Augmentation of Soil with the Nematophagous Fungi *Hirsutella rhossiliensis* and *Arthrobotrys haptotyla*

B. A. Jaffee

Department of Nematology, University of California at Davis, One Shields Avenue, Davis 95616-8668.
Accepted for publication 14 January 2000.

ABSTRACT

Jaffee, B. A. 2000. Augmentation of soil with the nematophagous fungi *Hirsutella rhossiliensis* and *Arthrobotrys haptotyla*. *Phytopathology* 90:498-504.

In previous studies, growth of *Hirsutella rhossiliensis* from pelletized assimilative hyphae was reduced by other soil organisms. In the current study, sensitivity to this biotic inhibition was compared when the fungus was added to soil as pelletized hyphae or as fungus-parasitized nematodes. The hypothesis was that the natural inoculum, the parasitized nematode, would be less sensitive than the artificial inoculum, pelletized hyphae. The soil was heated to 60°C for 2 h to remove putative antagonists or was not heated. The soil was packed into vials (17 cm³) and kept at 20°C in the laboratory or packed into cages (PVC pipe sealed at the ends with 480-µm pore mesh, 80 cm³) and buried 22 cm deep in a vineyard. After 2 or 4 weeks, assay nematodes were added to the vials or cages (recovered from the vineyard), respectively. The assay nematodes

were extracted from soil after 2.0 or 2.5 days and examined for adhesive conidia of *H. rhossiliensis*. Consistent with the hypothesis, *H. rhossiliensis* was quite sensitive to biotic inhibition when formulated as pelletized hyphae but was insensitive to biotic inhibition when formulated as parasitized nematodes. These data suggest that the activity of the *H. rhossiliensis* pellet could be increased if the pellet better mimicked the natural inoculum. Similar experiments with the nematode-trapping fungus *Arthrobotrys haptotyla*, however, exhibited an opposite trend: *A. haptotyla* was more sensitive to biotic inhibition when added to soil as fungus-parasitized nematodes than as pelletized hyphae. Results from laboratory and field experiments were similar.

Additional keywords: alginate pellets, bacterivorous nematodes, biological control, enchytraeids, fungivorous nematodes, omnivorous nematodes, plant-parasitic nematodes.

To add the nematophagous fungus *Hirsutella rhossiliensis* to soil (and thereby attempt to achieve short-term biological control of plant-parasitic nematodes), researchers have sometimes used fungus-parasitized nematodes as inoculum (10,24). Selection of this inoculum form was justified as follows. First, the fungus-parasitized nematode is an important stage in the life history of *H. rhossiliensis*, and large numbers of fungus-parasitized nematodes occur naturally in some fields (18). Second, although the conidium is the infective propagule for *H. rhossiliensis*, conidia are ineffective when added to soil and must be produced in situ to adhere to and infect nematodes (18). Third, fungus-parasitized nematodes serve as foci for sporulation (i.e., for in situ production of conidia). Finally, addition of parasitized nematodes to soil has caused significant proportions of healthy nematodes to become parasitized (10,24).

H. rhossiliensis-parasitized nematodes can be produced in the laboratory (10,24), but obtaining and storing the large number of parasitized nematodes necessary for treating an agricultural field is impractical. Because the fungus-parasitized nematode seems to consist of assimilative hyphae contained in host cuticle, Lackey et al. (17) focused on assimilative hyphae in their development of a substitute inoculum form. Assimilative hyphae were grown in shake culture, rinsed free of media, and embedded and dried in alginate pellets. Like the parasitized nematode, the pellet acts as a focus for sporulation. Moreover, sporulation, whether from the parasitized nematode or the pellet, does not require added nutrients. Instead, sporulation involves converting previously assimilated

food into new hyphae, phialides, and conidia. One might argue that supplemental nutrients would enhance sporulation, but *H. rhossiliensis* is a weak competitive saprophyte (13). I therefore suspect that more aggressive saprophytes would use the added nutrients and perhaps attack or otherwise inhibit *H. rhossiliensis*.

Given that sporulation from the alginate pellet does not depend on capture of new nutrients in the soil, I assumed that pellet efficacy would not be limited by competition in particular or by biotic inhibition in general. By biotic inhibition, I mean competition, predation or parasitism, antibiosis, mechanical damage, and any other negative influence from another organism. However, enchytraeids and other organisms greatly reduced sporulation of *H. rhossiliensis* from alginate pellets in the laboratory (9), in microplots (12), and in tomato fields and vineyards (4). Whether biotic inhibition also limits sporulation from *H. rhossiliensis*-parasitized nematodes is unknown.

