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Do Organic Amendments Enhance the Nematode-Trapping Fungi *Dactylellina haptotyla* and *Arthrobotrys oligospora?*

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Abstract: Soil cages (polyvinyl chloride pipe with mesh-covered ends) were used to determine how the quantity of two organic amendments affected the nematode-trapping fungi *Dactylellina haptotyla* and *Arthrobotrys oligospora,* which were studied independently in two different vineyards. Each cage contained 80 cm^3 of field soil (120 g dry weight equivalent), fungal inoculum (two alginate pellets, each weighing 1.9 mg and containing assimilative hyphae of one fungus), and dried grape or alfalfa leaves (0, 360, or 720 mg equivalent to 0, 4,500, or 9,000 kg/ha) with a C:N of 28:1 and 8:1, respectively. Cages were buried in the vineyards, recovered after 25 to 39 days, and returned to the laboratory where fungus population density and trapping were quantified. *Dactylellina haptotyla* population density and trapping were most enhanced by the smaller quantity of alfalfa amendment and were not enhanced by the larger quantity of alfalfa amendment. *Arthrobotrys oligospora* population density was most enhanced by the larger quantity of alfalfa amendment, but *A. oligospora* trapped few or no nematodes, regardless of amendment. Trapping and population density were correlated for *D. haptotyla* but not for *A. oligospora.*

Key words: Arthrobotrys oligospora, biological control, *Dactylellina haptotyla, Heterodera schachtii, Steinernema glaseri.*

Although organic amendments to soil may stimulate nematode-trapping fungi and thereby enhance biological control of plant-parasitic nematodes (Linford et al., 1938; Stirling, 1991; Wang et al., 2002), quantitative data supporting this idea are limited. Part of the problem is that fungus population density, and especially trapping, are difficult to quantify in soil. Moreover, trapping usually cannot be inferred simply from suppression of nematodes following organic amendment because such amendments can suppress nematodes in many different ways (Wang et al., 2002; Widmer et al., 2002). One also cannot assume that increases in fungus population density will mean increases in trapping; this may be true for some fungi but not for others (Jaffee, 2003).

The difficulties in quantifying both fungus population density and trapping in field soil are reduced by using 'soil cages', i.e., pipe sections that are packed with soil and amendment, buried in the field, recovered, and assayed in the laboratory (Jaffee, 2002). Soil cages enable the researcher to control the distribution of fungal inoculum and the organic amendment, to replicate and incubate experiments in the field while greatly reducing sampling variability and effort, and finally to measure trapping in undisturbed field soil (intact cores) under controlled, laboratory conditions.

A recent study used soil cages to quantify how nematode-trapping fungi responded to grape leaf amendment in two vineyards (Jaffee, 2002). In one vineyard (site 4), inoculum of the fungus *Dactylellina haptotyla,* which produces adhesive knobs, was also added to the cages. The data from site 4 were inconsistent: grape leaf amendment stimulated *D. haptotyla* population density and trapping in 1 year but not in another. A second vineyard (site 5c) contained a resident population of *Arthrobotrys oligospora,* which produces adhesive networks. The data from site 5c were also inconsistent: grape leaf amendment stimulated *A. oligospora* population density in 1 year but not in another. In contrast to *D. haptotyla, A. oligospora* apparently trapped few or no nematodes in either year.

The reasons for these inconsistent effects on trapping and population density are unclear, and the study described in the current paper explored whether amendment quantity or quality affected consistency. A second objective was to collect more data on the relationship between fungus population density and trapping (Jaffee, 2003). A third objective was to find an amendment that would stimulate trapping by *A. oligospora.*

Materials and Methods

Vineyard soil: Sites 4 and 5cw were mature vineyards near Lodi, California (Jaffee, 1999, 2002, 2003). Site 4 soil was a loamy sand, $pH_{calcium\ chloride}$ 6.5, and organic matter <1%. Site 5cw soil was a sandy loam, $pH_{calcium\ chloride}$ 5.7, and organic matter <1%. Soil was collected adjacent to vines and 5 to 20 cm deep. The soil was passed through a 6-mm sieve to remove roots and other debris and was stored at 10 °C for less than 10 days before experiments were started.

Fungi: In naming fungi, this paper follows the generic concept of Scholler et al. (1999). Thus, *Arthrobotrys* is used for species that produce adhesive networks and *Dactylellina* is used for species that produce stalked, adhesive knobs.

Site 4 soil contained few nematode-trapping fungi but supported the establishment of *D. haptotyla* when that fungus was introduced as assimilative hyphae embedded in alginate pellets (Jaffee 2002, 2003). *Dactylellina haptotyla* was worth studying because trapping by this fungus can be directly quantified (nematodes with adherent knobs can be extracted from soil) and be-

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cause the fungus is capable of trapping large proportions of nematodes (Jaffee, 2000, 2003). Pellets containing *D. haptotyla* (ATCC 204099) were produced as previously described (Jaffee, 1999), and each dry pellet contained about 0.6 mg of hyphae, weighed 1.9 mg, and was 2.1 mm in diam.

