Trichoderma hamatum Effects on Seed and Seedling Disease Induced in Radish and Pea by *Pythium* spp. or *Rhizoctonia solani*

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ABSTRACT

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In laboratory studies, treatment of pea or radish seeds with conidia of *Trichoderma hamatum* in a Methocel slurry protected seeds and seedlings from *Pythium* spp. or *Rhizoctonia solani*, respectively, nearly as effectively as fungicide seed treatments. By comparison, treatment of radish or pea seeds with *Chaetomium globosum* was less effective than *T. hamatum*. A combination of *C. globosum* and *T. hamatum* was less effective on peas than was *T. hamatum* alone. In soils planted with seeds treated with *T. hamatum*, numbers of Trichoderma propagules increased approximately 100-fold; replanting this soil once or twice with untreated seeds resulted in lower disease incidence than planting in soils originally planted with *C. globosum*-treated or untreated seeds. In soils containing *T. hamatum*, lower densities of *R. solani* and *Pythium* were present than in soils without *T. hamatum*. *Trichoderma hamatum* and *C. globosum* grew abundantly on treated seeds, while *T. harzianum* grew little.

Additional key words: biological control, soil fungi, fungicides, *Pisum sativum*, *Raphanus sativus*, mycoparasitism.

The use of biological agents to control soilborne pathogenic fungi is an attractive possibility. There have been many reports of successful uses of biological control agents (1,4,6,8,9,11,13,15, 20–24), but no agent has been used commercially thus far, partly because application of an excessively large amount of the biocontrol agent may be required (1,6,11,22), or in the case of seed treatments, the biocontrol agents may be effective for only a short time after planting (4,8,15,23).

Microorganisms, apparently responsible for biocontrol, have been isolated from suppressive soils (5,14,18). When these are added to conducive soils, they induce suppressiveness. These microorganisms have the potential of being superior biocontrol agents.

In this work, we describe a seed treatment with *Trichoderma hamatum* (Bon.) Bain which was isolated from a *Rhizoctonia*-suppressive soil (Chet and Baker, *unpublished*). The objectives of the research were to test the effectiveness of this antagonist when applied as a seed treatment against *Phythium* spp. or *Rhizoctonia solani* Kühn which induce damping-off of peas and radishes, respectively, and to determine the effect of such treatment on the suppressiveness of the soil associated with the seeds.

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MATERIALS AND METHODS

Soil. All experiments were performed in a clay soil obtained near Fort Collins, Colorado. The soil had the following characteristics: pH 7.4, >2% lime; conductivity, 1.4 mmhos/cm; organic matter 2.2%; 38 μg/g N (as NO_3); 25 μg/g P; 421 μg/g K; 3.4 μg/g Zn; 8.9 μg/g Fe; 1.7 μg/g Mn; and 3.5 μg/g Cu.

Microorganisms. Isolate R3 of *R. solani* (14) and *Pythium* spp. indigenous to the soil described above were used as pathogens in this study. This soil contained approximately 150 propagules per gram of *Pythium* as determined by dilutions on a selective medium

(16). Of these, approximately 50% were low-temperature and 50% were high-temperature types as determined by Pieczarka and Abawi's procedure (17). Of nine pure cultures obtained from discrete colonies at 20 C (low-temperature isolates), two were identified as *P. ultimum* Trow., five produced sporangia similar to that of *P. ultimum* but no oogonia, and two produced only a few small sporangia. Of 10 pure cultures obtained from discrete colonies at 37 C (high-temperature isolates), one was identified as *P. aphanidermatum* (Edson) Fitzp., seven as *P. oligandrum* Drechsler, and two contained too few fruiting bodies to identify.

Biocontrol agents used were *Trichoderma hamatum*, isolated from a *Rhizoctonia*-suppressive soil from Colombia, South American (Chet and Baker, *unpublished*), *T. harzianum* Rifai which was isolated from soil near Fort Collins, CO (14), and *Chaetomium globosum* Kze. NRRL 6296 (8).

