Effect of Mycelial Preparations of *Trichoderma* and *Gliocladium* on Populations of *Rhizoctonia solani* and the Incidence of Damping-Off

J. A. Lewis and G. C. Papavizas

Soil scientist and plant pathologist, respectively, Soilborne Diseases Laboratory, Plant Protection Institute, U.S. Department of Agriculture, Beltsville, MD 20705

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ABSTRACT

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Mycelial preparations of eight of 14 isolates of Trichoderma spp. and *Gliocladium virens* reduced survival of *Rhizoctonia solani* at least 50% in pathogen-infested beet seed in soil and in soil infested with sand/cornmeal inoculum of the pathogen. All isolates reduced saprophytic growth of *R. solani* from infested beet seed into soil. Isolates of *T. hamatum* and *G. virens* were more effective than those of *T. harzianum* and *T. viride*. Population densities of all isolates increased 10^4–10^6-fold during 3 wk of incubation after mycelial preparations were added to soil. Conidial preparations of isolates, added to soil in amounts equal to propagules in mycelial preparations, did not reduce survival of *R. solani* or its growth through the soil. Mycelial preparations, but not conidia, of most isolates of *Trichoderma* spp. and *G. virens* prevented damping-off of cotton, sugar beet, and radish seedlings in the greenhouse. There was no correlation between population density of antagonists and pathogen survival or damping-off. There was a highly significant negative correlation between stand in all three crops and pathogen survival in soil.

Direct application of antagonists continues to be the principal method for introducing microorganisms into soil for biological control of soilborne plant pathogens (17). In most studies, conidia or nondescript biomass on various types of organic substrates have been applied to soil (4,5,15,17,20). Although some success has been obtained with these preparations, it is possible that many potential antagonists are ineffective when used in these forms. Recently, we demonstrated that population densities of isolates of *Trichoderma* Pers. ex Fr., *Gliocladium* Corda, *Aspergillus* Mich. ex Fr., and *Talaromyces* C. R. Benjamin increased up to 10^6-fold in natural soils amended with mycelial preparations of young cultures on a food base (bran) rather than with conidial preparations (11). Populations of isolates of *Trichoderma* spp. and *G. virens* Miller, Giddens, & Foster also increased in soils amended with fermentor biomass preparations that consisted mostly of chlamydospores (10,16). Several recent studies also suggested the potential importance of the nature of propagules used in biological control (4,20). Fermentor biomass preparations of isolates of *Trichoderma* spp. and *G. virens*, abundant in chlamydospores, were more effective than conidia in reducing inoculum of *R. solani* Kühn in soil and preventing potato diseases caused by the pathogen (1). Damping-off caused in zinnia by *Pythium ultimum* Trow and *R. solani* in a soilless mix was significantly less after amendment of the growing medium with a young mycelial preparation of an isolate of *G. virens* on bran than with a conidial preparation (12). Pregerminated conidia of isolates of *Trichoderma* applied with a fluid drill gel resulted in low incidence of infection by *R. solani* on radish in the field (13).

The objective of this investigation was to compare the effect of young mycelial preparations and conidial preparations of several potential antagonists on the survival and saprophytic growth of *R. solani* in soil and on the damping-off of cotton, sugar beet, and radish caused by this pathogen in the greenhouse.

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MATERIALS AND METHODS

Preparation of pathogen inoculum. Isolate R-23 of *R. solani* (AG-4), was isolated from the hypocotyl of a diseased cotton plant at Beltsville, MD, and maintained on potato-dextrose agar (PDA). Three types of inocula were prepared (19). A medium that consisted of quartz sand (to pass a sieve with 2-mm openings), ground corn meal, and water (25:1:5, w/w/v), was mixed, autoclaved, seeded with an agar disk from a plate culture of *R. solani*, and incubated for 12 days before addition to soil. Flasks with beet (*Beta vulgaris* L.) seed and water (1:1, w/v), were autoclaved, seeded with *R. solani*, incubated for 28 days, and air dried. A medium was also prepared that contained millet (*Pennisetum glaucum* L.) seed in place of beet seed.

