Effect of Soilborne *Pseudomonas* spp. on the Biological Control Agent, *Trichoderma hamatum*, on Pea Seeds

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ABSTRACT

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In some soils, *Trichoderma hamatum*, applied as conidia to peas, colonizes seed coats and protects them from seed rots caused by *Pythium* spp. However, it fails to protect seeds in New York soils with low iron (<4 μg/g of soil) availability. This failure is caused by antagonism by fluorescent soilborne pseudomonads that colonize seed coats rapidly. Short, rod-shaped bacteria were observed in association with lysed germlings of *T. hamatum* on treated seeds after planting in soil. Addition of pseudomonads to seeds treated with *T. hamatum* caused *T. hamatum* to be ineffective as a biocontrol agent when these seeds were planted in steamed soil containing iron at 1 μg/g of soil and amended with *P. ultimum*. Conversely, planting seeds treated with both *T. hamatum* and pseudomonads in quartz sand with approximately 7 μg of extractable iron per gram of soil resulted in no decrease in efficacy of *T. hamatum*. Increasing iron levels in field soil to 8 μg of iron per gram of soil by the addition of ferrous oxalate resulted in effective control of seed rots by *T. hamatum*. Ferric EDTA was ineffective because it was toxic to *T. hamatum*. Various inorganic iron salts were ineffective when added as seed treatments; extraction of these seeds 5 days after planting gave low Fe levels (<4 μg/g). Growth of *T. hamatum* together with pseudomonads on a medium with little available iron (King's B) resulted in fluorescent zones around pseudomonad colonies that were inhibitory to *T. hamatum*. Growth of the fungus was inhibited by addition of partially purified fluorescent pigment to cultures in King's B broth; this inhibition could be overcome by addition of 100 μM Fe^{++} or Fe^{+++}. Seed-colonizing pseudomonads grow well even if seeds are treated with antibacterial antibiotics. Seeds treated with the *T. hamatum* and germinated in the absence of pseudomonads for 12–18 hr and then transplanted to field soils were protected against seed rot.

Additional key words: antagonism.

There have been numerous reports of seed treatment with biological agents for controlling soil-inhabiting, plant pathogenic fungi (2,10,11,17,18,28,29,30). Practical biological control has been plagued by erratic results; attempts to repeat successful first results in other places often fail (12,23). Harman et al (8,9) showed that an isolate of *Trichoderma hamatum* (Bon) Bain obtained from a Rhizoctonia-suppressive soil (3) prevented seed rots in soils from Colorado. However, the same isolate failed to protect in New York soils. In Fort Collins clay loam soils this *T. hamatum* isolate grew readily on seeds and became established in the soil, but neither protected seeds from attack by *Pythium* nor became established in soil obtained near Geneva, New York. Pseudomonads are the major bacterial species found on germinating seeds in New York (14) and Colorado soils. Pseudomonads and their siderophores are antagonistic to soil fungi, particularly under conditions of iron deprivation (22).

The objectives of this study were to investigate interactions between *T. hamatum* and *Pseudomonas* spp. on germinating pea seeds in New York soils, and to determine how this interaction altered effectiveness of *T. hamatum* as a biological seed protectant.

MATERIALS AND METHODS

Soil. Two New York field soils were used in these experiments. One was an Arkport fine sandy loam, the other a Honeoye fine sandy loam. They will henceforth be referred to as Arkport and Honeoye soils, respectively. Soil pH was determined in a 1:1 (w/v) soil-water suspension. After allowing the sample to settle 15–30 min the calomel electrode was placed in the clear supernatant, while the glass electrode was placed in the partially settled soil suspension. Soils were also analyzed according to the procedures described by MacDonald et al (19) and their characteristics are presented in Table 1. For comparison, the characteristics of the Fort Collins clay loam soil (henceforth called Fort Collins soil) used by Harman et al (8) are also presented. For all experiments, moist soils were stored in covered containers prior to use. Both soils were naturally infested with *Pythium* spp. High levels of this pathogen were obtained by cropping these soils with wheat and/or oats for 3 wk, followed by incorporation of the crop into the soil, and incubation for an additional 2 wk. This procedure increased numbers of *Pythium* 50- to 100-fold. Soils so cropped were blended with unamended soils to give levels of *Pythium* capable of rotting approximately 80% of nontreated pea seeds. Usual mixes contained one part of cropped soil to 10–50 parts of unamended soil; *Pythium* levels were 30–50 propagules per gram of soil.

