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Trophic interactions between bacterial-feeding nematodes in plant rhizospheres and the nematophagous fungus *Hirsutella rhossiliensis* to suppress *Heterodera schachtii*

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Abstract

Trophic exchanges in soil food webs may suppress populations of pest organisms. We hypothesize that the suppressive condition of soils might be enhanced by manipulating components of the food web. Specifically, by enhancing populations of bacterial-feeding nematodes, propagule density of the nematophagous fungus *Hirsutella rhossiliensis* should increase and constrain populations of *Heterodera schachtii*, a plant-parasitic nematode. The rhizospheres of *Crotalaria juncea* and *Vicia villosa* stimulated population growth of the bacterial-feeding nematode, *Acrobeloides bodenheimeri*, but not of the nematodes *Caenorhabditis elegans* or *Rhabditis cucumeris*. The rhizospheres of *Tagetes patula*, *Eragrostis curvula*, and *Sesamum indicum* had no effect on any of the bacterial-feeding nematodes investigated. *Acrobeloides bodenheimeri* was most susceptible to parasitism by the nematophagous fungus *H. rhossiliensis* with 35% of individuals being parasitized in a laboratory assay. In three separate trials, parasitism of *H. schachtii* by *H. rhossiliensis* was not enhanced when populations of *A. bodenheimeri* were amplified in a suitable rhizosphere.

Introduction

Suppressive soils occur where biotic and/or abiotic factors restrict the growth and abundance of soil-borne plant pathogens (Schneider, 1982). Biotic factors constrain pest densities through parasitism, predation, antibiosis, or competition. The degree of regulation depends on the density and activity of the biological agent and/or its target. In some cases, although antagonists of plant pathogens may be present in a soil, the number and distribution of the antagonists may be insufficient to constrain the target pathogen.

The distribution and abundance of predators and parasites is limited, to a large extent, by the availability of suitable prey and hosts (i.e., food). Oscillations in the number of antagonists may arise from predator-prey (Begon and Mortimer, 1986) or host-parasite interactions (Hassell and May, 1989) and may result in an inconsistent degree of suppression of the pathogen. For example, by the time antagonists respond numerically to increased food availability, populations of the target already may have caused severe plant damage and economic loss. Thus, a strategy for more sustainable pest control in agriculture is to provide a relatively consistent degree of plant protection by maintaining sufficient populations of antagonists.

Bacteria, fungi, and animal predators that are antagonistic to nematodes may protect plants from parasitic nematodes (Duddington, 1957; Giuma and Cooke, 1974; Linford, 1937; Oostenbrink, 1960). These antagonists may respond numerically to densities of their hosts, although the extent to which many of these antagonists rely on nematodes is unclear as certain fungal antagonists grow saprophytically (Cooke, 1962). For instance, Nicolay and Sikora (1991) found that the proportion of eggs of *Heterodera schachtii* that were

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parasitized by fungi in the field was weakly, positively correlated with *H. schachtii* density. Similarly, in microcosms, the density of *Drechmeria coniospora* was greater in soils amended with organic matter and bacterial-feeding nematodes (Van den Boogert, 1994).

For obligate parasites of nematodes, rates of parasitism may be density dependent (Jaffee et al., 1992). Hence, as host density increases, the probability that an antagonist will encounter and infect a host increases. As the number of infected nematodes increases, the density of propagules increases leading to a greater frequency of encounters between parasites and hosts. So, to augment populations of parasites, additional hosts must become available. To sustain populations of parasites, the dynamics of the antagonists must be decoupled from the dynamics of the target nematode species, especially when parasite propagules are short lived. Thus, populations of antagonists might be sustained by managing alternate host species.

Many nematodes in soils are not plant parasites but are grazers of bacteria or fungi (Griffiths, 1989; Henderson and Katznelson, 1961) and may be hosts for antagonists of plant-parasitic nematodes. Populations of bacterial-feeding nematodes might be managed by incorporating organic matter to stimulate the growth of soil bacteria (Linford, 1937), by introducing select species to the soil, and/or by growing appropriate plant species to enhance bacterial-feeding nematode populations in the rhizosphere (Griffiths, 1990).

One particular antagonist that might respond numerically to enhanced numbers of bacterial-feeding nematodes is the nematophagous fungus *Hirsutella rhossiliensis*. The fungus exhibits density-dependent parasitism in the laboratory when interacting with known hosts (Jaffee et al., 1992). *Hirsutella rhossiliensis* produces phialides which bear sticky spores. Spores that contact nematodes adhere to the cuticle (transmission). Nematodes become infected if the spore germinates, a germ tube penetrates the cuticle, and an infection bulb forms within the nematode's body. Following infection, the nematode body is assimilated by trophic hyhae and ∼4 days later the nematode dies. Parasitism is completed when hyphae emerge from the cadaver and produce new phialides and spores. Although the fungus can be cultured on media in the laboratory, it probably does not grow saprophytically in soil (Jaffee and Zehr, 1985).