Antagonist isolates and preparation of cultures. Ten isolates of *Trichoderma* spp. and four isolates of *G. virens* from the Soilborne Diseases Laboratory collection were used. These included three isolates of *T. viride* Pers. ex Gray (T-1-R4, T-1-R9, and TS-1-R3), four of *T. harzianum* Rifai (WT-6-24, Th-5, Th-58, and Th-23-R9), three of *T. hamatum* (Bonord.) Bain (TRI-4, Tm-23, 31-3), and four of G. virens (Gl-3, Gl-9, Gl-17, and Gl-21). Five of the isolates (T-1-R4, T-1-R9, TS-1-R3, WT6-24, and Th-23-R9) were benomyl-resistant and developed from wild types by UV irradiation or chemical mutagenesis (18,20).

Cultures were maintained on V-8 juice agar (200 ml of V-8 juice, 800 ml of water, 1 g of glucose, 20 g of agar, and 6.0 ml of 1.0 N NaOH) in the light (~700 μEin/m^2/sec) at 23–26 C. Conidial suspensions for introduction into media or soil were prepared from 9-day-old colonies growing on this medium. For mycelial preparations, a modification of the wheat bran culture for antagonists was used (4,5). Wheat bran (100 g) was passed through a sieve with 1-mm openings, mixed with water (100 ml), and autoclaved in 1-L Erlenmeyer flasks for 1 hr (11). After the medium had cooled, ten milliliters of a spore suspension, counted with a hemacytometer, were added to each flask to provide 10^7 conidia per 100 g of bran. The cultures were incubated for 3 days at 25 C. The

moistened 3-day-old bran cultures are hereafter referred to as mycelial preparations. Conidial preparations, which consisted of moistened, autoclaved bran with conidia added to equal the numbers of propagules in the mycelial preparations, were added to soil without incubation. In one experiment, conidia and mycelium were added to soil without bran. In contrast to mycelial preparations in which mycelia colonized the bran substrate (2), free mycelia were prepared by adding conidia of various isolates to potato-dextrose broth (1 g/L) in shake flasks. The cultures were incubated for 2 days, and the mycelium was filtered and washed free of excess nutrients before being added to the soil.

Survival and saprophytic growth of *R. solani* in soil. *R. solani* is usually found in soil fractions as mycelium embedded in organic debris (14,19). To simulate *R. solani* embedded in organic debris in soil, beet seed infested with R-23 were added to a nonsterile loamy sand (pH 6.4, 0.4% organic matter) at 4.0 g per 600 g soil. At this time, either mycelial or conidial preparations of various antagonists also were added to provide 0.5% bran and 5×10^3 propagules per gram of soil. Soils were moistened to about −0.3 bars (9% water) as determined with a pressure plate extractor and 600-g portions were placed in glass beakers which were covered with polyethylene film punctured with a needle to permit gas exchange. Soils were maintained at the appropriate moisture at 21–23 C. To determine residual survival of *R. solani* in beet seeds, seeds were retrieved after 1, 2, 3, and 6 wk of incubation from 150 g of soil on a sieve with 1.4-mm openings, washed, and 10 seeds were placed on each of five plates of 2% water agar with antibiotics (19). The characteristic, branched growth of *R. solani* was detected as described previously (19) on plates after 20–24 hr of incubation at 23–25 C. Survival was expressed as the percentage of beet seed that remained infested with *R. solani*. To determine the saprophytic growth of *R. solani* from infested beet seed into soil, autoclaved, noninfested beet seed (1.0 g) were added to the sieved soil portions (150 g) as a bait to trap hyphae of the pathogen which grew into the soil (14,19). Soils were incubated 3 days, beet seeds were retrieved, washed, plated, and observed as above. The results, expressed as the percentage of beet seeds colonized, reflected the growth or saprophytic activity of *R. solani* from infested beet seed into the soil.

