Mechanism of Biological Control of Preemergence Damping-off of Pea by Seed Treatment with *Trichoderma* spp.

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ABSTRACT

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Application of conidia of isolates of *Trichoderma harzianum* or *T. koningii* to pea seed reduced the incidence of preemergence damping-off of peas induced by a *Pythium* sp. Germination of sporangia of the *Pythium* sp. or chlamydospores of *Fusarium oxysporum* f. sp. *cucumerinum* in the spermosphere of peas treated with the isolates of *Trichoderma* was comparable to that of nontreated controls. Addition of asparagine or glucose to the seed did not nullify biological control of preemergence damping-off, suggesting that competition for nutrients (nitrogen and/or carbon) was not involved. Also, several factors precluded mycoparasitic interactions as likely candidates in antagonism. Such interactions were observed infrequently and occurred 24 hr or more after mycelial contact in dual culture. Germination of conidia of *Trichoderma* spp. required > 10–14 hr of incubation at 26 C, whereas sporangia of the *Pythium* sp. germinated within 90 min. The seed coat was colonized by *Pythium* sp. in 10 hr, and the embryo was infected 24–48 hr after inoculation. Time restraints and lack of extensive mycoparasitism of the *Pythium* sp. suggest that this mechanism of antagonism was not operative in biological control. However, the antagonists produced a factor that resulted in plasmolysis of hyphal tip cells of the *Pythium* sp. before contact of mycelium in dual culture. Culture filtrates of isolates of *Trichoderma* spp. inhibited growth of the pathogen in vitro but not of *Rhizoctonia solani* or *T. harzianum*. The operation of this toxic factor in the spermosphere provides a likely explanation for the phenomena observed with biological control.

Treatment of pea seeds with isolates of *Trichoderma* spp. has effectively controlled Pythium damping-off (20,30,43). Many investigators (6,10–13,16,20,21,29,32,36) have also reported that seed or soil treatment with isolates of *Trichoderma* spp. reduced damping-off induced by *Rhizoctonia solani* Kühn. These reports suggested that a considerable increment of control of *R. solani* was achieved through mycoparasitism. Isolates of *Trichoderma* spp. produced cell-wall-degrading enzymes that provided a means of penetration into the hyphae of *R. solani* (6,7). In addition, mycoparasitism appears to be responsible for the substantial decrease in the inoculum density of *R. solani* with a corresponding increase in population density of *Trichoderma* spp. observed during monoculture of certain hosts (29).

Despite the similarity in disease expression in preemergence damping-off induced by *R. solani* and by *Pythium* spp. (14), we found it difficult to generate suppressiveness in soil to the latter pathogen when isolates of *Trichoderma* spp. were mixed into conducive soil; only seed treatment was successful in control (20,31). In contrast, suppressiveness to *R. solani* was induced by both seed and soil treatment (6,20,28). The objective of this research was to determine the biological control mechanisms associated with seed treatment with isolates of *Trichoderma* spp. for reduction of damping-off induced by a *Pythium* sp.

MATERIALS AND METHODS

Soil. Nunn sandy loam soil was used in all experiments. The soil characteristics were: pH 7.3, >1% lime; conductivity, 0.4 mmole/cm; organic matter, 1%; 1 μg of nitrogen (as NO_3) per gram of soil; 9 μg of phosphorus per gram; 198 μg of potassium per gram; 0.5 μg of extractable zinc per gram; and 3.2 μg of extractable iron per gram. Soil was sieved through a 4-mm-mesh screen and stored air-dried in covered containers before use. The air-dried soil contained about 25 propagules per gram of a pathogenic *Pythium* sp. as determined by plating on *Pythium*-selective medium (33) and by the soil drop method (41). Isolations were made from pea and cucumber seedlings grown in that soil and showing symptoms of damping-off. The *Pythium* sp. isolated from these hosts produced only the asexual stage in culture, and we designated it isolate N1.

***Trichoderma* isolates and species.** *Trichoderma harzianum* Rifai T-12 and *T. koningii* Oudem. T-8, provided by G. E. Harman (New York State Agricultural Experiment Station, Geneva), were isolated from a New York soil. *T. harzianum* T-95 was derived from an isolate effective in inducing soil suppressiveness to *R. solani* (6). *T. viride* Pers. ex S. F. Gray T-1 and T1-R1 and *T. harzianum* W6 and W6-15, previously observed to be efficient biocontrol agents against *P. ultimum* Trow (35,36), were provided by G. C. Papavizas (Soilborne Disease Laboratory, USDA, Beltsville, MD).

Seed treatment and greenhouse experiments. Pea seeds (*Pisum sativum* L. 'Laxton Progress') were surface-disinfested in 1.5% sodium hypochlorite solution for 25 min, washed in tap water, and air-dried. Conidia of *Trichoderma* spp. were collectd from cultures grown on malt extract and yeast agar (0.2% [w/v] yeast extract, 2% [w/v] malt extract, 2% [w/v] agar), washed from media in sterile H_2O, and sieved through four layers of sterile cheesecloth. Conidial suspensions were centrifuged at 2,500 *g* for 15 min and resuspended in sterile water three times to remove remaining nutrients. Conidia were suspended in 10% (w/v) Pelgel (Nitrogen Co., Milwaukee, WI) at about 3×10^8 conidia per milliliter, of which 95% germinated on water agar. Aliquots (0.75 ml) of conidial suspensions were applied three times to 50 g of pea seed (total, 2.25 ml/50 g of seed) 24 hr before sowing.

