Influence of a Fungus-Feeding Nematode on Growth and Biocontrol Efficacy of *Trichoderma harzianum*

Yeoung-Seuk Bae and Guy R. Knudsen

Soil Science Division, University of Idaho, Moscow 83844.
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ABSTRACT

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A fungivorous nematode, *Aphelenchoides* sp., was isolated from field soil by baiting with mycelium of the biocontrol fungus *Trichoderma harzianum* ThzID1, and subsequently was maintained on agar cultures of the fungus. Interactions between the nematode and the green fluorescent protein-producing transformant, *T. harzianum* ThzID1-M3, were investigated in both heat-treated (80°C, 30 min) and untreated field soil. ThzID1-M3 was identified in soil by epifluorescence microscopy. When ThzID1-M3 was added to soil as an alginate pellet formulation, addition of the nematode (10 per gram of soil) significantly reduced radial growth and recoverable populations of the fungus, and the effect was greater in heat-treated soil than in untreated soil. Addition of ThzID1-M3 to soil pretreated with the nematode (10 per gram of soil) stimulated nematode population growth for approximately 10 to 20 days, whereas nematode populations decreased in the absence of added *Trichoderma* sp. When sclerotia of *Sclerotinia sclerotiorum* were added to soil (10 per 200 g of soil) with ThzID1-M3 (40 pellets per 200 g of soil), addition of *Aphelenchoides* sp. (2,000 per 200 g of soil) reduced the number of sclerotia colonized by ThzID1-M3. These results suggest that fungivorous nematodes may be a significant biotic constraint on activity of biocontrol fungi in the field.

Interactions between biocontrol fungi and soil microanimals have largely been neglected in studies relating to the ecology and biocontrol efficacy of fungal agents. Nematodes are the most abundant soil-inhabiting animals. There are more than 80,000 described species, and population densities typically are high in the top 5 cm of soils, with substantial seasonal differences in agricultural soils (26,29,30). One way that nematologists have characterized the functional role of nematodes in soil communities is according to the nature of their food and method of feeding (25). Identified trophic groups include fungivores, bacterivores, omnivores, predators, and plant parasites (12,25). Fungivorous nematodes have a stylet, and feed on mycelia of many different species of soil fungi, including beneficial fungi as well as plant pathogens (5,10). Numerous species of fungivores have been found in soils (25). The most common genera found in agricultural soils are *Aphelenchoides*, *Aphelenchus*, *Tylenchus*, and *Ditylenchus* (10,12, 17,30); some species within these genera are plant parasites. For example, *Ditylenchus destructor*, which is parasitic on carrot, potato, alfalfa, radish, and sweetpotato, also can be cultured on a variety of filamentous fungi (10). Fungivores within this group of nematodes usually are found at lower densities than are bacterivores or plant parasites (12,27). However, if suitable fungi as food are available, populations of fungivores may rapidly increase. Hofman and s'Jacob (17) reported that numbers of the mycophagous nematodes *Aphelenchoides* sp., *Aphelenchus avenae*, and unidentified microbivorous nematodes increased several-fold within a few days after being added to flax roots that had been precolonized by *Rhizoctonia solani*. Some plant ectoparasitic nematodes also may be fungivorous, as the need arises (25).

Isolates of the soil fungus *Trichoderma harzianum* Rifai have been described as potential biocontrol agents against several soil-borne plant pathogens (15,16,21,28). A variety of methods for application of biocontrol fungi to soil have been developed, with the goal of enhancing proliferation and establishment of the agent (11,22,23). Incorporation of spores or hyphal biomass into alginate pellets, with or without a nutrient source or other additives, has several advantages, including a relatively long shelf life of the formulated agent and ease of application (8,11,23). When a fungal biocontrol agent is formulated in this manner, the introduced fungus may rapidly grow from the source, form a hyphal network, and potentially contact and colonize target propagules of a soil-borne pathogen or other substrata (3,22). Knudsen and Bin (20) reported a hyphal density of 10 to 70 mm mm^{-3} of soil, with a maximum of 30 mm of hyphal radial extension, within 7 to 14 days for *T. harzianum*.