The effect of mycelial preparations of antagonists on survival of free mycelium of *R. solani* in soil also was determined. Soils (600 g) were amended with 12-day-old sand/cornmeal inoculum of the pathogen at a rate of 2%. This inoculum of R-23 contained only mycelium and no sclerotia. At this time, mycelial preparations of the antagonists to provide 0.5% bran and 5×10^3 propagules per gram of soil also were added. After 1, 2, 3, and 6 wk of incubation, autoclaved, noninfested beet seeds (1.0 g) were added to 150-g portions of soil as a bait for indigenous *R. solani*. After 3 days, beet seed colonization was assayed. The percentage of beet seeds colonized was an index of the amount of mycelium of *R. solani* that survived in the soil.

Assay for colony-forming units of *Trichoderma* spp. and *G. virens*. After the survival and saprophytic growth of *R. solani* were determined, 5-g portions of sieved soil were used to assay for population densities of the antagonists. Serial dilutions were prepared immediately and 1-ml aliquots were spread on the appropriate medium (five plates per replicate per dilution). For biotypes of *Trichoderma* that are insensitive to benomyl (T-1-R4, T-1-R9, TS-1-R3, WT-6-24, and Th-23-R9), a benomyl-amended, semiselective medium (TME-ben 10) was used (22). Populations of all other isolates were determined on TME medium without benomyl. Fungal colonies were counted on the agar plates after 7–10 days of incubation at 23–26 C under continuous fluorescent light and populations were reported as colony-forming units per gram of soil.

Effect of antagonists on damping-off. One-kilogram (dry weight equivalent) portions of nonsterile loamy sand were infested with R-23 on millet seed at a rate of 0.01%. Mycelial preparations of each of 14 isolates of *Trichoderma* spp. and *G. virens* were added concurrently with *R. solani* or 1 wk later to soil batches to provide 5×10^3 propagules per gram of soil and 0.5% bran. In one test, conidial preparations also were added at a comparable rate of propagules and bran. Soils were placed in plastic flats ($18 \times 12 \times 6.5$ cm), incubated for 1 wk, and planted with thiram-treated seed (0.9 g active ingredient a.i. per kilogram of seed) of cotton (*Gossypium hirsutum* L. 'Stoneville 213'), sugarbeet (*Beta vulgaris* L. 'USH-20'), and radish (*Raphanus sativus* L. 'Scarlet Globe'). The flats were planted (two rows in each) to cotton (five seeds per row) sugar beet and radish (eight seeds per row) and were maintained in the greenhouse at 21–23 C. Plant stands were counted at 1, 2, and 3 wk after planting and inoculum of *R. solani* in the soil was assayed at 3 wk according to the beet seed colonization method.

Statistical analyses. Each experiment, performed twice with five replications, contained appropriate controls. All data were analyzed for significant differences by using Duncan's multiple range test and, where applicable, linear regression analysis using the Statistical Analysis System computer program. Percentage values were analyzed after arcsin-transformation of the raw data.

RESULTS

Effect of antagonist preparations on survival and saprophytic growth of *R. solani* in soil. After 3 wk of incubation, mycelial preparations of isolates TRI-4, Tm-23, and Gl-21 significantly reduced survival of *R. solani* in infested beet seed in soil and prevented growth of the fungus from the beet seed into the soil (Table 1). Isolates added to soil as conidia alone, conidial

TABLE 1. Effect of preparations of isolates of *Trichoderma hamatum* and *Gliocladium virens* on survival and saprophytic growth of *Rhizoctonia solani* and on antagonist proliferaton in soils[x]

	R. solani (R-23)		
Isolate and preparation[y]	Survival in infested beet seed (%)	Saprophytic growth (% beet seed colonization in soil)	Antagonist population (colony-forming units/g soil)
Control	97 a[z]	89 a	3×10^3
Bran	93 a	92 a	6×10^3
T. hamatum			
Tm-23			
Conidia	97 a	92 a	5×10^2
Conidial prepration	96 a	95 a	1×10^3
Mycelium	94 a	83 a	4×10^3
Mycelial preparation	33 b	0 b	2×10^8
TRI-4			
Conidia	95 a	94 a	2×10^3
Conidial preparation	98 a	90 a	6×10^2
Mycelium	88 a	78 a	2×10^5
Mycelial preparation	2 b	0 b	4×10^9
G. virens			
Gl-21			
Conidia	99 a	90 a	1×10^3
Conidial preparation	91 a	89 a	6×10^3
Mycelium	92 a	76 a	6×10^4
Mycelial preparation	25 b	0 b	1×10^{10}

[x]Assay performed 3 wk after addition of *R. solani* and isolates of *T. hamatum* and *G. virens* to soil.