Pea seeds were divided into two groups of 100 g each—those treated with Pelgel solution containing conidia of *Trichoderma* and those treated with Pelgel solution without conidia. To test for competition for seed exudates between *Trichoderma* and *Pythium*, the Pelgel solution used for treating one-half of the seed in each group contained 4.5 and 1.1 mg of D-glucose and L-asparagine per gram of seed, respectively.

Six seeds were placed in plastic pots (80 mm deep, 110 mm top diameter) containing 200 g of raw soil or soil supplemented with

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inoculum of isolate N1, which added about 10 sporangia per gram of air-dried soil (see below). Soils were then moistened to about −0.3 bar (15% [w/v]), covered with transparent Mylar (DuPont Co., Wilmington, DE) sheets to reduce evaporation, and incubated for 7 days at 19 or 26 C. After incubation, the incidence (%) of damping-off was determined. Each treatment was replicated six times in a randomized factorial design and the experiment was repeated three times, with similar results.

Germination assay of sporangia of isolate N1 and chlamydospores of *Fusarium oxysporum* f. sp. *cucumerinum* in soil. Isolate N1 was grown in rolled oat and water medium (19) at 25 C for 7 days in the dark. Mycelial mats were removed and mixed in raw soil, as described by Lifshitz and Hancock (28). The infested soil was air-dried, ground, and sieved through a 1-mm-mesh screen. The inoculum density of isolate N1 was estimated at 10^3 sporangia per gram of air-dried soil by the soil drop method (41). Chlamydospores of *F. o.* f. sp. *cucumerinum* Snyd. & Hans. were obtained by use of a procedure developed by B. Sneh and M. Dupler (*unpublished*). The fungus was grown in a potato-dextrose broth (PDB, Difco) supplemented with 0.2% (w/v) yeast extract at 27 C for 5 days in the dark. Mycelial mats were removed and washed thoroughly in running distilled water over four layers of cheesecloth to remove microconidia. The washed mats were blended for 1 min and mixed in raw soil. Soil was moistened to about 15% (v/w) and incubated for 4 wk in plastic bags. At the end of the incubation period, the propagule density (primarily chlamydospores) was estimated as 2×10^5 colony-forming units per gram of air-dried soil by the plate dilution method on *Fusarium*-selective medium (26).

Pea seeds were planted in small vials containing 2 g of raw soil infested with either chlamydospores of *F. o.* f. sp. *cucumerinum* or sporangia of isolate N1. The vials were moistened (0.4 ml of water per 2 g of air-dried soil), covered with plastic to reduce evaporation, and incubated at 26 C in the dark. Sporangium germination was assayed 1.5 hr after sowing by the soil smear technique described by Nash et al (34). Chlamydospore germination was assayed 18 hr after sowing by staining soil particles with 0.3% calcofluor white M2R solution (Polysciences, Inc., Warrington, PA) and viewing under an ultraviolet light microscope for fluorescent germ tubes (37). At least 100 sporangia or chlamydospores were observed from each vial, and at least three vials were examined in each treatment.

In vitro hyphal interaction between *Trichoderma* spp. and isolate N1. A cellophane membrane, washed in boiling, distilled water, was placed on the surface of water agar (7). A water agar disk with mycelium of each *Trichoderma* spp. was placed on one end of the cellophane membrane, and a disk with mycelium of isolate N1 was placed on the other end. The plates were incubated at 26 ± 1 C for 4 days. Cellophane membranes from the interaction area were removed periodically, and the hyphal interactions were observed under a phase-contrast microscope at magnifications of ×200 and ×400.

Production of toxic metabolites by *Trichoderma* spp. *Trichoderma* spp. were grown in 8.5-cm-diameter petri dishes containing 15 ml of autoclaved PDB. Cultures were incubated for 7 days at 26 ± 1 C. Mycelial mats were removed, and the growth medium was filtered through a sterile membrane (0.45 μm pore size, Nuclepore Corp., Pleasanton, CA).

Portions of the culture filtrate were autoclaved or stored for 4 days at 4 C. Then, 3 ml of the filtrate was placed in petri dishes (52 mm diameter) and approximately 12 ml of 1% water agar was added and mixed with the filtrate. The agar was allowed to gel, and a 4-mm plug of water agar containing isolate N1 was placed in the center of each dish. Cultures were incubated at 28 C, and the growth rate after 30–35 hr was measured as the mean of two colony diameters on each plate.

