Parasitism of Nematodes by the Fungus *Hirsutella rhossiliensis* as Affected by Certain Organic Amendments


Abstract: Experiments were conducted to determine whether the addition of organic matter to soil increased numbers of bacterivorous nematodes and parasitic activity of the nematophagous fungus *Hirsutella rhossiliensis*. In a peach orchard on loamy sand, parasitism of the plant-parasitic nematode *Criconemella xenoplax* by *H. rhossiliensis* was slightly suppressed and numbers of *C. xenoplax* were not affected by addition of 73 metric tons of composted chicken manure/ha. In the laboratory, numbers of bacterivorous nematodes (especially *Acrobeloides* spp.) and fungivorous nematodes increased but parasitism of nematodes by *H. rhossiliensis* usually decreased with addition of wheat straw or composted cow manure to a loamy sand naturally infested with *H. rhossiliensis*. These results do not support the hypothesis that organic amendments will enhance parasitism of nematodes by *H. rhossiliensis*.

Key words: bacterivorous nematode, biocontrol, biological control, *Criconemella xenoplax*, density-dependent parasitism, fungivorous nematode, *Hirsutella rhossiliensis*, nematode, nematophagous fungus, organic amendment.

Linford et al. (18) reported that addition of chopped pineapple plants to soil suppressed root-knot nematodes in Hawaiian pineapple fields. They suggested that the amendment supported sequential increases in bacteria, bacterivorous nematodes, and nematode-trapping fungi. The presumed increase in density of trapping fungi in response to total nematode density was thought to suppress all hosts, including root-knot nematodes (trapping fungi generally possess broad host ranges). Although Linford et al. (18) did not use the term density-dependent parasitism, that concept clearly was central to their understanding of the system. Density-dependent parasitism occurs when the probability of a host being parasitized increases with host density (10,22).

Cooke (7) and Pramer (19) noted that Linford et al. (18) were too hasty in concluding that parasitism by trapping fungi was influenced chiefly by nematode density. Cooke (8) found little relationship between parasitism by trapping fungi and nematode density and concluded that captured nematodes supplement saprophytism. Linford et al. (18) and Drechsler (9) had assumed that these fungi behave largely as obligate parasites in soil, saprophytism being a laboratory artifact. Cooke (8) proposed that endoparasitic fungi (fungi that parasitize nematodes with adhesive or ingested spores rather than with traps) depended more on nematodes as a food source than did trapping fungi and thus were more likely to exhibit density-dependent parasitism. His prediction with respect to endoparasitic fungi was supported by a recent study with the fungus *Drechmeria coniospora* (1).

Field observations and laboratory experiments (10,12,13,15) indicate that another endoparasitic fungus, *Hirsutella rhossiliensis*, also parasitizes nematodes in a density-dependent manner. The spores of *H. rhossiliensis* adhere to vermiform nematodes, which are directly penetrated and subsequently killed by the fungus. New spores are produced from the cadaver. The fungus has a broad host range, including species of *Heterodera* and *Meloidogyne* (4,24, 25), *Pratylenchus* (24,27), *Criconemella* (11), *Anguina* (5), *Ditylenchus* (4,24), *Xiphinema* (6), *Aphelenchoides* (4,24), *Steinernema* (26), *Heterorhabditis* (26), *Anaplectus* (24), and *Cephalobus* (24).

Our objective in the present study was to
examine the concept of Linford et al. (18) as applied to *H. rhossiliensis*. We asked, "Does addition of organic matter to soil increase numbers of bacterivorous nematodes and thus the parasitic activity of *H. rhossiliensis*?"

**Materials and Methods**

*Field experiment:* The field site was a mature peach (*Prunus persica*) orchard on 'Nemaguard' rootstock in Merced County, California. The characteristics of the loamy sand, designated M in previous studies, were described elsewhere (11). The soil contained *H. rhossiliensis* and the plant-parasitic nematode *Criconemella xenoplax*. In previous studies in this orchard, *C. xenoplax* composed more than 95% of the nematodes parasitized by *H. rhossiliensis* (11-14).