[y]Conidia were added directly to soil at 5×10^3/g soil. Conidial preparations were conidia on bran mixed immediately with soil (1:200, w/w) to give antagonist density of 5×10^3 conidia per gram of soil. Mycelium was grown on potato-dextrose broth for 2 days and added to soil at 5×10^3 propagules per gram of soil. Mycelial preparations were 3-day-old cultures of antagonist on bran added to soil (1:200, w/w) to give antagonist density of 5×10^3 propagules per gram of soil.

[z]Numbers in each column for each isolate followed by same letter do not signficantly differ from each other according to Duncan's multiple range test, $P = 0.05$.

preparations, or mycelium not grown on bran did not affect survival or growth of *R. solani* into soil. The addition of mycelium growing on bran was necessary for a preparation to be effective. Increases of at least 10^4-fold in antagonist density also occurred with addition of mycelial preparations to soil but not with other preparations.

Since mycelial, but not conidial, preparations were effective against *R. solani*, a more detailed survey with mycelial preparations of 14 isolates was performed. Ten of the 14 isolates significantly reduced survival of *R. solani* in pathogen-infested beet seed (Table 2). Isolates of *T. hamatum* and *G. virens* were very effective, whereas two isolates of *T. viride* (T-1-R4, T-1-R9) and two isolates of *T. harzianum* (WT-6-24 and Th-5) did not reduce survival. To various degrees, all isolates prevented growth of the pathogen from infested beet seed into soil. Isolates of *T. hamatum* and *G. virens* were more effective than those of *T. harzianum* and *T. viride*. All isolates that reduced survival of *R. solani* in beet seed also reduced pathogen survival when it was added to soil as free mycelium (in sand/cornmeal medium). Survival of *R. solani* in beet seed was correlated ($P = 0.01$) with the ability of the pathogen to grow into and colonize the soil ($r = 0.748$) and with the survival of the pathogen added as mycelium in sand/cornmeal ($r = 0.930$). The population density of all isolates of antagonists increased 10^4–10^6-fold during the 3-wk incubation period. Values for colony-forming units per gram of soil ranged from 4×10^7 for WT-6-24 to 2×10^9 for Gl-9. Numbers of each isolate were similar in noninfested and pathogen-infested soils. There was no correlation between colony-forming units in soil and extent of survival and saprophytic growth of *R. solani*.

TABLE 2. Effect of mycelial preparations of isolates of *Trichoderma* spp. and *Gliocladium virens* on survival and saprophytic growth of *Rhizoctonia solani* in soil[x]

	R. solani (R-23)		
Isolate[y]	Survival in infested beet seed (%)	Saprophytic growth (% beet seed colonization in soil)	Colonization of beet seed in soil amended with pathogen in sand corn meal (%)
Control	95 a[z]	95 a	89 ab
Bran	93 ab	94 a	100 a
T. viride			
T-1-R4	77 bc	41 b	76 b
T-1-R9	78 bc	45 b	92 ab
TS-1-R3	15 de	0 d	0 c
T. harizanum			
WT-6-24	78 bc	46 b	85 ab
Th-5	83 bc	16 cd	77 b
Th-58	56 c	32 bc	3 c
Th-23-R9	73 c	46 b	76 b
T. hamatum			
TRI-4	6 e	0 d	5 c
Tm-23	14 de	6 d	0 c
31-3	6 e	0 d	3 c
G. virens			
Gl-3	17 de	0 d	0 c
Gl-9	18 de	3 d	0 c
G1-17	18 de	0 d	0 c
Gl-21	33 d	3 d	5 c

[x] Assay performed 3 wk after addition of *R. solani* and isolates of *Trichoderma* spp. and *G. virens* to soil.