To compare the effect of toxic metabolites on growth of *T. harzianum* T-12, isolate N1, and *R. solani* (6), a 4-mm plug of agar containing thallus of T-12 was placed in a 250-ml Erlenmeyer flask containing 125 ml of PDB, incubated at 28 C for 3 days, and shaken at a rate of 100 rpm. Mycelial mats were removed, the extract of T-12 was sterilized by filtration, and agar dishes were prepared as described above. Dishes were seeded with 4-mm agar plugs containing thallus of T-12, N1, or *R. solani*. Water agar dishes were seeded with one of each of the three fungi to serve as controls. Cultures were incubated at 28 C, and the growth rate was measured as the mean of two colony diameters taken after 30–35 hr.

RESULTS

Effect of isolates of *Trichoderma* spp. on incidence of *Pythium*-induced damping-off. *T. harzianum* T-95 and T-12 and *T. koningii* T-8 significantly ($P = 0.05$) reduced the incidence of seedling damping-off at 26 C (Table 1). At 19 C, however, only strain T-8 reduced disease incidence significantly ($P = 0.05$). Application of nutrients (glucose and asparagine) to the seeds had no effect on the activity of the *Trichoderma* spp. as biocontrol agents.

Germination of sporangia of *Pythium* sp. (isolate N1) and chlamydospores of *F. o.* f. sp. *cucumerinum* in the spermosphere. Pea seed exudates stimulated the germination of chlamydospores of *F. o.* f. sp. *cucumerinum* and sporangia of *Pythium* sp. to 87.8 and 94.0%, respectively, compared with 3.2 and 6.0% germination, respectively, in raw soil without seeds (Table 2). The presence of *Trichoderma* spp. on the seeds had no effect on sporangium or chlamydospore germination in soil, whether propagules were in raw soil or in the spermosphere of peas.

Seed colonization by *Pythium* sp. (isolate N1). Seed coats were colonized by isolate N1 within less than 12 hr of incubation in soil at

TABLE 1. Effect of seed treatment with conidia of *Trichoderma* spp.[a] and organic nutrients on incidence of Pythium damping-off

	Incidence of damping-off (%)[b]			
	Infested[c]		Not infested[d]	
Seed treatment	19 C	26 C	19 C	26 C
Without nutrients				
T. harzianum T-95	97.2 v	50.0 vw	77.8 vw	37.8 wxy
T. harzianum T-12	75.0 v	41.1 w	58.3 w	33.3 xyz
T. koningii T-8	47.2 w	33.3 w	41.7 w	27.8 yz
Pelgel-treated control	86.1 v	77.8 v	77.8 vw	58.3 vwx
Nontreated control	88.9 v	88.9 v	75.0 vw	61.1 v
With nutrients[e]				
T. harzianum T-95	91.7 v	52.8 w	80.6 vw	11.1 z
T. koningii T-8	41.7 w	30.6 w	4.44 w	22.2 yz
Control	97.2 v	91.7 v	94.4 v	75.0 vw

[a] For each isolate, 2.25 ml of conidial suspension in 10% Pelgel provided 1.4×10^7 conidia per gram of seed.
[b] In each column, values followd by the same letter do not differ significantly ($P = 0.05$) according to FLSD.
[c] Ten sporangia of *Pythium* sp. (isolate N1) were added to each gram of air-dried soil.
[d] Population density of *Pythium* spp. was about 25 colony-forming units per gram of soil in raw soil.
[e] Seeds were coated with 4.5 and 1.1 mg of D-glucose and L-asparagine, respectively, per gram.

TABLE 2. Influence of seed treated with isolates of *Trichoderma* on germination of chlamydospores of *Fusarium oxysporum* f. sp. *cucumerinum* and sporangia of *Pythium* (isolate N1) in soil[a]

	Germination (%)[b]	
Seed treatment	Chlamydospores	Sporangia
Nontreated	87.8 y	94.0 y
Pelgel-treated control	92.6 y	90.1 y
T. harzianum T-95	94.1 y	92.4 y
T. harzianum T-12	86.4 y	89.6 y
T. koningii T-8	88.8 y	96.4 y
Raw soil only[c]	3.2 z	6.0 z

[a] Chlamydospore density in raw soil was estimated at 2×10^5 colony-forming units per gram of air-dried soil, 2 g of soil per seed; germination was assayed 18 hr after sowing. Sporangium density in raw soil was about 1.2×10^4 colony-forming units per gram of air-dried soil, 2 g of soil per seed; germination was assayed 1.5 hr after sowing.
[b] In each column, numbers followed by the same letter do not differ significantly ($P = 0.05$) according to FLSD.
[c] No seed present.

19 or 26 C (Table 3). Colonization of embryos, however, was substantially delayed and reached only 5.0 and 24.0% at 19 and 26 C, respectively, after 24 hr of incubation. At both temperatures, a significant ($P = 0.05$) increase in embryo colonization incidence occurred between 24 and 48 hr. The increase in incidence of embryo colonization incidence was associated with the time when seed coats were broken at germination. Microscopic observations of seed coats after seeds were incubated 24 hr at 19 C showed coenocytic hyphae only on the outer side. Seed coats taken after seeds were incubated 48 hr at 19 C, however, were colonized by coenocytic hyphae on the inner as well as the outer side.