Seeds and seed treatments. We used seeds of the pea cultivars Dark Skin Perfection (Asgrow Seed Co., Kalamazoo, MI 49001), Lincoln (Seedway, Inc., Hall, NY 14463), or Venus (Asgrow). All of these cultivars with large wrinkled seeds are highly susceptible to seed rots caused by *Pythium* spp.

Seeds were treated with conidia of the isolate of *T. hamatum* used previously (8,9) at the rate of 10 mg of dried conidia per 50 g of seeds. Spores were suspended in 2% (w/v) Methocel A4C (Dow Chemical Co., Midland, MI 48640) at the rate of 10 mg of dried conidia per milliliter of Methocel prior to seed treatment and 1 ml of the spore suspension was used to treat 50 g of seeds. The isolate

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of *Trichoderma* used in these studies lacks sterile conidiophore appendages; this and other characteristics would allow it to be classified as either *T. hamatum* or *T. harzianum*. We are using *T. hamatum* in this work since this isolate was described by this binomial in previous work (3,4,8,9). This isolate has been deposited in the USDA collection (Northern Regional Research Center, Peoria, IL 61604) as NRRL 12568.

To study the effects of bacteria on *T. hamatum*, antibiotics were added to the slurry treatments of *T. hamatum*. They included streptomycin (AS-50; Pfizer, Inc., Terre Haute, IN 47808), 25,000 μg/ml seed treatment; rifampicin, 50, 500, and 5,000 μg/ml seed treatment; Vancomycin 2,000, 20,000 μg/ml seed treatment; and Polymixin B 20,000 μg/ml seed treatment (all from Sigma Chemical Co., St. Louis, MO 63178). Soluble iron was added either as $FeCl_3 \cdot 6H_2O$, $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, and $FeSO_4 \cdot 7H_2O$, or the ferric sodium salt of ethylene-diamine tetraacetic acid to suppress the effect of fluorescent pigments. Seeds were treated with captan at the recommended rate of 0.92 g a.i. per 100 g of pea seeds.

Five seeds were planted per 10 × 10 × 6-cm plastic box, at a depth of approximately 1 cm. Soil moisture was maintained at 14% by covering boxes with a tightly fitting lid. Two small holes allowed gas exchange. All incubations were at 25 C.

Microscopy. Small sections (1–2 mm square) were cut from seed coats of seeds germinating in the soil. They were stained by a modification of the method of Anderson and Slinger (1), by immersing them in a staining solution consisting of 5 mM europium chelate (europium (III) thenoyltrifluoroacetonate, Eastman Kodak Co., Rochester, NY 14650) and 0.025% (w/v) Calcofluor White M2R (Polysciences, Inc., Warrington, PA 18976), in 50% ethanol (1). After 2 min, the staining solution was removed and the specimen was washed in 50% ethanol followed by distilled water. Specimens were then examined with a Bausch & Lomb epifluorescent microscope equipped with a 5840-2 exciter filter, a Corning OG515 barrier filter, and a 500 nm dichoric mirror. With this combination, excitation light was between 300–400 nm (maximum intensity at 350–360 nm), and transmitted light was above 530 nm.

TABLE 1. The pH and nutrient status of soils used in this study of the interaction of *Trichoderma hamatum* and soilborne *Pseudomonas* spp.[a]

Soil	pH	P	K	Ca	Mg	Zn	Mn	Fe	$CaCO_3$
Arkport fine sandy loam	7.3	5.8	28	430	55	0.5	2.1	1	ND[c]
Honeoye fine sandy loam	7.0	7.6	44	1,440	180	0.5	4.9	1.6	<0.5
Fort Collins clay loam[b]	7.4	25	421	ND	ND	3.4	1.7	8.9	3.2

[a] Values represent concentration in μg/g for values other than pH.
[b] Data from Harman et al (10).
[c] ND = not done.

