. (6,9,27), and *Verticillium dahliae* Kleb. (15,18). Suppressiveness of certain soils to *Aphanomyces euteiches* Drechs., *Pythium* spp., and *R. solani* may be due to the activity of species of *Trichoderma* or *Gliocladium* (5,23). *T. viride* Pers. ex Gray also has been implicated in biological control of heart rot of pineapple caused by *P. parasitica* (23), but, to date, there have been no documented efforts to use *Trichoderma* and/or *Gliocladium* spp. to control diseases caused by other soilborne *Phytophthora* spp. However, the apparent activity of these fungi against other biflagellate zoospore-forming plant pathogens suggests the possible existence of isolates of *Trichoderma* or *Gliocladium* spp. active against *P. cactorum*. Therefore, the objective of our study was to design a system to identify such isolates and determine their potential for use as components in a broader integrated pest management system for control of root and crown rots of apple trees. Preliminary reports of this work have been published (28,29).

MATERIALS AND METHODS

Source of candidate biocontrol fungi. Soil was collected from orchards in Orleans County, NY, from the upper 5 cm of soil adjacent to the crowns of apparently healthy apple and cherry trees growing on wet or frequently flooded soil. Samples also were collected from fields in commercial production of snapbean and pea in Livingston County, NY, where soils previously were reported to be suppressive to Aphanomyces root rot of pea (2), and from a poorly drained area overgrown with wild grasses on Cornell University's Vegetable Research Farm near Geneva, NY. In each field, at least 20 soil samples were taken with a 2.5-cm-diameter soil auger along an arbitrary transect, then bulked and thoroughly mixed. In the laboratory, 10-g subsamples were decimally diluted in sterile distilled water, and 1-ml aliquots of soil suspension dispensed onto a modified version of TSM (7), a medium selective for *Trichoderma* and *Gliocladium* spp. Modified TSM contains (g/L): $Ca(NO_3)_2$, 1.0; KNO_3, 0.26; $MgSO_4 \cdot 7H_2O$, 0.26; KH_2PO_4, 0.12; $CaCl_2 \cdot 2H_2O$, 1.0; citric acid, 0.05; sucrose, 2.0; agar, 20.0; Igepal 630 (Alltech Associates, Inc., Deerfield, IL), 1.0 ml; chlortetracycline, 0.05; captan (50% wettable powder), 0.04; and vinclozolin, 0.0025. Conidia of candidate biocontrol fungi were mass transferred to half-strength malt agar (Difco Laboratories, Detroit, MI) after 5 days of incubation on the lab bench or at 11 C (Vegetable Research Farm soil). Isolates were identified to species based on color, size, and shape of phialospores; size, shape, and arrangement of phialides; presence or absence of chlamydospores; and appearance and odor of mycelium on the plates (24).

Selection of cold-tolerant candidate isolates. Five-mm-diameter disks cut from the edge of colonies actively growing on cornmeal agar (CMA; Difco) were transferred to the center of 90-mm-diameter petri dishes containing 20 ml of CMA. These cultures were incubated at 10 C, and radial growth from the disks was measured daily for 6 days. Two replicate plates were prepared for each isolate, and the experiment was repeated twice. Growth rate of each fungus was calculated, and isolates having a growth rate at 10 C that was at least 20% greater than that of other isolates at this temperature were considered to be cold tolerant.

Standard strains. Three strains of *Trichoderma* spp. with documented efficacy against *Pythium ultimum* Trow and other fungi were chosen as standards for comparison in all assays described below. These were *T. koningii* Ouden. (strain T8m; ATCC 20736), *T. harzianum* (strain T12m; ATCC 20737) (3,9), and *T. harzianum* (strain 1295-22; ATCC 20847), which was prepared via protoplast fusion (11).

Activity against *P. cactorum* in vitro. Five-millimeter-diameter disks obtained from the edge of an actively growing colony of *P. cactorum* (isolate NY 359) were paired on individual plates of CMA with similar disks of each candidate biocontrol isolate, approximately 4 cm apart. Plates were incubated at 19 C and observed daily for 4–6 days. Overgrowth of *P. cactorum* by the candidate biocontrol isolate, or any inhibition of radial growth of *P. cactorum* (apparent antibiotic activity) was noted. Two replicate plates were prepared for each combination, and the experiment was repeated twice.