Seed treatments and experimental design. All experiments were performed at 25 ± 1 C. Plastic containers 11 cm wide and 8 cm deep were used in all plantings. Soil was placed in these containers to a depth of 3 cm for peas (*Pisum sativum* L. 'Laxton's Progress') and 1.5 cm for radishes (*Raphanus sativus* L. 'Early Scarlet Globe'). After seeds were planted, pots were covered with plastic covers to reduce evaporation.

For tests with *Pythium* spp., four pea seeds were planted 1 cm deep in each container of the naturally-infested Fort Collins clay soil. Soil moisture was adjusted to 18% (−0.15 bars).

For tests with *R. solani*, 32 radish seeds were planted 1–3 mm deep in each container of soil. Soil moisture was adjusted to 14% (−0.5 bars); at this moisture content and with this host no damage from the resident *Pythium* spp. was noted. Immediately prior to planting, the soil in each container was inoculated with a pellet of *R. solani* containing 900 large (>589 μm) propagules (9).

All biocontrol agents were applied to seeds in suspensions containing 2% (w/v) Methocel A4C Premium (Dow Chemical Co., Midland, MI 48640) as a spreader and adhesive. Spore suspensions of *Trichoderma* spp. contained 3-8 × 10^8 conidia/ml, of which >95% germinated. Suspensions of *C. globosum* contained 8 × 10^5 ascospores per milliliter, of which approximately 40% germinated on Difco-Bacto Potato Dextrose Agar (PDA) (Difco Laboratories, Inc., Detroit, MI 48323). Twenty and 80 ml of spore-Methocel suspensions were applied per kilogram of pea and radish seeds, respectively. Seeds also were treated with both *T. hamatum* and *C. globosum*. In this case seeds were treated first with *T. hamatum* and then with *C. globosum*. As controls, seeds were not treated, were treated with Methocel alone, or were treated twice with Methocel.

Seeds also were treated with standard fungicides, all at the rate of 1.1 g a.i./kg of seeds. Radish seeds were treated with dusts of captan (*N*-[[trichloromethyl]thio]-4-cyclohexene-1,2-dicarboximide) or PCNB (pentachloronitrobenzene), (Olin Corp., Little Rock, AR 72203). Pea seeds were treated either with captan or fenaminosulf (Dexon) (sodium *p*-[dimethylamino] benzenediazosulfanate), (Mobay Chemical Corp., Chemagro Div., Kansas City, MO 64120).

In all experiments, five replicates of each seed treatment were planted and each experiment was done at least twice with similar results. The numbers of surviving seedlings were counted on the third or fourth day after planting, and then on every second or third day for as many as 9 additional days. *Pythium* spp. or *R. solani* could be isolated from diseased pea or radish seedlings, respectively. After this period, the plants were removed from the soil, the soil from all replicates of each treatment was mixed, soil was returned to plastic containers, and nontreated seeds of the same species were planted. The containers were again incubated, and counts of surviving seedlings were made as described above. In some experiments, after counts were made in the second planting cycle, soil was again removed from containers, mixed as described, and replanted with nontreated seeds of the same or a different species.

In some experiments, inoculum densities of *R. solani, Pythium* spp., and *Trichoderma* spp. were determined. The inoculum density of *R. solani* was assayed with a soil pelleting device (10) on Ko and Hora's medium (12), whereas *Trichoderma* and *Pythium* spp. were estimated using dilution-plate techniques on the selective media of Elad et al (7) and Mircetich (16), respectively.

Observational studies. Seeds coated with *T. hamatum* were either nongerminated or germinated on moist filter paper for 2 days. These seeds were coated with gold and viewed on the AMR 100 scanning electron microscope at the Harvard Museum of Comparative Zoology.