For the current study, 20 trees in one corner of the orchard were selected. Eleven kg of composted chicken manure plus rice hulls (30% moisture and 1.5% nitrogen based on dry weight), equivalent to 73 metric tons of chicken manure/ha, were added to a rectangular plot (1.5 × 1.0 m) on one side of each of 10 trees in April 1988. Each plot was considered a replicate. Composted chicken manure from Foster Farms, Livingston, California, was selected as the amendment because it is commercially available in the San Joaquin Valley of California. The closest side and center of each plot were 30 and 80 cm from the trunk, respectively. After addition to the surface of each plot, the compost was mixed into the soil with a shovel 25-30 cm deep. Plots adjacent to the 10 remaining trees were treated in the same manner, but compost was not added. The experimental design was completely random, with two treatments replicated 10 times.

Soil samples were collected 70-80 cm from the trunk and 0–33 cm or 34–66 cm deep with a soil auger (4.5 cm d) before addition of compost (week 0), and at weeks 3, 10, 30, and 45. Two 700-cm³ samples were combined per depth and plot. Nematodes were extracted from a 500-cm³ sub-sample by elutriation (2) and centrifugation in water and then in sucrose (454 g sucrose/liter) (16); a 38-μm-pore-d sieve was used. Numbers of parasitized and nonparasitized nematodes (almost entirely *C. xenoplax* and microbivores) were determined with an agar plate assay using NaOCl, as described elsewhere (11). Nematodes not added to agar plates were fixed in formalin, and the most common nematodes (other than *C. xenoplax*) in subsamples from three replicate plots per treatment, depth, and sample time were identified to species.

The number of colony-forming units of the plant-pathogenic fungus *Pythium ultimum* was determined in each soil sample (23). The concentrations of NH₃ and NO₃ (3,17) and the percentage organic matter were determined in one composite sample per treatment, depth, and sample period by the DANR Laboratory at the University of California at Davis.

*General procedures for laboratory experiments:* The effect of organic amendments on nematodes and parasitism by *H. rhossiliensis* was examined in three experiments with soil in vials. The soil had properties similar to the loamy sand in the field experiment and was collected from the rhizospheres of twelve 30-year-old almond trees adjacent to the peach trees used in the field experiment. This soil contained about 500 *H. rhossiliensis*-parasitized *C. xenoplax*/100 cm³ soil (corrected for extraction efficiency), which represented about 50% of the total *C. xenoplax* population. The soil was screened (2-mm-pore d) to remove large roots, leaves, and almond hulls and stored at 10 C for less than 1 month before use. Additional soil was collected for each experimental trial, because *H. rhossiliensis* declines in storage at 10 C (11).

Composted cow manure plus rice hulls, leaves of woolypod vetch (*Vicia pillosa*), and wheat straw were obtained from the Student Experimental Farm at the University of California at Davis. Composted chicken manure plus rice hulls was obtained from Foster Farms, Livingston, California.
Amendments were dried and heated at 80°C in a drying oven, ground in a Wiley Mill, and screened (0.83-mm-pore d) before addition to soil. The vetch and cow manure were moist when collected and were dried and heated for 2 days, but the wheat straw and chicken manure, already dry, were heated for only 2 hours.

Nematodes were extracted from soil by decanting and sieving (25-μm-pore d) followed by centrifugation in water and then in sucrose (454 g/liter); nematodes in sucrose were collected on a 25-μm-pore-d sieve and suspended in 10 ml of water.

**Laboratory experiment 1:** To determine which of the amendments caused the largest increase in microbivorous nematodes, soil was moistened to about 11.0% with distilled water and divided into 20-g samples (18.0 g dry weight soil). One of the four amendments was mixed into each sample (800-mg amendment per sample, equivalent to approximately 70 metric tons/ha). Samples that received no amendment were controls. Addition of dry organic matter affected the water status of the soil, and we attempted to standardize matric potential (based on subjective assessment) by adding distilled water; the percentage moisture was 24, 24, 19, 17, and 14% for the vetch, straw, cow manure, chicken manure, or control, respectively. Each sample was packed (bulk density = 1.2) into a 25-ml plastic vial, which was sealed with a lid having a 0.2-mm-d hole. The vials were placed in a moisture chamber at 20°C.

On day 0, 10, 27, 40, and 55, the nematodes from four vials per treatment were extracted, the suspension was adjusted to 10 ml, and 1 ml of suspension was placed in a Hawksley counting slide and examined at 140× magnification. Nematodes with and without spores of *H. rhossiliensis* attached were counted and separated into trophic groups: bacterivores, fungivores, predators, plant-parasites, and others, but were not identified to species or genus (in contrast to the field experiment and other laboratory experiments). The numbers of *C. xenoplax* and other nematodes parasitized by *H. rhossiliensis* also were determined by incubating aliquots of the suspension on agar as described for the field experiment, except that the nematode suspensions were not treated with NaOCl. There were four replicates per treatment per sample time, and the experiment was not repeated.