The objectives of this study were to measure the susceptibility of bacterial-feeding nematodes to *H. rhossiliensis*, to quantify the effect of plant rhizospheres on target populations of bacterial-feeding nematodes, and to assess the impact of plant × bacterial-feeding nematode × fungus interactions on populations of plant-parasitic nematodes. Specifically, we hypothesize that: 1) plant species differ in their ability to amplify bacterial-feeding nematode populations; 2) bacterial-feeding nematode species differ in their ability to establish populations in the rhizospheres of different plants; 3) bacterial-feeding nematode species differ in their susceptibility to infection by *H. rhossiliensis*; and, 4) the interaction of three components in a soil fold web (an appropriate rhizosphere, a bacterial-feeding nematode species, and *H. rhossiliensis*) will reduce the number of infective plant parasitic nematodes more than when any of the components are absent.

Materials and methods

Origin and isolation of nematodes

The bacterial-feeding nematodes *Acrobeloides bodenheimeri* Thorne, *Cruznema tripartitum* Zullini, and *Rhabditis cucumeris* Andrássy were isolated from agricultural research plots at the University of California at Davis. Nematodes were extracted and cultured as described in Venette and Ferris (1997). At the time of experimentation, all isolates had been maintained on nematode growth medium (NGM) (Sulston and Hodgkin, 1988) or NGM-gellan gum (Ferris et al., 1995) for approximately 2 years. A laboratory culture of *Caenorhabditis elegans* Dougherty var. Bristol (wild type strain N2) was included as a comparative standard. Fresh stock cultures of all nematode species were maintained with associated bacteria at room temperature (24 ± 3 °C) except for *R. cucumeris*, which was maintained at 17 °C due to its inability to reproduce consistently at ambient lab temperature. Voucher specimens of all nematodes are deposited in the UC Davis Nematology Collection with the following accession numbers: *A. bodenheimeri*- UCDNC 2908 and 2909; *C. elegans*- UCDNC 3032; *C. tripartitum*- UCDNC 2910 and 2911; and *R. cucumeris* UCDNC 3033.

The cyst nematode, *Heterodera schachtii* Schmidt, was reared in greenhouse cultures on sugar beet (*Beta vulgaris* L. cv. 'SSNB-2'). Cysts were extracted from the soil by sieving and were placed in Baermann funnels (Barker, 1985). Emerging second-stage juveniles (J2) were collected every 2 h (Jaffee et al., 1990) and

stored at 17 °C in aerated water for less than 24 h before use.

Impact of plant species on bacterial-feeding nematodes

Seeds of marigold (*Tagetes patula* L.), love grass (*Eragrostis curvula* (Schrad.) Nees), sesame (*Sesamum indicum* L.), sunn hemp (*Crotalaria juncea* L.) and woolypod vetch (*Vicia villosa* Roth var. *dasycarpa*) were sown in 100 mL, plastic pots of an autoclaved 2:1 mixture of sand and loam (final composition: 81% sand, 13% silt, 5% clay; pH 7.4; 0.5% OM). Twenty pots were prepared for each plant species and maintained in a growth chamber at 25 °C with 14 h light and 57% relative humidity. Plants were irrigated weekly with Hoagland's solution (Poorter and Bergkotte, 1992) and, as needed, with deionized water. After 12 days, seedlings were thinned to one plant per pot, and the soil was inoculated with 2 mL of freshly prepared microbial inoculum into 1-cm deep holes.

Microbial inoculum was prepared from soil planted with vetch that had been managed under organic farming practices in a Sustainable Agriculture Farming Systems Project (see Temple et al., 1994). Fifty g of soil was combined with 500 mL of sterile deionized water and shaken vigorously for 10 min. The soil slurry was centrifuged for 3 min at 750 *g* to remove vermiform nematodes and eggs. Debris-free supernatant was drawn out of each centrifuge tube with a sterile pipette and placed in a sterile collection vessel. Of this microbial inoculum, 9 mL were used to determine the concentration of colony forming units (CFU) through dilution plating into Petri dishes containing 1/10× nutrient agar and incubating the dishes for at least 72 h at ambient lab temperature. Bacteria and fungi were not identified.

Sixteen days after sowing seeds, soils were inoculated with bacterial-feeding nematodes from stock cultures. Nematodes were collected from NGM-gellangum cultures using protocols established by Eyre and Caswell (1991) or by gently rinsing the substrate with deionized water. All nematodes were concentrated by centrifugation at 750 *g* for 2 min. The supernatant was discarded and the nematodes were transferred to sterile deionized water. In the growth chamber, a pot received 100 individuals of a single nematode species placed into 1-cm deep holes. There were four replicates of each plant × nematode combination which were arranged in a randomized-block design.

After 20 d, microbivorous nematodes were extracted from the soil in each pot by sieving and sugar-flotation (Barker, 1985). Nematodes were counted and the identity of each species confirmed. Nematode population counts were not corrected for extraction efficiency. Bacteria and fungi from the treatments were not counted. The experiment was repeated with a fallow treatment included for each nematode species. Final nematode population data from both experiments were combined and subjected to an analysis of variance. Means separation tests were conducted using Duncan's multiple range test (α=0.05) on data that were log-transformed to stabilize variances.

