Survival of *Trichoderma harzianum* in Soil and in Pea and Bean Rhizospheres

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ABSTRACT

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A semiselective medium was developed for the direct isolation of *Trichoderma harzianum* and other *Trichoderma* spp. from soil, and for the quantitative estimation of its inoculum density in soil. The selective agar medium contained per liter: V-8 juice, 200 ml; glucose, 1 g; agar, 20 g; and water, 800 ml. The agar was autoclaved separately in 0.5 L of water and added to the V-8 juice after autoclaving. The medium, which was designated as TME, contained the following antimicrobial agents (μg/ml): neomycin sulfate, bacitracin, penicillin G, and chloroneb, 100 each; nystatin, 20; chlortetracycline HCl, 25; sodium propionate, 500; and pentachloronitrobenzene, 100. For benomyl-tolerant biotypes, the TME medium was supplemented with 10 μg/ml of benomyl. Conidia of *T. harzianum* that were added to soil without nutrient-supplying amendments survived for at least 130 days, but the duration and the percent survival depended on the isolate used. *T. harzianum* did not survive well in the rhizosphere of bean and pea seedlings when seed was coated with conidia of the fungus. It also did not increase in the rhizosphere of pea seedlings from conidia applied directly to soil 1 day before planting. Infusion of pea seed with the fungicide metalaxyl before coating it with conidia of *T. harzianum* improved survival of conidia in the rhizosphere compared with the survival in the rhizosphere from seed that received conidia only.

Additional key words: biological control.

Although there have been numerous recent attempts to use *Trichoderma* spp. for experimental biological control of certain plant diseases (1,3,5,7,13), few workers have attempted quantitative studies of the ecology and survival of the antagonist in soil and in the plant rhizosphere. The scarcity of information on the survival of *Trichoderma* spp. in soil is due, at least in part, to lack of precise techniques for its isolation and enumeration. Danielson and Davey (2) used the dextrose-peptone-yeast extract agar of Papavizas and Davey (10) to study the abundance and distribution of propagules of various *Trichoderma* spp. in forest soils. Liu and Baker (9) recently modified Martin's rose bengal-streptomycin agar by adding pentachloronitrobenzene (PCNB) at 100 μg/ml and used the medium to determine numbers of *Trichoderma* spp. propagules in soil. They also used this medium to study the influence of soil pH and matric potential on *Trichoderma harzianum* Rifai in the presence of organic matter from radishes, cucumbers, and *Rhizoctonia solani* mycelium. A methylene blue agar medium also was used in petri plates to count conidia of *T. viride* dispersed in barley flour (4).

The objectives of this study were to develop a selective culture medium for isolating and enumerating *T. harzianum* and other *Trichoderma* spp. and to study the survival of these antagonists in soil and in plant rhizospheres in the absence of added organic matter.

MATERIALS AND METHODS

Isolates of *T. harzianum*. Eight isolates of *T. harzianum* were used in this study: H. D. Wells, USDA-ARS, Georgia Coastal Plain Experiment Station, Tifton 31794, supplied isolates H-54, T-1, T-5, T-14, and WT-6 (his designations); and by ultraviolet (UV) light irradiation I developed biotypes T-1 (ben 100-1), WT-6-1, and WT-6-11 (*unpublished*). The numbers (or other designations) following Wells' numbers in my biotype designations indicate the sequence in each particular biotype series produced by UV irradiation. Conidia of WT-6 and biotypes derived from it are white, whereas those of other isolates are various shades of green. Stock cultures of the wild isolates and the induced biotypes were maintained on V-8 juice agar slants at 5 C. Conidia were obtained from 5-day-old cultures grown on V-8 juice agar at 25 C under continuous fluorescent light by adding a few milliliters of sterile water to the cultures and gently rubbing the surface with a sterile cotton-tipped applicator. Conidia were counted in a hemacytometer, and the volumes of the suspensions were adjusted to obtain the numbers of conidia indicated in each experiment.

Soils and plants used. The soil used for artificial infestation experiments and for rhizosphere studies in the greenhouse was a Rumsford sandy loam (pH 6.0) from Beltsville, MD. For isolation of *Trichoderma* spp. 10 natural soils from Maryland, Mexico, and Canada were also used. For the rhizosphere studies, peas (*Pisum sativum* L. 'Perfected Freezer') and snap beans (*Phaseolus vulgaris* L. 'Blue Lake') were used.