Germination of conidia. Conidia of *T. koningii* T-8 germinated faster than conidia of *T. harzianum* T-95 or T-12 when incubated on water agar medium (Table 4). For 50% germination, conidia of isolate T-8 required less than 10 hr at 26 C and 14–18 hr at 19 C. Conidia of isolate T-12 required 10–14 hr at 26 C and 22–26 hr at 19 C. Microscopic observations of seed coats treated with conidia of *Trichoderma* spp. and incubated in raw soil revealed that conidia germinated at a similar rate to that on water agar but only when not touched by others, i.e., germination rates of crowded conidia were substantially lower.

TABLE 3. Incidence of seed-coat and embryo colonization by *Pythium* sp. (isolate N1) during incubation in infested soil

Incubation (hr)	Incidence of colonization (%)[a]			
	Seed coat		Embryo	
	19 C	26 C	19 C	26 C
12	100	100	4.6 x	6.0 x
24	100	100	5.0 x	24.0 x
48	100	100	61.8 y	46.2 z

[a] In each column, values followed by the same letter do not differ significantly ($P = 0.05$) according to FLSD.

TABLE 4. Germination rate of conidia of *Trichoderma* spp. on 2% water agar

Temperature (C)	Time (hr)	Germination (%)[a]		
		T. harzianum T-95	*T. harzianum* T-12	*T. koningii* T-8
26	10	14.4	31.0	86.5
26	14	73.6	75.0	95.6
26	18	98.0	93.6	98.0
19	10	0	0	0
19	14	0.3	0.9	14.5
19	18	8.7	13.4	78.0
19	22	14.8	26.5	91.4
19	26	26.5	54.2	91.2

[a] Means of 150–200 conidia observed on media in three dishes.