[y] Mycelial preparations were 3-day-old cultures of antagonist on bran added to soil (1:200, w/w) to give antagonist density of 5×10^3 propagules per gram of soil.

[z] Numbers in each column followed by same letter do not signficantly differ from each other according to Duncan's multiple range test, $P = 0.05$.

Most of the reduction in pathogen survival in beet seed occurred within 1 wk of incubation. The rate of reduction in survival with five of the 14 isolates of *Trichoderma* spp. and *G. virens* is shown in Fig. 1. With all effective isolates, there was at least 50% reduction in survival at this time; with TRI-4 and 31-3 (*unpublished*), there was 90% reduction. Between 1 and 6 wk of incubation, there was generally no significant reduction in survival. Isolates that rapidly reduced survival of *R. solani* in infested beet seed also almost completely prevented its growth into the soil during the first week and essentially eliminated survival of pathogen mycelium.

Effect of antagonist preparations on damping-off caused by *R. solani*. In control flats without antagonists, or in bran-amended flats, millet seed infested with *R. solani* and added to soil at 0.01% essentially eliminated the stand of cotton, sugarbeet, and radish seedlings within 3 wk (Table 3). Mycelial preparations of most isolates prevented damping-off, and stands that resulted from many of these treatments (10 in cotton, 12 in sugar beet, and nine in radish) were comparable to those of the noninfested controls. In general, mycelial preparations of isolates of *T. hamatum* and *G. virens* were more effective than isolates of *T. harzianum* and *T. viride* in preventing damping-off caused by *R. solani*.

In cotton and radish, stands greater than those in the noninfested controls were not achieved with mycelial preparations of any isolate. However, the stands of sugar beet seedlings in soils amended with mycelial preparations of seven isolates (Th-23-R9, Tm-23, 31-3, G1-3, G1-9, G1-17, and G1-21) were more than 50% higher than the stand in noninfested control flats. Unlike emergence of cotton and radish, that of sugar beet was poor in noninfested soil and even at 1 wk the stand was only 49%, compared to 94% for cotton and radish. On moist filter paper, thiram-treated sugar beet seeds germinated 60–70%; so, in addition to being effective against *R. solani*, it appeared that mycelial preparations of isolates also suppressed other microflora that contributed to damping-off.

Experiments also were performed in which mycelial preparations were added to soils 1 wk after amendment with pathogen-infested millet. Stands of cotton, sugar beet, and radish at 1, 2, and 3 wk after planting were comparable ($P = 0.01$) to those of the previous experiment in which pathogen and antagonist were added to soil at the same time. Correlation coefficients (r) between

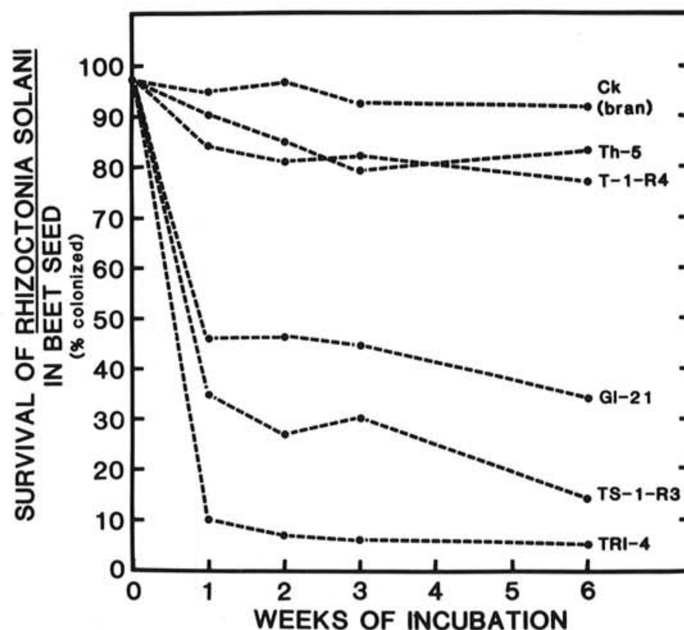

Fig. 1. Effect of selected isolates of *Trichoderma viride* (T-1-R4, TS-1-R3), *T. harzianum* (Th-5), *T. hamatum* (TRI-4), and *Gliocladium virens* (G1-21) on the survival of *Rhizoctonia solani* (R-23) in pathogen-infested beet seeds added to soil.