Selective medium for isolation of *Trichoderma* spp. from soil. A V-8 juice agar medium was the basal medium used for isolation of *Trichoderma* spp. from soil. The medium contains (per liter of liquid): 200 ml V-8 juice, 1 g glucose, and 20 g agar. The agar was autoclaved separately in 500 ml of water and mixed with the dilute V-8 juice after autoclaving. The pH of the medium after autoclaving was 3.8–4.0.

Of 23 antimicrobial agents tested, the following did not inhibit the germination or growth of conidia of *T. harzianum* and were selected for further testing: sodium propionate, nystatin (Mycostatin 4,960 units per milligram, Calbiochem-Behring Corp., La Jolla, CA 92307), chloroneb (1,4-dichloro-2,5-dimethoxybenzene, E. I. du Pont de Nemours & Company, Wilmington, DE 19898), penicillin G (potassium salt, >1,435 units per milligram), bacitracin (50 units per milligram, chlortetracycline HCl (Aureomycin®), and neomycin sulfate. Penicillin G, chlortetracycline HCl, and neomycin sulfate were obtained from ICN Nutritional Biochemicals, Cleveland, OH 44128. Various concentrations of the antimicrobial agents were added to the acid

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V-8 juice agar after autoclaving to formulate eight different media. After several preliminary tests, Trichoderma medium E (TME) was selected. This medium contained (per liter of V-8 juice agar): 100 mg each of neomycin sulfate, bacitracin, penicillin G, and chloroneb; 25 mg chlortetracycline HCl; 20 mg nystatin; and 500 mg sodium propionate. The last ingredient is partially effective against *Rhizopus* spp. and *Mucor* spp.

Survival of *T. harzianum* conidia in soil. Conidia of wild strains WT-6, T-1, T-5, T-14, and H-54 and of the biotype WT-6-1, which is tolerant to methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate (benomyl, 50% wettable powder, E. I. du Pont de Nemours & Company, Wilmington, DE 19898) (*unpublished*), were added to 100-g portions of air-dry soil in 400-ml beakers at 1×10^4/g. The conidia were added with enough water to adjust the moisture tension in all samples to −9 bars and the containers were covered with polyethylene film to prevent water evaporation.

The soils were assayed for *T. harzianum* at 0, 12, 35, 75, and 130 days with the dilution-plate method (dilution, 1:1,000). One-milliliter aliquots were removed from the final dilution while the liquid was agitated by a magnetic stirrer and spread on the TME medium (six plates per replication, five replications). The plates were incubated at 25 ± 2 C under continuous fluorescent light and colonies were counted after 6–7 days. For the biotype WT-6-1 the medium was supplemented with benomyl at 10 mg/L. This selective medium for benomyl-tolerant biotypes will henceforth be designated as TME-ben 10.

Survival of *T. harzianum* in the rhizosphere. Conidia were added to the seed or directly to soil before planting for studies on the establishment of *T. harzianum* in pea and bean rhizosphere. Conidia were harvested from 7-day-old cultures of V-8 juice agar by gently rubbing the colony surface to which 2 ml of 4% sterile methyl cellulose (MC) solution had been added. The conidial suspension was mixed thoroughly in a mixer. Aliquots were removed and diluted 1,000-fold in water to count the conidia. The original spore suspension in MC was then adjusted with additional MC to obtain the desired concentration of conidia per milliliter. The adjusted suspensions were applied to seed in amounts to obtain concentrations indicated in individual tests. Treated and untreated seed were planted in 15-cm-diameter plastic pots (one seed per pot) immediately after the seed had dried.

Adjusted aqueous suspensions of conidia also were added to the Beltsville soil at 5×10^3 and 1×10^4 per gram of air-dried soil and mixed thoroughly. Untreated seeds were planted as before. In another test, pea seed was first treated with the systemic fungicide *N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl)alanine methyl ester (CGA-48988, metalaxyl, CIBA-Geigy Corp., Greensboro, NC 27409). The metalaxyl was dissolved in acetone (1% active ingredient) and the seed was immersed in the solution for 20 min as described before (11). The seed was allowed to dry and was then coated with either of two concentrations of conidia (3.2×10^8 and 6.4×10^8/g of seed).