In addition to the endoparasitic *H. rhossiliensis*, several nematode-trapping fungi have been formulated as assimilative hyphae in alginate, and at least one, *Monacrosporium geophyropagum*, is inhibited by other soil organisms when growing from pellets (4). For *M. geophyropagum*, however, inhibition of population density rather than parasitic activity was measured (4). This was necessary because nematodes parasitized by *M. geophyropagum*, and most other trapping fungi, cannot be extracted from soil: the trap remains attached to the parent hypha, which does not separate from the soil during standard extraction.

Unlike parasitism by *M. geophyropagum*, parasitism by the nematode-trapping fungus *Arthrobotrys haptotyla* can be quantified directly. *A. haptotyla* traps nematodes on adhesive knobs, which are borne on slender stalks. Because the stalks break when soil is disturbed mechanically, nematodes with adherent knobs can be extracted from soil and counted in the same manner as nematodes

Corresponding author: B. A. Jaffee; E-mail address: bajaffee@ucdavis.edu

Publication no. P-2000-0321-02R

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with *H. rhossiliensis* adhesive conidia (3). Pelletized *A. haptotyla* has suppressed root-knot nematodes in the laboratory (3,5) and in field microplots (B. A. Jaffee, *unpublished data*). The sensitivity of *A. haptotyla* to biotic inhibition, whether formulated as hyphae in alginate or as fungus-parasitized nematodes, is unknown.

In this article, I ask "Is growth from the *H. rhossiliensis*-parasitized nematode, like growth from our alginate pellet formulation, sensitive to biotic inhibition?" If not, perhaps the pelletized formulation can be changed to better mimic the fungus-parasitized nematode and thereby increase its efficacy. This study also determines whether *A. haptotyla*, in the form of pelletized hyphae or parasitized nematodes, is sensitive to biotic inhibition.

MATERIALS AND METHODS

Soil and field site. A loamy sand (pH 7.0_{water}, organic matter content 0.4%) was collected from a 20-year-old vineyard previously designated site 4 (4). In this soil, biotic inhibition of *H. rhossiliensis* was substantial and caused mostly by organisms narrower than 20 μm and not by enchytraeids (4). Soil was collected adjacent to vines and 5 to 25 cm deep. Extensive sampling in 1998 and 1999 yielded no cyst nematodes (*Heterodera* spp.) or *A. haptotyla*; small numbers of *H. rhossiliensis* and large numbers of root-knot nematode (*Meloidogyne* spp.) were occasionally detected (4; B. A. Jaffee, *unpublished data*).

For vial experiments, soil was passed through a 2-mm sieve to remove large roots and debris; this was not necessary for the cage experiments because the volume of soil was substantially greater in cages than in vials. Half the soil was heated to 60°C for 2 h and then exposed to the air on the laboratory bench for 4 h. The remaining soil was not heated. This heat treatment eliminates nematodes, arthropods, and enchytraeids; although it reduces numbers of fungi and bacteria, substantial numbers survive and rapidly recolonize the soil (12). To prevent loss of antagonistic organisms, soil was stored moist at 10°C and used in experiments within 14 days of collection.

Fungi and nematodes. *H. rhossiliensis* (ATCC 46487, IMI 265748) and *A. haptotyla* (ATCC 204099) were maintained on quarter-strength cornmeal agar (CMA/4). *A. haptotyla* grows faster than *H. rhossiliensis* (B. A. Jaffee, *unpublished data*), and many procedures described below involved shorter incubation times for the former fungus.

Cysts of the sugar beet cyst nematode *Heterodera schachtii* were obtained from sugar beets grown in a greenhouse. To obtain second-stage juveniles, cysts were placed on Baermann funnels; the juveniles were collected every 30 min and stored at 10°C with aeration for less than 2 days. Eggs of the root-knot nematode *Meloidogyne javanica* were obtained from infected tomato plants grown in a greenhouse (2). Eggs were placed on Baermann funnels, and second-stage juveniles were collected and stored as described for *Heterodera schachtii*. Infective juveniles of the entomopathogenic nematode *Steinernema glaseri* were obtained from infected wax-moth larvae (15) and stored at 10°C for less than 1.5 months. As described below, the size of the nematodes affects their utility in the experiments. Infective juveniles of *S. glaseri* are relatively large (1,160 μm long and 45 μm wide) (19). Second-stage juveniles of *Heterodera schachtii* and *M. javanica* are substantially smaller than *S. glaseri*, but *Heterodera schachtii* (463 μm long and 22 μm wide) (20) is longer and especially wider than *M. javanica* (417 μm long and 14 μm wide) (25).