Previous research in this laboratory demonstrated effects of several abiotic (temperature, soil moisture, added nutrients) and biotic (potentially antagonistic bacteria) factors on hyphal growth and biocontrol efficacy of *T. harzianum* (6,8,20,21). Some of these experiments were conducted using partially sterilized soil, whereas other experiments in untreated soil were not able to clearly distinguish effects of introduced *T. harzianum* from possible effects of indigenous *Trichoderma* spp. Recently, we reported the cotransformation of *T. harzianum* ThzID1 with genes encoding production of green fluorescent protein (GFP), as well as β-glucuronidase and hygromycin B resistance (3). Expression of GFP allows detection of the introduced agent against a background of indigenous fungi and thus provides a useful tool for evaluating its growth and biocontrol activity in a natural soil community. We hypothesized that one constituent of the soil community, fungivorous nematodes, may adversely affect growth and biocontrol efficacy of the introduced agent. The objectives of this study were to determine (i) whether a fungivorous nematode influences growth and proliferation of ThzID1-M3 in soil, (ii) whether application of formulated ThzID1-M3 to soil stimulates population growth of known fungivorous and other nematodes, and (iii) whether a population of fungivorous nematodes can reduce the ability of ThzID1-M3 to colonize sclerotia of *Sclerotinia sclerotiorum* in soil.

Corresponding author: G. R. Knudsen; E-mail address: gknudsen@uidaho.edu

MATERIALS AND METHODS

Nematode isolation and identification. A quantity of Latahco silt loam soil was obtained from the University of Idaho Parker Farm near Moscow. The field had been cropped to potato ('Russet Burbank') for 2 years in succession. Soil analysis (University of Idaho Analytical Services Laboratory) indicated that the soil contained 20% sand, 20% clay, and 60% silt by weight. Soil pH in soil/water (2:1) solution was approximately 5.9. Soil was sieved through a 2-mm mesh and air dried prior to use. *T. harzianum* ThzID1 was grown in potato dextrose broth shake culture for 3 days at approximately 22°C. Mycelium was harvested by filtering with four layers of cheesecloth, then washed with sterile distilled water. A sample of the mycelial mat (approximately 5 g) was enclosed in nylon mesh (0.1-mm mesh size) and buried in approximately 1 kg of soil. After 3 days of incubation at approximately 22°C, the mycelium was harvested and nematodes were extracted from the mycelium with a Baermann funnel. The isolated nematodes were sorted by morphological characteristics using a compound microscope. Based on the morphological characteristics of fungal feeders (13,25), a single male and a single female from each of five morphologically distinct types were transferred onto potato dextrose agar (PDA) plates of *T. harzianum* and incubated at 25°C. Nematodes of one morphological type showed rapid growth on ThzID1 cultures. To eliminate possible microbial contaminants, nematodes were subcultured on ThzID1 cultures on *Trichoderma* sp. selective medium (TSM; 9), then subcultured again on PDA cultures of ThzID1. These nematodes were used in subsequent experiments. For identification, ≈100 nematodes were heat killed (60°C, 10 min) and observed at ×1,000 magnification. Images were captured with a digital video camera and compared with reference drawings and photographs (13,24). Based on the following characteristics, we identified the nematode as a member of the genus *Aphelenchoides*: shape vermiform with nondistinct cuticular segmentation; relatively short stylet with small distinct knobs; esophagus is a slender precorpus leading to a distinct, well-developed median bulb, and isthmus united imperceptibly with the beginning of the intestine; male tail arcuate with a small pointed terminus, presenting three pairs of caudal papillae, spicules paired and thorn-shaped; female tail tapering and conical with a small terminus.
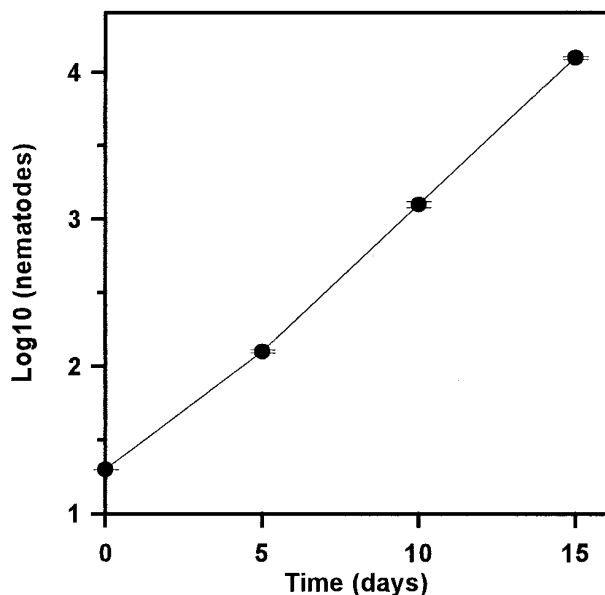

Fig. 1. Numbers of *Aphelenchoides* sp. in cultures with *Trichoderma harzianum* grown at 25°C. Nematodes were extracted 0, 5, 10, and 15 days after transfer onto the fungal cultures.