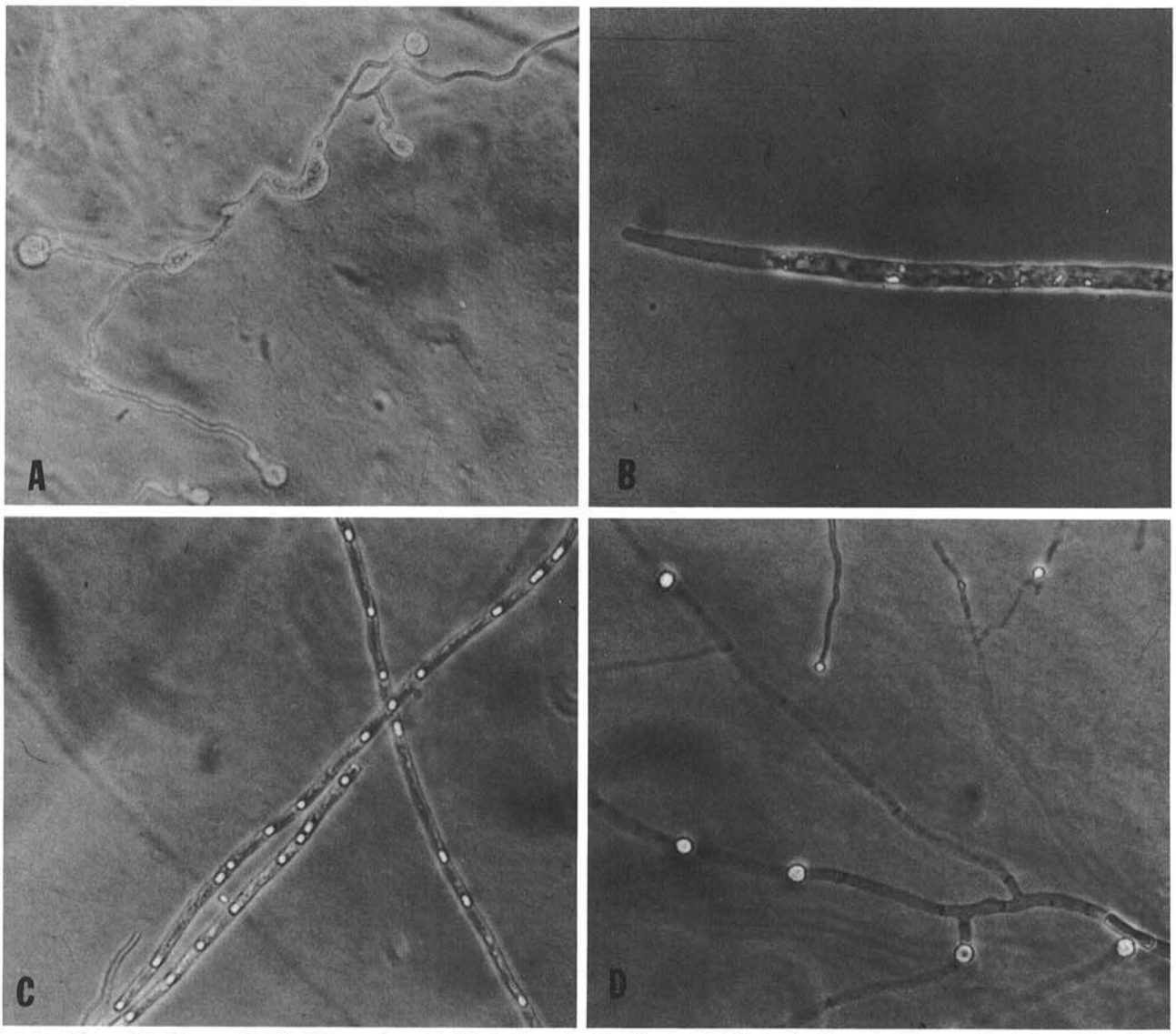

Fig. 1. Interactions of *Pythium* sp. (isolate N1) growing with *Trichoderma koningii* (isolate T-8) in dual culture. **A,** Abnormal hyphal swelling of isolate N1. **B,** Empty hyphal tip of isolate N1 (×410). **C,** Abnormal vacuolation of hyphae of isolate N1 (×150). **D,** Chlamydospores of *T. koningii* (×250) produced abundantly in dual culture with isolate N1.

In vitro interaction between *Trichoderma* spp. and *Pythium* sp. (isolate N1). No evidence of growth inhibition was observed macroscopically when fungi were grown in dual culture on cellophane and water agar medium until hyphal contact was made. During the following 24 hr, no lysis or coiling interaction that would have suggested cell wall lysis (9) was observed. Several abnormalities were noted, however. Hyphae of isolate N1 close to thalli of *Trichoderma* spp. showed an unusual vacuolation and coagulation of protoplasm. Protoplasm retreated from hyphal tips, abnormal vacuolation occurred, and abundant cross-septations and hyphal swellings were observed (Fig. 1A–C). At later stages of incubation, hyphae of *Trichoderma* advanced over the colony of isolate N1, which by then had only empty hyphae. *Trichoderma* spp. sporulated abundantly in areas of hyphal interactions and some (isolates T-1, T1-R1, and T-8) formed chlamydospores (Fig. 1D). Sporangia of isolate N1 germinated within 45–90 min, and the amount of germination was not affected by the presence of any *Trichoderma* spp. The germ tubes of sporangia (hyphae) were affected as described above, however. No apparent differences in activity against sporangia were observed among the isolates of *Trichoderma* tested.

The filtrates from nutrient medium supporting growth of all isolates of *Trichoderma* spp. tested were inhibitory to growth and sporangium germination of isolate N1 (Table 5). The greatest inhibition of hyphal growth rate was induced by filtrates of *T. harzianum* T-12 (96.3% inhibition, compared with the control), followed by *T. koningii* T-8 (65.4% inhibition, compared with the control). Extract obtained from *T. harzianum* T-12 inhibited growth of isolate N1 but not of *T. harzianum* T-12 or *R. solani*. No inhibition of isolate N1 by extracts of *Trichoderma* spp. was observed after autoclaving for 10 min. Inhibitory effects were also not observed after storage of extract of *Trichoderma* at 4 C for 4 days.

DISCUSSION

Biological control induced by seed treatment with *Trichoderma* spp. against pathogenic species of *Pythium* could result from mycoparasitism, competition for nutrients in seed exudates, and/or antibiosis.

Harman et al (20) suggested that mycoparasitism was the principal mechanism of Pythium damping-off when seeds were coated with *T. hamatum*. Their suggestion was based on evidence that in the presence of *Pythium* spp., *T. hamatum* was capable of producing the hydrolytic enzymes β-1,-3-glucanase and cellulase and on observation of hyphal parasitism in vitro on *Pythium* spp. (7). Another indication of the involvement of mycoparasitism was provided by Elad et al (11), who showed that the ability of isolates of *T. harzianum* to control damping-off caused by *P. aphanidermatum* was correlated to the level of hydrolytic enzyme production in soil. We also observed that isolates of *Trichoderma* spp. could parasitize hyphae of isolate N1 in vitro.

Considerations related to the time sequence of the host-pathogen interaction, inoculum potential relationships, and colocation of the biocontrol agent and pathogen in the infection court suggest that mycoparasitism is not a likely mechanism in the control of Pythium damping-off by seed treatment with at least some isolates of *Trichoderma* spp. A fundamental observation leading to this conclusion is that seed treatment with isolates of *Trichoderma* spp. was effective in controlling both Pythium and Rhizoctonia damping-off, whereas mixing these same biocontrol agents in soil suppressed *R. solani* but not *Pythium* spp. (6,20,28). How can this phenomenon be explained?