All treatments were set up in quadruplicate in a randomized block design. All rhizosphere experiments were done in a growth room at 25 ± 0.5 C and plants received 16 hr of light per day. Light was provided by cool-white fluorescent tubes and 25-W incandescent lamps. Light at ~800 μEin/m^2/sec was supplied to the upper leaves.

Plants from each pot were carefully removed from the soil after the plants grew for the lengths of time specified in each experiment. Roots (including the cotyledons for peas) were allowed to air-dry at room temperature for 2 hr, gently tapped to remove superfluous oil, and placed in sterile tap water. Appropriate rhizosphere dilutions were prepared and plated on the TME medium as described by Horst and Herr (6). In another experiment with pea seed treated with WT-6-1 or T-1(ben 100-1), another new benomyl-tolerant biotype of *T. harzianum* (*unpublished*), cotyledons were recovered from the soil and assayed separately from the roots on the TME-ben10 medium. Roots were cut 10 cm from the cotyledons and assayed as before. Rhizosphere assay plates were incubated for 6–7 days at 25 ± 2 C under continuous fluorescent light and the colonies were counted.

TABLE 1. Recovery of *Trichoderma* spp. from various soils by using the dilution plate method on Trichoderma medium E (TME) and on rose bengal-streptomycin-pentachloronitrobenzene medium (RBS-PCNB)[y]

Soil and origin	Soil pH	Colony forming units per gram of soil (×10^3) on indicated medium	
		TME	RBS-PCNB
Loamy sand, Maryland	5.8	9.8 a[z]	5.6 abc
Silty clay loam, Mexico	6.4	9.2 a	1.0 cd
Muck soil, Canada	6.7	7.0 ab	0.2 d
Muck soil, Canada	6.5	6.8 ab	0.8 d
Silty clay loam, Mexico	6.7	6.6 ab	0.6 d
Sandy loam, Maryland	6.0	6.2 ab	0.6 d
Silty clay, Mexico	6.9	4.0 bcd	2.6 bcd
Silty clay, Mexico	6.3	3.8 bcd	3.2 bcd
Sandy loam, Mexico	7.4	3.6 bcd	3.4 bcd
Muck soil, Canada	6.7	1.4 cd	0.6 d

[y] Martin's medium as modifed by Liu and Baker (9).
[z] Values followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

RESULTS

Selective medium. The new TME medium was compared with Martin's rose bengal-streptomycin agar (RBS) as modified by Liu and Baker (9) by adding 100 μg/ml of PCNB to RBS. Soil dilutions (1:1,000) were prepared from 10 soils naturally infested with *Trichoderma* spp. The TME plates were incubated under continuous fluorescent light and those of RBS-PCNB in the dark. Significantly more colonies of *Trichoderma* spp. were recovered from five of 10 soils on the TME medium than on the RBS-PCNB medium (Table 1). Recovery from the other five soils tested was equal on both media. The isolates recovered from the Mexican soils belonged to *T. harzianum* and *T. viride* Pers. ex S. F. Gray. *T. viride* and *T. polysporum* were recovered from the Canadian soils and *T. harzianum* and *T. hamatum* (Bon.) Bain. from the two Maryland soils.

The colonies of *Trichoderma* were well delineated and easy to count on both media. Contamination with bacteria was not a problem on the TME medium. Where *Rhizopus* spp. and *Mucor* spp. were present in the soils or in the air during preparation of dilutions, TME plates became contaminated with spreading colonies of the two fungi which prevented, to some extent, accurate colony counts despite the fact that sodium propionate was partially inhibitory to *Mucor* and *Rhizopus*. Since no colonies of such contaminants appeared on the RBS-PCNB medium, PCNB was added to the TME medium at 0, 25, 50, and 100 μg a.i./ml after autoclaving and the media were used to isolate *Trichoderma* from five of the 10 soils in Table 1 that were infested with *Mucor* and *Rhizopus*. The PCNB added to TME medium at 50 and 100 μg a.i./ml prevented development of *Rhizopus* and *Mucor* on the plates. At 25 μg a.i./ml the fungicide reduced both the number and size of colonies of these soil fungi. Even at 50 μg/ml, however, the fungicide significantly reduced recovery of *Trichoderma* spp. from all soils tested. Addition of benomyl (10 μg a.i./ml) to the TME medium after autoclaving greatly improved its ability to isolate benomyl-resistant biotypes from soil (Fig. 1). For instance, the benomyl-resistant biotype WT-6-1 was recovered from soil with practically no interference from other fungi. The nontolerant WT-6 did not develop colonies on the TME-ben10 medium.