stands after different times of antagonist addition were 0.781, 0.727, and 0.806 for cotton, sugar beet, and radish, respectively.

At time of harvest (3 wk), soil was assayed for inoculum density of *R. solani* by using the beet seed colonization method. A highly significant negative correlation ($P = 0.01$) between stand and inoculum density in soil was found. Mycelial preparations of isolates that prevented damping-off also reduced pathogen inoculum in soil; the correlation coefficients (r) between plant stand and pathogen density were −0.96, −0.92, and −0.94 for cotton, sugar beet, and radish, respectively.

An experiment was performed to determine the effectiveness of conidial preparations in preventing damping-off in the systems studied. Two isolates previously shown to be effective in preventing disease (TRI-4 and Gl-21) and two which were ineffective (T-1-R4 and WT-6-24) were added to pathogen-infested soils either as mycelial or conidial preparations. There was no significant damping-off of cotton, sugar beet, or radish with mycelial preparations of TRI-4 and Gl-21, but considerable damping-off occurred with mycelial preparations of T-1-R4 and WT-6-24 (Table 4). In addition, conidial preparations of the four isolates did not prevent damping-off in any of the crops, and disease with these treatments was as severe as that in the pathogen-infested controls.

Correlation between experimental data. Statistical analyses were performed to determine whether there was a correlation between damping-off caused in the greenhouse by *R. solani* and survival and saprophytic growth of the pathogen in soil. Correlations also were determined for damping-off and antagonist density. There was a highly significant negative correlation ($P = 0.01$) between stands in all three crops and pathogen survival in soil (Table 5). Similarly, there was a significant negative correlation ($P = 0.05$) between stand and saprophytic growth of *R. solani* from infested beet seed into the soil. However, there was no correlation between plant stand and antagonist density in soil.

TABLE 3. Effect of mycelial preparations of isolates of *Trichoderma* spp. and *Gliocladium virens* on cotton, sugar beet, and radish stands in soil infested with *Rhizoctonia solani* and on saprophytic growth of the pathogen

Isolate[x]	Plant stand (%) at 3 wk			Saprophytic growth (% beet seed colonized in soil)[y]
	Cotton	Sugar beet	Radish	
Control (noninfested)	92 ab[z]	39 bcd	79 ab	8 f
R. solani	0 e	3 e	1 f	94 a
R. solani and bran	0 e	4 e	4 f	72 ab
T. viride				
T-1-R4	55 cd	24 cd	39 def	33 de
T-1-R9	36 d	21 de	31 ef	37 cde
TS-1-R3	34 d	29 cd	51 de	52 bcd
T. harzianum				
WT-6-24	4 e	4 e	36 def	59 bc
Th-5	76 bc	48 bc	60 cde	19 ef
Th-58	76 bc	51 bc	70 abc	9 f
Th-23-R9	90 ab	63 ab	66 bcd	14 ef
T. hamatum				
TRI-4	74 bc	53 abc	73 abc	18 ef
Tm-23	90 ab	69 a	73 abc	1 f
31-3	94 ab	68 a	88 ab	1 f
G. virens				
Gl-3	92 ab	70 a	78 ab	1 f
Gl-9	94 ab	61 ab	85 ab	1 f
Gl-17	92 ab	64 a	89 ab	0 f
Gl-21	100 a	60 ab	90 a	0 f

[x] Mycelial preparations were 3-day-old cultures of antagonist on bran added to soil (1:200, w/w) to give antagonist density of 5×10^3 propagules per gram of soil.
[y] Assay performed at harvest after 3 wk of plant growth.
[z] Numbers in each column followed by same letter do not signficantly differ from each other according to Duncan's multiple range test, $P = 0.05$.