Population growth rate of *Aphelenchoides* sp. on *T. harzianum* agar cultures. The population growth rate of *Aphelenchoides* sp. was investigated in agar culture with *T. harzianum*. The medium used was TSM, which inhibits growth of contaminant bacteria, many fungi, and non-stylet-bearing nematodes (9), but supports limited colony growth of *Trichoderma* spp. *Aphelenchoides* sp. adults, 10 males and 10 females, were placed on TSM agar plates on which *T. harzianum* had been allowed to grow for 3 days. The plates were incubated at 25°C in the dark. After 5, 10, and 15 days, plates were destructively sampled by inverting the contents onto four layers of tissue paper (Kimwipes) in a Baermann funnel filled with sterile distilled water. Nematodes were extracted over a 6-day period, and total counts were obtained with a dissecting microscope. There were three replicates of each treatment for each sample time and the experiment was conducted twice.

Effect of *Aphelenchoides* sp. on radial growth of *T. harzianum* in soil. Latahco silt loam soil was either air dried as above ("untreated soil"), or air dried and then heat treated ("heat-treated soil") by wetting with sterile water to a soil moisture potential of –100 kPa, then placing ≈1-kg samples at 80°C for 30 min. The latter treatment effectively eliminated populations of indigenous nematodes and *Trichoderma* spp. as well as most indigenous fungi and bacteria (data not shown). Both untreated and heat-treated soils were adjusted a soil moisture content of –50 kPa by adding either sterile water or water containing *Aphelenchoides* sp. from ThzID1 cultures (1,000 nematodes per 100 g of dry soil, air-dried basis).

The strain of *T. harzianum* used in this experiment was ThzID1-M3, which was transformed with genes encoding hygromycin B resistance, β-glucuronidase (GUS), and GFP (3). Alginate pellets containing hyphal biomass of ThzID1-M3 plus wheat bran and polyethylene glycol 8000 were formulated as described previously (20,22). Pellets were approximately 1.8 mm in diameter. Colony growth of ThzID1-M3 was measured by previously described methods (3). Glass petri dishes (15-cm diameter) were half filled with soil (treatments as described above and a glass microscope slide precoated with 1.8% water agar was placed on the soil surface. A single ThzID1-M3 pellet was attached to the slide surface with cyanoacrylate glue. Dishes then were filled with additional soil, placed in a covered plastic container lined with moist paper towels, and incubated at 25°C for 3, 5, 7, or 14 days. At each sample time, glass slides were removed and hyphal growth on the glass surface was examined using epifluorescence microscopy. Colony radii were quantified by measuring captured video images at magnifications of ×250 or ×400. There were four replicates per treatment, and the experiment was conducted twice.

Effect of *Aphelenchoides* sp. on recoverable numbers of *Trichoderma* propagules, and nematode population dynamics in soil. Untreated or heat-treated soil as described above was mixed with sterile distilled water or nematode suspensions of *Aphelenchoides* sp. to provide a matric potential of –50 kPa and nematode population levels of 0 or 500 per 50 g of soil. Glass containers (250-ml) were filled with 50 g of either untreated or heat-treated soil, with or without nematodes. Then, either 0 or 20 ThzID1-M3 pellets were added (i.e., a 2-by-2-by-2 factorial design). The containers were covered with aluminum foil and incubated at 25°C in the dark for 5, 10, 20, or 30 days. There were three replicates per treatment for each sample time, and the experiment was conducted twice. At each sample time, recoverable populations of ThzID1-M3 and total recoverable *Trichoderma* spp. were determined as follows. The soil in the glass container was thoroughly mixed by shaking, after which a 1-g sample was removed and suspended in 9 ml of sterile distilled water. Serial dilutions were made and aliquots (100-μl) were spread on duplicate plates of TSM amended with hygromycin B at 100 μg/ml for enumeration of ThzID1-M3, and with unamended TSM for total *Trichoderma* spp. Plates were incubated at 25°C, and colonies were counted after 7 to 10 days. *Trichoderma* spp. were identified

to genus by colony color and conidiophore morphology. To enumerate nematode populations, a 20-g soil sample was obtained from each container and placed on four layers of tissue paper in a Baermann funnel filled with sterile distilled water. Extracts were collected over a 6-day period and counts of all nematodes present (including any indigenous nematodes in untreated soil) were made using a dissecting microscope.

Effect of *Aphelenchoides* sp. on colonization of sclerotia of *S. sclerotiorum* by ThzID1-M3. Mycelial disks of *S. sclerotiorum* grown on PDA for 7 days were transferred to sterilized sliced carrots in 2-liter Erlenmeyer flasks. After 6 to 8 weeks at 16°C, sclerotia were harvested, rinsed with sterile distilled water, and air dried for 2 to 3 days. Sclerotia were attached to plastic toothpicks with cyanoacrylate glue and allowed to dry overnight. All sclerotia were then surface disinfested (10% ethanol, 10% bleach in water) for 1 min and stored at 4°C before use.