Susceptibility of bacterial-feeding nematodes to paratism by Hirsutella rhossiliensis *on agar*

Hirsutella rhossiliensis (strain IMI 265748) was cultured on one-quarter-strength corn meal agar (1/4-CMA, Difco Laboratories, Detroit, MI). The fungal isolate is morphologically and pathogenically similar to other isolates of *H. rhossiliensis* (Tedford et al., 1994). Six plugs of mycelium, each 1 cm^2, were arranged in a circular pattern (approximately 6 cm diameter) in each of twenty-five 100×15 mm Petri dishes filled with 1/4-CMA. Cultures were incubated for three weeks at ambient lab temperature. Approximately 300 individuals of a single bacterial-feeding nematode species in less than 0.5 mL of 4.5 m*M* KCl were added to a dish with sporulating *H. rhossiliensis*. There were five replicate dishes for each nematode species. All vermiform developmental stages of the nematodes were represented. J2 of *H. schachtii* were applied at the same rate to an additional five dishes to serve as susceptible controls.

After 2 days at room temperature, all individuals from each Petri dish were rinsed from the surface with 4.5 m*M* KCl and transferred to Petri dishes containing MOPS (Sigma, St. Louis, MO, USA)-KOH buffered water agar (1.5% w:w Bacto-agar; pH 7). After an additional 2 days of incubation on water agar, the percentage of nematodes of each species with hyphae emerging from their bodies was determined.

The experiment was repeated. Data from both experiments were combined and arcsine transformed for analysis of variance. Differences between means were tested using Duncan's multiple range test (α=0.05).

Amplification of soil suppressiveness

This experiment was conducted three times. Specific details for each trial will follow after an account of the general procedures used. The experiment was a complete factorial design involving 48 pots filled with the autoclaved sand-loam mix. Half were maintained without plants (fallow), and half were planted with species having rhizospheres that supported greater nematode numbers (as determined above). For both sets of treatments, half were inoculated with *H. rhossiliensis*, and half were not. For each treatment combination, half were inoculated with *A. bodenheimeri*, and half were not. This provided six replicates of each treatment combination. All treatments received J2 of *H. schachtii*. The efficacy of the tri-trophic level interaction in regulating or suppressing the plant-parasitic nematode species was determined using a bioassay with a susceptible host plant. All pots were completely randomized in a greenhouse maintained at 23 ± 6 °C and were watered as needed with sterile deionized water or sterile Hoagland's solution. To minimize disturbance of *H. rhossiliensis*, plants were watered by filling dishes around the base of the pots.

Data for each experiment were analyzed using both factor-effects and cell-means models for analysis of variance. Data from different trials were not pooled because different protocols were used for each experiment. Differences between means were tested using Duncan's multiple range test (α=0.05).

In the first experiment, we used vetch grown in 1-L polystyrene cups to provide a conducive rhizosphere for *A. bodenheimeri*. Four days after planting vetch, seedlings were thinned to one per cup. All cups, with and without vetch, were inoculated with 5 mL of fresh microbial inoculum prepared as above, containing 1.89 ($\pm$0.2 SE)$\times 10^6$ CFUs mL^{-1}, placed into 3-cm deep holes. Four days later, all cups were inoculated with 1000 J2 of *H. schachtii* placed into 3-cm deep holes. Into the same holes, appropriate cups received 2000 individuals of *A. bodenheimeri* (in all vermiform stages), and/or 700 individuals of the entomopathogenic nematode *Steinernema glaseri* (Steiner) colonized by *H. rhossiliensis.*

Steinernema glaseri was prepared as an inoculum source for *H. rhossiliensis* by placing approximately 50000 infective juveniles of the nematode into sand cultures of the fungus (Jaffee et al., 1990). Infective juveniles were exposed to the fungus for 4 days. The nematodes were extracted from the soil using a modified sieving and sugar-centrifugation technique (Jaffee et al., 1988). Parasitized nematodes were used as inoculum because spores added to the soil do not adhere to nematodes (Jaffee et al., 1990). Assuming infected *S. glaseri* produce 893 conidia/nematode (Timper and Brodie, 1995), conidial density was 625 cm^{-3} of soil which was 1/2 the density used by Tedford et al. (1995) but was nearly twice the density used by Tedford et al. (1994).

Twenty days after the introduction of *H. schachtii*, vetch plants were cut at the soil line and removed. One cabbage seedling (*Brassica oleracea* L. var. 'Savoy Chieftain') was transplanted into each cup to serve as a bioassay for survival and infectivity of *H. schachtii*. After two weeks, cabbage plants were removed from the soil, and root systems were stained with acid fuchsin (Byrd et al., 1983). The number of developing juveniles of *H. schachtii* in each root system was counted. Nematodes were also extracted from the soil in the pots by sieving and sugar-centrifugation (Jaffee et al., 1988). The number of parasitized and healthy individuals were counted for each species.