Site 5cw soil contained small numbers (<10 propagules/g of soil) of the following trapping fungi: *Arthrobotrys oligospora, A. eudermata, A. thaumasia,* and *D. ellipsospora. Arthrobotrys oligospora* (ATCC MYA-2480) was emphasized because it occurs naturally in site 5cw soil and is presumably adapted to that soil environment. In addition, *A. oligospora* is a commonly encountered and commonly studied nematode-trapping fungus (Stirling, 1991). Trapping by *A. oligospora,* however, cannot be directly quantified because trapped nematodes cannot be extracted from soil; trapping is inferred based on reduced extraction of nematodes (Jansson, 1982).

Organic amendments: A previous study (Jaffee, 2002) used only grape leaves (C:N of 28 to 30:1) at a rate equivalent to 4,500 kg/ha. In the current study, two organic amendments (grape leaves and alfalfa leaves, both without pesticide treatment) and two rates (equivalent to 4,500 and 9,000 kg/ha) were compared. Grape leaves were collected in November 2000 for experiments 4a and 5a (Table 1) and in October 2002 for experiments 4b and 5b, cut into small segments, air dried, and stored at room temperature; each dried segment was about 60 mm^2 in area and 5 mg in mass. Grape leaf C:N was 28:1 in experiments 4a and 5a (1.5% N and 42.2% C) and 27:1 in experiments 4b and 5b (1.6% N and 42.7% C). Carbon and nitrogen contents of leaves were determined by the University of California DANR Analytical Laboratory using the Carlo Erba combustion method.

Vigorously growing alfalfa shoots were collected in June 2001 for experiments 4a and 5a and in June 2002 for experiments 4b and 5b; alfalfa leaves were removed and leaflets were separated, air dried, and stored at room temperature; each dried leaflet was about 100 mm^2 in area and 10 mg in mass. Although the alfalfa leaflets were initially larger than the grape leaf segments, the alfalfa leaflets fragmented more frequently than did grape leaf segments when mixed with soil. As expected, the dried alfalfa leaves contained more nitrogen than did the grape leaves; alfalfa leaf C:N was 8:1 in experiments 4a and 5a (6.0% N and 44.8% C) and also in experiments 4b and 5b (5.8% N and 44.9% C).

Experiments: Independent experiments were conducted at site 4 and site 5cw with *D. haptotyla* and *A. oligospora,* respectively (Table 1). For experiments 4a and 4b, the water content of the collected site 4 soil was adjusted to 8.0% (grams of water/100 grams of dry soil), which was equivalent to about −16 kPa based on the water release curve for site 4 (Jaffee, 1999); this water level was selected so that the soil was moist at the start of the experiment but not so moist that it would compact excessively when placed in cages. After the moistened soil was mixed, dry grape or alfalfa leaves (0.0 mg, 180 mg, or 360 mg) were mixed into 60-g portions of soil (dry weight equivalent); on a dry weight basis, the soil received 0%, 0.3%, or 0.6% organic amendment. Each 60-g portion of soil was then packed into a polyvinyl chloride (PVC) pipe (about 1.5 g of soil/cm^3), which was sealed at one end with fiberglass window screen (hole diam. 2 mm). Each pipe or section was 3 cm long and had an inner diameter of 3.9 cm. A wood applicator was inserted into the center of the soil mass, one pellet of *D. haptotyla* was added, and the hole was filled in. Two pipes or sections were then taped together to form one cage, with screen covering the two outer ends. Therefore, each cage was 6 cm long and contained 80 cm^3 of soil (120 g dry weight equivalent); 0 mg, 360 mg, or 720 mg dry alfalfa or grape leaves; and two *D. haptotyla* pellets. Each cage was immediately sealed in a plastic bag and kept cool until it was buried in the field. There were six replicate cages for each of the five treatments (no leaf amendment or small or large quantities of grape or alfalfa leaves, all with *D. haptotyla* pellets). Cages without *D. haptotyla* pellets were not included because I lacked sufficient resources to double the size of the experiment and because I wanted to determine whether organic amendments enhanced *D. haptotyla* population density and trapping, both of which were directly assessed. Inclusion of treatments with organic amendments but without *D. haptotyla* would indicate how organic amendments affected resident natural enemies, but I assumed that trapping fungi naturally present in the soil would not confound the results because so few natural en-

TABLE 1. Experiments conducted in this study.