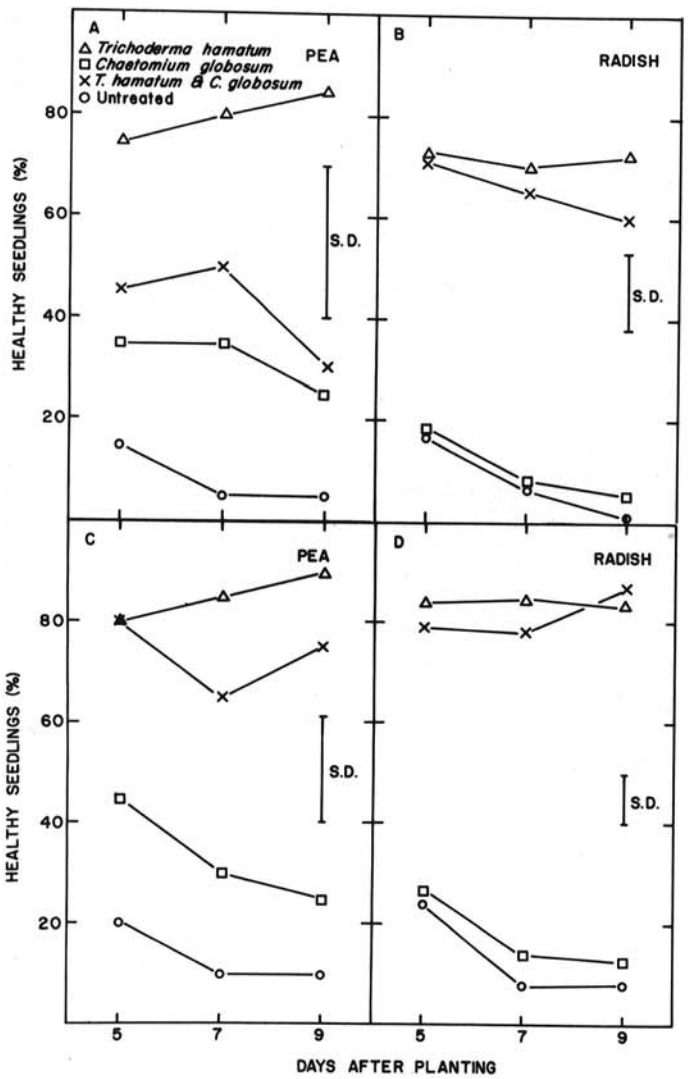

Fig. 1. Percentage of healthy pea (A and C) or radish (B and D) seedlings at various intervals after planting. Seedlings grew either from seeds treated with various biocontrol agents (A and B) or from nontreated seeds in soil in which seeds of the same species had been planted with treated seeds in the previous planting cycle (C and D). Peas were planted in soil containing *Pythium* spp. indigenous to Fort Collins clay soil, while radishes were planted in soil to which *Rhizoctonia solani* had been added. The bars labeled S.D. represent the overall standard deviation for the experiment shown.

RESULTS

Treatment of radish or pea seeds with *T. hamatum* resulted in less seedling disease than occurred with nontreated or with Methocel-treated seeds (Figs. 1A, B). Damping-off due to *Pythium* spp. was less with pea seeds treated with *C. globosum* than with nontreated seeds, but was significantly greater than with seed treated with *T. hamatum* (Fig. 1A). Neither Methocel nor *T. harzianum* protected pea seeds against *Pythium* (data not shown), and neither *C. globosum* (Fig. 1B) nor Methocel protected radish seeds against *R. solani*. In another experiment (data not shown) with less damping-off than in the experiment shown in Fig. 1A (11 days after planting only 45% of nontreated seeds were attacked), *C. globosum* appeared as effective as *T. hamatum* in protecting

against *Pythium* spp. (90–95% of the pea seedlings were alive 6 days after planting). However, postemergence damping-off occurred on seedlings produced by *C. globosum*-treated seeds (11 days after planting 50% of the seedlings were alive), but not on seedlings from *T. hamatum*-treated seeds.

Treatment of pea seeds with both *T. hamatum* and *C. globosum* was less effective than treatment with *T. hamatum* alone (Fig. 1A), but no significant difference was found between radish seeds treated either with *T. hamatum* or the mixture (Fig. 1B).

After seedlings were removed, the soil mixed, and replanted with nontreated seed, residual protection occurred only with soil originally planted with *T. hamatum*-treated or *T. hamatum*-plus *C. globosum*-treated seeds (Figs. 1C, D).