In the second experiment, the same complete factorial design was used. However, to avoid a possible confounding effect from vetch as a potential host to *H. schachtii* (Goodey et al., 1965), sunn hemp was used instead. Eleven days after sowing, sunn hemp plants were inoculated with 2 mL of microbial inoculum containing 2.65 ($\pm$0.2 SE)$\times 10^6$ CFUs mL^{-1}, prepared as above. Two days later, plants were thinned to one per pot. The next day, 650 healthy individuals of *A. bodenheimeri* with all vermiform stages represented and/or 230 individuals of *H. rhossiliensis* infected *A. bodenheimeri* were placed into 3-cm deep holes in appropriate pots. Infected individuals were prepared by substituting *A. bodenheimeri* for *S. glaseri* in the protocol given above and extending exposure time to one week. Nematode species were changed based on the assumption that conidia produced from *A. bodenheimeri* cadavers might infect healthy *A. bodenheimeri* more effectively. Assuming infected *A. bodenheimeri* produce as many conidia as *S. glaseri*, conidial densities in this second experiment should have been more comparable to the densities used by Tedford et al. (1994).

To improve survivorship of the cyst nematode in soils without *H. rhossiliensis*, the inoculation of all pots with *H. schachtii* was delayed until 17 days after the introduction of *A. bodenheimeri*. At that time, sunn hemp plants were cut at the soil line, and all pots were inoculated with 1000 J2 of *H. schachtii*. Cabbage seedlings were transplanted one week later and grown

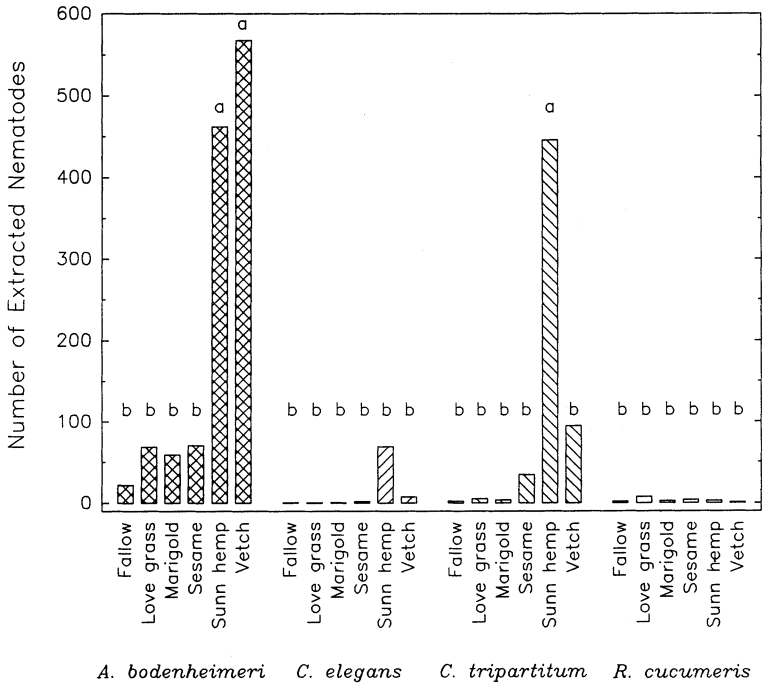

Figure 1. Interaction of bacterial-feeding nematodes with rhizospheres of five plant species and a fallow control. Bars with the same letter are not statistically different (p>0.05) as determined by a protected Duncan's multiple range test.

for three weeks before roots were harvested, stained, and examined for presence of *H. schachtii*.

In the third experiment, the same complete factorial design was used and was conducted in 15 cm diam (350 cc) clay pots. *Hirsutella rhossiliensis* was applied as dry pelletized hyphae (Lackey et al., 1992) in an attempt to increase the density, and homogenize the distribution, of conidia. Pelletized hyphae were manually incorporated into the soil of 24 pots at a rate of 0.37 mg cc^{-1} soil which is approximately equal to the recommended rate of one pellet 4 cc^{-1} soil (Lackey et al., 1992). Soil without fungi was used to fill the remaining pots. Ten mL of microbial inoculum containing 3.53 ($\pm$1.1 SE)$\times$ 10^6 CFU mL^{-1} were applied to the soil surface of each pot. *Acrobeloides bodenheimeri* were applied (1600 individuals/pot) into 3-cm deep holes and two pre-germinated sunn hemp seedlings were transplanted into the appropriate 24 pots. An additional six pots of the treatment with sunn hemp, *A. bodenheimeri*, and *H. rhossiliensis* were also prepared. After 2 days plants were thinned to one per pot. One week later, the extra six replicates were destructively sampled to ensure sporulation of the pellets and parasitim of the bacterial-feeding nematodes. Pellets were collected by wet-sieving, and nematodes were collected by sieving and sugar-centrifugation. Pellets were examined at 80$\times$ magnification and nematodes were placed on water agar. After 3 days at room temperature, the proportion of nematodes with hyphae emerging was determined.

Five weeks after transplanting sunn hemp was cut and removed. After 10 days to allow the root systems to die, all pots were inoculated with 1000 J2 of *H. schachtii* into 3-cm deep holes. A week following inoculation, cabbage seedlings were transplanted and allowed to grow for three weeks. Roots were rinsed of soil and stained. The number of nematodes in each developmental stage was counted for each root system.