Experiment	Fungus added	Site	Number of days cages were in field[a]	Soil temperature[b]	Degree days (base 10 °C)	Rain or irrigation[c]
4a	*Dactylellina haptotyla*	4	25	11,25,18	191	4/0
4b	*D. haptotyla*	4	34	11,21,16	191	42/5
5a	*Arthrobotrys oligospora*	5cw	29	13,24,19	245	19/1
5b	*A. oligospora*	5cw	39	10,21,15	195	66/7

[a] Cages were buried in the field on 25 March 2002, 24 March 2003, 28 March 2002, and 27 March 2003 for experiments 4a, 4b, 5a, and 5c, respectively.
[b] Minimum, maximum, and mean soil temperature (°C) at 20-cm depth.
[c] Total rain or irrigation (mm)/number of days with rainfall or irrigation > 5 mm. Site 5cw was irrigated once during experiment 5a; no other irrigations occurred during this study.

emies were detected in preliminary assays (Jaffee, unpubl. data). The cages and two temperature loggers (Hobo-Temp model, Onset Computer Corporation, Pocasset, MA) were buried in vineyard 4 the same day they were prepared. Cages were buried between vines in blocks and were oriented with open ends at the top and bottom, with about 2 cm between adjacent cages in a block and with about 19 cm between the top of the cage and the soil surface. Soil temperature was recorded hourly.

Experiments with *A. oligospora* in site 5cw soil were similar to those with *D. haptotyla* in site 4 soil except that *A. oligospora* rather than *D. haptotyla* pellets were added and site 5cw soil was packed at 9% water content (−28 kPa). To convert water content to water potential for site 5cw soil, the water release curve was determined with a pressure plate; at 0, −10, −30, −100, −500, and −1,500 kPa, the water content was 31.8%, 14.6%, 8.2%, 6.5%, 3.5%, and 2.7%, respectively. In contrast to experiments with *D. haptotyla,* those with *A. oligospora* included controls without pellets.

Cages were removed from the field after 190 to 250 degree days (base 10 °C). This was sufficient time for organic amendments to affect the organisms but not so much time that the effect might disappear (Cooke, 1963b; Jaffee, 2002). Rainfall also influenced when cages were collected because cages are difficult to assay if the soil has not drained for 2 days after a heavy rain. Once removed from soil, cages were sealed in plastic bags and returned to the laboratory in an ice chest.

Quantification of trapping, nematodes, and enchytraeids: In the laboratory, each cage was separated into its two sections. Although the soil had fused into a cylinder, it usually fractured at the plane where the two sections were taped together. Top sections were stored at 10 °C until they could be assessed for fungus population density and water content. Bottom sections were used to quantify trapping by adding, recovering, and examining assay nematodes. Assay nematodes rather than resident nematodes were used to obtain controlled and reproducible estimates of trapping, and selection of assay nematode species requires explanation. The assay nematode should ideally have the following characteristics: (i) it should be susceptible to the control agents in question; (ii) it should be economically important or at least representative of an economically important pest nematode; (iii) to avoid confusion with resident nematodes, the assay nematode should not be naturally present in soil, else exposure time in soil and therefore the probability of encountering a trap would be unknown; (iv) to reduce assessment time, the assay nematode should be easy to detect in the extracted sample; and (v) the assay nematode should be uniform and easy to obtain in large numbers.

Although economically important and susceptible to both *D. haptotyla* and *A. oligospora,* a *Meloidogyne* species (root-knot nematode) was not used because *Meloidogyne* spp. occur naturally in both soils and because individuals of *Meloidogyne* spp. are relatively easy to miss in extracted samples. *Heterodera schachtii* (the sugarbeet cyst nematode) was used to assay for trapping by *D. haptotyla* because it is in many ways similar to root-knot nematodes but is not naturally present in site 4 soil and is much easier to detect in extracted samples. Cyst nematodes are less susceptible than root-knot to fungi that use adhesive traps (Jaffee, 1998) but are sufficiently susceptible for the purposes of this study. Second-stage juveniles (J2) of *H. schachtii* were obtained by placing cysts on Baermann funnels; the collected J2 were aerated and added to soil within 6 hours.

The entomopathogenic nematode *Steinernema glaseri* was used to assay for trapping by *A. oligospora* because it is susceptible to *A. oligospora* (Koppenhöfer et al., 1996) and does not occur naturally in site 5 soil. Infective juveniles (IJ) of *S. glaseri* were obtained from nematode-infected wax moths (Kaya and Stock, 1997) and were stored at 10 °C for less than 1 month before addition to soil.