TABLE 2. Interactions of selected pseudomonad isolates with *Trichoderma hamatum* on seed coats of pea seeds planted in steamed field soil or quartz sand[a]

Fungal/chemical[b] seed treatment	Pseudomonad seed[c] treatment (isolate number)	Sand or soil treatment	Seedling emergence (%)[d] Sand	Soil
None	None	None	-	80 AB
T. hamatum	2	None	-	96 A
T. hamatum	3	None	-	92 A
T. hamatum	10	None	-	76 AB
Captan	None	*P. ultimum*	76 AB	76 AB
T. hamatum	1	*P. ultimum*	80 AB	-
T. hamatum	2	*P. ultimum*	84 AB	32 DE
T. hamatum	3	*P. ultimum*	96 A	36 CDE
T. hamatum	10	*P. ultimum*	-	44 CD
None	1	*P. ultimum*	28 C	-
None	2	*P. ultimum*	28 C	-
None	3	*P. ultimum*	40 C	-
T. hamatum	None	*P. ultimum*	84 AB	64 BC
None	None	*P. ultimum*	24 C	12 E

[a] Field soil was Honeoye fine sandy loam after steaming at 100 C for 30 min and contained 1.6 μg/g Fe. Quartz sand contained 7.1 μg/g Fe. Soil or sand treatments so designated contained 1–2 *Pythium* sporangia per gram.
[b] *T. hamatum* was added at the rate of 10 mg conidia in 1 ml 2% (w/v) Methocel A4C per 50 g seeds. Captan was added at the rate of 460 mg a.i. per 40 g pea seeds.
[c] Seeds were treated by dipping them in a suspension of 10^6/ml starved cells of *Pseudomonas* spp. in sterile water.
[d] Means in each column followed by dissimilar letters are significantly different ($K = 100$) according to Waller and Duncan's multiple range test.

Isolation and characteristics of pseudomonads from seed coats. Numbers of *Pseudomonas* spp. on seed coats of germinating pea seeds were estimated 48 hr after planting. Bacteria were washed from the seed coat surface by mixing the seed in 10 ml of 0.1% water agar. Dilutions of this initial suspension were plated in the selective medium of Grant and Holt (7). The characteristics of representative isolates were determined according to the methods outlined by Stainer et al (27) and affinity with different species was determined according to Bergey's Manual (5).

Interactions of pseudomonad isolates and their fluorescent pigments with *T. hamatum*. Individual isolates of bacteria obtained from naturally infested seed coats were selected to study their effect on *T. hamatum* in soil. Seeds treated with *T. hamatum* were dipped into a suspension of starved bacterial cells. Bacteria were starved by removing cells from a 24-hr-old nutrient broth culture by centrifugation and resuspending them in sterile mineral salts solution (31) for 2 hr. Starved cells were diluted to a density of approximately 10^6/ml prior to seed inoculation. Seeds so treated were planted in steam-pasteurized (100 C for 20 min) soil or in quartz sand amended with approximately two *Pythium* propagules (sporangial inoculum) per gram of soil. Sporangia production was induced in an isolate of *Pythium ultimum* (P_4 of Pieczarka and Abawi [24]) by culture on bean pod medium. Sporangia were harvested aseptically. Seeds treated with *T. hamatum* and then inoculated with pseudomonads were also planted in pasteurized soil or sand, which had not been infested with *P. ultimum*. The soil contained 1 μg of iron per gram of soil, while the sand contained iron at approximately 7 μg per gram of sand.

We also added Nu-Iron (a preparation of ferrous oxalate containing 29% Fe; Cities Service Co., Atlanta, GA 30302) to nontreated Arkport soil at the rate of 400 mg/kg. In other tests, we added Sequestrene 330 (an EDTA complex containing 10% Fe; Ciba-Geigy Corp., Greensboro, NC 27409) to soil at various levels.

Interactions were also studied in vitro. Petri dishes (9 cm) containing King's B medium (15) were inoculated with *T. hamatum* at the center and up to six isolates of fluorescent pseudomonads were inoculated at separate points equidistant on the circumference of a circle 4 cm from the center of the plate. Plates were amended with iron (as $FeCl_3$) at 0, 1, or 10 μg/ml.

Fluorescent pigments were obtained from a *Pseudomonas* (isolate 10) inhibitory to *T. hamatum* (Table 2). This bacterium was grown in shake cultures on succinate plus salts medium (22) for 30 hr at 25 C, bacterial cells were removed, and the pigment was partially purified by the acetone precipitation procedure described by Misaghi et al (22). To test effects of the pigment on growth of *T. hamatum*, 4 ml of King's B (15) medium were added to 25-ml flasks, aliquots of partially purified pigments were added, and volumes were brought to 5 ml. The flasks were then inoculated with *T. hamatum*, incubated with shaking for 48 hr, and the dry weight of the mycelium was then determined. Other flasks were treated similarly, but sufficient iron was added as $FeCl_3$ or $FeSO_4$ to give solutions containing 100 μM Fe.