All three nematodes are susceptible to *H. rhossiliensis* (21,23,24). In contrast, *S. glaseri* and *M. javanica* are quite susceptible to *A. haptotyla*, but *Heterodera schachtii* is not (3; B. A. Jaffee, *unpublished data*); the adhesive knobs adhere to and infect *Heterodera schachtii*, but adherence and infection is substantially less with *Heterodera schachtii* than with the other two nematodes (3).

Fungal inoculum. *Pelletized hyphae.* Assimilative hyphae were grown in shake culture, embedded in alginate, coated with sand,

and dried as described previously (4,7,17). *A. haptotyla* pellets were heavier (1.9 versus 1.1 mg) and larger (2.1 versus 1.9 mm) than those of *H. rhossiliensis*. The approximate mass of dry hyphae per pellet, based on the number of pellets produced per unit of dry hyphae, was 0.6 mg for *A. haptotyla* and 0.3 mg for *H. rhossiliensis*. Pellets were stored in glass vials at -1°C for 2 to 20 days.

Parasitized nematodes. Parasitized nematodes were obtained from CMA/4 cultures or sand cultures. In the former case, 1,000 *S. glaseri* in 0.1 ml of distilled water were added to the culture when the fungus had covered $\approx 75\%$ of the agar surface in a petri dish 9 cm in diameter. After 3.0 days for *H. rhossiliensis* or 2.5 days for *A. haptotyla*, parasitized nematodes were moved individually from the surface of the agar to soil with a needle. Infective juveniles of *S. glaseri* are especially useful for this kind of experiment because they are susceptible to many nematophagous fungi (11), easily produced in large numbers (15), relatively large (19) and therefore easier to transfer than are small nematodes, and produce many infective structures per parasitized nematode (11,24).

To obtain larger numbers of parasitized nematodes than could be transferred from CMA/4 cultures, sand cultures in petri dishes (10 cm in diameter, 8 cm high) were prepared as described previously (10,24), except that the sand was moistened with potato dextrose broth instead of V8-juice broth. In this method, large numbers of healthy nematodes are added to fungus-colonized sand and recovered after sufficient time for infection and colonization. Healthy *Heterodera schachtii* or *S. glaseri* nematodes were added to 1- to 4-month-old *H. rhossiliensis* cultures and extracted after 3 days (*Heterodera schachtii*) or 5 days (*S. glaseri*). Healthy *S. glaseri* nematodes were added to 3-week-old *A. haptotyla* cultures and extracted after 7 days; shorter and longer times gave unsatisfactory results (B. A. Jaffee, *unpublished data*). Extraction involved wet sieving and centrifugation in water and sucrose (14). Sand cultures worked well for *H. rhossiliensis* but inconsistently for *A. haptotyla* (B. A. Jaffee, *unpublished data*).

Laboratory experiments in vials. The water content of heat-treated and nonheat-treated soil was adjusted to 9.5% (grams of water per 100 g of dry soil) by adding distilled water and mixing. The fungal inoculum was mixed into 24.0 g of soil (dry weight equivalent), which was packed into 25-ml plastic vials (17 cm³ of soil, bulk density = 1.41 g of dry soil per cm³). Each vial was sealed with a plastic lid containing a 1-mm hole for gas exchange. Vials were placed on moist paper towels in a plastic box.

In the *H. rhossiliensis* laboratory experiment, three kinds of inoculum were added to soil: pelletized hyphae (three pellets per vial in both trials), *H. rhossiliensis*-parasitized *Heterodera schachtii* from sand cultures (230 per vial in trial 1 and 150 per vial in trial 2), and *H. rhossiliensis*-parasitized *S. glaseri* from CMA/4 cultures (10 per vial for trials 1 and 2). Parasitized *Heterodera schachtii* were added in dilute KCl (0.36 ml of 4 mM KCl per vial), whereas parasitized *S. glaseri* and pellets were transferred by hand; the quantity of inoculum was selected to achieve $\approx 50\%$ parasitism of assay nematodes (discussed below). Before the soil was mixed and packed, 0.36 ml of dilute KCl was added if needed, so the final soil-water content in all vials was 11.0%, equivalent to approximately -9 kPa, based on the water release curve of this soil (4).