First, inoculum densities of the two pathogens found in nature are exceedingly different. *R. solani* was isolated from field soils below 1–10 propagules per gram (22,25), whereas inoculum densities of *Pythium* spp. were considerably higher (41). *R. solani* compensates for such low inoculum densities by having a relatively high linear growth rate (2). Evidence summarized by Baker and Drury (3) also suggests that propagules of *R. solani* respond to seed exudates at some distance from the host infection court. This provides *Trichoderma* spp. with the opportunity to "intercept" this pathogen as it grows toward the host infection court; relatively few propagules of *R. solani* touch the seed coat, thereby avoiding antagonism in the volume of the spermosphere. In contrast, propagules of *Pythium* spp. may have to touch the infection court (42) to succeed in infecting the host; relatively high inoculum densities, in this case, ensure that an adequate proportion of the total inoculum in the soil is adjacent to the seed. Therefore, biocontrol agents must interpose a barrier between the propagules of the pathogen and the host, namely, a coating of the agent on the seed.

Trichoderma spp. parasitized hyphae of isolate N1 in vitro; the time restraints, however, are critical for mycoparasitism to occur. Hyphal coilings, suggesting early stages of mycoparasitism, were observed rarely and occurred only after 24 hr from the time mycelial contact was made. This imposes a time limit for mycoparasitism to occur, since our results (Table 3) confirm the observations of Stasz et al (42) that seed coat colonization by *Pythium* spp. occurs within 10 hr and substantial embryo infection occurs within 24–48 hr in a susceptible pea cultivar. In addition, the time available for biocontrol agents to parasitize *Pythium* spp. is mediated by the time required for germination of propagules of *Trichoderma* spp. Whereas sporangium germination occurred within 90 min after introduction into the infection court, conidia of *T. harzianum* required 10–14 hr for over 50% germination at 26 C (Table 4); these isolates still protected seed from damping-off at this temperature (Table 1), however. It is therefore doubtful that mycoparasitism fully explains the mechanism of antagonism involved in seed treatment with *Trichoderma* spp. for control of Pythium damping-off.

Competition for nutrients in seed exudates also is a candidate for the mechanism of biological control of Pythium damping-off by seed treatment. The concentration of seed exudates affects successful infection, as evidenced by the following: Presumed removal of exudates by presoaking of seeds reduced disease incidence (15, 39–41), whereas amendments of carbohydrates or dead seeds to the soil increased disease incidence (17,23,30). Environmental factors that increased seed exudates also increased

TABLE 5. Effect of extracts[a] from nutrient medium supporting growth of isolates of *Trichoderma* on mycelium growth and sporangium germination of *Pythium* sp. (isolate N1)

Trichoderma isolate	Fresh extract[b]		Four-day-old extract[b]	Autoclaved extract[b]
	Linear growth rate[c] (mm/day)	Sporangium germination[d] (%)	Linear growth rate[c] (mm/day)	Linear growth rate[c] (mm/day)
T-95	21 x	0 x	29 w	33 w
T-1	23 x	0 x	29 w	···
T1-R1	18 x	0 x	29 w	···
W6	21 x	0 x	27 w	···
W6-15	17 x	0 x	28 w	···
T-8	13 y	0 x	26 w	32 w
T-12	5 z	0 x	23 w	30 w
Control[e]	30 w	96 w	28 w	32 w

[a] Fresh extract: *Trichoderma* spp. growth medium filtered through membrane (0.45 μm pore size) and diluted 1:10 with 1% (w/v) water agar (49 C); for sporangium germination assay, 1 ml of medium filtrates supplemented with 0.1 ml of sterile potato-dextrose broth and 0.1 ml of isolate N1 sporangium suspension in sterile distilled water (10^4 sporangia per milliliter). Four-day-old extract: *Trichoderma* spp. growth medium filtered as for fresh extract but stored for 4 days at 4 C before being diluted in water agar (1:10). Autoclaved extract: *Trichoderma* spp. growth medium autoclaved for 15 min at 43.9 g/cm^2, then diluted in water agar (1:10).

[b] In each column, values followed by the same letter do not differ significantly ($P = 0.05$) according to FLSD.

[c] Based on two perpendicular diameters of colony of isolate N1 after 24 hr of growth at 26 C from 4-mm disk cut from margin of 2-day-old culture.

[d] Based on three observations of at least 100 sporangia incubated for 90 min at 26 C and 100% relative humidity.

[e] For linear growth, 1% (w/v) agar only; for sporangium germination, 1 ml of sterile water with 0.1 ml of potato-dextrose broth and 0.1 ml of sporangial suspension in sterile water.

incidence, i.e., oxygen levels (5), soil temperature (27), and soil moisture (24). Further, host variety differences in susceptibility to *Pythium* were directly related to differences in the amount of seed exudation (23,38). Quantitative rather than qualitative differences in exudation appear to be most important in disease development with bean (38) and soybean cultivars (23). Since the correlation between quantity of seed exudates and disease incidence is well established, we may assume that partial removal of seed exudates by a biocontrol agent applied on seed could reduce the inoculum potential (2) of *Pythium* spp. Our study, however, suggests that competition for seed exudates is not an important antagonistic mechanism between *Trichoderma* spp. and isolate N1, based on the following: Addition of nutrients (glucose and asparagine) to the seeds did not affect successful disease control (Table 1), and the presence of *Trichoderma* spp. on seed did not reduce germination of sporangia of isolate N1 or chlamydospores of *F. o.* f. sp. *cucumerinum* in the spermosphere (Table 2). These observations suggest that nutrients required for germination, penetration, and infection by the pathogen were not limited by the activity of *Trichoderma* spp. on the seed coat.