A 200-g amount of either untreated or heat-treated soil was added to glass screwcap jars (9-cm diameter by 9-cm depth). Forty pellets of ThzID1-M3 were added to each glass jar and thoroughly mixed with soil; then, 10 sclerotia were randomly placed in the soil at a depth of approximately 2 cm. Soil was adjusted to a moisture content of –50 kPa with sterile distilled water or nematode suspension (to reach 2,000 nematodes per jar). The jars were closed and incubated at 25°C for 14 days in the dark. All jars contained sclerotia and ThzID1 pellets, so that the experimental design was a factorial experiment with two levels of soil treatment (untreated and heat-treated) and two levels of nematode infestation (0 and 2,000 nematodes per jar). There were five replicates per treatment, and the experiment was conducted twice. After 14 days, sclerotia were recovered, surface sterilized, and placed on PDA containing streptomycin at 50 μg/ml to avoid bacterial contamination. After incubation at 25°C for 7 days, sclerotia were observed to determine whether *S. sclerotiorum*, *Trichoderma* spp., or other fungi were growing from them. When hyphae of *Trichoderma* spp. were observed, they were examined for GFP activity using epifluorescence microscopy and proportions of sclerotia colonized by indigenous (non-GFP) *Trichoderma* spp. versus ThzID1-M3 were recorded.

Statistical analysis. Factorial analyses of variance (ANOVA) of all data except growth data of *Aphelenchoides* sp. were performed using Statistical Analysis System software (SAS Institute Inc., Cary, NC). Statistical analyses and inferences were based on log transformations for all population data and based on arcsin transformation for proportion data. Each experiment was conducted twice; treatment by experiment interactions was not significant; therefore, results from both sets of experiments were pooled. Treatment effects were considered to be statistically significant at the α = 0.05 level. Means and standard errors of the means were calculated for the number of colony forming units (log transformed) of *Trichoderma* spp. population and for the number of total nematodes observed at each sampling time.

RESULTS

Population growth rate of *Aphelenchoides* sp. on *T. harzianum* agar cultures. Growth of *Aphelenchoides* sp. on *T. harzianum* cultured on TSM was approximately exponential (Fig. 1), with an intrinsic population growth rate of ≈0.43 nematodes nematode^{-1} day^{-1}. Ratios of male/female and adult/juvenile were determined for a randomly selected sample after 15 days. The male/female ratio was approximately 1:1.5; the adult/juvenile ratio was approximately 1:1.4.

Effect of *Aphelenchoides* sp. on radial growth of *T. harzianum* in soil. Data were analyzed over all sample times. Each of the main effects (soil, nematode, sample time) had a statistically significant effect on radial growth of ThzID1-M3 (P_{soil} = 0.001, $P_{nematode}$ = 0.002, and $P_{sample\ time}$ = 0.001). Colony radius was less in untreated soil compared to heat-treated soil, was less when

nematodes were present, and generally increased over time in all treatments. The soil–sample time interaction was significant (P = 0.01); thus, soil effects on radial growth were greatest at the 14-day sample time. However, mean colony radius in the heat-treated soil was higher than that in untreated soil at every sample time. The soil–nematode interaction was not significant (P = 0.21). In heat-treated soil, mean colony radius at day 14 was 9.8 mm in the absence of *Aphelenchoides* sp. compared with 6.8 mm with the nematode present (Fig. 2). In untreated soil, mean colony radius at day 14 averaged 5.2 mm in the absence of *Aphelenchoides* sp. compared with 4.3 mm with the nematode present (Fig. 2).

Effect of *Aphelenchoides* sp. on population growth of *Trichoderma* and nematode population dynamics in soil. Mean recoverable numbers (log transformed) of ThzID1-M3 are shown in Figure 3. Over all sample times, soil treatment and presence of nematodes were statistically significant in their effect on numbers of recoverable propagules of ThzID1-M3 (P_{soil} = 0.0001, $P_{nematode}$ = 0.0001). Recoverable numbers of ThzID1-M3 were higher in heat-treated than in untreated soil at all sampling times, regardless of whether nematodes were present or absent (Fig. 3), and addition of the nematode significantly reduced recoverable populations of ThzID1-M3 (Fig. 3). Interactions between factors were not significant (P > 0.05).

Increased population densities of total recoverable *Trichoderma* spp. in both soils resulted from the addition of ThzID1-M3, with higher population levels attained in heat-treated soil (P = 0.001, Fig. 4). In untreated soil, mean numbers of total *Trichoderma* spp. were approximately 10- to 15-fold higher when ThzID1-M3 was added (Fig. 4). Treatment with *Aphelenchoides* sp. in both soils significantly reduced total populations of *Trichoderma* spp. (P = 0.001; Fig. 4). However, this effect was confounded by a significant soil–nematode interaction (P = 0.01), because low levels of *Trichoderma* spp. apparently were introduced as contaminants along with the nematodes (which had been reared on *Trichoderma* spp. cultures) (Fig. 4).