In the *A. haptotyla* laboratory experiment, two kinds of inoculum were added to soil: pelletized hyphae (3 or 2 per vial in trials 1 and 2, respectively) and parasitized *S. glaseri* (10 per vial in trials 1 and 2) from CMA/4 cultures. Parasitized *S. glaseri* and pellets were added by hand, and final water content was 11.0%, as described for *H. rhossiliensis*.

For both fungi, viability of fungal inoculum was assessed by placing samples (50 pellets, 20 parasitized *S. glaseri*, or 400 parasitized *Heterodera schachtii* [only for *H. rhossiliensis*]) on CMA/4 and examining for growth daily for 5 days. All inoculum was viable.

After 14 days at 20°C and without light, a bioassay was used to quantify the relative abundance of *H. rhossiliensis* adhesive co-

nidia or *A. haptotyla* adhesive knobs. Assay nematodes (600 in 0.25 ml of 4 mM KCl) were added to the surface of the soil in each vial. Nematodes were extracted from the soil in the vials 2 days later by wet sieving and sucrose centrifugation (14). The nematode suspension was adjusted to 10 ml, and 2 ml was examined with a dissecting microscope at 70 to 140× magnification. In the case of *A. haptotyla*, the sample was shaken vigorously before the 2-ml subsample was removed (shaking separated the nematodes from the sometimes abundant hyphae). Assay nematodes were scored for the presence or absence of adherent *H. rhossiliensis* conidia or *A. haptotyla* knobs. Resident nematodes in the sample were identified to trophic group (plant parasite, bacterivore, fungivore, predator, or omnivore) (26).

In the *H. rhossiliensis* laboratory experiment, *Heterodera schachtii* was used as the assay nematode because it is susceptible to the fungus and is not naturally present in the soil. At extraction, the parasitized *Heterodera schachtii* used as fungal inoculum at time 0 were degraded, and the *Heterodera schachtii* used as assay nematodes were not (8); therefore, confusion between these two kinds of *Heterodera schachtii*, loss of parasitized assay nematodes because of degradation (8), and secondary spread of the fungus from parasitized assay nematodes were not problems. In the *A. haptotyla* experiment, *M. javanica* was used in trial 1 because it is more susceptible than is *Heterodera schachtii* to the fungus (3). However, *M. javanica* was difficult to examine in extracted samples (it is smaller than *Heterodera schachtii* and rapidly became difficult to recognize when parasitized by *A. haptotyla*; B. A. Jaffee, unpublished data), so *Heterodera schachtii* was used in trial 2.

Field experiments in cages. Field experiments were conducted to determine whether patterns observed in the laboratory also occurred in the field. The field experiments used cages, as described previously (4,12). Briefly, a cage consisted of two sections of PVC pipe with nylon mesh (480-µm openings) glued to one end of each section. Fungal inoculum was mixed into heat-treated or nonheat-treated soil, and the soil (8.0% water content, -11 kPa) was packed into each section (quantities of inoculum are provided below). Finally, the two sections were taped together to form a cylinder (3.9-cm inner diameter and 6 cm long, 80 cm³ of soil) with mesh at each end. Bulk density was 1.49 g of dry soil/cm³.

Immediately after the sections were taped together, the cage was sealed in a plastic bag and placed at 10°C. The cages were taken to the field in an ice chest, removed from plastic bags, and buried in soil within 4 h of packing. Each cage was oriented vertically, with mesh at top and bottom and with the top 19 cm from the soil surface. The distance between adjacent cages was 3 to 5 cm. The cages were buried in six blocks, each of which was located in the vine row and midway between consecutive vines; this area was not cultivated. Each block contained four cages (a factorial combination of pellets or parasitized nematodes in heat-treated or nonheat-treated soil).

Temperature recorders (Hobo-Temp model; Onset Computer Corporation, Pocasset, MA) were buried with the cages in blocks 1 and 6 of each experiment, and temperature was recorded hourly. Accumulated heat units (degree days, base 10°C) and mean, minimum, and maximum temperatures were determined. Rainfall data were obtained from the University of California Integrated Pest Management Data Base (University of California, Davis).