Preparation of inocula. Inoculum of *P. cactorum* was prepared with minor modifications of previously described procedures (21). Briefly, V-8 juice broth (200 ml of V-8 juice, 2 g of $CaCO_3$, and 800 ml of distilled water) was mixed with fine vermiculite (3:5, v/v) in an Erlenmeyer flask and autoclaved for 15 min on 2 successive days. Four to six disks from the margin of a colony of *P. cactorum* growing on CMA were transferred to the flask, and the flask was incubated on the lab bench for 2–3 wk. Immediately before use, this mixture was placed on four layers of cheesecloth in a vacuum funnel and washed thoroughly with approximately 2 L of running tap water. Inoculum was considered to be free of V-8 juice broth and consisted primarily of vermiculite infested with oospores, sporangia, and mycelial fragments of *P. cactorum*.

Inoculum of each candidate biocontrol isolate consisted of hyphae, conidia, and chlamydospores grown in a mixture of peat and wheat bran. Fifty milliliters of commercial peat was mixed with 50 ml of food-grade wheat bran in a 250-ml Erlenmeyer flask, then moistened with 50 ml of distilled water, and autoclaved for 15 min on 2 successive days. After cooling, four to six 5-mm-diameter disks from the margin of a colony of the candidate isolate growing on CMA were transferred to the peat/bran preparation and incubated on the lab bench for 7–10 days.

Dose response for *P. cactorum*. Potting medium was prepared by mixing pasteurized sandy loam soil and fine vermiculite (1:2, v/v), and the medium was infested with washed vermiculite inoculum of *P. cactorum* at a rate of 2% (v/v). Infested potting medium then was serially diluted with uninfested potting medium to achieve concentrations of 1, 0.5, 0.25, 0.125, and 0.0625% vermiculite inoculum. Five sprouted seeds from open-pollinated McIntosh apples were planted in 0.65-L plastic pots filled with infested or uninfested potting medium, five replicate pots per inoculum concentration, and arranged in randomized complete blocks in a greenhouse maintained at 18–20 C. Pots were watered with tap water as needed for 2 wk until emerged seedlings had four to six true leaves. Pots then were moved to watertight containers and flooded with tap water to a depth of approximately 1 cm to induce the production and dispersal of zoospores and subsequent infection by *P. cactorum*. Seven to 10 days after the conclusion of flooding (72-hr flooding duration), potting medium was removed from each pot by washing in running tap water, and disease severity was evaluated for each pot based on a visual assessment of root decay of all seedlings in each pot (0–5 scale, where 0 = <1%, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–89%, and 5 = >90% root rot), total plant weight of all seedlings in each pot, and the number of seedlings with hypocotyl lesions (considered to be analogous to crown lesions on mature plants). The experiment was repeated three times.

Evaluation of biocontrol isolates in vivo. Candidate biocontrol isolates tolerant of low temperature and exhibiting antibiosis to *P. cactorum* in vitro were selected for evaluation of their potential for biocontrol of Phytophthora root rot in apple seedlings in greenhouse trials. Tolerance of all candidate isolates to the fungicide metalaxyl in vitro also was tested by growing the isolates on CMA containing a range of concentrations of the fungicide. Disks containing actively growing hyphae of candidate biocontrol isolates were placed on CMA containing metalaxyl at 0.039–10 mg/L, obtained by incorporating serially diluted aqueous stock solutions of technical-grade metalaxyl into autoclaved CMA before plates were poured. Four replicate plates for each isolate × concentration combination were prepared and incubated at 19 C; radial growth was compared to that on unamended CMA daily for 4 days.

Potting medium was prepared by mixing pasteurized sandy loam soil and fine vermiculite (1:2, v/v) and infesting with washed vermiculite inoculum of *P. cactorum* at a rate of 0.25% or 2%, v/v, depending on the experiment. Treatments consisted of soil mix that was uninfested; infested with peat/bran inoculum of each biocontrol candidate alone (0.6%, v/v; 10^4–10^6 colony-forming units/g fresh weight of soil); infested with inoculum of *P. cactorum* alone; and infested with each biocontrol candidate × *P. cactorum* combination. All infested soil was held at room temperature for 8–10 hr before use, then sampled and assayed by dilution plates on TSM for initial population densities of the biocontrol candidate. Sprouted apple seeds were planted and seedlings were flooded as outlined above. During flooding, leaf-disk baits of Mazzard (*Prunus avium* L.) or Mahaleb (*P. mahaleb* L.) cherry were floated on the surface water in each pot. After the conclusion of flooding (72 hr), leaf disks were collected from the soil surface, rinsed briefly with tap water, blotted on paper towels, and plated onto P_5ARPH medium (13), selective for *Phytophthora* spp. The number of leaf disks from which *P. cactorum* was isolated after 72 hr of incubation at 22 C was used as a relative measure of zoospore activity of *P. cactorum* in the potting mix. Disease severity was evaluated for each pot as outlined above. Because some isolates were collected earlier than others, they were evaluated for biocontrol efficacy in separate greenhouse trials. Each experiment was repeated at least once.