Assay nematodes in 0.5 ml dilute (4 mM) KCl were added to the exposed soil surface of bottom sections, except that 1.0 ml was used for experiment 4a because the soil was dry. Bottom sections received 1,767 *H. schachtii* J2 (experiment 4a), 1,665 *H. schachtii* J2 (experiment 4b), 86 *S. glaseri* IJ (experiment 5a), or 107 *S. glaseri* IJ (experiment 5b). Fewer *S. glaseri* than *H. schachtii* were added because *S. glaseri* IJ are larger than *H. schachtii* J2 and have a substantially higher extraction efficiency. Using wet sieving and sugar flotation, my laboratory typically recovers about 50% to 60% of added *S. glaseri* and 25% to 35% of added *H. schachtii* (Jaffee, unpub. data). Bottom sections were then sealed in plastic bags, and after 2.8 days at 20 °C nematodes were extracted by wet sieving followed by sugar flotation (Jenkins, 1964). For experiments with *D. haptotyla,* the extracted sample was reduced to 10 ml and shaken vigorously to separate nematodes from hyphae and debris. Then 1 ml was examined with a dissecting microscope at ×70 to ×140 magnification to determine the number of *H. schachtii* with and without adhering knobs. Other nematodes, identified to trophic group (Yeates et al., 1993), and enchytraeids were also counted. If the number of assay nematodes in the first milliliter examined was less than 20, an additional milliliter was examined so that the percentage of assay nematodes with knobs was based on examination of at least 20 nematodes in each sample. For experiments with *A. oligospora,* the extracted sample was reduced to 20 ml and shaken vigorously. All 20 ml were examined at ×30 to ×70 magnification, and *S. glaseri* with and without adhering hyphae and enchytraeids were counted. The sample was then reduced to 10 ml, 1 ml of which was examined to quantify numbers of other nematodes by trophic group.

The percentage of *H. schachtii* with and without

knobs was calculated and was used as a measure of trapping activity within each sample and without reference to a control that received no *D. haptotyla.* This was possible because nematodes with *D. haptotyla* can be extracted from soil (Jaffee, 1998) and because *D. haptotyla* has not been detected and other knob-forming fungi have only been rarely detected in this soil (Jaffee, unpub. data). In contrast, nematodes caught in adhesive networks are not readily extracted from soil, and researchers have used reduction in extraction from soil as a measure of trapping by *A. oligospora* (Jansson, 1982). To calculate reduction, one must compare extraction in the presence and absence of the fungus. Consequently, treatments without pellets were included in experiments 5a and 5b. Reduction in extraction was calculated as x/y *100, where x is the number of *S. glaseri* recovered from the section and y is the mean number of *S. glaseri* extracted from sections receiving no pellets or leaf amendments. The extracted *S. glaseri* were also examined for adhering adhesive hyphae (portions of networks), but such nematodes were never seen.

Quantification of fungus population density and soil water content: Soil in the top section of each cage was placed in a plastic bag and mixed, and 10 g (dry weight equivalent) was placed in a sterile 125-ml flask. The volume in the flask was increased to 50 ml with sterile distilled water, and the flask was sealed and shaken for 8 minutes on a wrist-action shaker. A 10-fold dilution series was then prepared, and 0.1 ml of each dilution was deposited on each of five replicate petri dishes containing quarter-strength corn meal agar; each dish therefore received about 0.02, 0.002, or 0.0002 g of soil, depending on the dilution. Each dish also received about 1,000 bait nematodes (*S. glaseri* IJ) to increase the chance of detecting nematode-trapping fungi. After 3 weeks at 22 °C, the dishes were examined at ×30 to ×70 magnification. Nematode-trapping fungi were identified (Cooke and Godfrey, 1964), and the qualitative pattern of detection was converted into a quantitative estimate with a most probable number program (Klee, 1993). Soil water content (grams of water/100 grams of dry soil) was determined by weighing a 15-g soil sample before and after drying.

Statistical analyses: SAS release 8.02 (SAS Institute Inc., Cary, NC) was used. Before analysis, counts and percentages were transformed (log and arcsine, respectively). Analysis of variance was used to determine the significance of main effects, interactions between main effects, and treatment effects. When main effects were significant ($P \leq 0.05$), Duncan's multiple-range test was used to separate means ($P = 0.05$). Although experiment 4b was essentially a repeat of 4a and 5b was a repeat of 5a, the experiments were analyzed separately and are presented separately because some results differed between years. Block effects were seldom significant and are not presented. Correlation analysis was used to determine the relationship between fungus population density and trapping.

Results

Weather: Because spring 2003 was wetter and cooler than spring 2002 (Table 1), cages were left in the field longer in 2003. Degree-day accumulation was identical in experiment 4a and 4b but was substantially less in 5b than in 5a (Table 1).

Experiments with D. haptotyla: In experiment 4a, the smaller but not the larger quantity of alfalfa leaves enhanced *D. haptotyla* population density (Fig. 1A); grape leaves also enhanced *D. haptotyla* population density but less than did the smaller quantity of alfalfa leaves (Fig.