An additional test was performed to determine the average percentage of conidia of three strains (T-1, T-5, WT-6) of *T. harzianum* recovered on the TME medium with the dilution plate method. Aqueous suspensions of conidia from 7-day-old colonies on V-8 juice agar were added to the Beltsville soil at 10^4 conidia per gram of soil. The conidia were mixed thoroughly with the soil and dilutions (1:1,000) were made immediately after addition of conidia to soil on the TME medium containing 0, 25, 50, and 100 μg a.i./ml of PCNB. The average percentage of conidia of strain T-1 recovered on the TME medium amended with four concentrations of PCNB was 86, 64, 60, and 56% (with a range of 6%, $P = 0.05$) for

0, 25, 50, and 100 μg a.i./ml of PCNB in the TME medium, respectively; for strain T-5 the values were 96, 82, 86, and 98% (range, 15%); and for strain WT-6 the values were 70, 62, 68, and 88% (range, 8%). *Rhizopus* and *Mucor* were effectively suppressed by the two highest concentrations of PCNB. No *Trichoderma* colonies developed on plates from the noninfested control soil.

Survival of conidia in soil. With the exception of the UV light-induced biotype WT-6-1 (resistant to benomyl), the colony-forming units (cfu) of all isolates of *T. harzianum* recovered from soil was reduced considerably after 12 days of incubation (Fig. 2). Most reduction was observed with isolate H-54; recovery of cfu after 75 days of incubation corresponded to 8% only of the original number of conidia added to soil. The number of cfu of WT-6-1 recovered on the 12th day of incubation on the TME-ben10 medium exceeded the number of conidia added to soil (10^4/g) at zero time. Recovery of cfu after 35, 75, and 130 days of incubation was 50, 20, and 3% of the original number of conidia of WT-6-1 added, respectively. After 35 days in soil, cfu of isolates T-14, WT-6, T-1, and T-5 ranged from 13 to 35% of the original number of conidia added. Population density dropped to less than 1,000 cfu per gram of soil after 130 days in soil.

Failure of *T. harzianum* to establish in the rhizosphere. For peas, sample assays included soil adhering to cotyledons (and seed coats), tap root, and secondary roots. For beans, sample assays included soil adhering to tap roots and secondary roots from the point of seed attachment. Counts of cfu of WT-6 on the TME medium from these "rhizosphere" samples of both plants were high after 2.5 wk (Table 2) indicating the presence, but not an increase of *T. harzianum* cfu in the composite samples. Almost twice as many colonies were observed after 2.5 wk in the samples from seedlings that came from seed treated with 6×10^8 conidia per gram of seed than from seed coated with half as many conidia. After 5 wk, population densities were reduced and there were no differences between the two seed lots treated with WT-6.

A similar experiment was performed with peas only. The seed was coated with conidia of WT-6 at five concentrations from 0 to 6.8×10^7 conidia per gram of seed (Table 3). The size of the population recovered from the composite rhizosphere (soil adhered to cotyledons and roots) 2 wk after planting depended on the number of conidia added to seed; and the size of the population at 4 wk fell to about one-third or one-half of that recovered at 2 wk. The numbers of cfu recovered from a gram of rhizosphere soil at 2 wk, however, was considerably less than the number of conidia originally added per individual seed.

In a third experiment 10^4 conidia of the benomyl-tolerant biotypes WT-6-1 and WT-6-11 were added to soil directly and 1 day later untreated pea seed was planted. No white colonies of *Trichoderma* (similar to WT-6 and its biotypes) were recovered from the rhizosphere of peas grown in control soil (soil that did not receive the WT-6-1 or WT-6-11) at 10, 20, and 40 days after planting (Table 4). In no case did the rhizosphere soil from beans or peas contain populations of *Trichoderma* (WT-6-1 or WT-6-11)

TABLE 2. Survival of *Trichoderma harzianum* isolate WT-6 in bean and pea rhizosphere[x] from seed coated with conidia of the antagonist