To determine whether *H. rhossiliensis* or *A. haptotyla* was naturally present in nonheat-treated soil, four additional sections were packed with nonheat-treated soil at the start of each experiment. The sections were sealed in plastic and kept at 20°C. After 14 days, each section was assayed as described below.

Experiments were started in March and April 1999 (Table 1). The decision to terminate an experiment depended on fungus biology and rainfall. *A. haptotyla* grows faster than does *H. rhossiliensis* (B. A. Jaffee, unpublished data), and I assumed that its numbers and activity would increase and then decrease more rapidly than would those of *H. rhossiliensis*. I intended to terminate experiments after ≈140 degree days (equivalent to 14 days at 20°C) for *A. haptotyla* and ≈200 degree days for *H. rhossiliensis*. However, the bioassay requires moderate soil moisture, and lengths of experiments were sometimes increased or decreased to avoid rain or to allow drainage after rain.

After removal from the vineyard, cages were carried to the laboratory in an ice chest and stored overnight at 10°C. The next morning, each cage was carefully separated into two sections, and each section was placed in a plastic bag with the mesh end down; soil was disturbed as little as possible because disturbance reduces activity of *H. rhossiliensis* (18) and possibly of other fungi. Assay nematodes in 0.5 ml (in trial 1) or 1.0 ml (in trial 2) of 4 mM KCl were added to the surface of the soil in each section; a greater volume was used in trial 2 because the soil was substantially drier. The assay nematode added to the top section was *S. glaseri* (≈100 added per section), and the assay nematode added to the bottom section was *Heterodera schachtii* (≈1,700 added per section). I assumed that fungal abundance in top and bottom sections was similar and that using different assay nematodes would increase the chance of detecting treatment differences. If, for example, *A. haptotyla* was very abundant in a sample, *Heterodera schachtii* would reveal greater differences among treatments than would *S. glaseri* (because *S. glaseri* is susceptible and highly motile, parasitism could be near 100% regardless of treatment). Conversely, if *A. haptotyla* was not abundant, *S. glaseri* would reveal greater differences than would *Heterodera schachtii* (because *Heterodera schachtii* is not very susceptible, parasitism could be near 0% regardless of treatment). Although this argument is less compelling for *H. rhossiliensis*, two assay nematodes also were used. Fewer *S. glaseri* than *Heterodera schachtii* were added because the extraction efficiency is much greater for the former nematode. After assay nematodes were added, the sections were sealed in plastic bags and placed in a moisture chamber at 20°C.

Nematodes were extracted after 2.0 days (*S. glaseri*) or 2.5 days (*Heterodera schachtii*) using the same procedures described for the vial experiments. Assay nematodes and resident nematodes in the extracted suspension were counted as described for the vial experiments, except that the entire suspension was examined, and both nematodes and enchytraeids were counted when *S. glaseri* was the assay nematode.

Experimental design and statistical analyses. All experiments were repeated (trials 1 and 2), but trials were analyzed separately because of differences in inoculum quantity (in laboratory and field) and environment (in field). The design was randomized completely for laboratory experiments and was a randomized complete block for field experiments. A statistical interaction between soil treatment

TABLE 1. Background information on field experiments with *Hirsutella rhossiliensis* and *Arthrobotrys haptotyla*

Fungus	Trial	Date in field	No. of days in field	Temperature (°C)			No. of degree days ^a	No. of rain days ^b
				Mean	Minimum	Maximum		
<i>H. rhossiliensis</i>	1	29 March 1999	28	16	8	24	158	2
<i>H. rhossiliensis</i>	2	14 April 1999	27	19	14	22	233	1
<i>A. haptotyla</i>	1	31 March 1999	21	15	9	23	105	2
<i>A. haptotyla</i>	2	16 April 1999	20	19	15	22	181	0

^a Base 10°C.

^b Days with >5 mm of rain.

(heat treated versus nonheat treated) and fungus inoculum (pellets versus parasitized nematodes) was hypothesized, and an analysis of variance was used to test for this interaction. The primary data (percentage of assay nematodes with conidia or knobs) were arcsine transformed to achieve normality in distribution. In the laboratory experiment, independent variables were soil treatment, inoculum, and the interaction of soil treatment and inoculum. In the field experiment, independent variables were soil treatment, inoculum, the interaction of soil treatment and inoculum, and block. When the interaction between soil treatment and inoculum was significant, further analyses of variance were conducted by soil treatment. Data on numbers of resident nematodes and enchytraeids in soil were log transformed and subjected to analysis of variance. Additional description of the statistical analyses is provided in the Results. SAS release 7.00 (SAS Institute Inc., Cary, NC) was used throughout. Statistical significance was determined at $P \leq 0.05$. Block effects were sometimes significant but are not discussed.