Analysis of data. Infestation of the potting mix with *P. cactorum* and biocontrol isolate was treated as a qualitative variable in analysis of variance, with plant weight as the dependent variable. Correlation coefficients between all quantitative variables (incidence of recovery of *P. cactorum* from leaf-disk baits, incidence of hypocotyl infection, total plant weight, and initial population density of individual biocontrol isolates) were calculated. The Waller-Duncan exact Bayesian *K*-ratio LSD rule was used to evaluate differences between plant weight means and number of hypocotyl lesions in pots of each treatment. A chi-square test for independence was used to compare frequency distributions of pots within each treatment that were assigned qualitative root rot ratings (26).

RESULTS

Dose response for *P. cactorum*. The minimum concentration of vermiculite inoculum of *P. cactorum* in the potting medium that resulted in a high level of disease and mortality of apple seedlings was 0.25% (Fig. 1). Above that level, total plant weight was not significantly reduced by increasing inoculum concentration of *P. cactorum* inoculum until the 2% level was reached. Based on this finding, both the 2 and 0.25% level of inoculum were used in subsequent greenhouse trials. Isolates selected as a result of their performance at these concentrations were tested further at 0.125% level of inoculum.

Screening of candidate isolates. When paired on CMA, nearly all 67 isolates originally tested overgrew and sporulated on *P. cactorum*; hence, this was not a useful test for distinguishing

promising biocontrol isolates. In contrast, only 27 isolates exhibited cold tolerance and antibiosis to *P. cactorum* in vitro and, thus, were selected for further study in vivo. None of the standard strains exhibited antibiosis; nonetheless, all three were tested for their ability to protect apple seedlings, and none was effective. Of the 23 isolates collected from soil on the Cornell Vegetable Farms, 10 satisfied initial selection criteria; however, they were ineffective in increasing plant weight or decreasing root damage of apple seedlings, so no further data on these isolates are shown. Six of 30 isolates from apple and cherry orchards met these same selection criteria; one such isolate, identified as *T. harzianum* Rifai, provided biocontrol of *P. cactorum* on apple seedlings, and increased plant weight was obtained with another isolate of *T. harzianum* in the absence of pathogen. Eleven of the 14 isolates collected from soil suppressive to *Aphanomyces* exhibited cold tolerance and antibiosis to *P. cactorum* in vitro, and seven provided biocontrol of *P. cactorum* in vivo. Effective biocontrol isolates from this soil were identified as *T. harzianum* (two isolates), *T. hamatum* (Bonord.) Bain (one isolate), *T. viride* (one isolate), and *Gliocladium virens* Miller (three isolates).

At the 2% inoculum concentration of *P. cactorum*, significant increases in plant weight and marked reductions in the incidence of hypocotyl lesions and severity of root damage caused by *P. cactorum* were obtained with six of 11 isolates tested in one set of experiments (Fig. 2A–C). For example, in the presence of isolate 036 of *T. viride*, the incidence of hypocotyl infection on test seedlings was significantly reduced (Fig. 2A). In the presence of isolate 035 of *G. virens*, total plant weight was 181% greater than that of seedlings grown in potting medium infested with *P. cactorum* alone (Fig. 2B). Treatment variances in each experiment were consistent and similar, so statistical analysis was done on bulked data from all experiments. Analysis of variance indicated that infestation of the potting soil with a biocontrol isolate and with the 2% inoculum concentration of *P. cactorum* each influenced total plant weight of apple seedlings ($P = 0.05$). A chi-square test indicated a lack of independence between the qualitative root rot rating and presence of biocontrol fungi in potting mix infested with *P. cactorum* ($\chi^2 = 367.4$, df = 80); there was an interaction between infestation of the potting mix with pathogen and biocontrol agent and the number of seedlings per pot in each root decay category. For all biocontrol candidates, total plant weight was negatively correlated with the number of leaf disks colonized by *P. cactorum* (a measure of zoospore activity) and the number of hypocotyl lesions in each pot (Table 1). Significant positive correlations were found between zoospore activity of *P. cactorum* and the number of hypocotyl lesions in each pot. Significant positive correlations were found between zoospore activity of *P. cactorum* and the number of hypocotyl lesions that developed on plants in the same pots, although there was no correlation between initial population densities of biocontrol candidate isolates and zoospore activity during the flooding period.