Addition of ThzID1-M3 stimulated populations of nematodes in untreated soil, regardless of whether *Aphelenchoides* nematodes
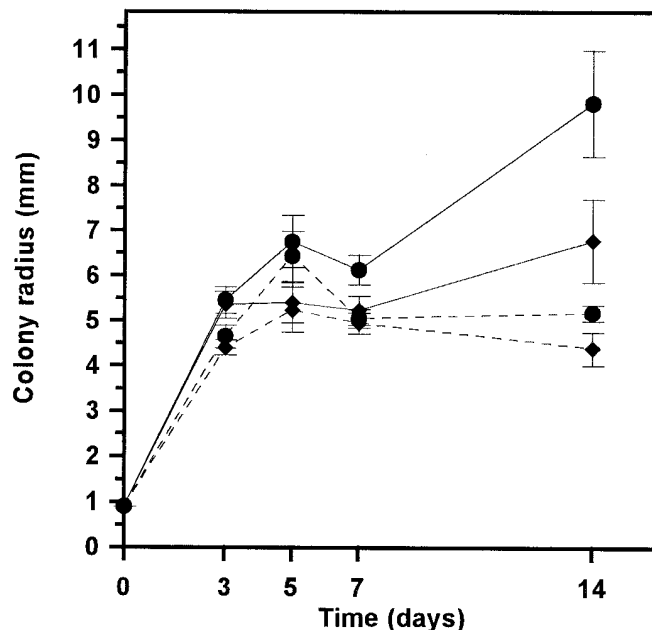

Fig. 2. Effect of *Aphelenchoides* sp. on radial growth of *Trichoderma harzianum* isolate ThzID1-M3 originating from alginate pellets in heat-treated (solid line) or untreated (dotted line) soil at 0, 3, 5, 7, and 14 days. *Aphelenchoides* sp. was applied to soil at initial levels of 0 (●) or 10 (◆) per gram of dry soil. Radial growth was measured at a magnification of ×250 or ×400 under epifluorescence microscopy. Vertical bars represent ±1 standard error of the mean.

were added (Fig. 5). Indigenous nematodes initially were at undetectable levels and did not increase when ThzID1-M3 was not added (Fig. 5). When individuals of *Aphelenchoides* sp. were added to untreated soil, numbers of total recoverable nematodes decreased over time if ThzID1-M3 was not also added (Fig. 5). Results were similar for the heat-treated soil, except that indigenous nematodes apparently were eliminated by the heat treatment (Fig. 5).
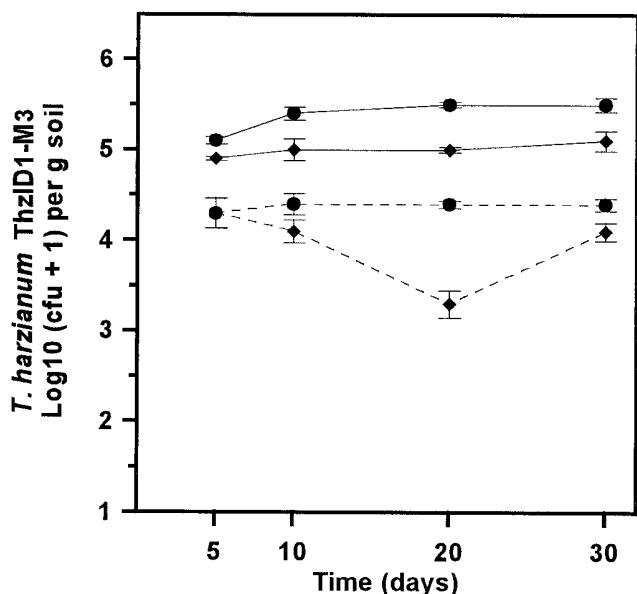

Fig. 3. Effect of *Aphelenchoides* sp. on populations of *Trichoderma harzianum* isolate ThzID1-M3 in heat-treated (solid line) or untreated (dotted line) soil at 5, 10, 20, and 30 days. *Aphelenchoides* sp. was applied to soil at initial levels of 0 (●) or 10 (◆) per gram of dry soil, with 20 alginate pellets (per 50 g of dry soil) of ThzID1-M3. Vertical bars represent ±1 standard error of the mean.