RESULTS

Environment in laboratory vials versus field cages. With a constant temperature of 20°C for 14 days, degree days in the laboratory always totaled 140. In the field, soil temperatures were usually less than 20°C, but the experiments ran for ≈ 3 weeks (*A. haptotyla*) or 4 weeks (*H. rhossiliensis*), and degree days in cages usually exceeded 140 (Table 1). Rainfall was light to moderate in the vineyard (Table 1), and neither the vineyard nor the vials were irrigated. Weed roots often grew into cages, but roots were largely absent from vials.

Fig. 1. *Hirsutella rhossiliensis* activity in laboratory vials as affected by inoculum and soil. *H. rhossiliensis* pelletized hyphae or parasitized nematodes (*Steinernema glaseri* or *Heterodera schachtii*) were added to soil (heat treated or nonheat treated) and packed in vials. After 14 days at 20°C, ≈ 600 assay nematodes (healthy *Heterodera schachtii*) were added to each vial. Two days later, assay nematodes were extracted from the soil and examined for adhering conidia of *H. rhossiliensis*. The experiment was repeated (trials 1 and 2). Each value is the mean (plus standard error) of six replicate vials.

***H. rhossiliensis*.** Significant statistical interactions occurred in all trials of all experiments. When *H. rhossiliensis* was added to soil as pelletized hyphae, the percentage of assay nematodes with conidia was much greater in heat-treated than in nonheat-treated soil; this was true in the laboratory (Fig. 1) and in the field (Fig. 2). However, when *H. rhossiliensis* was added to soil as parasitized nematodes, the percentage of assay nematodes with conidia was similar in heat-treated and nonheat-treated soil (Figs. 1 and 2). *H. rhossiliensis* was not detected in soil not receiving fungus inoculum.

***A. haptotyla*.** Significant statistical interactions occurred in most trials, but the interactions differed from those for *H. rhossiliensis*. When *A. haptotyla* was added to soil as pelletized hyphae, the percentage of assay nematodes with knobs was never greater in heat-treated than in nonheat-treated soil (Figs. 3 and 4B and D); in one case, the percentage was greater in nonheat-treated soil (Fig. 3B). However, when *A. haptotyla* was added to soil as parasitized nematodes, the percentage of assay nematodes with knobs was greater in heat-treated than in nonheat-treated soil (Figs. 3A and 4B and D), except in one case where the trend was the same but the difference was not significant (Fig. 3B). Statistical interactions could not be assessed in field experiments using pellets as inoculum and *S. glaseri* as the assay nematode because all of the assay nematodes acquired knobs in both heat-treated and nonheat-treated soil (Fig. 4A and C); however, when parasitized nematodes were used, the percentage of assay nematodes with knobs was greater in heat-treated than in nonheat-treated soil (Fig. 4A and C). *A. hap-*

Fig. 2. *Hirsutella rhossiliensis* activity in field cages as affected by inoculum and soil. *H. rhossiliensis* pelletized hyphae or parasitized nematodes (*Steinernema glaseri*) were added to soil (heat treated or nonheat treated) and packed in cages, which were buried in a vineyard. After 4 weeks, the cages were recovered and separated into two sections (top and bottom). Healthy *S. glaseri* assay nematodes (≈ 100) were added to the top section, and healthy *Heterodera schachtii* assay nematodes ($\approx 1,700$) were added to the bottom section. After 2.0 days (*S. glaseri*) or 2.5 days (*Heterodera schachtii*), assay nematodes were extracted from the soil and examined for adhering conidia of *H. rhossiliensis*. The experiment was repeated (trials 1 and 2). Each value is the mean (plus standard error) of six replicate cages.

totyla was not detected in soil not receiving fungus inoculum. Overall, *A. haptotyla* was less sensitive to biotic inhibition when added to soil as pellets than as parasitized nematodes.

Resident nematodes and enchytraeids. Data from vials treated with *H. rhossiliensis*-parasitized *Heterodera schachtii* were similar to data from vials treated with *H. rhossiliensis*-parasitized *S. glaseri*, and only the latter data are shown to simplify the presentation. In vials containing heat-treated soil, resident nematode numbers were almost always zero (Fig. 5); thus, the interaction between soil treatment and inoculum was not examined. In nonheat-treated soil, resident nematode numbers (by trophic group) did not statistically differ in vials containing pellets versus parasitized nematodes (Fig. 5). Enchytraeids were rarely observed in vials (data not shown).