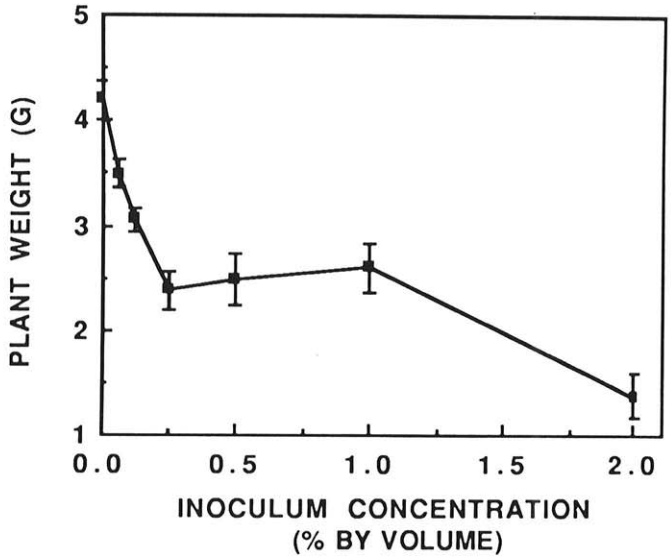

Fig. 1. Dose response curve for apple seedlings exposed to varied concentrations of inoculum of *Phytophthora cactorum*. Colonized vermiculite inoculum was incorporated into pasteurized potting medium at the indicated concentrations and seedlings subsequently flooded for a single 72-hr period, as described in the text. Points represent means of 12 observations each, obtained by bulking the data from three repeated greenhouse trials; bars represent standard error of each mean.

At 0.25% inoculum concentration of *P. cactorum*, significant increases in plant weight and reductions in root damage caused by *P. cactorum* were obtained with eight of 23 isolates tested (Table 2). With the most effective isolate, 041 of *G. virens*, total plant weight was 31% greater than that of seedlings grown in potting medium infested with *P. cactorum* alone. Similar to results obtained with the 2% inoculum concentration, analysis of variance indicated that infestation of the potting mix with a biocontrol isolate and with the 0.25% inoculum concentration of *P. cactorum* each influenced total plant weight of apple seedlings ($P = 0.05$). A chi-square test indicated a lack of independence between the qualitative root rot rating and presence of biocontrol fungi in potting mix infested with *P. cactorum* ($\chi^2 = 244.9$, df = 80); there was an interaction between infestation of the potting mix with pathogen and biocontrol agent and the number of seedlings per pot in each root decay category. However, disease pressure (as measured by total plant weight and the incidence of hypocotyl infection of plants grown in the presence of *P. cactorum* alone) was less at the 0.25% inoculum concentration than at 2%; therefore, the contrast among treatments was not as pronounced at this lower inoculum level. Continuous with this trend, relatively low levels of disease occurred on plants grown solely in the presence of a 0.125% concentration of inoculum of *P. cactorum*; hence, there was no significant effect of any candidate biocontrol isolate tested at this lowest inoculum concentration (data not shown).

When grown in potting medium infested with only a biocontrol isolate, total plant weight of apple seedlings was significantly greater than when grown in potting medium alone. For example, total plant weight of seedlings grown in potting medium infested with *G. virens* 035 was 6.1 g, whereas that of plants in uninfested potting medium was 5.2 g. This increase in total plant weight was consistent for most of the isolates tested.

DISCUSSION

In the present study, we designed a system to identify isolates of *Trichoderma* and *Gliocladium* spp. with potential to reduce crown and root rot of apple caused by *P. cactorum*. Because disease development often is associated with cool, wet soil in the fall and spring, it was important to find a biocontrol agent capable of growth and proliferation at relatively low temperatures (10 C). An antibiotic produced by *G. virens* has been implicated in biocontrol of *P. ultimum* (12), thus, antibiotics from *Trichoderma* spp. and *G. virens* also may be active against the related fungus *P. cactorum*. Therefore, it was desirable to identify isolates with antibiotic activity against *P. cactorum* in vitro, particularly because this is an easily identifiable characteristic. The above criteria were used initially to select potential biocontrol agents in the laboratory. Greenhouse trials were a more rigorous test of the biocontrol capability of the isolates, and some that showed potential for biocontrol of *P. cactorum* in vitro failed in vivo. In these tests, relative efficacy among potential biocontrol isolates was much easier to discriminate at higher inoculum concentrations of *P. cactorum*, when disease severity in the absence of biocontrol isolates was greatest. Relative efficacy among the biocontrol isolates also may be different in nonsterile potting mix.