Effect of *Aphelenchoides* sp. on colonization of sclerotia by ThzID1-M3. Mean proportions of sclerotia colonized by ThzID1-M3 are shown in Figure 6. Both main effects (soil, $P = 0.0002$; nematode, $P = 0.004$) had significant effects on colonization by ThzID1-M3, although proportions of sclerotia colonized by indigenous *Trichoderma* spp. (i.e., *Trichoderma* spp. other than ThzID1-M3) did not differ significantly among treatments ($P = 0.25$). Numbers of sclerotia colonized by ThzID1-M3 were higher overall in heat-treated soil than in untreated soil, and addition of *Aphelenchoides* sp. resulted in lower numbers, in both soils, of sclerotia colonized by ThzID1-M3 compared with untreated controls. The soil–nematode interaction was not significant.

DISCUSSION

The effectiveness of biocontrol fungi against soilborne plant diseases may depend in part on their ability to proliferate through the three-dimensional soil space via hyphal growth originating from the introduced source (21,22). There are many biotic and abiotic factors, including temperature, soil moisture, nutrients, and microorganisms or microanimals, that may affect the growth and efficacy of biocontrol fungi in soil (6,8,18,20). We demonstrate here that a fungal-feeding nematode potentially can reduce the growth and biocontrol efficacy of *T. harzianum* in soil.

Fungivorous nematodes such as *Aphelenchoides* sp. and *Aphelenchus avenae* are associated with saprophytic, pathogenic, and mycorrhizal fungi in soil (5,10,19). Several fungus-feeding nematodes can be cultured on media with an appropriate fungus. For example, the potato rot nematode *Ditylenchus destructor* fed and reproduced on 64 out of 115 soil-inhabiting fungi representing 40 genera and 8 orders (10). In our study, we isolated *Aphelenchoides* sp. by baiting soil from a field previously cropped to potato with mycelium of *T. harzianum*. *Aphelenchoides* spp. are reported to be common and abundant in potato fields (17). Although our method may have selected for nematodes that were able to aggressively feed on mycelium of *T. harzianum*, we did not examine other soil fungi for their potential food value for this nematode. *Aphelenchoides* sp. successfully reproduced on PDA or
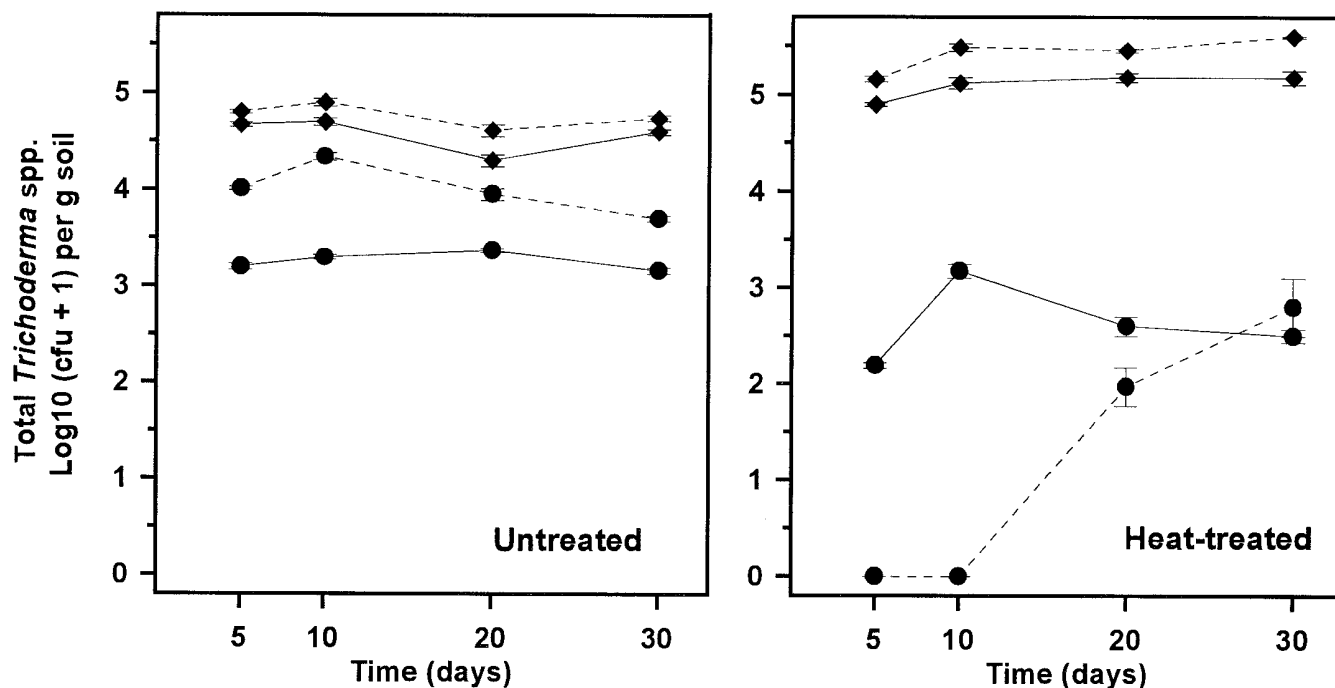