Increases in *Trichoderma* propagule density were accompanied by lower levels of both *Pythium* spp. and *R. solani* (Table 1). Soils in which seeds treated with *T. hamatum* were planted had approximately 100-fold higher levels of *Trichoderma* spp. after two plantings than did soils that were originally planted with seeds not treated with this agent. Levels of *R. solani* were 4- to 10-fold lower in soils planted with *T. hamatum*-treated seeds than in nontreated controls. *Pythium* did not increase significantly in soils planted with pea seeds coated with *T. hamatum* alone, whereas pea seeds coated with *C. globosum* or with *T. hamatum* and *C. globosum* showed large increases (Table 1).

When soils in which radishes had been cropped twice were planted with peas, less damping-off occurred when the first planting of radishes was treated with *T. hamatum* or *T. hamatum* plus *C. globosum* than when seeds were nontreated or treated with *C. globosum* alone (Fig. 2).

Comparative tests with chemical seeds treatments indicated that *T. hamatum* protected both radish and pea seeds against preemergence damping-off (3-day counts) as well as PCNB, captan, or fenaminosulf (Figs. 3A, B). There was significantly less postemergence damping-off of radish seedlings treated with *T. hamatum* than in those treated with either PCNB or captan (Fig. 3B). *Trichoderma hamatum* did not, however, protect pea seedlings against postemergence damping-off as well as either captan or fenaminosulf, although protection was still evident (Fig. 3A). In the second planting, peas were not severely damaged in soils originally planted with seeds treated with *T. hamatum*, captan, or fenaminosulf (Fig. 3C), whereas radish seeds were protected from attack by *R. solani* only in soils originally planted with *T. hamatum*-treated seeds. In the third planting, pea seeds planted in soils originally planted with either fenaminosulf- or *T. hamatum*-treated seeds were not severely damaged, while those peas planted in soils originally planted with either captan-treated or nontreated seeds were all rotted by *Pythium* (Fig. 3E).

Scanning electron micrographs of *T. hamatum*-coated seeds showed conidia distributed over and attached to the seed coat (Fig. 4A). Similar seeds after 2 days of incubation in a moist chamber were covered with mycelium and spores of *T. hamatum* (Fig. 4B).

DISCUSSION

The Fort Collins soil used in these investigations was conducive to the development of damping-off, but its characteristics, especially the alkaline soil reaction, were not favorable for development of suppressiveness (5,14). *Trichoderma,* the antagonist responsible for suppressiveness in this soil, is inhibited at an alkaline pH. Further, the propagules of *R. solani* used in inoculation were large. Induction of suppressiveness in soil containing such propagules has been difficult (5). Thus, the test conditions in this study were not favorable for biocontrol. The success of biocontrol with seed treatment with *T. hamatum* is even more impressive, because it was achieved in spite of, rather than because of, the test conditions.

Regardless of the unfavorable soil conditions, treatment of seeds with *T. hamatum* appeared more effective than most other attempts at biocontrol. *Trichoderma hamatum* provided control

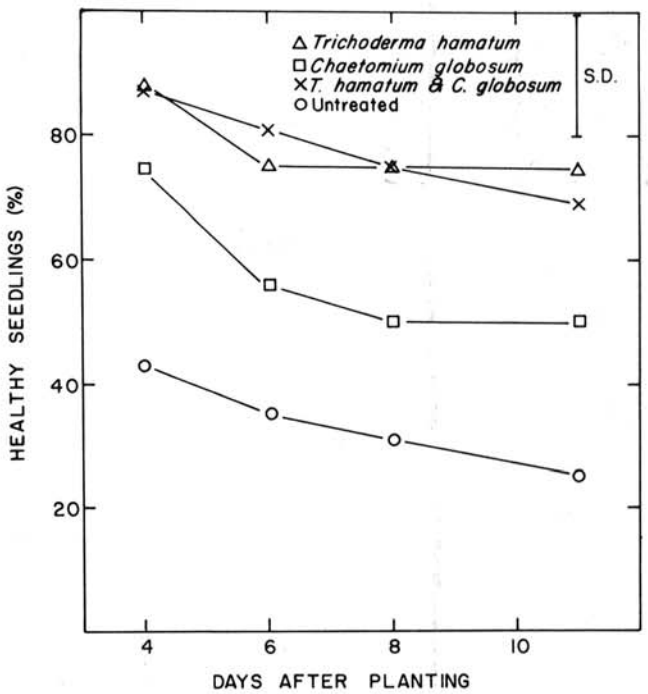

Fig. 2. Percentage of healthy pea seedlings at various intervals after planting. All seeds were nontreated and were planted in soil previously planted twice: first with radish seeds treated with various biocontrol agents, and then planted 7 days after the first seeding with nontreated radish seeds in the same soil. The soil contained *Pythium* spp. indigenous to the Fort Collins clay soil used. The bars labeled S.D. represent the overall standard deviation for the experiment.