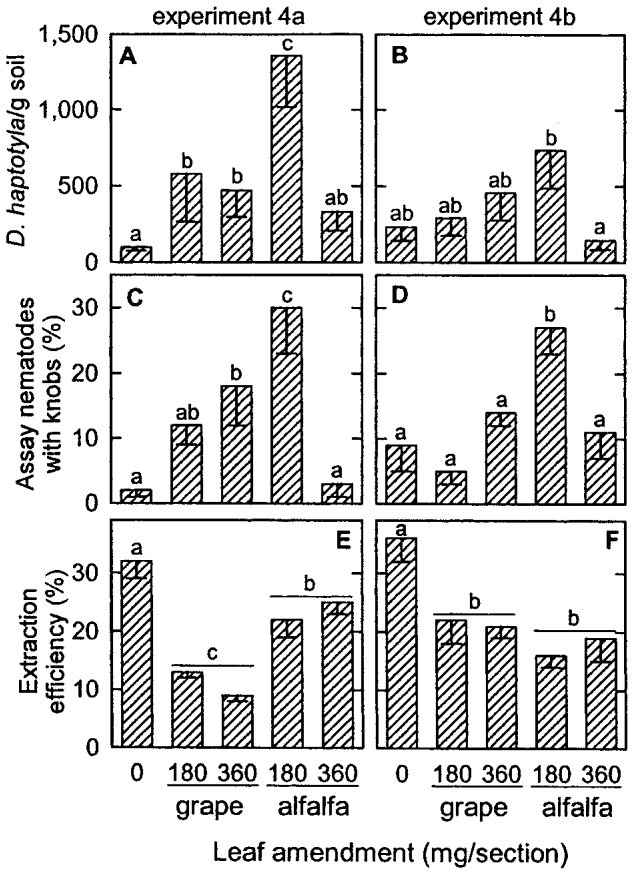

Fig. 1. Effect of organic amendments on the population density of *Dactylellina haptotyla,* trapping of assay nematodes by *D. haptotyla,* and extraction efficiency of assay nematodes (experiments 4a and 4b). Soil containing pellets of *D. haptotyla* and one of three levels of an organic amendment (0, 360, or 720 mg of grape leaves or alfalfa leaves) was packed into soil cages (two sections/cage; 60 g of soil/section; and 0, 180, or 360 mg of amendment/section). The cages were buried in the field, recovered after 24 days (experiment 4a) or 35 days (experiment 4b), and then returned to the laboratory for analyses. Values are means of six replicate cages; vertical lines indicate one standard error. Mean separation was done with Duncan's multiple-range test, and bars within the same panel and with the same lowercase letters are not different ($P > 0.05$). For Fig. 1A–D, mean separation refers to specific combinations of amendment and amendment level; for Fig. 1E,F, mean separation refers to amendment averaged over amendment levels because the effect of amendment level was not significant.

1A). In experiment 4b, *D. haptotyla* population density was again greater with the smaller than with the larger quantity of alfalfa leaves (Fig. 1B), but other comparisons were not significant. In a combined analysis of experiments 4a and 4b, *D. haptotyla* population density was greater with the smaller quantity of alfalfa leaves than with the other treatments. Small numbers (usually <10 propagules/g of soil) of the following nematode-trapping fungi were occasionally detected: *A. eudermata, A. thaumasia, A. oligospora, D. leptospora,* and *Nematoctonus* sp.

Trapping of assay nematodes by *D. haptotyla* was enhanced by the smaller quantity of alfalfa leaves in both experiments and by the larger quantity of grape leaves in the first experiment; other effects were not significant (Fig. 1C,D).

Extraction efficiency for *H. schachtii* assay nematodes was 32% to 35% in the nonamended soil, which is similar to that with heat-treated soil (Jaffee, unpub. data) and therefore indicates that the nonamended soil contained few antagonists of *H. schachtii* J2. Extraction efficiency was substantially less in the amended soil, especially in grape leaf-amended soil in experiment 4a (Fig. 1E). Many grape leaf fragments but few alfalfa leaf fragments were evident when nematodes were extracted from soil in experiments 4a and 4b.

Organic amendments in experiments 4a and 4b always increased numbers of bacterivorous nematodes (Fig. 2A,B) and usually increased numbers of fungivorous nematodes (Fig. 2C,D) and omnivorous nematodes (Fig. 2E,F). Numbers of fungivorous nematodes tended to be greater with grape leaves than alfalfa leaves (Fig. 2C,D), but the trend was opposite with bacterivorous nematodes (Fig. 2A,B). As with *D. haptotyla* population density and trapping, numbers of omnivorous nematodes were more enhanced by the smaller than the larger quantity of alfalfa (Fig. 2E,F). Plant-parasitic nematodes detected in experiments 4a and 4b included species of *Meloidogyne, Mesocriconema,* and *Trichodorus;* numbers were small and variable and were unaffected by the organic amendments (Fig. 2G,H). Enchytraeids were enhanced by both kinds of amendments, but more by alfalfa than grape leaves (Fig. 2I,J).