Most effective biocontrol isolates were collected from a soil previously identified as suppressive to another biflagellate zoospore-forming fungus, *A. euteiches*. The remainder were from orchard soil where healthy trees existed in sites conducive to

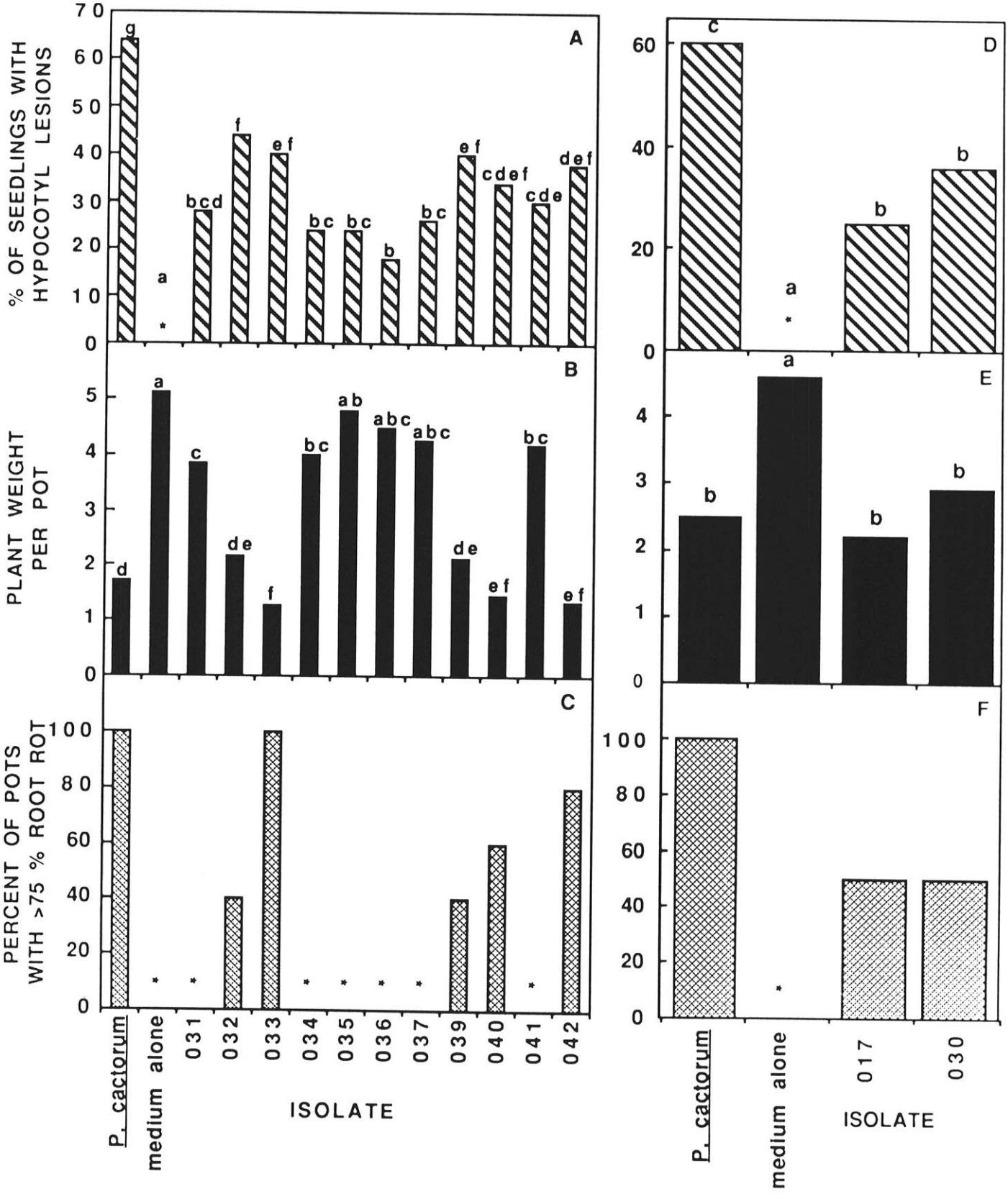

Fig. 2. Extent of biocontrol of *Phytophthora cactorum* on apple seedlings obtained with 13 isolates of *Trichoderma* and *Gliocladium* spp. in two different sets of experiments. **A and D,** Incidence of hypocotyl lesions. **B and E,** Total plant fresh weight. **C and F,** Incidence of severe root rot. Seedlings were grown in soil mix infested with vermiculite inoculum of *P. cactorum* (2%, v/v) and flooded for a single 72-hr period, as described in the text. Means within an individual figure not identified by a common letter are significantly different ($P = 0.05$) according to the Waller-Duncan exact Bayesian K-ratio LSD rule. Values represent data obtained from 10 observations (two repeated experiments, five replicate pots per experiment, five seedlings per pot); * = zero.

TABLE 1. Correlation coefficients between dependent variables in seven greenhouse trials of biocontrol fungi with a 2% concentration of vermiculite inoculum of *Phytophthora cactorum*

	Total plant weight per pot	Qualitative root damage rating	Incidence of hypocotyl infection per pot	Zoospore activity of *P. cactorum*[a]
Initial cfu of biocontrol candidate	−0.09*[b] $N = 354$	0.11** $N = 354$	0.06 $N = 354$	0.06 $N = 357$
Zoospore activity of *P. cactorum*[a]	−0.57*** $N = 353$	0.60*** $N = 353$	0.63*** $N = 353$	
Incidence of hypocotyl infection per pot	−0.70*** $N = 354$	0.79*** $N = 354$		
Qualitative root damage rating	−0.87*** $N = 354$			

[a]Zoospore activity indicated by the number of Mazzard or Mahaleb cherry leaf disks supporting growth of *P. cactorum*.
[b]*, **, and *** indicate probability levels of 0.1, 0.05, and 0.0001, respectively.

TABLE 2. Effect of treatment with biocontrol candidates from various soils on plant weight of apple seedlings and number of hypocotyl lesions caused by 0.25% inoculum of *Phytophthora cactorum*