Resident nematode numbers in cages were larger than those in vials (Fig. 6 versus Fig. 5). Resident nematodes invaded the heat-treated soil in cages, but their numbers were often less in heat-treated than in nonheat-treated soil (Fig. 6C, D, H, I, K, N, O, Q, U, V, and W). The interaction between soil treatment and inoculum was significant only twice (Fig. 6E and X). Bacterivorous nematodes were more abundant in cages containing pellets than in cages containing parasitized nematodes, regardless of soil treatment or fungus (Fig. 6A, G, M, and S); the same was true for enchytraeids (Fig. 6F, L, R, and X). Numbers of other nematode trophic groups were greater when pellets rather than parasitized nematodes were added in two cases (Fig. 6I and O).

DISCUSSION

As hypothesized, form of fungus inoculum greatly influenced *H. rhossiliensis* activity in soil. Thus, the *H. rhossiliensis*-parasi-

tized nematode performed equally well in heat-treated (putative antagonists removed) and in nonheat-treated (putative antagonists in place) soil. In contrast, assimilative hyphae in alginate pellets performed better in heat-treated than in nonheat-treated soil. This difference seems easy to explain: the natural form of *H. rhossiliensis* inoculum is adapted to resist biotic inhibition, whereas the artificial form is not.

Which organisms inhibit *H. rhossiliensis* growth from pellets in this field soil is unknown. Enchytraeids, including *Enchytraeus crypticus*, ingest and consume fungi (1,9), and more enchytraeids entered cages containing pellets than those containing parasitized nematodes. However, enchytraeid numbers were small in this vineyard, and exclusion of enchytraeids in a previous study did not improve pellet performance (4). Stylet-bearing fungivorous and omnivorous nematodes may decrease fungus activity, but their numbers were not consistently or substantially greater in soil containing pellets than in soil containing parasitized nematodes (if they consumed the fungus, their numbers should have increased). Bacterivorous nematodes that are not hosts to the fungus also may suppress *H. rhossiliensis* by removing conidia from phialides (22), and bacterivorous nematodes were more abundant in soil containing pellets than in soil containing parasitized nematodes. Perhaps the pellets as opposed to the parasitized nematodes leak nutrients that support a bacterial bloom, which in turn supports bacterivorous nematodes. If many of these nematodes are not hosts, they could reduce *H. rhossiliensis* activity.

Fig. 3. *Arthrobotrys haptotyla* activity in laboratory vials as affected by inoculum and soil. A. *haptotyla* pelletized hyphae or parasitized nematodes (*Steinernema glaseri*) were added to soil (heat treated or nonheat treated) and packed in vials. After 14 days at 20°C, ~600 assay nematodes (healthy *Meloidogyne javanica* in trial 1 and healthy *Heterodera schachtii* in trial 2) were added to each vial. Two days later, assay nematodes were extracted from the soil and examined for adhering knobs of *A. haptotyla*. Each value is the mean (plus standard error) of 8 (trial 1) or 10 (trial 2) replicate vials.

Fig. 4. *Arthrobotrys haptotyla* activity in field cages as affected by inoculum and soil. A. *haptotyla* pelletized hyphae or parasitized nematodes (*Steinernema glaseri*) were added to soil (heat treated or nonheat treated) and packed in cages, which were buried in a vineyard. After 3 weeks, the cages were recovered and separated into two sections (top and bottom). Healthy *S. glaseri* assay nematodes (~100) were added to the top section, and healthy *Heterodera schachtii* assay nematodes (~1,700) were added to the bottom section. After 2.0 days (*S. glaseri*) or 2.5 days (*H. schachtii*), assay nematodes were extracted from the soil and examined for adhering knobs of *A. haptotyla*. The experiment was repeated (trials 1 and 2). Each value is the mean (plus standard error) of six replicate cages.

The present experiments with *H. rhossiliensis* share some features with an earlier experiment in which the fungus could not invade and colonize dead nematodes in soil unless the soil had been heat treated but could invade and colonize living nematodes regardless of soil treatment (13). In the present experiments, *H. rhossiliensis* was sensitive to biotic inhibition when added to soil in an unnatural form but insensitive when added in a natural form. Together, the two studies indicate that *H. rhossiliensis* is a specialized parasite that loses its competitive advantage when conditions are unsuitable.