Plant and seed treatment	Conidia added per gram of seed ($\times 10^8$)	Colony forming units[y] ($\times 10^3$) per gram of air-dry rhizosphere soil at indicated week after planting[z]	
		2.5 wk	5 wk
Bean			
None	0	0 a	0 a
WT-6	3	155 c	23 b
WT-6	6	253 d	38 b
Pea			
None	0	0 a	0 a
WT-6	3	96 b	18 b
WT-6	6	164 c	17 b

[x] Includes soil adhering to roots, seed coats, and cotyledons.
[y] Recovered on the Trichoderma medium E.
[z] In each column, values followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

TABLE 3. Survival of *Trichoderma harzianum* isolate WT-6 in the rhizosphere[x] of pea seedlings from seed coated with four concentrations of conidia of the antagonist

Conidia added per gram of seed	Colony forming units[y] ($\times 10^3$) per gram of air-dry rhizosphere soil at indicated week after planting[z]	
	2 wk	4 wk
0.0	0.0 a	0.0 a
2.3×10^5	0.6 a	0.0 a
11.4×10^6	45.4 b	13.9 b
2.3×10^7	45.0 b	22.4 bc
6.8×10^7	67.7 c	34.1 c

[x] Includes soil adhering to roots, seed coats, and cotyledons.
[y] Recovered on the Trichoderma medium E.
[z] In each column, values followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

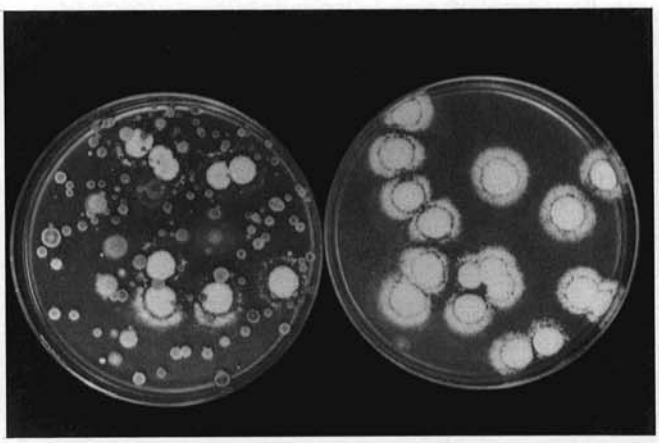

Fig. 1. Isolation of *Trichoderma harzianum* (biotype WT-6-1) from soil by the dilution plate method. Left, on TME isolation medium; right, on the TME medium containing benomyl at 10 μg a.i./ml.

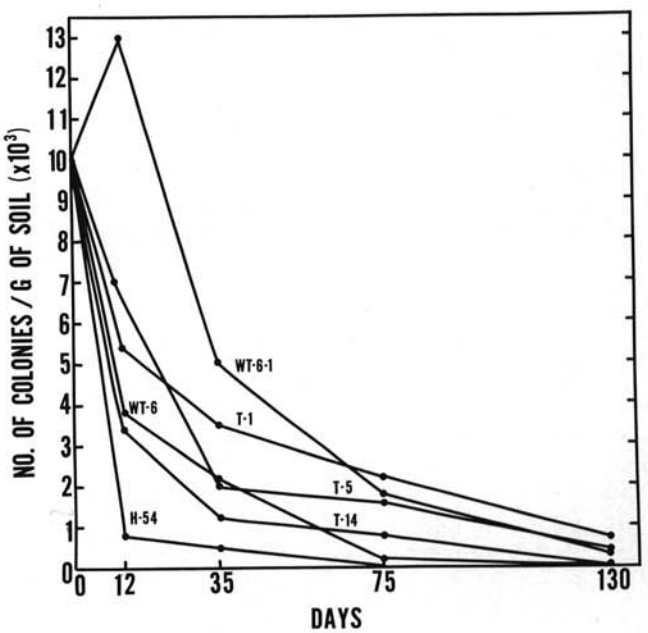

Fig. 2. Survival in soil of *Trichoderma harzianum* (strains T-1, T-5, T-14, H-54, WT-6, and UV-induced biotype WT-6-1) as indicated by the number of colonies recovered on a special selective Trichoderma medium after 130 days of incubation in soil.

greater than the unplanted, infested soil on an equal weight basis, even 40 days after planting.