**Laboratory experiment 2:** Based on the results from the previous experiment, the effect of straw and cow manure on parasitism of nematodes by *H. rhossiliensis* was examined. Nonamended soil or soil amended with straw or cow manure was packed into 25-ml plastic vials and incubated at 20°C as described. Soil moisture was 15, 20, or 25% for the control, manure-amended, or straw-amended soil, respectively. To quantify the parasitic activity of *H. rhossiliensis*, each vial was inoculated with 600 healthy second-stage juveniles (*J2*) of *Heterodera schachtii* in 0.5 ml of 4.5 mM KCl. The *J2* were added to the soil surface of four vials per treatment on day 21, 42, 63, 85, and 105, and soil was extracted 66 hours later as in laboratory experiment 1. Because *H. schachtii* was not naturally present in the soil, all of the *H. schachtii* recovered were added to the soil and exposed to the fungus for 66 hours. On day 0, four vials per treatment that did not receive *H. schachtii* were extracted to determine initial nematode numbers.

The nematode suspension was adjusted to 10 ml, and 1 ml was examined at 140× magnification to determine the number of nematodes by trophic group and the percentage of *H. schachtii* *J2* and microbivorous nematodes with spores. The numbers of *C. xenoplax* and other nematodes parasitized by *H. rhossiliensis* were determined by incubating aliquots of the suspension on agar as in the first laboratory experiment. In addition, the first 20 specimens of microbivorous nematodes in each sample were identified to genus and in some cases to species on day 85 and 105. This experiment was repeated (trials 1 and 2).

**Laboratory experiment 3:** The effect of quantity of organic amendment was examined. In the first trial, soil amended with 0, 50, 100, 200, 400, or 800 mg of cow ma-
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nure or straw per vial was packed and incubated at 20 °C as in the first laboratory experiment. Soil moisture was 11.0% in unamended soil and 13.6% or 16.3% in soil amended with the highest levels of manure and straw, respectively. Vials amended with intermediate levels of manure or straw had intermediate levels of soil moisture. On day 65, all vials were inoculated with H. schachtii J2 and extracted 66 hours later. The numbers of microbivorous nematodes and the percentage of H. schachtii J2 and microbivorous nematodes with spores of H. rhossiliensis were determined as in the second laboratory experiment. Twenty randomly selected nematodes per treatment were identified to genus and sometimes to species. There were five replicate vials per treatment. Experiment 2 was repeated (trial 2), except that the levels of amendment were 0, 25, 50, 100, 200, or 400 mg per vial.

Statistical analysis: Field data (log transformed for counts of nematodes and fungi, and arcsine transformed for percentages) were subjected to repeated-measures analysis of variance with the general linear model procedures of SAS (21). Treatment, time, and the interaction of treatment and time were independent variables for each depth; the effect of depth was determined in a separate analysis. For the laboratory experiments, inferences were based on means and standard errors.

Results

Field experiment: Numbers of C. xenoplax were unaffected (P > 0.09) by treatment (± compost), time, or treatment × time, but were fewer (P < 0.01) in the top soil (0–33 cm deep) than at the greater depth (34–66 cm) (Fig. 1A). Numbers of microbivorous nematodes were unaffected (P > 0.08) by treatment, time, or treatment × time but were greater (P < 0.01) in the top soil than below (Fig. 1B). The percentage of C. xenoplax parasitized by H. rhossiliensis was smaller (P = 0.05) in amended soil than in nonamended soil in the top soil but not below; parasitism was greater (P < 0.01) in soil from the lower depth than in the top (Fig. 1C). Parasitized nematodes other than C. xenoplax were rare. In the top soil, numbers of colony-forming units of P. ultimum were suppressed by the amendment. Suppression was affected by time, as indicated by a significant (P < 0.01) interaction of treatment × time (Fig. 1D). In soil from the lower depth, neither treatment nor treatment × time affected P. ultimum (P > 0.16), but time was marginally significant (P = 0.06). In treated plots, NH₄ peaked at week 3 and then declined (Fig. 1E); NO₃ increased to much higher concentrations in the topsoil than in the lower depth and decreased only slowly (Fig. 1F). In untreated plots, NH₄ and NO₃ concentrations remained low, but NH₄ increased slightly on week 3 (Fig. 1E,F). At week 45, the percentage organic matter in the topsoil was 1.0 and 1.3 in untreated and treated plots, respectively; in the lower depth, the percentage organic matter was 0.5, regardless of treatment.