Experiments with A. oligospora: In experiments 5a and 5b, *A. oligospora* population density was always substantially greater when it was added to soil than when it was not (Fig. 3A,B) and was further enhanced by the addition of any organic amendment in experiment 5a (Fig. 3A) and by the larger quantity of alfalfa leaves in experiment 5b (Fig. 3B). Although other nematode-trapping fungi were detected, greater than 99% of the nematode-trapping fungi detected in soil receiving *A. oligospora* pellets were *A. oligospora.*

In soil that did not receive *A. oligospora* pellets, organic amendments enhanced the population densities of resident nematode-trapping fungi. For example, in experiment 5b and in soil that did not receive *A. oligospora* pellets, the population density (mean ± SE) of nematode-trapping fungi was 5 ± 5 (nonamended soil), 30 ± 9 (180 mg grape leaves), 62 ± 17 (360 mg grape leaves), 32 ± 8 (180 mg alfalfa leaves), and 86 ± 25 (360 mg alfalfa leaves). Although *A. oligospora* was the most common trapping fungus in these treatments, the following fungi were also detected: *A. conoides, A. eudermata, A. musiformis, A. superba, A. thaumasia, D. ellipsospora, D. haptotyla, Myzocytium* sp., and *Nematoctonus* sp.

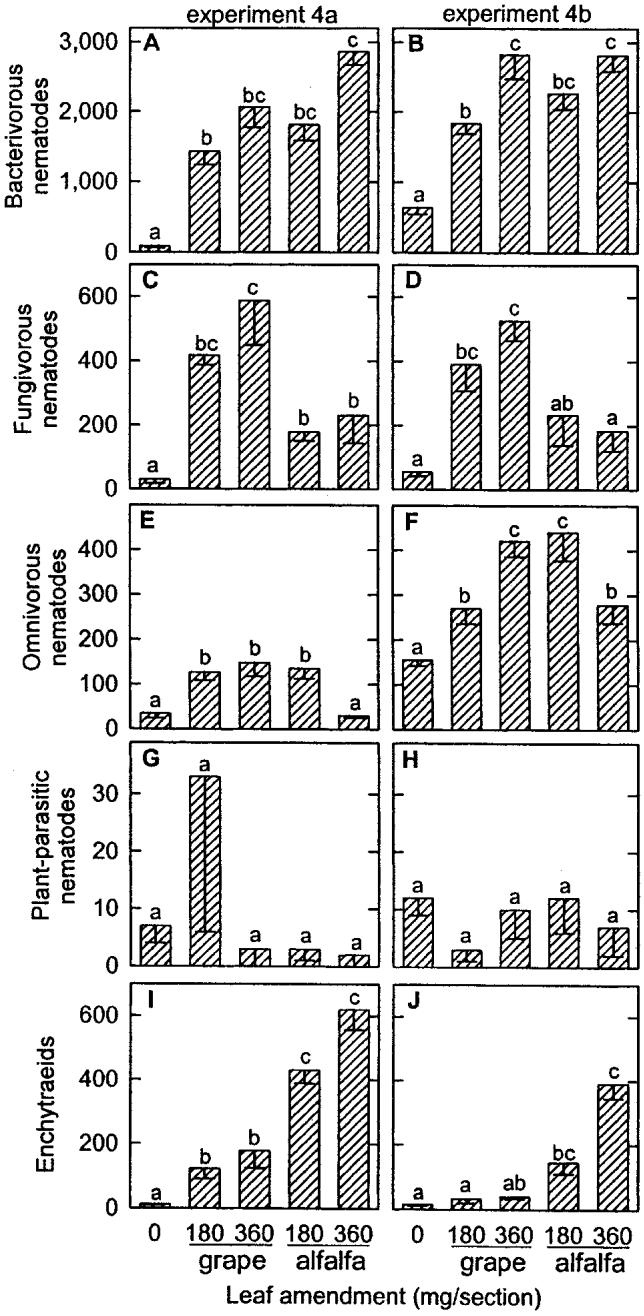

FIG. 2. Effect of organic amendments on resident nematodes and enchytraeids in experiments 4a and 4b. Values indicate mean numbers per section (60 g of soil/section and two sections/cage) from six replicate cages; vertical lines indicate one standard error. Mean separation was done with Duncan's multiple-range test, and bars within the same panel and with the same lowercase letters are not different ($P > 0.05$).