Results

Impact of plant species on bacterial-feeding nematodes

Species of bacterial-feeding nematodes differed in their final population densities ($p\leq$0.001; Figure 1). Averaged across all plants, 230 *A. bodenheimeri*, 100 *C. tripartitum*, 14 *C. elegans*, and 3 *R. cucumeris* were recovered per 100 cc soil. Only the densties of

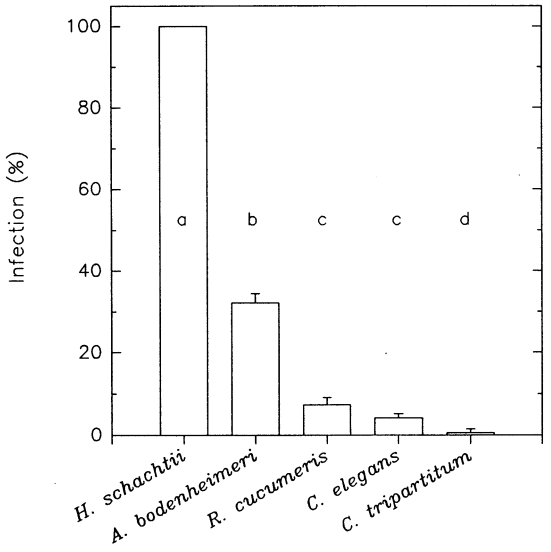

Figure 2. Susceptibility of nematode species to parasitism by *Hirsutella rhossiliensis* (± standard error). Bars with the same letter are not statistically different ($p>0.05$) as determined by a protected Duncan's multiple range test. *Heterodera schachtii* is an obligate plant parasite; all other species are bacterial feeders.

Table 1. Number of *Acrobeloides bodenheimeri* per 100 cc soil either healthy or infected by *Hirsutella rhossiliensis*. (Data from Experiment 1)[a]

Treatments			Healthy	Infected
-V	-Hr	-Ab	0b	0b
-V	-Hr	+Ab	703b	0b
-V	+Hr	-Ab	0b	0b
-V	+Hr	+Ab	1051b	0b
+V	-Hr	-Ab	0b	0b
+V	-Hr	+Ab	3502a	0b
+V	+Hr	-Ab	0b	0b
+V	+Hr	+Ab	3789a	159a

[a] On day 0, vetch (V) was planted into 1-L polystyrene cups and on day 4 inoculated with a microbial slurry prepared from soil. On day 8, 1000 healthy *H. schachtii*, 2000 *A. bodenheimeri* (Ab), and *H. rhossiliensis* (Hr, in the form of 700 parasitized *S.glaseri*) were applied into holes. Vetch was cut at the soil line on day 28. Nematodes were extracted from the soil 6 days later. Means within a colunm followed by the same letter are not statisticalty different ($p>0.05$) as determined by a protected Duncan's multiple range test.

C. elegans and *R. cucumeris* were similar ($p\leq0.05$). Plant species influenced densities of bacterial-feeding nematodes ($p\leq0.001$). Nematode densities (averaged across all nematode species) on vetch and sunn hemp were similar, collectively averaging 200 individuals per 100 cc soil, and were greater than densities in fallow soil ($p\leq0.001$). Nematode numbers associated with sesame, love grass, and marigold collectively averaged 20 individuals per 100 cc soil. These densities did not differ from the fallow soil ($p>0.05$). An interaction existed between plant species (or fallow) and nematode species ($p\leq0.001$). The combinations of vetch and *A. bodenheimeri*, sunn hemp and *A. bodenheimeri*, and sunn hemp and *C. tripartitum* yielded the greatest number of nematodes (Figure 1). Collectively, these treatment combinations provided an average of 493 individuals per 100 cc soil. All other plant and nematode combinations rendered no more nematodes than the fallow controls (Figure 1).

Susceptibility of bacterial-feeding nematodes to parasitism by H. rhossiliensis *on agar*

After nematodes were exposed to sporulating hyphae of *H. rhossiliensis*, adherent spores were observed on the cuticles of all species. Spores seemed particularly abundant on the head and tail regions of the bacterial-feeding nematodes. After 2 days on water agar, hyphae began to emerge from nematode cadavers. Following Jaffee et al. (1992), parasitism was measured as the proportion of all individuals in the population (active and inactive) from which hyphae emerged. All individuals of *H. schachtii*, the susceptible control, were parasitized by the fungus (Figure 2). Substantially fewer of the bacterial-feeding nematodes were parasitized compared to *H. schachtii* ($p<0.05$). Of the bacterial-feeders, *A. bodenheimeri* was the most susceptible. The other nematode species showed less than 10% parasitism (Figure 2).

Amplification of soil suppressiveness

In the experiment using vetch, numbers of *A. bodenheimeri* were greater ($p\leq0.001$) when vetch was present ($\bar{x}$=1800) than when vetch was absent ($\bar{x}$=410). Numbers of *A. bodenheimeri* were not affected by the presence of *H. rhossiliensis* (+Hr, $\bar{x}$=2100 vs. -Hr, $\bar{x}$=2500). Signiricant parasitism of *A. bodenheimeri* only occurred in the presence of vetch (4% vs. 0%; $p<0.05$) (Table 1).