Isolate and no.	Plant weight (g)[z]	No. of hypocotyl lesions
None	2.59 a	0.01 a
Gliocladium virens 041	2.09 b	1.8 bcd
Trichoderma harzianum 017	2.05 bc	1.4 b
G. virens 035	2.04 bc	2.3 cde
T. hamatum 037	1.97 bcd	1.9 bcde
T. harzianum 043	1.96 bcd	1.2 bc
T. koningii 25	1.87 bcde	2.2 cde
T. viride 24	1.81 bcde	2.3 cde
T. viride 036	1.77 bcde	2.1 bcde
T. viride 14	1.71 de	2.6 e
T. harzianum 030	1.63 e	2.2 cde
T. koningii 23	1.59 e	2.1 bcde
P. cactorum alone	1.59 e	2.0 bcde
	LSD = 0.33	LSD = 0.8

[z]Bulked data from three greenhouse trials; means of 15 observations of five plants each. Values in a column followed by the same letter are not significantly different ($P = 0.05$) according to the Waller-Duncan exact Bayesian K-ratio LSD rule.

significant development of Phytophthora root and crown rot. In contrast, no effective isolates were obtained from a site where *Phytophthora* spp. or related pathogens would not be expected to reside in large numbers. We conclude that selection of potential biocontrol strains should be done from soils known or likely to be suppressive to the disease in question, as others have found in working with different pathogens (17). In our study, effective isolates represented four different species among two genera, supporting the concept that biocontrol capability is an isolate-specific characteristic that has little or no relation to taxonomic rank within a group of related organisms.

Phytophthora and *Pythium* are closely related fungi, so it would seem reasonable that isolates effective against one of these would also be effective against the other. However, none of the standard strains were effective against *P. cactorum*, even though one or more of these are highly effective against *Pythium* spp., *R. solani*, and *Alternaria* spp. (9,10,30). Thus, biocontrol of *Phytophthora* spp. apparently requires attributes not required for biocontrol of the diverse group of other fungi noted above, or characteristics not found in the genetically unique strains developed via protoplast fusion techniques.