Fungitoxic action of *Trichoderma* spp. on pathogenic *Pythium* spp. was first reported by Allen and Haenseler (1,18) and later by Dennis and Webster (8,9). Nevertheless, antibiosis was not considered in later reports on biological control of Pythium damping-off by *Trichoderma* spp. (4,7). In our study, toxic metabolites were produced in vitro by seven effective biocontrol isolates of *Trichoderma* (Table 5). In addition, stress signs were obvious in hyphae of *P. ultimum* grown in dual culture 24 hr before a mycoparasitism reaction was observed (Fig. 1). This was noted, however, only at a microscopic level and would not have been evident by macrobiotic observations, conventionally used in screening. Because the protoplasm in the hyphal tips of isolate N1 responds to the *Trichoderma*-produced factor by being disorganized and "thrown into confused flight," we suggest it be named the routing factor.

The lack of growth inhibition of *T. harzianum* T-12 by metabolites derived from the same fungus suggests that the factors inhibitory to isolate N1 were not staling substances. Culture filtrates were inhibitory to radial growth of isolate N1 but not to *R. solani*, again suggesting that biological control mechanisms of *Trichoderma* spp. against the two pathogens are dissimilar.

The saprophytic activity of *Pythium* spp. in soil generally is restricted to early colonization of substrate in advance of other microorganisms. The inability of *Pythium* spp. to compete as a secondary colonizer was attributed to their great sensitivity to toxic metabolities produced by other microorganisms (4,15). Because the interaction between *Trichoderma* spp. and isolate N1 occurred on dead seed coats, and because such isolates were capable of producing metabolites toxic to the pathogen, we suggest that production of toxic metabolites by *Trichoderma* spp. on seed coats is the principal mechanism of biological control of Pythium damping-off.