So reasonable are the *H. rhossiliensis* data that one might wish to generalize: Perhaps all endoparasitic and nematode-trapping fungi do best when introduced to soil as parasitized nematodes. But the generalization cannot stand because *A. haptotyla* behaved quite differently. *A. haptotyla* in pellets seemed insensitive to biotic inhibition, whereas *A. haptotyla*-parasitized nematodes were often sensitive. One speculative explanation involves bacterivorous nematodes, whose numbers increased, as they did in the *H. rhossiliensis* experiments, when pellets were added to soil. In the case of *A. haptotyla*, one would have to argue that many of these nematodes were hosts and therefore supported a numerical response by the fungus. Such a numerical response has been de-

scribed for *Monacrosporium ellipso sporum*, which also produces adhesive knobs on stalks (6). The *A. haptotyla* pellets also were larger than the *H. rhossiliensis* pellets, and the decrease in surface area to volume may decrease sensitivity to other soil organisms. The sensitivity of *A. haptotyla*-parasitized nematodes to biotic inhibition is difficult to explain; perhaps *A. haptotyla* rapidly degrades the nematode's cuticle and, thus, loses its protection. Perhaps the parasitized nematode is less central to *A. haptotyla* than to *H. rhossiliensis*.

The sensitivity of other nematode-trapping fungi to biotic inhibition has been assessed in several recent studies. As noted earlier, pelletized *M. gephyropagum* was inhibited by soil organisms in microplot, tomato, and vineyard soils (4,12). In another study (16), *M. gephyropagum* and four other nematode-trapping fungi were added to a noncultivated soil in vials in the form of parasitized nematodes; the fungi performed equally well in heat-treated and nonheat-treated soil, but the soil had been air-dried. Perhaps air-drying suppresses those organisms that inhibit the fungi.

Fig. 5. Resident nematode numbers (by trophic group) at the end of laboratory experiments as affected by heat treatment of soil and fungal inoculum. Data from vials treated with *Hirsutella rhossiliensis*-parasitized *Heterodera schachtii* were similar to data from vials treated with *H. rhossiliensis*-parasitized *Steinernema glaseri*; only the latter data are shown. Numbers have been expressed per 100 cm³ of soil to facilitate comparisons with numbers from cages. Values are the means of 6 to 10 replicate vials.

Fig. 6. Resident nematode numbers (by trophic group) and enchytraeids at the end of field experiments as affected by heat treatment of soil and fungal inoculum. Numbers have been expressed per 100 cm³ of soil to facilitate comparisons with numbers from vials. Values are the means of six replicate cages.

As experimental tools, vials and cages have similarities and differences, advantages and disadvantages. Both enable sampling of soil that has not been disturbed since the start of the experiment. Both reduce heterogeneity among patches of soil and therefore reduce the number of replicates needed to obtain acceptable variance (the gain may not be worthwhile, however, if heterogeneity among soil patches is considered important). In contrast to vials, cages include temporal changes in temperature and soil water and also allow invertebrates (especially enchytraeids, mites, collembolans, and nematodes) to move in and out of the soil patches. By allowing these invertebrates to aggregate around resources and to move away from depleted resources, cages are probably more realistic than are vials. In the current study, however, data patterns (percentage of assay nematodes with adhesive conidia or knobs) were similar in vials and cages; it follows that the organisms inhibiting growth of *H. rhossiliensis* from pellets are probably not very motile.

Finally, these results suggest that *H. rhossiliensis* pellets, but not *A. haptotyla* pellets, would perform better if they better resembled parasitized nematodes. Pellets and parasitized nematodes of *H. rhossiliensis* differ in size (pellets are much larger), in being dried or not (pellets are dried, and parasitized nematodes are not), in the presence of a cuticle or other barrier (the pellet has none), and in the condition of the assimilative hyphae (those in pellets are fragmented in a blender, and those in nematodes are intact). Of these differences, the latter two may be most important. Assimilative hyphae are thin walled and may be especially vulnerable if fragmented and not separated from other soil organisms by a barrier.

ACKNOWLEDGMENTS

I thank J. Techlenburg for allowing me to work in his vineyard and E. Caswell-Chen and J. VanderGheynst for many useful comments on an earlier version of this manuscript.

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