The number of *H. schachtii* penetrating and developing in the roots of the assay seedling was affected by the presence of vetch ($p<0.001$) and *H. rhossiliensis*

Table 2. Average number of fourth-stage juveniles and adult females of *Heterodera schachtii* observed in the roots of an assay plant. (Data from Experiment 1)[a]

Treatments		+Ab	-Ab
+V	+Hr	0.0c	0.0c
+V	-Hr	0.2c	0.3c
-V	+Hr	1.2bc	2.8b
-V	-Hr	5.9a	2.8b

[a] 0n day 0, vetch (V) was planted into 1-L polystyrene cups and on day 4 inoculated with a microbial slurry prepared from soil. On day 8, 1000 healthy *H. schachtii*, 2000 *A. bodenheimeri* (Ab), and *H. rhossiliensis* (Hr, in the form of 700 parasitized *S.glaseri*) were applied into holes. Vetch was cut at the soil line on day 28 and a cabbage seedling was transplanted into each cup. Roots were rinsed and stained 6 days later. Across rows and columns, means followed by the same letter are not statistically different (p>0.05) as determined by a protected Duncan's multiple range test.

Table 3. Average number of fourth-stage juveniles and adult females of *Heterodera schachtii* observed in the roots of an assay plant. (Data from Experiment 3)[a]

Treatments		+Ab	-Ab
+Sh	+Hr	3.7d	0.3d
+Sh	-Hr	16.2bc	7.3cd
-Sh	+Hr	7.7cd	4.5d
-Sh	-Hr	32.3a	22.5b

[a] On day 0, soil was mixed with pelletized hyphae of *H. rhossiliensis* (approximately 1 pellet 4 cc^{-1} soil) and was used to fill 350 cc clay pots. Soil without fungi was used to fill remaining pots. Soils received microbial inoculum. Then, 1600 *A. bodenheimeri* (Ab) were applied into holes and two sunn hemp (Sh) seedlings were transplanted. In 2 days, plants were thinned to one per pot. On day 35, sunn hemp was cut at the soil line. On day 45, 1000 healthy *H. schachtii* were added. After one week, roots were rinsed and stained. Across rows and columns, means followed by the same letter are not statistically different (p>0.05) as determined by a protected Duncan's multiple range test.

(p<0.05) but not by the presence of *A. bodenheimeri* (Table 2). Interactions between main effects were not significant. Fewer *H. schachtii* penetrated and developed within roots of the assay plant in treatments with vetch than in treatments without vetch (p<0.001) In the presence of *H. rhossiliensis*, fewer *H. schachtii* were found in assay roots than in the absence of the fungus (p<0.05). The presence of *A. bodenheimeri* in treatments with *H. rhossiliensis* did not improve the capacity of *H. rhossiliensis* to reduce the number of *H. schachtii* (p>0.05).

In the experiment using infected *A. bodenheimeri* as the inoculum source for *H. rhossiliensis* and sunn hemp as the stimulatory crop, none of the main factors significantly affected the number of fourth-stage juveniles and adult females of *H. schachtii* in the roots of the assay plant (data not shown). Across all treatments, an average of 48 (±2.7 SE) *H. schachtii* were recovered in the roots, more than in the first experiment.

In the final experiment using pelletized hyphae as the inoculum source for *H. rhossiliensis* and sunn hemp as the stimulatory crop, all hyphal pellets recovered from the soil 9 days after the start of the experiment had sporulated and 15% (±3% SE) of *A. bodenheimeri* were parasitized. At the end of the experiment, the presence of the fungus, plant, and bacterial-feeding nematode affected the number of *H. schachtii* in roots (p<0.01 for each factor; Table 3). There was also a sifnificant interaction between the plant and the fungus (p=0.01). Fewer fourth-stage juveniles and adult females of *H. schachtii* were observed when *H. rhossiliensis* was present (4 vs. 20) or when sunn hemp was present (7 vs. 17). More *H. schachtii* were in roots when *A. bodenheimeri* was present (15 vs. 9). As in the experiment with vetch, the added presence of *A. bodenheimeri* did not improve the capacity of *H. rhossiliensis* to reduce the number of *H. schachtii* (p>0.05). In fact, the number of *H. schachtii* tended to increase when *A. bodenheimeri* was also present, though this trend was not significant (Table 3).

Table 4. Reproductive success of four bacterial-feeding nematode species across 5 plant rhizospheres and fallow soil

	N[a]	%[b]	GM(λ)[c]
A. bodenheimeri	41	36.6	1.263
C. tripartitum	44	22.7	NA
C. elegans	44	6.8	0.424
R. cucumeris	42	0.0	1.03×10^{-4}

[a] Total number of pots.
[b] Percentage of pots with final population levels greater than the inoculum dose.
[c] Geometric mean of finite rates of population increase, a measure of physiological homeostasis for a species (Venette and Ferris, 1997).