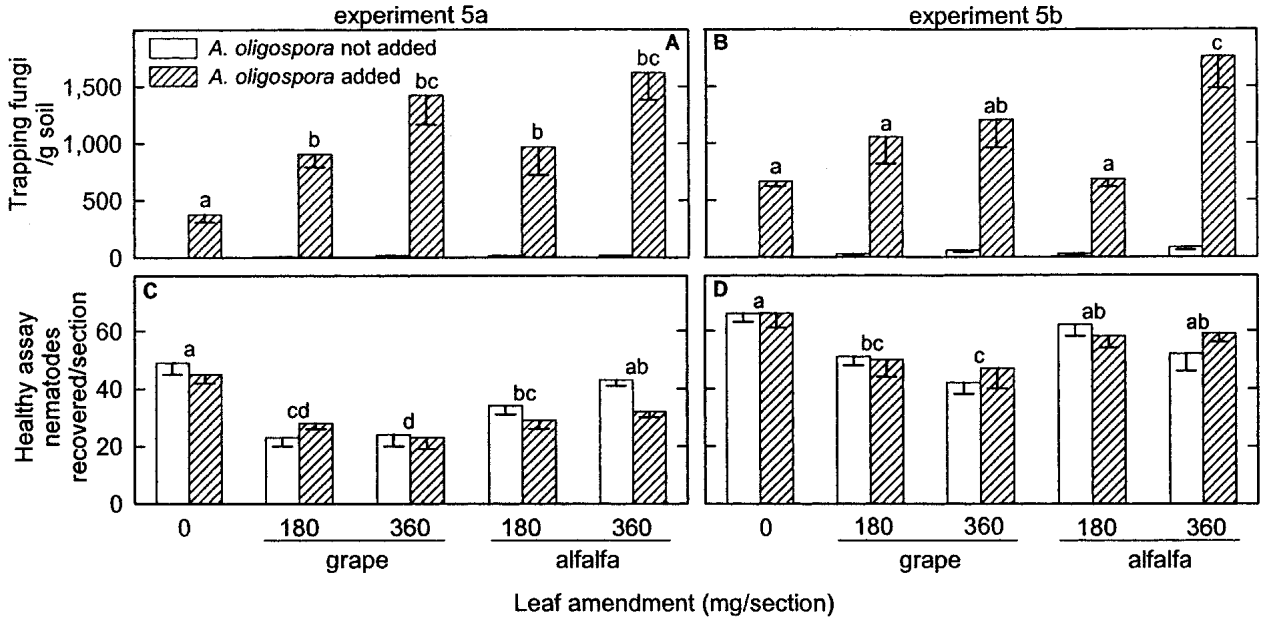

FIG. 3. Effect of organic amendments and *Arthrobotrys oligospora* pellets on the population density of nematode-trapping fungi and on the number of healthy assay nematodes recovered (experiments 5a and 5b). Soil without or with pellets of *A. oligospora* and one of three levels of an organic amendment (0, 360, or 720 mg of grape leaves or alfalfa leaves) was packed into soil cages (two sections/cage; 60 g of soil/section; and 0, 180, or 360 mg of amendment/section). The cages were buried in the field, recovered after 29 days (experiment 5a) or 39 days (experiment 5b), and then returned to the laboratory for analyses. Values are means of six replicate cages; vertical lines indicate one standard error. Mean separation was done with Duncan's multiple-range test, and bars within the same panel and with the same lowercase letters are not different ($P > 0.05$). In Fig. 3A,B the mean separation refers only to cages with pellets because cages without pellets had much smaller numbers of nematode-trapping fungi than did those with pellets. In Fig. 3C,D the mean separation refers to the effects of specific combinations of amendment and amendment level averaged over pellet level because the effect of pellets was not significant.

The number of healthy assay nematodes extracted was similar in soils with and without *A. oligospora* pellets (Fig. 3C,D), and the statistical analysis therefore ignores the effect of pellet and focuses on the effect of amendment. Almost all assay nematodes extracted from soil appeared healthy and lacked adherent hyphae. The number of healthy assay nematodes recovered was consistently reduced by grape leaves and sometimes reduced by alfalfa leaves (Fig. 3C,D). The extraction efficiency for *S. glaseri* assay nematodes in nonamended soil was 55 ± 3% in experiment 5a and 62 ± 3% in experiment 5b. Note that fewer healthy nematodes were detected in experiment 5a (Fig. 3C) than 5b (Fig. 3B) because fewer assay nematodes were added in experiment 5a.

Arthrobotrys oligospora pellets did not affect resident nematodes or enchytraeids (Fig. 4), except in two cases: more fungivorous nematodes were present in experiment 5b (Fig. 4D) and more omnivorous nematodes were present in experiment 5a (Fig. 4E) when the larger quantity of alfalfa leaves was added with pellets rather than without pellets. In all other cases, there was no effect of pellet nor was there a significant interaction between pellet and treatment, and a second analysis was done, which included treatment and block but excluded pellet and the interaction term as independent variables. As in experiments 4a and 4b, organic amendments and especially alfalfa enhanced numbers of bacterivorous nematodes (Fig. 4A,B), fungivorous nematodes (Fig. 4C,D), and omnivorous nematodes (Fig. 4E,F). Plant-parasitic nematodes were suppressed by alfalfa amendments in experiment 5a (Fig. 4G) but not in experiment 5b (Fig. 4H). Enchytraeids were enhanced by organic amendments and especially by alfalfa in both experiments (Fig. 4I,J).

Correlations between trapping and population density: The mean percentage of assay nematodes with adherent knobs was correlated with *D. haptotyla* population density in experiments 4a and 4b and in a combined analysis including data from two previously published experiments (Fig. 5). The percentage of assay nematodes trapped by *A. oligospora* was unrelated to *A. oligospora* population density ($P > 0.05$) because large increases in *A. oligospora* population density were not associated with any change in recovery of healthy assay nematodes.