The mechanism of biological control by isolates in the present study was not investigated. However, several mechanisms have been proposed for other biocontrol agents of soilborne plant pathogens. For instance, microorganisms in suppressive container medium created a nutrient sink, which deprived *P. ultimum* of nutrients needed for germination of infective propagules (4). *T. hamatum* has been shown to directly parasitize hyphae of *R. solani*, thereby effecting biocontrol (5,6). Species of *Trichoderma* and *Gliocladium* also have been shown to produce various toxic metabolites and antibiotics, which may be implicated in biological control (8). We used evidence of antibiosis in vitro as a preliminary screen for potential biological control capability of our isolates; those not exhibiting antibiosis were not tested further. However, we acknowledge that such a screening method may have eliminated effective biocontrol agents.

Apple seedlings grown in potting medium amended with biocontrol candidates were consistently larger and more vigorous than seedlings grown in unamended potting medium. Increased plant growth in the presence of *Trichoderma* spp. may be due to the elimination of minor pathogens in the rhizosphere (10). However, the sandy loam soil used in the potting mix was pasteurized before infestation, thus, minimizing the likelihood of contamination by other pathogens or plant growth-promoting rhizobacteria (16). An alternative possibility for such increased growth was suggested by Windham et al (32), who found that *Trichoderma* spp. produced a growth-regulating factor that increased the rate of seed germination and dry weight of shoots and stems of tomato.

Metalaxyl-tolerant isolates identified with the system we have developed may be suitable for inclusion in an integrated control program of chemical and cultural control measures, such as rootstock selection, site modification, or treatment with appropriate systemic fungicides, to reduce damage on apple trees due to infection by *Phytophthora* spp. For example, Ohr et al (22) presented evidence that *Trichoderma* spp. could be integrated with methyl bromide fumigation for control of *Armillaria mellea* (Vah.:Fr) P. Kumm. on citrus. Field tests of our biocontrol fungi are currently under way to validate the preliminary greenhouse studies presented herein. Integration of *Trichoderma* and *Gliocladium* with current chemical and cultural means of controlling *P. cactorum* on apple warrants further consideration.

LITERATURE CITED

1. Abd-el Moity, T. H., and Shatla, M. N. 1981. Biological control of white rot disease of onion (*Sclerotium cepivorum*) by *Trichoderma harzianum*. Phytopathol. Z. 100:29-35.
2. Blume, M. C. 1978. *Thielaviopsis basicola*: A new component of the pea root-rot complex in New York. M.S. thesis. SUNY College of Environmental Science and Forestry, Syracuse, NY. 189 pp.
3. Chao, W. L., Nelson, E. B., Harman, G. E., and Hoch, H. C. 1986. Colonization of the rhizosphere by biological control agents applied to seeds. Phytopathology 76:60-65.
4. Chen, W., Hoitink, H. A. J., Schmitthenner, A. F., and Tuovinen, O. H. 1988. The role of microbial activity in suppression of damping-off caused by *Pythium ultimum*. Phytopathology 78:314-322.
5. Chet, I., and Baker, R. 1981. Isolation and biocontrol potential of *Trichoderma hamatum* from soil naturally suppressive to *Rhizoctonia solani*. Phytopathology 71:286-290.
6. Chet, I., Harman, G. E., and Baker, R. 1981. *Trichoderma hamatum*: Its hyphal interactions with *Rhizoctonia solani* and *Pythium* spp. Microb. Ecol. 7:29-38.
7. Davet, P. 1979. Technique pour l'analyse des populations de *Trichoderma* et de *Gliocladium virens* dans le sol. Ann. Phytopathol. 11:529-533.
8. Elad, Y., Kalfon, A., and Chet, I. 1983. Control of *Rhizoctonia solani* in cotton by seed-coating with *Trichoderma* spp. Plant Soil 66:279-281.
9. Hadar, Y., Harman, G. E., and Taylor, A. G. 1984. Evaluation of *Trichoderma koningii* and *T. harzianum* from New York soils for biological control of seed rot caused by *Pythium* spp. Phytopathology 74:106-110.
10. Harman, G. E., and Hadar, Y. 1983. Biological control of *Pythium* species. Seed Sci. Technol. 11:893-906.
11. Harman, G. E., Taylor, A. G., and Stasz, T. E. 1989. Combining effective strains of *Trichoderma harzianum* and solid matrix priming to improve biological seed treatments. Plant Dis. 73:631-637.
12. Howell, C. R., and Stipanovic, R. D. 1973. Gliovirin, a new antibiotic from *Gliocladium virens*, and its role in the biological control of *Pythium ultimum*. Can. J. Microbiol. 29:321-324.