Fig. 4. Effect of *Aphelenchoides* sp. on total populations of *Trichoderma* spp. in heat-treated or untreated soil at 5, 10, 20, and 30 days. *Aphelenchoides* sp. was applied to soil at initial levels of 0 (dotted line) or 10 (solid line) nematodes per gram of dry soil, with alginate pellets of ThzID1-M3 at levels of 0 (●) or 20 (◆) pellets per 50 g of dry soil. Vertical bars represent ±1 standard error of the mean.

TSM cultures of *T. harzianum*, and exhibited exponential growth on TSM with *T. harzianum* isolate ThzID1 as a food source. Within 10 days after inoculating fungal cultures with nematodes, there were no aerial mycelia observable and, after approximately 10 days, the amount of visible mycelium in culture plates appeared to be greatly diminished.

By using heat-treated soil in some experiments, we tried to eliminate the possibility of direct effects of indigenous nematodes or fungi either on the growth and biocontrol efficacy of ThzID1-M3 or on the growth of *Aphelenchoides* sp. Addition of the nematode significantly reduced radial growth and recoverable populations of ThzID1-M3 added to soil as an alginate pellet formulation both in heat-treated and untreated soil. The addition of ThzID1-M3 to soil pretreated with the nematode stimulated nematode population growth for ≈10 to 20 days, whereas nematode populations decreased in the absence of ThzID1-M3 in both soils. When *Aphelenchoides* sp. and ThzID1-M3 were added to soil together, total nematode populations were lower in untreated soil than in heat-treated soil. It is not clear whether this result was caused by the presence of other microorganisms or by lower growth and proliferation of the food source (ThzID1-M3) in untreated soil. Untreated soil may contain nematophagous fungi, bacteria, and predacious nematodes (4,12). Field soils may harbor many other fungivorous nematodes, including *A. avenea*, which may occur at high densities (5,7). Cultivation of crops influences the diversity and population of nematodes in the field (12,30). Some fungi, such as *Aspergillus* and *Penicillium* spp., also produce nematicidal substrates that can cause direct lethal effects in soil (1). Addition of ThzID1-M3 pellets significantly stimulated the population growth of indigenous nematodes. These might be fungivores, bacterivores, or other nematodes, but quite likely they mainly are fungivores stimulated by ThzID1-M3 addition. In our experiments, we observed many nematodes associated with *Trichoderma* hyphae around pellets and presumably feeding, and numerous nematode eggs were seen to be adhering to hyphae. These results suggest that populations of fungivores may rapidly increase following addition of biocontrol fungi in the field, consequently inhibiting the growth and proliferation of the added fungi. Compared with overall results from heat-treated soil, the effects of *Aphelenchoides* sp. on ThzID1-M3 were reduced in untreated soil.

These results support the contention that heat-treated soil has reduced populations of competitive or antagonistic microflora that may influence the growth of introduced biocontrol agents as well as that of the nematodes.

Colonization of sclerotia of *S. sclerotiorum* by ThzID1-M3 was significantly reduced by the addition of the nematode in both soils. Some soil insects may stimulate colonization of sclerotia by *Trichoderma* spp. by damaging sclerotia in soil (2,14). However, our results suggest that nematodes did not stimulate colonization
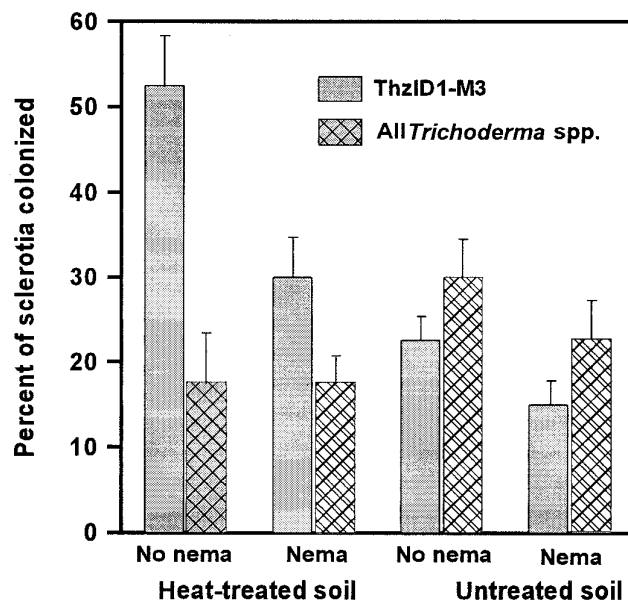