LITERATURE CITED

1. Allen, M. C., and Haenseler, C. M. 1935. Antagonistic action of *Trichoderma* on *Rhizoctonia* and other soil fungi. Phytopathology 25:244-252.
2. Baker, R. 1978. Inoculum potential. Pages 137-157 in: Plant Disease. An Advanced Treatise. Vol. 2. How Disease Develops in Populations. J. G. Horsfall and E. B. Cowling, eds. Academic Press, New York. 436 pp.
3. Baker, R., and Drury, R. 1981. Inoculum potential and soilborne pathogens: The essence of every model is within the frame. Phytopathology 71:363-372.
4. Barton, R. 1961. Saprophytic activity of *Pythium mamilatum* in soils. III. Factors restricting *P. mamilatum*. Trans. Br. Mycol. Soc. 44:105-118.
5. Brown, G. E., and Kennedy, B. W. 1966. Effect of oxygen concentration on *Pythium* seed rot of soybean. Phytopathology 56:407-411.
6. Chet, I., and Baker, R. 1981. Isolation and biocontrol potential of *Trichoderma hamatum* from soil naturally suppressive to *Rhizoctonia solani*. Phytopathology 71:286-290.
7. Chet, I., Harman, G. E., and Baker, R. 1981. *Trichoderma hamatum*: Its hyphal interactions with *Rhizoctonia solani* and *Pythium* spp. Microb. Ecol. 7:29-38.
8. Dennis, C., and Webster, J. 1971. Antagonistic properties of specific groups of *Trichoderma*. I. Production of non-volatile antibiotics. Trans. Br. Mycol. Soc. 57:25-30.
9. Dennis, C., and Webster, J. 1971. Antagonistic properties of specific groups of *Trichoderma*. II. Production of volatile antibiotics. Trans. Br. Mycol. Soc. 57:41-48.
10. Elad, Y., Chet, I., and Henis, Y. 1981. Biological control of *Rhizoctonia solani* in strawberry fields by *Trichoderma harzianum*. Plant Soil 60:245-254.
11. Elad, Y., Chet, I., and Henis, Y. 1982. Degradation of plant pathogenic fungi by *Trichoderma harzianum*. Can. J. Microbiol. 28:719-725.
12. Elad, Y., Chet, I., and Katan, J. 1980. *Trichoderma harzianum*: A biological agent effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. Phytopathology 70:119-121.
13. Elad, Y., Kalfan, A., and Chet, I. 1982. Control of *Rhizoctonia solani* in cotton by seed-coating with *Trichoderma* spp. spores. Plant Soil 66:279-281.
14. Flentje, N. T., and Saksena, H. K. 1964. Pre-emergence rotting of peas in South Australia. III. Host-pathogen interaction. Aust. J. Biol. Sci. 17:665-675.
15. Garrett, S. D. 1956. Biology of Root-infecting Fungi. Cambridge University Press, New York. 293 pp.
16. Hadar, Y., Chet, I., and Henis, Y. 1979. Biological control of *Rhizoctonia solani* damping-off with wheat bran culture of *Trichoderma harzianum*. Phytopathology 69:64-68.
17. Hadar, Y., Harman, G. E., Taylor, A. G., and Norton, J. M. 1983. Effects of pregermination of pea and cucumber and seed treatment with *Enterobacter cloacae* on rots caused by *Pythium* spp. Phytopathology 73:1322-1325.
18. Haenseler, C. M., and Allen, M. C. 1934. Toxic action of *Trichoderma* on *Rhizoctonia* and other soil fungi. (Abstr.) Phytopathology 24:10.
19. Hancock, J. G. 1977. Factors affecting soil populations of *Pythium ultimum* in the San Joaquin Valley of California. Hilgardia 145:107-122.
20. Harman, G. E., Chet, I., and Baker, R. 1980. *Trichoderma hamatum* on seedling disease induced in radish and pea by *Pythium* spp. or *Rhizoctonia solani*. Phytopathology 70:1107-1172.
21. Henis, Y., Ghaffar, A., and Baker, R. 1978. Integrated control of *Rhizoctonia solani* damping-off of radish: Effect of successive plantings, PCNB and *Trichoderma harzianum* on pathogen and disease. Phytopathology 68:900-907.
22. Henis, Y., Ghaffar, A., Baker, R., and Gillespie, S. L. 1978. A new pellet soil sampler and its use for the study of population dynamics of *Rhizoctonia solani* in soil. Phytopathology 68:371-376.
23. Keeling, B. L. 1979. Soybean seed rot and the relation of seed exudate to host susceptibility. Phytopathology 64:1445-1447.
24. Kerr, A. 1964. The influence of soil moisture on infection of peas by *Pythium ultimum*. Aust. J. Biol. Sci. 17:676-685.
25. Ko, W. H., and Hora, F. K. 1971. A selective medium for the quantitative determination of *Rhizoctonia solani* in soil. Phytopathology 61:707-710.
26. Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Rev. Plant Prot. Res. 8:114-125.
27. Kraft, J. M., and Erwin, D. C. 1967. Stimulation of *Pythium aphanidermatum* by exudates from mung bean seeds. Phytopathology 57:866-868.
28. Lifshitz, R., and Hancock, J. G. 1981. An enrichment method to estimate potential seedling disease caused by low densities of *Pythium ultimum* inocula in soils. Plant Dis. 65:828-829.
29. Liu, S., and Baker, R. 1980. Mechanism of biological control in soil suppressive to *Rhizoctonia solani*. Phytopathology 70:404-412.
30. Liu, S., and Vaughan, E. K. 1965. Control of *Pythium* infection in table beet seedlings by antagonistic microorganisms. Phytopathology 55:986-989.
31. Locke, J. C., Papavizas, G. C., and Rubel, M. K. 1979. Biological control of Pythium seed rot of peas with *Trichoderma* conidia. (Abstr.) Phytopathology 69:536.
32. Marshal, D. S. 1982. Effect of *Trichoderma harzianum* seed treatment and *Rhizoctonia solani* inoculum concentration on damping-off of snap bean in acid soils. Plant Dis. 66:788-789.
33. Miretich, S. M., and Kraft, J. M. 1973. Efficiency of various selective media in determining *Pythium* population in soil. Mycopathol. Mycol. Appl. 50:151-161.
34. Nash, S. M., Christon, T., and Snyder, W. C. 1961. Existence of *Fusarium solani* f. sp. *phaseoli* as chlamydospores in soil. Phytopathology 51:308-312.

35. Papavizas, G. C., and Lewis, J. A. 1983. Physiological and biocontrol characteristics of stable mutants of *Trichoderma viride* resistant to MBC fungicides. Phytopathology 73:407-411.
36. Papavizas, G. C., Lewis, J. A., and Abde-El Moity, T. H. 1982. Evaluation of new biotypes of *Trichoderma harzianum* for tolerance to benomyl and enhanced biocontrol capabilities. Phytopathology 72:126-132.
37. Scher, F. M., and Baker, R. 1983. A fluorescent microscopic technique for viewing in soil and its application to studies of a *Fusarium*-suppressive soil. Soil Biol. Biochem. 15:715-718.
38. Schroth, M. N., and Cook, R. J. 1964. Seed exudation and its influence on pre-emergence damping-off of bean. Phytopathology 54:670-673.
39. Short, G. E., and Lacy, M. L. 1976. Factors affecting pea seeds and seedling rot in soil. Phytopathology 66:188-192.
40. Short, G. E., Loria, R., and Lacy, M. L. 1977. Effect of seed quality and seed treatment on yield of pea. J. Am. Soc. Hortic. Sci. 102:258-260.
41. Stanghellini, M. E., and Hancock, J. G. 1970. A quantitative method for the isolation of *Pythium ultimum* from soil. Phytopathology 60:551-552.
42. Stasz, T. E., Harman, G. E., and Marx, G. A. 1980. Time and site of infection of resistant and susceptible germinating pea seeds by *Pythium ultimum*. Phytopathology 70:730-733.
43. Wright, J. M. 1956. Biological control of soil-borne *Pythium* infection by seed inoculation. Plant Soil 8:132-140.