Discussion

Soils contain complex detrital food webs involving numerous taxa. Interactions between groups at different trophic levels are modulated by edaphic factors and the myriad interrelationships between other web components. However, not all species at the same

trophic position are equally suitable as prey for other organisms in the web. Although our soil microcosms contain only a small subset of the numerous potential taxa, our results suggest that the presence and abundance of particular species may alter the rate and degree of carbon flow. The rhizospheres of various plant species may selectively enhance population levels of bacterial-feeding nematodes. The stimulatory effect of rhizospheres on bacterial-feeding nematodes has been demonstrated with numerous plant species (e.g., Griffiths et al., 1992; Griffiths, 1990, 1994). In rhizosphere soil, taxa in the family Cephalobidae frequently dominate the bacterial-feeding nematode trophic group (Griffiths et al., 1991, 1992). Our results are consistent with those observations. Sunn hemp and vetch enhance numbers of bacterial-feeding nematodes especially *A. bodenheimeri* (Cephalobidae), while the other plant species have no measurable effect. In part, this result may be due to edaphic conditions. For example, temperatures in the growth chamber exceeded 24.6 °C the upper thermal tolerance of *R. cucumeris* (Venette and Ferris, 1997). Other species may have been stressed. In this experiment, the percentage of all pots with final populations greater than the inoculum dose corresponds with a measure of physiological homeostasis across temperatures (Table 4).

Even if edaphic conditions are suitable for nematode reproduction, our Petri dish assays suggest that many species of bacterial-feeding nematodes are relatively resistant to infection by *H. rhossiliensis*. In soil, the rates of parasitism may be considerably different. In the assay, nematodes were exposed to high spore densities on a medium which facilitates nematode dispersion. Under such conditions, transmission of spores is nearly assured. We also infer that the conditions of the assay facilitated parasitism, based on the degree of parasitism of *H. schachtii*. The results obtained for the susceptible control gives us confidence in the negative results obtained for the bacterial-feeding nematode species. Consequently, in soils these nematodes are unlikely to lie more susceptible to infection by *H. rhossiliensis* than *H. schachtii* and are probably not a major source of carbon for the fungus.

At least four intrinsic mechanisms may exist for bacterial-feeding nematodes to escape parasitism by *H. rhossiliensis*. First, in soil, behavioral attributes of certain bacterial-feeding nematodes may reduce the probability of spore transmission. Since spore transmission by the fungus is passive, the probability and degree of transmission is largely a function of nematode movement (Tedford et al., 1992). Species of bacterial-feeding nematodes differ in their relative locomotive activity (Ferris et al., 1995), with members of the Cephalobidae being generally less active than members of the Rhabditidae.

Second, physical barriers may prevent the fungus from penetrating the nematode cuticle. Infective juveniles of the entomopathogenic nematode *Heterorhabditis bacteriophora* possess a cuticular sheath. If the sheath is experimentally removed, parasitism increases substantially (Timper et al., 1991). Presumably, the infection peg is unable to penetrate the cuticle after it pierces the sheath. Germinating hyphae are rapidly depleted of nutrients and die without affecting the nematode. The general significance of this mechanism for other bacterial feeding nematodes is unknown. However, dauer larvae (an alternative to the third juvenile developmental stage) are characterized, in part, by the presence of a cuticular sheath and are common among members of the Rhabditidae (Riddle, 1988). Dauer larvae, a "survival" stage, generally only appear as populations become large or food-limited.

Third, the cuticle of certain bacterial-feeding nematodes may not have the proper chemical components to initiate spore attachment or germination. Timper and Brodie (1991) and Tedford et al. (1994) have commented on the degree of specificity between isolates of *H. rhossiliensis* and various nematode hosts. Jansson (1994) reported differences in the adhesion of conidia of *Drechmeria coniospora*, an endoparasitic fungus, to different species/isolates of bacterial-feeding nematodes. Bernard and Arroyo (1990) discussed the suitability of *C. tripartitum* and other rhabitid nematodes and the unsuitability of cephalobid nematodes as hosts for *Macrobiotophthora vermicola*.

Fourth, developmental rates of the fungus may attribute to the survival of bacterial-feeding nematodes. Following spore adhesion, fungal penetration of the nematode body cavity is not instantaneous. At 20 °C, approximately 15 h are required for 50%, and 20 h for 50%, of adherent conidia to germinate and form an infection bulb within the nematode host (Tedford et al., 1995). The time required for infection varies with temperature. However, at 20 °C, many bacterial-feeding nematode species require less than 20 h to molt between stages (Ferris et al., 1996). If a nematode with adherent spores is able to molt before the spores germinate, the nematode survives. Spores adhering to the shed cuticle are removed from the pool of infective propagules.

In our study, differences in rates of parasitism occur among bacterial-feeding nematodes despite sub-

stantial acquisition of spores. We cannot explain the low parasitism of *C. tripartitum*. Microscopic examiation of healthy *C. tripartitum* from stock cultures did not reveal an obvious cuticular sheath. Differences in development rates (Ferris et al., 1996) probably account for differences in infection rates of *A. bodenheimeri*, *R. cucumeris*, and *C. elegans*. Based on requisite development times, fourth-stage juveniles and adults of these species are more likely to be parasitized than earlier developmental stages. Although all vermiform stages were present in our assays, stage distributions were not quantified. From these assays, *A. bodenheimeri* showed the most promise for serving as an alternate host for *H. rhossiliensis*.