Transplant experiments. Seeds, treated with *T. hamatum* or not treated, were planted in moist quartz sand containing neither *Pythium* nor *Pseudomonas* spp., incubated for various lengths of time, and then transplanted to Honeoye soil infested with both organisms. Other seeds were planted first in Honeoye soil, and transferred to quartz sand after various lengths of time. All experiments described in this work contained five replicates of each treatment and all results were confirmed in separate experiments.

RESULTS

Microscopic observations. Conidia of *T. hamatum* germinated and gave rise to a dense hyphal mat on the surface of pea seed coats within 24 hr in both the Arkport or the Honeoye soils, if there was little competition from bacteria (Fig. 1A). Occasionally hyphae of *T. hamatum* could be seen coiled around pythiacous hyphae (Fig. 1C). However, in both Arkport and Honeoye soils, bacteria proliferated near germinating conidia of *T. hamatum* and hyphae frequently lysed. Bacteria were concentrated near germlings of *T. hamatum* (Fig. 1B). Hyphae of *T. hamatum* were not observed near or in large colonies of bacteria which formed on the seed coat.

Soil analyses. Soil pH was similar for Honeoye, Arkport, and Fort Collins clay loam soils. With the exception of P and Mn, the Fort Collins soil contained higher levels of other ions and of $CaCO_3$. The Fort Collins soil contained sixfold more $CaCO_3$ than Honeoye soil, 5.5- to ninefold more Fe, sixfold more Zn, and 9.5- to 15-fold more K than either the Arkport or Honeoye soils (Table 1).

Pseudomonads isolated from seed coats. The number of pseudomonads isolated from seeds 48 hr after planting in Fort Collins, Arkport, or Honeoye soils ranged from 3 to 19×10^5 per seed coat, and did not differ significantly among these soils.

Characteristics of pseudomonad isolates found on seed coats of seeds in Arkport and Honeoye soils were also similar. Thirty-seven isolates from seeds in Fort Collins soil were examined. Eight were characteristic of *P. fluorescens* and 21 others resembled *P. fluorescens*, except for their inability to utilize certain carbohydrates. The remaining eight isolates failed to liquify gelatin, would not grow at 41 C, and except for two, were capable of growth on trehalose. Their affiliation is therefore more uncertain. A total of 31 (of 37) isolates produced fluorescent pigments. Forty-nine isolates from Arkport soil were examined. Five were characteristic of *P. fluorescens*, and 33 others resembled *P. fluorescens*, except for their inability to utilize one or more of the carbohydrates tested, while 11 others were of more uncertain affiliation. A total of 39 isolates produced fluorescent pigments on King's B medium.

Effects of pseudomonads and iron on *T. hamatum* in vitro. Fluorescent pseudomonad isolates were examined in vitro for interaction with *T. hamatum*. Zones of inhibition of growth of *T. hamatum* extended up to 1 cm from fluorescent pseudomonad colonies producing fluorescent pigments on King's B medium. If 1 or 10 μg of Fe^{+++} was added per gram of the medium, prior to inoculation, the fluorescent pigment was not produced and growth of *T. hamatum* on this medium was not inhibited. Filter-sterilized culture filtrates from pseudomonad cultures growing in iron-deficient King's B broth also proved inhibitory to growth of *T. hamatum* when added to wells in agar plates of King's B medium, but if ferric iron was first added to those filtrates, they then failed to inhibit *T. hamatum*.

Fluorescent pigments from pseudomonad isolate 10 were produced, partially purified, and their effects on *T. hamatum* were determined. Absorption peaks of the partially purified siderophore in water at pH 7.0 were at 210 and 408 nm; the 210-nm peak had shoulders at 230 and 260 nm. Pigment solutions inhibited growth of *T. hamatum* in a concentration-dependent manner. Growth inhibition by pigments was abolished by the addition of Fe^{++} or Fe^{+++} (Fig. 2).

Seeds treated with *T. hamatum* were dipped into pigment solution and germinated between moist paper towels. *T. hamatum* grew well and sporulated on nontreated seeds, but failed to grow on pigment-treated seeds.