The most common species of microbivorous nematodes were Acrobeloides bodenheimeri and Acrobeles complexus. Other common species were Acrobeloides buetschlii, A. uberrinus, Cervidellus serratus, Panagrolaimus subelongatus, Eudorylaimus monohystera, and Rhabditis spp. Neither treatment nor time greatly affected the species observed, although Panagrolaimus subelongatus, A. uberrinus, and a Rhabditis sp. were encountered somewhat more commonly in plots amended with manure.

Laboratory experiment 1: Few bacterivorous and fungivorous nematodes were present on day 0, and they remained low in unamended soil (Fig. 2A,B). In soil amended with vetch, no bacterivorous nematodes were observed after day 27. A substantial increase in bacterivorous nematodes occurred between day 27 and 55 in cow manure- and straw-amended soil; a moderate increase occurred in soil amended with chicken manure. Fungivorous nematodes increased in some vials but not in others amended with straw and cow manure, as indicated by the high variances (Fig. 2B), but remained few in other treatments. Predaceous nematodes with or without spores and microbivorous nema-
Fig. 1. Effect of composted chicken manure on numbers of nematodes and fungi and on nitrogen status in a peach orchard. Manure was mixed into the top 25–30 cm of soil on 14 April 1988. Soil samples were collected from 0–33 cm or from 34–66 cm; the week 0 sample was collected 1 hour before incorporation of the manure. A) Numbers of Criconemella xenoplax (Cx). B) Numbers of microbivorous nematodes. C) Percentage of C. xenoplax parasitized by Hirsutella rhossiliensis (Hr). D) Numbers of colony forming units of Pythium ultimum. E) Concentrations of NH$_4$-N. F) Concentrations of NO$_3$-N. Values are the means of 10 replicate plots.

Numbers of C. xenoplax parasitized by H. rhossiliensis tended to be greater in the control than in the other treatments and declined in all treatments over time (Fig. 2C).
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Although species of nematodes other than C. xenoplax were parasitized by H. rhossiliensis, the number was never greater than five per vial.

Laboratory experiment 2: Results of both trials were similar, and data were combined for presentation. Numbers of bacterivorous and fungivorous nematodes remained small in unamended soil but increased in soil amended with straw or cow manure (Fig. 3A,B). Predaceous nematodes were observed rarely. Most (>84%) of the bacterivorous nematodes at day 85 or 105 in straw- or cow manure-amended soil were Acrobeles spp. (A. bodenheimeri, A. buetschlii, and A. tricornis); the remainder were species of Cervidellus and Plectus. Fungivorous nematodes included Apheleonchoides sp. and Apheleonchoides sp.

The number of C. xenoplax parasitized by H. rhossiliensis was greater in unamended than in amended soil at time 0 and declined in all treatments over time (Fig. 3C). No species other than C. xenoplax was parasitized by H. rhossiliensis.

The percentage of H. schachtii J2 with spores of H. rhossiliensis was greatest in unamended soil, intermediate in straw-amended soil, and least in cow manure-amended soil (Fig. 3D). This percentage tended to decline with time in straw-amended soil. Microbivorous nematodes with spores were seldom observed.

Laboratory experiment 3: In both trials, the numbers of bacterivorous nematodes after 65 days were greater in soil amended with cow manure than in unamended soil (Fig. 4A,D); Acrobeles spp., Mononchus sp., Rhabditis sp., and Apheleonchoides sp. Numbers of fungivorous nematodes (A. tricornis, A. buetschlii, and A. bodenheimeri) composed more than 50% of these nematodes across all treatments. Also present were Diploscapter sp., Panagrolaimus sp., and Eu-rodorylaimus sp. Numbers of fungivorous nematodes (A. tricornis, A. buetschlii, and A. bodenheimeri) composed more than 50% of these nematodes across all treatments. As in cow manure-amended soil, C. xenoplax numbers were greater with addition of cow manure, especially in trial 1 (Fig. 4B,E). Numbers of bacterivorous nematodes usually were less in soil amended with straw than in soil amended with cow manure but were greater than in unamended soil (Fig. 4A,D); as in cow manure-amended soil, A. tricornis, A. buetschlii, and A. bodenheimeri composed more than 50%
of the bacterivorous nematodes across all treatments receiving straw. Other species present were similar to those in cow manure-amended soil, except that species of Acrobeles, Panagrolaimus, and Cylindrolaimus were not observed. Numbers of fungivorous nematodes (Aphelenchoides sp. and Aphelenchus sp.) were correlated with the amount of straw added in trial 2 (Fig. 4E) but not in trial 1 (Fig. 4B).