Fig. 6. Effect of *Aphelenchoides* sp. on colonization of sclerotia of *Sclerotinia sclerotinia* by *Trichoderma harzianum* (ThzID1-M3 versus total *Trichoderma* spp.) in heat-treated or untreated soil. *Aphelenchoides* sp. was applied to soil at initial levels of 0 or 10 nematodes per gram of dry soil, along with two alginate pellets of ThzID1-M3 per 10 g of soil. Ten sclerotia were randomly distributed in 200 g of soil, and colonization was assessed after 14 days. Vertical bars represent ±1 standard error of the mean.
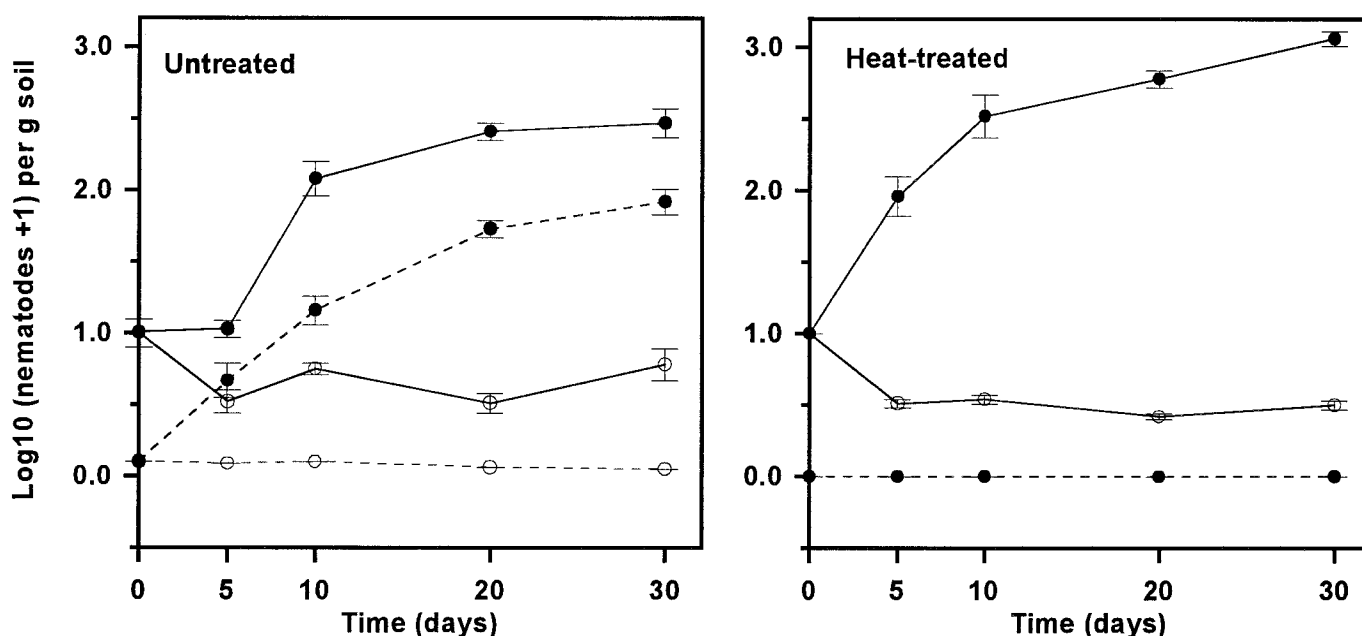

Fig. 5. Effect of *Trichoderma harzianum* isolate ThzID1-M3 on populations of nematodes in heat-treated or untreated soil at 5, 10, 20, and 30 days. *Aphelenchoides* sp. was applied to soil at initial levels of 0 (dotted line) or 10 (solid line) nematodes per gram of dry soil, with alginate pellets of ThzID1-M3 at levels of 0 (●) or 20 (◆) pellets per 50 g of dry soil. Vertical bars represent ±1 standard error of the mean.

of sclerotia by *Trichoderma* spp. in soil. Fungivorous nematodes migrate to and reproduce at locations in the soil where food is available in large quantities (17). The introduced *Trichoderma* sp. formulated in pellets forms a high-density hyphal net in soil (3,20), and this transient growth of *Trichoderma* spp. may attract nematodes and serve as an appropriate food source. In conclusion, the results of our study suggest that fungivorous nematodes may, in some circumstances, be significant constraints on the efficacy of biocontrol fungi, so that further investigation of interactions between biocontrol agents and indigenous nematodes under field conditions is warranted.

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