Interaction of pseudomonads and iron with *T. hamatum* on seeds planted in soil. *T. hamatum* protected seeds from *P. ultimum* in pasteurized Honeoye soil or quartz sand that had been reinfested with sporangia of *P. ultimum* in the absence of pseudomonads (Table 2). However, fluorescent pseudomonads isolated from the seed coats of germinating pea seeds and inoculated onto the *T. hamatum*-treated pea seeds reduced emergence from soil, but not from sand. Emergence of seedlings from seeds that had been treated with *T. hamatum* from pasteurized noninfested soil was not affected by inoculation with

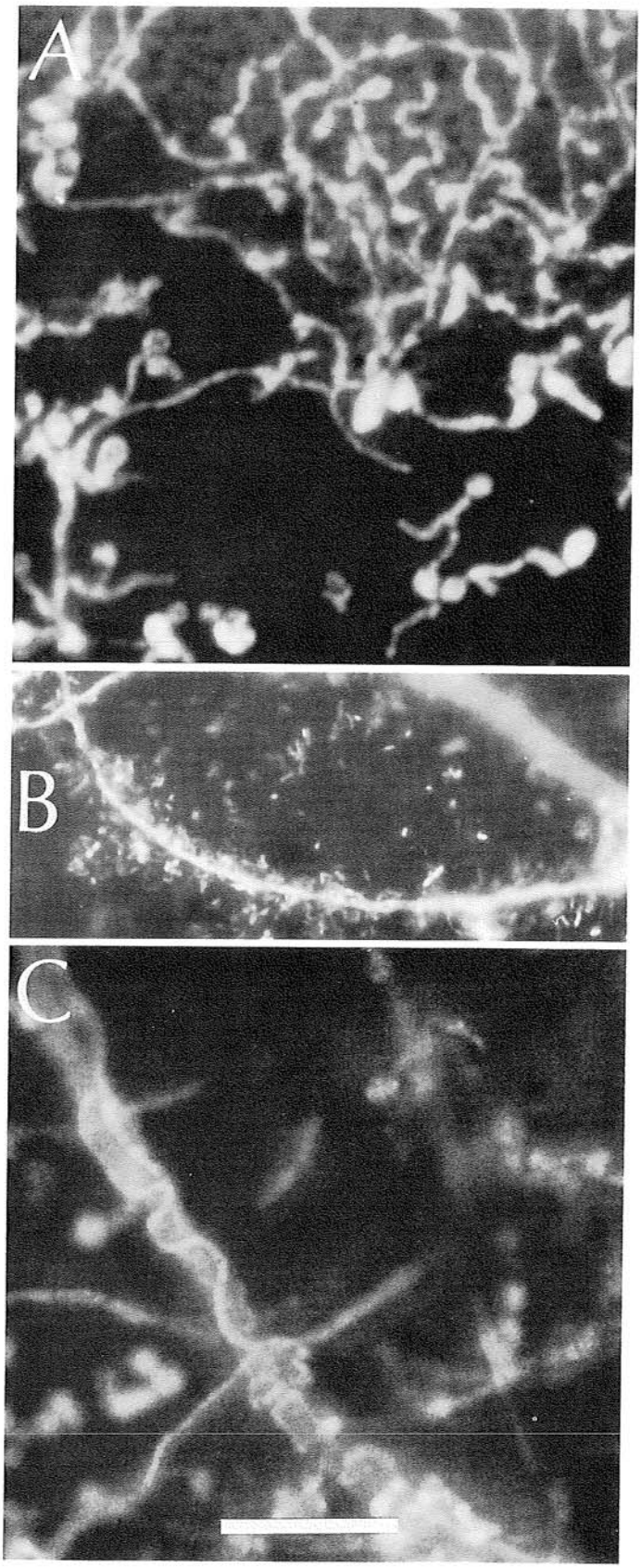

Fig. 1. Fluorescence photomicrographs of interactions of *Trichoderma hamatum* with other microorganisms on pea seed coats. **A,** Germinating conidia of *T. hamatum* in the absence of bacterial proliferation. **B,** Rod-shaped bacteria aligned along a hyphal fragment and, **C,** *T. hamatum* hyphae coiling around a pythiaceous hypha. Bar represents 40 μm.

these pseudomonads. Seeds that had been inoculated with pseudomonads alone and planted in sand infested with *P. ultimum* were rotted (Table 2).

Attempts were made to increase soluble iron concentrations around seeds treated with *T. hamatum* by adding iron in various forms to seed treatments. None of these treatments consistently had a significant effect on performance of *T. hamatum*. Quantitative analyses of extractable iron from these seeds at 0 and 48 hr indicated that very little (<4 μg/g soil) soluble iron remained in any of the treatments after 48 hr.