In trial 1, the percentage of H. schachtii J2 with spores increased with addition of low quantities of cow manure or straw and then decreased as either amendment increased (Fig. 4C). In trial 2, only a marginal increase in parasitism occurred with low levels of amendment (Fig. 4F); as in trial 1, parasitism was inversely correlated with quantity of amendment when level of amendment exceeded 100 mg for straw or 50 mg for cow manure. Microbivorous nematodes with spores were seldom observed.

Nematodes may have been introduced to the soil with the organic amendments, even though the amendments were heated to 80 C. Based on observation of nematodes from unamended soil in experiments 2 and 3, the following nematodes were naturally present: Criconemella xenoplax, Acrobeles bodenheimeri, A. buetschlii, A. tricornis, Aphelenchoides sp., Aphelenchus sp., Cruznema sp., Cylindrolaimus sp., Mononchus sp., Rhabditis sp., and Tylenchus davainei.

**DISCUSSION**

Organic amendments usually suppressed rather than stimulated parasitism of nematodes by H. rhossiliensis in the field.
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**Fig. 4.** Effect of quantity of straw and cow manure amendments on numbers of microbivorous nematodes and parasitism of nematodes by *Hirsutella rhossiliensis*. A loamy sand naturally infested with *H. rhossiliensis* was amended with organic matter and incubated in vials (18 g dry weight soil and 15 cm³ soil per vial) for 65 days at 20°C. The experiment was performed twice (trials 1 and 2). A,D) Numbers of bacterivorous nematodes. B,E) Numbers of fungivorous nematodes. C,F) Percentage of *Heterodera schachtii* juveniles (Hs J2) with spores of *H. rhossiliensis* (Hr). Each value is the mean of five replicate vials; vertical bars = 1 SE.

and in the laboratory. The mechanism of suppression was not clear, however, and experiments like these are difficult to interpret because the amendments can affect many processes. In addition to stimulating antagonists of nematodes via density-dependent parasitism (18), organic matter may be the source of ammonia and other nematicidal and fungicidal compounds (20); may alter soil porosity and soil water and thus influence several aspects of the biology of nematodes and *H. rhossiliensis* (25); and may stimulate fungivorous nematodes and other antagonists of fungi.
Suppression of *P. ultimum* in the field experiment demonstrated that the suppression was not specific to *H. rhossiliensis.*

Low quantities of organic amendments did appear to stimulate parasitism in one laboratory trial (Fig. 4C). The stimulation, however, was not reproducible (Fig. 4E). Perhaps by affecting several processes simultaneously, the organic amendments both enhanced and suppressed parasitism, and the net effect was suppression unless levels of organic matter were low.

In two laboratory experiments, amendments decreased the number of parasitized *C. xenoplax* detected with the agar plate assay at time 0, suggesting a direct effect on extraction. Many parasitized *C. xenoplax* detected with this assay had hyphae growing from their bodies, and such external hyphae could become entwined or otherwise interact with organic matter during extraction. External hyphae would not be a problem with the second and more important quantification procedure (in which healthy *H. schachtii* J2 were added to soil and recovered and examined for spores after 66 hours) because these nematodes are not in soil long enough for growth of external hyphae. We have no evidence that organic matter differentially affected the extraction of *H. schachtii* J2 with and without spores.

Inadequate information on the many possible influences of organic matter and on the host status of bacterivorous nematodes limits understanding of the results. Amendments decreased the number of bacterivorous nematodes in the laboratory experiments, but even though the host range of *H. rhossiliensis* is broad, one cannot assume that all bacterivorous nematodes are hosts. Some species of bacterivorous nematodes acquire spores but fail to become infected (P. Timper, pers. comm.). Thus, the probability of suppression would be reduced. In the present study, however, we observed few microbivorous nematodes with spores.

Organic matter may have stimulated *H. rhossiliensis,* had experimental conditions been different. For example, the soil used in this study already contained high levels of *H. rhossiliensis,* and enhancement of this fungus may have occurred had the initial levels been lower. Other kinds of organic matter and different soils also may have provided more encouraging results.

**Literature Cited**