TABLE 1. Effects of seed treatment with *Trichoderma hamatum* or *Chaetomium globosum* on numbers of propagules of fungi in soil after two successive crops of either radish or pea seedlings

Seed treatment[a]	Crop	Propagule density[b] (propagules/gram soil)		
		Rhizoctonia solani	*Phythium* spp.	*Trichoderma* spp.
None	Radish	3.6 D	186 A	300 A
Trichoderma hamatum	Radish	0.33 A	257 A	50,000 C
Chaetomium globosum	Radish	2.6 C	213 A	100 A
T. hamatum + *C. globosum*	Radish	0.9 B	227 A	20,000 B
None	Pea	...[c]	2,300 C	100 A
Trichoderma hamatum	Pea	...[c]	667 A	8,000 AB
Chaetomium globosum	Pea	...[c]	3,233 D	140 A
T. hamatum + *C. globosum*	Pea	...[c]	1,775 B	10,000 AB

[a] Seeds were treated with spore suspensions in 2% Methocel. *T. hamatum* suspensions contained $3–8 \times 10^8$ conidia per milliliter and *C. globosum* suspensions contained 8×10^8 ascospores per milliliter. Twenty and 80 ml of spore suspensions were added per kilogram of pea and radish seeds, respectively.
[b] Numbers followed by dissimilar letters, within columns, are significantly different, $P = 0.05$.
[c] Soil was not inoculated with *R. solani*.

nearly equivalent to various fungicide seed treatments (Figs. 2A, B) and gave better control than *C. globosum* or *T. harzianum*. An initial seed treatment with *T. hamatum* protected seeds through two subsequent cycles of planting, even though the last two plantings were with nontreated seeds. Use of *T. hamatum* as a seed treatment allows its use in small quantities; other agents have been most effective when applied in quantities too large to be economically feasible (1,6,11,22). The fact that activity of *T. hamatum* is retained for a time after planting suggests that control of root rots and other soilborne diseases might be obtained by using this seed treatment.

Trichoderma hamatum acts as a mycoparasite of both *Pythium* spp. and of *R. solani* (authors, *unpublished*), and this may be its principal mode of action. This suggestion is supported by the direct observation that mycoparasitism by *T. hamatum* occurs on both pathogens with no apparent evidence of in vitro antibiosis.