On agar plates and as amendments to seed treatments, none of the iron amendments adversely affected *T. hamatum* with the exception of ferric EDTA. EDTA itself markedly retarded growth of *T. hamatum* at 10 and 100 μg/ml in culture and microscopic examination indicated that seeds treated with EDTA at 10 and 100 μg/ml were more sparsely colonized with hyphae of *T. hamatum* than seeds treated with only 1 μg/ml.

The iron content of the Arkport soil was increased by adding ferrous oxalate. In the absence of added iron, seed rot was severe regardless of seed treatment. *T. hamatum* protected seeds from rot, however, if the soil iron content was increased to 8 μg/g soil (Table 3). Addition of ferric EDTA to soil resulted in no increase in the efficacy of *T. hamatum*, presumably because of the toxicity noted above.

Effects of antibiotics as seed treatments. None of the antibiotics tested enhanced effectiveness of *T. hamatum* as a seed treatment or suppressed pseudomonad colonization of the seed coat. Even on nontreated seeds, a high percentage of antibiotic resistant pseudomonads occurred in the seed coat population.

Transplant experiments. Seeds planted in quartz sand and then transplanted to Honeoye soil 12–18 hr later were protected by *T. hamatum*. Most nontreated seeds were rotted when this procedure was followed (Table 4).

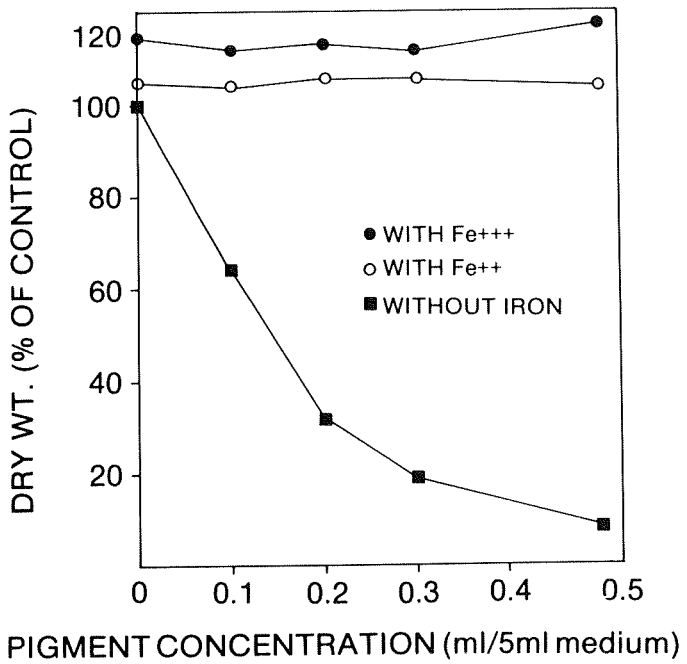

Fig. 2. Dry weight of *Trichoderma hamatum* grown in King's B medium containing various amounts of partially purified fluorescent pigment from a pseudomonad inhibitory to *T. hamatum*. Some flasks contained sufficient $FeSO_4$ (Fe^{++}) (o) or $FeCl_3$ (Fe^{+++}) (•) to make 100 M solutions of these salts.

TABLE 3. Effects of adding iron to Arkport fine sandy loam on the ability of *Trichoderma hamatum* to protect germinating pea seeds from attack by *Pythium* spp.

Seed treatment	Soil treatment	Available iron in soil (μg/g)	Emergence[b] (%)
None	None	1	40 A
T. hamatum	None	1	44 A
None	Nu-Iron[d]	8	40 A
T. hamatum	Nu-Iron	8	68 B

[a] Pea seeds were treated with *T. hamatum* by adding 10 mg conidia in 1 ml of 2% (w/v) Methocel A4C per 50 g of seeds.
[b] Numbers followed by dissimilar letters are significantly different ($K = 100$) according to Waller and Duncan's multiple range test.
[c] Number of pseudomonads per seed 48 hr after planting.
[d] Nu-Iron is a preparation of ferrous oxalate containing 29% Fe that was added to soil at the rate of 400 mg/kg.