Because *H. rhossiliensis* responds numerically to increases in host density, the presence of alternate hosts should have increased the degree of infection of plant-parasitic nematodes. Increasing the number of *A. bodenheimeri* should have stimulated even greater infection of *H. schachtii*. However, in all three experiments, the number of plant-parasitic nematodes entering the root of an assay plant in soil containing *H. rhossiliensis* was either unaffected or enhanced by the presence of *A. bodenheimeri*. The obvious question is: Why? We can discount methodological error (i.e. counting *A. bodenheimeri* in roots as *H. schachtii*) because only the number of fourth-stage juveniles and young adults of *H. schachtii* were quantified. Morphologically, these stages are clearly distinct from bacterial-feeding nematodes. Discounting the efffects of bacterial-feeding nematodes on plant growth (via enhanced nitrogen mineralization) and the speculative consequences for plant-parasitic nematodes, two other explanations exist.

First, bacterial-feeding nematodes may not be ideal hosts for *H. rhossiliensis*. From the perspective of a parasite, an ideal host is a susceptible organism that requires few propagules to become infected and yields substantially more propagules than were "consumed" during infection. Given the mechanisms by which bacterial-feeding nematodes can avoid parasitism, these nematodes are certainly less ideal than plant-parasitic nematodes. If more spores are contacted and removed than are produced after parasitism, poor or resistant hosts could deplete the number of viable propagules from the soil (Timper and Brodie, 1995). Under such conditions, the soil would become less suppressive to plant parasites.

Other research also suggests that bacterial-feeding nematodes may diminish or fail to improve the effectiveness of *H. rhossiliensis* to control plant-parasitic nematodes. In a series of microcosm studies, after incorporating various organic amendments into the soil to amplify populations of microbivorous (i.e. fungal- and bacterial-feeding) nematodes, parasitism of *C. xenoplax* and *H. schachtii* by *H. rhossiliensis* declined substantially (Jaffee et al., 1994). Jaffee et al. (1994) observed few microbivorous nematodes with adherent conidia. The organic amendments may have had a detrimental effect on the fungus (Hoitink and Fahy, 1986). However, an analysis of the data pooled from several trials also suggests a negative relationship, following the equation $Y = 41.3e^{-0.0036X}$ (r^2=0.54, p<0.0001), between the mean number of bacterial-feeding nematodes (X) and the percentage of assay nematodes infected (Y). The presence of the bacterial-feeding nematode *Teratorhabditis dentifera* also failed to increase the effectiveness of *H. rhossiliensis* against *Pratylenchus penetrans* infecting potato (*Solanum tuberosum*) (Timper and Brodie, 1995). In their study, Timper and Brodie (1995) noted the presence of dauer stages of *T. dentifera* which seemed impervious to infection.

Second, *H. rhossiliensis* and *A. bodenheimeri* may not have interacted in the soil. In the initial test using vetch, parasitism of *A. bodenheimeri* ocurred at a very low rate (4%) and only when vetch was present. In the absence of strong directional cues (e.g., as provided by bacteria in the rhizosphere), movement of bacterial-feeding nematodes might be limited. Nematode inactivity could create a situation in which we assume the fungus and nematode interact, but really they do not (Tedford et al., 1992).

The interpretation of our experiments designed to enhance soil suppressiveness is complicated by the fact that very few plant-parasitic nematodes were recovered or could be confidently identified in the assay plants. Second and third-stage juveniles of *H. schachtii* that may have been present in roots were excluded from analyses because they could not be distinguished from free-living nematodes that might also occur in damaged roots. Alternatively, survival of *H. schachtii* J2 in soil may be limited. Data from Jaffee and Muldoon (1995) suggest that 8% of J2 may die or become inactive each day in the absence of antagonists in soil. In our experiment, by the time the known host was planted, 44-83% of the J2 may have expired. Other J2 may have entered the vetch or sunn hemp roots. Nematodes in these roots could not be quantified because the roots did not release the stain. Consequently, comparisons may be tenuous between treatments with vetch or sunn hemp and treatments without those plants.

Nevertheless, in three separate trials, despite methodological adjustments intended to maximize the effects of our treatments, we were unable to enhance soil suppressiveness to *H. schachtii* using bacterial-feeding nematodes as alternate hosts for *H. rhossiliensis*. Our early experiments demonstrate that numbers of particular bacterial-feeding nematode species can be manipulated by growing particular plants and that not all of these nematodes are equally susceptible to parasitism by *H. rhossiliensis*. Bacterial-feeding nematodes are a diverse group. Consequently, our results do not dispute the findings of other authors who were able to enhance suppressiveness following the addition of organic matter (e.g. Linford, 1937; Van den Boogert et al., 1994). A successful outcome may depend upon which saprophytic nematodes increase numerically and which antagonists are present. Our results caution against the assumption that all bacterial-feeding nematodes are equivalently suitable hosts or prey for nematode antagonists.

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