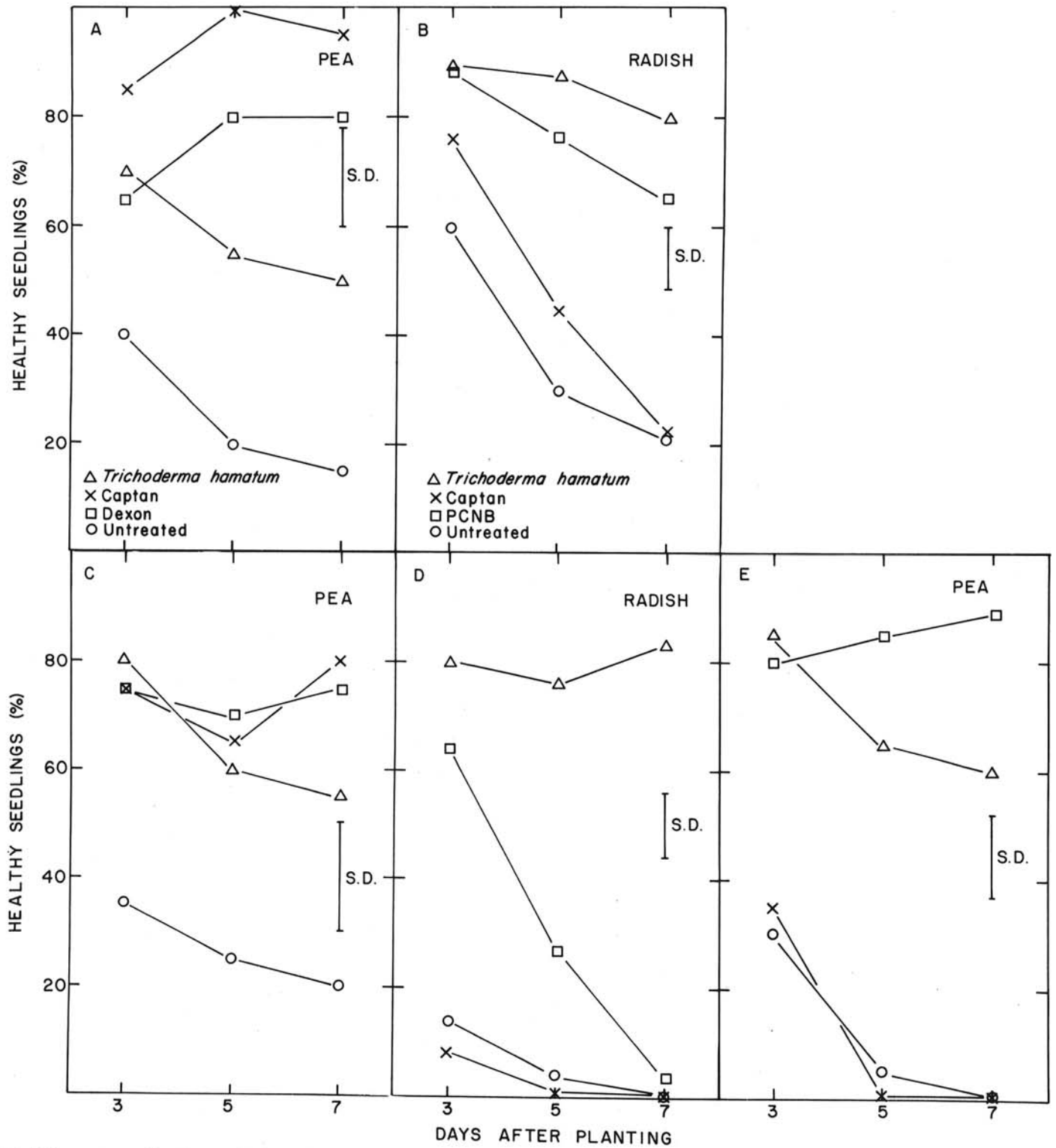

Fig. 3. Percentages of healthy pea (A, C, and E) or radish (B and D) seedlings at various intervals after planting. Seedlings grew either from treated seeds (A and B), from nontreated seeds in soil in which seeds of the same species had been planted with treated seeds in the preceding generation (C and D), or from nontreated seeds in soil planted with treated seeds two generations ago (E). Peas were planted in soil containing *Pythium* spp. indigenous to Fort Collins clay soil, while radishes were planted in similar soil to which *Rhizoctonia solani* had been added. Bars labeled S.D. represent the overall standard deviation for the experiment shown.

Trichoderma hamatum limited densities of *R. solani,* but merely prevented large increases in the numbers of *Pythium* spp.

Trichoderma hamatum colonizes seed coats completely, and may control seed damage by physical occupation of this substrate and by utilization of seed exudates produced by the germinating seeds. Such a mechanism has been suggested for *C. globosum* (4,8), our isolate of which has no known antibiotic or mycoparasitic ability.