In the fourth experiment recovery of the two benomyl-tolerant biotypes, T-1(ben100-1) and WT-6-1, on the TME-ben10 medium was negligible from the rhizosphere samples taken 10 cm or more from the cotyledon attachment point (Table 5). Large numbers of colonies of both biotypes were recovered when the rotting cotyledons and seed coats were used as the "rhizosphere" samples. The number of colonies of both biotypes recovered declined sharply between the 2.5- and 8-wk samplings.

In the *T. harzianum*-metalaxyl test, in which the fungicide was added to pea seed with acetone before applying conidia of WT-6 at 3.2×10^8 and 6.4×10^8 per gram of seed, more colonies of isolate WT-6 were recovered from the rhizosphere of seedlings grown from seeds treated with metalaxyl before coating with conidia, than were recovered from the rhizosphere of seedlings from seed that received no fungicide (Table 6). For example, after 2.5 wk, 1.5×10^4 and 5.1×10^4 cfu/g of rhizosphere soil were recovered near seedlings from seeds treated with WT-6 alone and from those from seeds treated with metalaxyl and *T. harzianum*, respectively. The equivalent counts for the high WT-6 concentration alone and with the metalaxyl treatment were 4×10^4 and 15×10^4, respectively.

DISCUSSION

It is very difficult or perhaps even impossible to develop a culture medium that could be used for selective recovery of all *Trichoderma* spp. from soil without interference from various other soil fungi. The new Trichoderma-selective medium (TME) described in this paper gave satisfactory results in this research, but it does have disadvantages. First, even with the sodium propionate, a mild fungicide (mold preventative) (14), the medium allows growth and spread of *Mucor* spp., *Rhizopus* spp., and other Mucorales that, when fully developed, obscure colonies of *Trichoderma* and hinder the counting. This may be corrected by the addition of PCNB (25–100 μg/ml) to the medium. However, PCNB, even at 25 μg a.i./ml, may reduce the number of cfu of *Trichoderma* spp. recovered depending on the relative sensitivities of different strains. This may explain why the RBS-PCNB medium (9) was less efficient than TME for recovering cfu from soil. When contaminants such as *Rhizopus* spp. are not a problem TME should be used without PCNB for best recovery. Second, chloroneb at 100 μg a.i./ml may be inhibitory to some *Gliocladium* isolates (J. Krikun and G. C. Papavizas, *unpublished*). If it is desirable to recover *Gliocladium* from soil, chloroneb should be omitted from the medium. Third, the medium requires V-8 juice, a proprietary product that may not be available outside the United States.

Addition of benomyl to the TME medium made it selective for biotypes that have tolerance to benomyl. UV light-induced, benomyl-tolerant biotypes of *T. harzianum* were recovered on dilution plates without interference from soil saprophytes. The use of benomyl for tolerant biotypes may be used as an excellent model for studying the ecology and survival of *Trichoderma* in soil and rhizosphere with little or no interference from other soil saprophytes. Such studies are now in progress.

The study on the survival of *T. harzianum* conidia in soil presented circumstantial evidence that some conidia can survive as long as or longer than 4 mo without a food base. Although it is not possible to determine whether cfu come from the original conidia added, the lack of proliferation of the isolates of *T. harzianum* used in the rhizosphere of peas and beans tends to support this view. Theoretically, soil fungistasis should prevent conidia from germinating and preserve them in soil for a long time (8). It is possible that some conidia are lysed in soil without first germinating; or they may germinate in response to nutrients released by soil and subsequently lyse in the absence of adequate food bases needed to sustain growth and sporulation. Further studies are needed to illucidate the dynamics of germination and growth of conidia in soil.

The rhizosphere studies indicated that *T. harzianum* did not establish in the rhizosphere of bean and pea seedlings. This conclusion is supported by the following observations: even when the rhizosphere samples assayed included the roots and decaying seed coats and cotyledons, the number of cfu recovered per gram of rhizosphere soil on dilution plates was always considerably less than the number of conidia added per individual seed, which indicated reduced survival, lack of sporulation in the rhizosphere, or both; when conidia of *T. harzianum* were added to the soil before planting, but not to the seed, the rhizosphere populations never

TABLE 4. Establishment of *Trichoderma harzianum* biotypes WT-6-1 and WT-6-11 in the rhizosphere[v,w] of bean and pea seedlings grown in soil infested[x] with the biotypes 1 day before planting