DISCUSSION

The mycoparasitic isolate of *T. hamatum* used in these studies has previously shown considerable promise for biological control of seed rot induced by *Pythium* and *Rhizoctonia solani* and damping-off (4,8,9). It was surprising that it performed so poorly in laboratory trials with field soil in New York. Such anomalous behavior has been characteristic of many promising biological agents (12,21,23).

This work demonstrates that seed-colonizing pseudomonads are largely responsible for the failure of *T. hamatum* as a seed protectant in soils from New York. Small, rod-shaped bacteria were seen in areas of the seed coat on which *T. hamatum* developed poorly. Conversely, robust growth and mycoparasitic activity by *T. hamatum* occurred in pasteurized New York soils in the absence of visible colonization of the seed coat by bacteria. Also, seeds were protected by *T. hamatum* in pasteurized Honeoye soil infested with *P. ultimum*. However, if seeds were preplant inoculated with pseudomonad isolates, *T. hamatum* was unable to protect seeds (Table 2). If *T. hamatum* is protected from pseudomonads for 12–18 hr after planting, it can protect seeds from seed rot (Table 4). Apparently, if the agent becomes established on seeds, it is less susceptible than if pseudomonads colonize seeds as soon as or before conidia of *T. hamatum* germinate.

Our data indicate that pseudomonads inhibited *T. hamatum* only when little iron was available. In vitro experiments demonstrated that extracellular products produced by fluorescent pseudomonads under conditions of iron deprivation were responsible for inhibition of *T. hamatum*. Addition of iron to media supporting growth of pseudomonads or to their fluorescent pigments abolished this inhibition. Both the Honeoye and Arkport soils used in these experiments had low levels of available iron (1.0–1.6 μg/g). Addition of ferrous oxalate to these soils permitted *T. hamatum* to protect seeds (Table 3). Also, although pseudomonads reduced efficacy of *T. hamatum* in steamed soil containing little available iron, they did not affect *T. hamatum* in sand containing approximately 7 μg/g iron (Table 2). These data together suggest that pseudomonads inhibit *T. hamatum* through the production of siderophores. These compounds are inactive, and are probably not produced, if iron is readily available. Siderophores act by tightly chelating available iron and making it unavailable to other organisms (16,22).

TABLE 4. Effect of germination of pea seeds in quartz sand for various lengths of time before gentle washing and transplanting into Arkport fine sandy loam

Seed treatment	Germination time (hr) in quartz sand prior to transplanting	Emergence[a] (%)
None	0	32 C
T. hamatum	0	24 C
None	12	44 C
T. hamatum	12	72 B
None	18	36 C
T. hamatum	18	80 AB
None	Not transplanted	96 A
T. hamatum	Not transplanted	84 AB

[a] Numbers followed by the same letter are not significantly different ($K = 100$) according to Waller and Duncan's multiple range test.

The Fort Collins soil contained 5.5- to 9-fold more iron than the NY soils and *T. hamatum* protected seeds in Fort Collins soil even though the two soils from NY and the Fort Collins soil contained quantitatively and qualitatively similar pseudomonad populations. Generally iron levels are low in soils with higher pH values, but Gough et al (6) suggested that in alkaline, calcarious, soils such as the Fort Collins soil, iron may exist as ferrous carbonate. Although not soluble at alkaline pH, ferrous carbonate would become soluble as pH dropped, as in the vicinity of a germinating seed where organic acids might be produced in association with intense microbial activity.

Antagonism by soil pseudomonads towards certain fungi has been the subject of a number of recent reports. In culture, rod-shaped bacteria (possibly pseudomonads) were observed by scanning electron microscopy in association with necrotic *Phytophthora cinnamoni* (20). Smiley (26) has discussed the isolation of a *Pseudomonas* spp. antagonistic to the take-all fungus from the rhizoplane of wheat, and Scher and Baker (25) have described the isolation of another *Pseudomonas* antagonistic to *Fusarium oxysporum* f. sp. *dianthii*. An isolate of *Pseudomonas fluorescens* produced, under conditions of soluble iron deprivation, the intracellular toxin pyrrolnitrin, which was active against *Rhizoctonia solani* (13). In another study, extracellular products comprising an integral part of the iron transport system in plant growth promoting rhizobacteria (*P. fluorescens-putida* group) may have been responsible for the inhibition of low grade pathogens and hence increased plant growth by these bacteria (16). Siderophores of *Pseudomonas* spp. have been shown to be inhibitory to several soil-inhabiting fungi, but this inhibition could be reversed by addition of iron (22).

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