DISCUSSION

Mycelial preparations of *Trichoderma* spp. and *G. virens* containing young actively-growing hyphae either embedded in (or in possession of) a food base (bran) were more effective than conidial preparations in both reducing *R. solani* activity and

TABLE 4. Effect of mycelial and conidial preparations of isolates of *Trichoderma viride* (T-1-R4), *T. harzianum* (WT-6-24), *T. hamatum* (TRI-4), and *Gliocladium virens* (Gl-21) on cotton, sugar beet, and radish seedling stands in soil infested with *Rhizoctonia solani*[x]

Isolate and preparation[y]	Plant stand (%) at 3 wk		
	Cotton	Sugar beet	Radish
Control (noninfested)	96 a[z]	53 a	76 a
R. solani	2 b	8 c	3 c
R. solani and bran	6 b	13 bc	8 bc
T. viride (T-1-R4)			
Mycelial preparation	25 b	21 b	31 b
Conidial preparation	18 b	16 bc	9 bc
T. harzianum (WT-6-24)			
Mycelial preparation	18 b	11 bc	14 bc
Conidial preparation	16 b	21 b	29 bc
T. hamatum (TRI-4)			
Mycelial preparation	86 a	63 a	70 a
Conidial preparation	10 b	8 c	15 bc
G. virens (Gl-21)			
Mycelial preparation	94 a	70 a	83 a
Conidial preparation	20 b	21 b	38 b

[x] Assay performed 3 wk after addition to soil of *R. solani* and isolates of *Trichoderma* spp. and *G. virens*.
[y] Mycelial preparations were 3-day-old cultures of antagonist on bran added to soil (1:200, w/w) to give antagonist density of 5×10^3 propagules per gram of soil. Conidial preparations were conidia on bran mixed immediately with soil (1:200, w/w) to give antagonist density of 5×10^3 conidia per gram of soil.
[z] Numbers in each column followed by same letter do not significantly differ from each other according to Duncan's multiple range test, $P = 0.05$.

TABLE 5. Correlation coefficients (r) between experimental variables and cotton, sugar beet, and radish stands in the greenhouse

Variable	Correlation coefficient (r)[v]		
	Cotton stand	Sugar beet stand	Radish stand
Survival[w] (%)	−0.921 **	−0.979 **	−0.967 **
Survival[x] (%)	−0.931 **	−0.982 **	−0.959 **
Saprophytic growth[y] (%)	−0.715 *	−0.770 *	−0.757 *
Population[z] (cfu/g soil)	0.286	0.403	0.383

[v] Asterisks * and ** indicate significant correlation at $P = 0.05$ and highly significant correlation at $P = 0.01$, respectively. Minuses indicate negative correlation.
[w] Survival of *Rhizoctonia solani* in infested sugar beet seed after 3 wk of incubation in soil.
[x] Colonization of sugar beet seed in soil amended with pathogen in sand corn meal.
[y] Saprophytic growth of *R. solani* in soil as measured by beet seed colonization.
[z] Population density (colony-forming units per gram of soil at 3 wk) of antagonists added to soil as mycelial preparations.

survival in soil and preventing damping-off. In this report and previously (11), we also showed that mycelial preparations were more effective than conidial preparations, conidia, or free mycelium of antagonists in stimulating population proliferation. We suggested that hyphae already occupying the food base do not appear to be subject to fungistasis and that intimate contact between mycelium and food base enabled the antagonists to grow relatively unimpeded through the soil. The activity of mycelial preparations in enhancement of antagonist density coupled with suppression of pathogen and prevention of damping-off may be explained by the principle of substrate possession described by Bruehl (2). However, it was of interest that our results showed no correlation between antagonist populations and pathogen survival or damping-off. Although population increase of augmented microorganisms continues to be a criterion for successful biological control, few studies have established a relationship between these two parameters. The specific activities of the antagonist that occur during its growth are probably more important in pathogen suppression than the population proliferation of the antagonist. These activities may be enzyme (5) or antibiotic production (3), or mycoparasitism (7,21). We have recently demonstrated parasitic activity of mycelial preparations and fermentor biomass of *Trichoderma isolates* against *R. solani* in soil (*unpublished*).