Trichoderma biotype and host plant	Colony forming units[y] ($\times 10^3$) per gram of air-dry rhizosphere soil at indicated week after planting[z]		
	10 wk	20 wk	40 wk
None			
Unplanted	0.0 a	0.0 a	0.0 a
Bean	0.0 a	0.0 a	0.0 a
Pea	0.0 a	0.0 a	0.0 a
WT-6-1			
Unplanted	7.3 cd	8.0 d	6.4 c
Bean	8.0 d	8.5 d	6.7 c
Pea	5.3 bc	4.7 bc	6.0 c
WT-6-11			
Unplanted	6.6 c	6.3 cd	5.4 bc
Bean	3.1 b	3.0 b	3.6 b
Pea	7.7 d	2.2 b	5.1 bc

[v] Includes soil adhering to roots, seed coats, and cotyledons.
[w] From seed not coated with *T. harzianum*.
[x] The two biotypes were added to soil at 10^4 conidia per gram.
[y] Recovered on the Trichoderma medium E-ben10.
[z] In each column, values followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

TABLE 5. Survival of *Trichoderma harzianum* biotypes T-1(ben100-1) and WT-6-1 in the rhizosphere of pea seedlings from seed coated[w] with conidia of the antagonists

Seed treatment	Sampled portions of the rhizosphere	Colony forming units[x] ($\times 10^3$) per gram of air-dry rhizosphere soil at indicated week after planting[y]		
		2.5 wk	5 wk	8 wk
T-1(ben100-1)	Roots[y]	0.0	0.3	0.8
	Cotyledons[z]	153.0	130.0	15.0
WT-6-1	Roots	0.3	0.1	2.6
	Cotyledons	374.0	56.0	24.0

[w] Conidia of the two biotypes were added to the seed at 2.6×10^7 per gram.
[x] Recovered on the Trichoderma medium E-ben10.
[y] Rhizosphere soil removed from roots 10 cm or more from decomposing cotyledons.
[z] Rhizosphere soil removed from decomposing cotyledons only.

TABLE 6. Survival of *Trichoderma harzianum* isolate WT-6 in pea rhizosphere[x] from seed treated with the fungicide metalaxyl and coated with conidia of the antagonist

Seed treatment	Number of conidia added per gram of seed ($\times 10^8$)	Colony forming units[y] ($\times 10^3$) per gram of air-dry rhizosphere soil at the indicated week after planting[z]	
		2.5 wk	5 wk
None (control)	0	0 a	0 a
Metalaxyl	0	0 a	0 a
WT-6	3.2	15 ab	8 a
WT-6	6.4	40 bc	26 ab
Metalaxyl + WT-6	3.2	51 c	29 bc
Metalaxyl + WT-6	6.4	150 d	41 c

[x] Includes soil adhering to roots, seed coats, and cotyledons.
[y] Recovered on the Trichoderma medium E.
[z] In each column, values followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

exceeded those recovered from the unplanted soil indicating a lack of rhizosphere effect; very few or no colonies were recovered from the rhizosphere soil from roots removed 10 cm away from the seed, the treated area. These results support the conclusions of Rovira (12) who stated that "There is little doubt about the ability of some seed inoculants, e.g., *Rhizobium* spp. and *Azotobacter* spp., to move from the seed to the root, but as the differentiation of most seed, rhizosphere, and soil bacteria is difficult no definite conclusions can be drawn on the contribution of the seed coat microflora to the rhizosphere population." The present findings with *T. harzianum* may also explain why this antagonist needs a suitable food base to establish in soil and in the rhizosphere for effective biological control of root diseases (1,3,12).

Populations of *T. harzianum* in the rhizosphere of seedlings from seed that was coated with the antagonist after it had been infused with the fungicide were not reduced when pea seed were treated with metalaxyl, a systemic fungicide effective against *Phytophthora* and *Pythium*. In fact, more colonies of *T. harzianum* developed in the rhizospheres of seedlings from treated seed than in those from funigicide-free seed. Metalaxyl is not toxic to *T. harzianum* (*unpublished*). The fungicide may have eliminated or reduced soil saprophytes that compete with *T. harzianum* for food bases. Since the soil used was acid (pH 6.0) to begin with, increased acidity in the rhizosphere as a result of adding metalaxyl to seed may not account for the increase. Additional research is needed to establish the beneficial or detrimental effects of fungicidal seed treatments on populations of *Trichoderma* in soil and in the rhizosphere.

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