The ability of *T. hamatum* to be effective in small amounts as a seed treatment is probably associated with its ability to grow and sporulate on seeds (Fig. 4) and thereafter to become established in large numbers in soil (Table 1). This ability is in contrast with that of *T. harzianum* which is unable to colonize seeds, to protect seeds, or to parasitize *Pythium* spp. *T. hamatum* produces cellulase (Chet and Baker, *unpublished)* while *T. harzianum* has little cellulolytic ability. Cellulase should aid colonization of seed coats by providing glucose for the fungus; *C. globosum* also has cellulolytic ability and also colonizes seed coats very well. Further, *Pythium* cell walls contain cellulose and this may permit parasitism of this organism by *T. hamatum,* but not by *T. harzianum.*

Seed-treatment fungicides protected radish seeds through the first planting cycle in the case of radishes and through the second cycle when applied to peas. This is surprising, especially with captan, because this chemical decomposes rapidly in moist environments (3). However, fungicides probably have a longer period of efficacy than that induced during their initial and residual toxicity because they lower the inoculum potential of pathogens. Peas appeared to be protected longer than radishes, but this is probably due to the development of resistance to *Pythium* seed rot within 3 days of planting (19); comparable resistance to *R. solani* does not develop in radish seedlings. Thus, a reduction in apparent efficacy of a chemical (eg, captan) might be seen in radishes one planting cycle earlier than occurs with peas. Fenaminosulf protected pea seeds even through the third cycle. This is not surprising because it is water-soluble, and would be expected to leach from seeds into soil, whereas the other chemicals are insoluble and should not leach. Once in the soil, fenaminosulf persisted and protected plants for at least 5 wk (2).

Our results demonstrated that seed treatments with *T. hamatum* are nearly as effective as chemical seed treatments. Moreover, this agent became established in soil and protected subsequent generations of seedlings from attack. This indicates that seed treatments with this antagonist may be effective against soilborne diseases of older plants (eg, root rots).

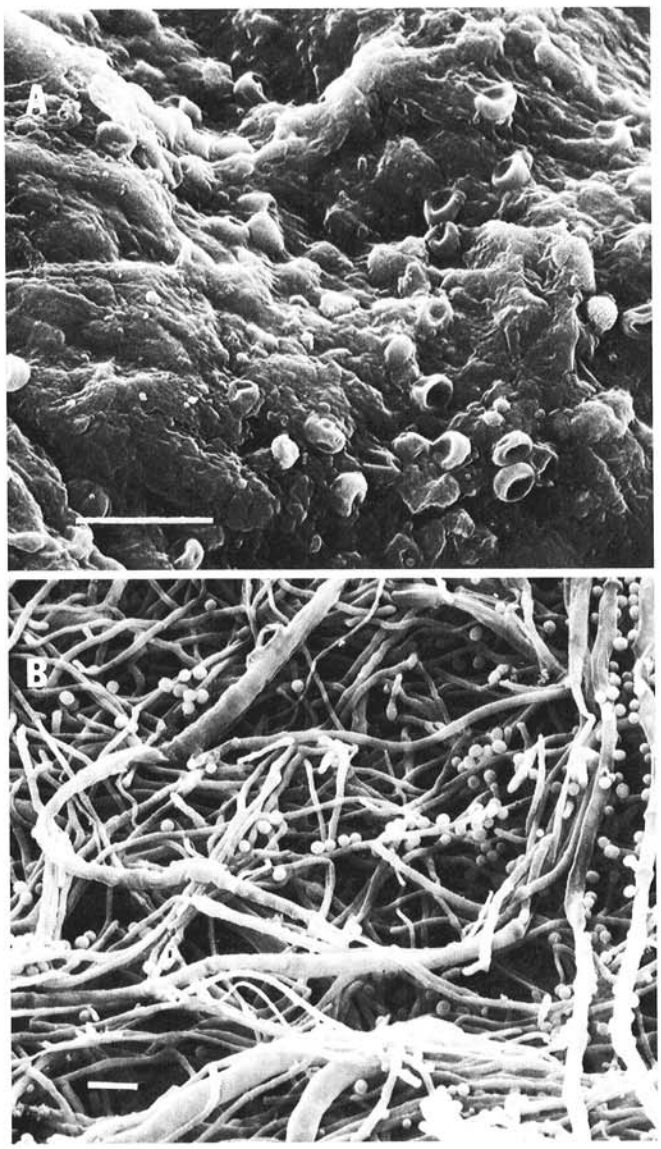

Fig. 4. A, Spores of *Trichoderma hamatum* on treated radish seed before germination. **B,** Hyphae and spores of *T. hamatum* on radish seed coat after 2 days of incubation in a moist chamber. In both photographs the bar on the lower left corner represents 10 μm.

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