Our observations emphasize that a major obstacle to biological control by direct soil augmentation may be the inappropriate method for mass culturing and delivery to soil of effective propagules of the biological control fungus. In the past, relatively little attention was given to the type of propagules in preparations. For example, the nature of antagonists was indicated in only a few of the 13 cases of successful field biological control of soilborne pathogens performed in various parts of the world from 1971 to 1980 in which antagonists were added to soil (17). Conidia of biological control fungi, especially those of *Trichoderma* and *Gliocladium*, have been used extensively in studies because they are abundantly produced on agar media and are easy to handle. However, except for possible use as seed treatments, our results suggest that conidia are inappropriate for introducing antagonists into soil for biological control. Consequently, potentially effective biological control fungi may be overlooked or discarded in screening tests when conidia alone are used.

In addition to mycelial preparations, other propagule forms and preparations should be considered in biological control studies. Twelve-day-old wheat bran preparations of *T. harzianum*, although not described exactly, were more effective than conidial preparations in reducing bean disease caused by *Sclerotium rolfsii* Sacc. (4). Although mycelial and conidial preparations of the *T. harzianum* isolate WT-6-24 were generally ineffective in disease control and pathogen density reduction in the present investigation, 20-day-old ground oat preparations did significantly reduce saprophytic activity of *R. solani* in soil (20). We showed that both liquid and solid fermentation preparations, including those of wheat bran and oat kernels contained large amounts of chlamydospores (8,9,16), which appeared to be more effective than conidia in pathogen and disease control (1,10). The form of the pathogen propagule may also be important in studying disease control with biological control preparations. Recently, it was suggested that the response of *S. rolfsii* to *Trichoderma* depended on the developmental stage, physiological state, and age of the pathogen (7). For example, *R. solani* can exist in soil in several forms (14,19). In this study, isolates effective against the pathogen reduced its survival when it was added to soil embedded in organic matter (beet seed) or as free mycelium (in sand/cornmeal) as well as preventing its growth from a food base into soil. These observations would suggest that mycelial preparations of antagonists may be able to attack and destroy a pathogen that exists in soil in various forms (e.g., as mycelium, "barrel cells," or sclerotia).

Of the three *Trichoderma* species studied, our results suggest that isolates of *T. hamatum* were more effective than those of *T. harzianum* and *T. viride*. In laboratory, greenhouse, and field tests, isolates of all species, but especially those of *T. hamatum* and *T. harzianum* have been implicated as effective biological control agents (1,4,5,7,15,20). It is recognized that biological control can be specific and depends on a wide range of soils, environmental factors, location, and season in addition to crop and pathogen. Our results also demonstrated that there was a highly significant correlation ($P = 0.01$) between the ability of isolates of *Trichoderma* and *Gliocladium* to prevent damping-off and reduce pathogen density in soil. Recently, it was shown that a high correlation existed between the ability of isolates of *Trichoderma* to prevent bean disease caused by *S. rolfsii* in the greenhouse and germination of pathogen sclerotia on soil plates in the laboratory (6).

A major advance in biological control research would be the establishment of a rapid, easy screening test performed in the laboratory which could be correlated to biological control activity in the field. Although most biological control studies are suggested by laboratory plate assays that show antibiosis or mycoparasitism on a specific medium (3,15,21), generally there is little or no correlation between these phenomena and the ability to reduce or prevent disease in greenhouse or field. An assay that can be standardized to include natural soil, a defined pathogen, and a known antagonist may be a more productive approach. The beet seed colonization method we used to determine pathogen survival and activity may not be as rapid as a dilution plate technique, but it is simple, can be performed in the laboratory, and as many as 150 samples can be processed per day. Such an assay may be developed for other pathogens with a high probability of success.

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