13. Jeffers, S. N., and Martin, S. B. 1986. Comparison of two media selective for *Phytophthora* and *Pythium* species. Plant Dis. 70:1038-1043.
14. Jeffers, S. N., Aldwinckle, H. S., Burr, T. J., and Arneson, P. A. 1982. *Phytophthora* and *Pythium* species associated with crown rot in New York apple orchards. Phytopathology 72:533-538.
15. Jordan, V. W. L., and Tarr, H. S. 1978. Inoculum suppression of *Verticillium dahliae*. Ann. Appl. Biol. 89:139-141.
16. Kloepper, J. W., and Schroth, M. N. 1981. Plant growth-promoting rhizobacteria and plant growth under gnotobiotic conditions. Phytopathology 71:642-644.
17. Linderman, R. G., Moore, L. W., Baker, K. F., and Cooksey, D. A. 1983. Strategies for detecting and characterizing systems for biological control of soilborne plant pathogens. Plant Dis. 67:1058-1064.
18. Marois, J. J., Johnston, S. A., Dunn, M. T., and Papavizas, G. C. 1982. Biological control of Verticillium wilt of eggplant in the field. Plant Dis. 66:1166-1168.
19. Matheron, M. E., Young, D. J., and Matejka, J. C. 1988. Phytophthora root and crown rot of apple trees in Arizona. Plant Dis. 72:481-484.
20. Mircetich, J. S. M., and Browne, G. T. 1987. Phytophthora root and crown rot of deciduous fruit trees: Progress and problems in etiology, epidemiology, and control. Pages 64-95 in: Proc. Commemorative Symposium on Changes and Opportunities in Fruit Production, Protection, and Utilization. N. E. Looney, ed. The Summerland Res. Stn. Agric. Canada, Summerland, B.C., Canada.
21. Mircetich, S. M., and Matheron, M. E. 1976. Phytophthora root and crown rot of cherry trees. Phytopathology 66:549-558.
22. Ohr, H. D., Munneke, D.E., and Bricker, J. L. 1973. The interaction of *Armillaria mellea* and *Trichoderma* spp. as modified by methyl bromide. Phytopathology 63:965-973.
23. Papavizas, G. C. 1985. *Trichoderma* and *Gliocladium*: Biology, ecology, and potential for biocontrol. Annu. Rev. Phytopathol. 23:23-54.
24. Rifai, M. A. 1969. A revision of the genus *Trichoderma*. Mycol. Pap. 116. Commonwealth Mycological Institute, Kew, Surrey, England. 56 pp.
25. Ruppel, E. G., Baker, R., Harman, G. E., Hubbard, J. P., Hecker, R. J., and Chet, I. 1983. Field tests of *Trichoderma harzianum* Rifai aggr. as a biocontrol agent of seedling disease in several crops and Rhizoctonia root rot of sugar beet. Crop. Prot. 2:399-408.
26. SAS Institute, Inc. 1985. SAS User's Guide: Statistics. 1985 ed. SAS Institute Inc., Cary, NC. 956 pp.
27. Sivan, A., Elad, Y., and Chet, I. 1984. Biological control effects of a new isolate of *Trichoderma harzianum* on *Pythium aphanidermatum*. Phytopathology 74:498-501.
28. Smith, V. L., Wilcox, W. F., and Harman, G. E. 1988. A system for screening *Trichoderma* for potential biocontrol of *Phytophthora cactorum*. (Abstr.) Phytopathology 78:1523.
29. Smith, V. L., Wilcox, W. F., and Harman, G. E. 1988. Control of Phytophthora root and crown rot of apple seedlings by *Trichoderma* spp. (Abstr.) Phytopathology 78:1511.
30. Vannacci, G., and Harman, G. E. 1987. Biocontrol of seed-borne *Alternaria raphani* and *A. brassicicola*. Can. J. Microbiol. 33:850-856.
31. Wilcox, W. F., Agnello, A. M., Kovach, J., and Stiles, W. C. 1989. Cornell Recommendations for Commercial Tree-Fruit Pest Management. Cornell University, Ithaca, NY. 68 pp.
32. Windham, M. T., Elad, Y., and Baker, R. 1986. A mechanism for increased plant growth induced by *Trichoderma* spp. Phytopathology 76:518-521.