DISCUSSION

Because they were studied separately and in different vineyards, *D. haptotyla* and *A. oligospora* will be discussed separately and with few comparisons. The *A. oligospora* data are puzzling. Its population density was greatly increased by adding a small quantity of fungal inoculum to field soil and was further increased by organic

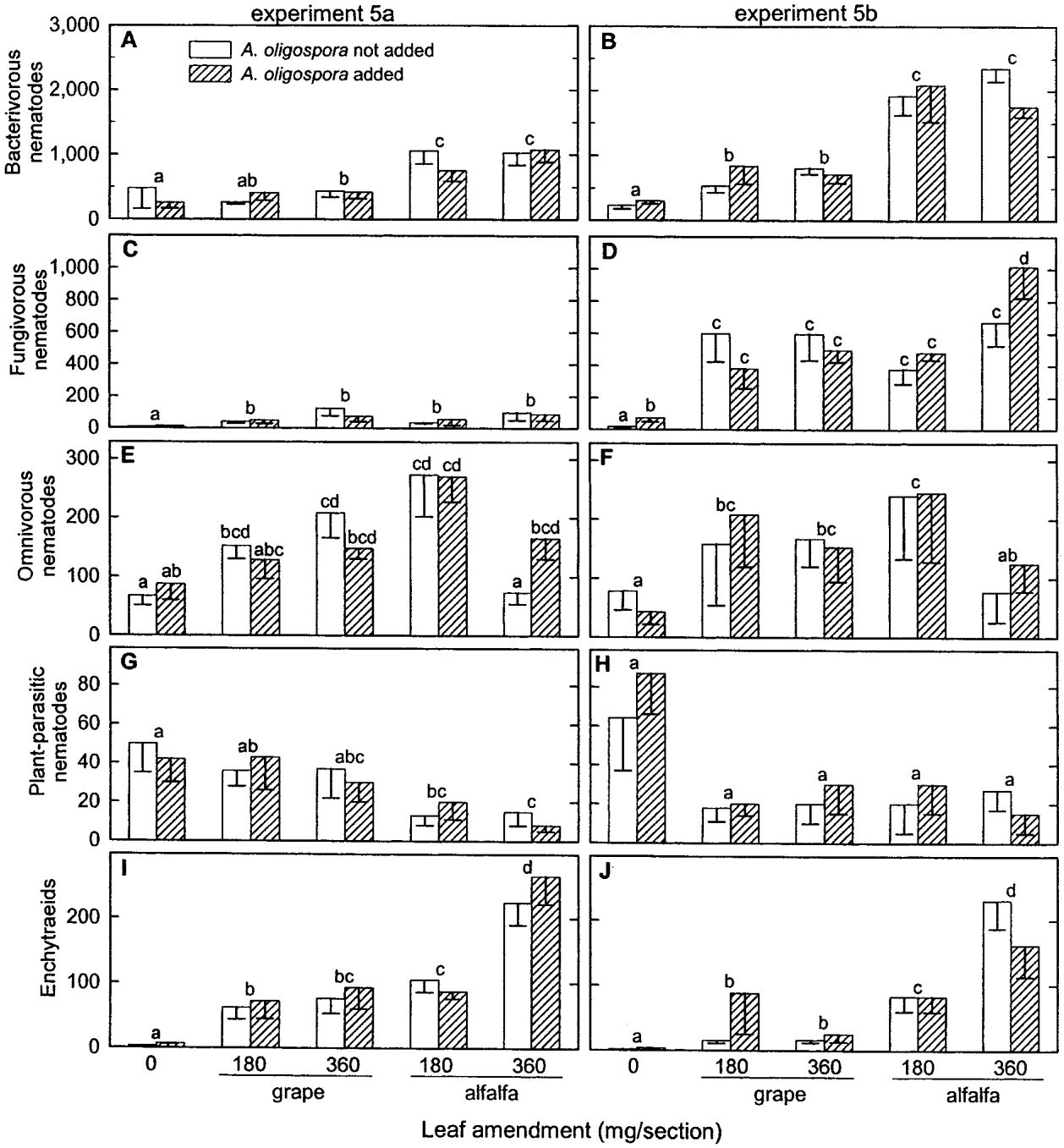

FIG. 4. Effect of organic amendments and *Arthrobotrys oligospora* pellets on resident nematodes and enchytraeids in experiments 5a and 5b. Values indicate mean numbers per section (60 g of soil/section and two sections/cage) from six replicate cages; vertical lines indicate one standard error. Mean separation was done with Duncan's multiple-range test, and bars within the same panel and with the same lowercase letter are not different ($P > 0.05$). In all panels but D and E, the mean separation refers to the effects of specific combinations of amendment and amendment level averaged over pellet level because the effect of pellets was not significant. In panels D and E, the interaction between pellet and alfalfa level was significant, and the mean separation refers to specific combinations of amendment, amendment level, and pellet level.

amendment, but it apparently trapped no assay nematodes. Either *A. oligospora* produced few traps, the traps were short lived, or the traps failed to contact or adhere to assay nematodes in soil.

In other studies, *A. oligospora* and related network-forming fungi did suppress nematodes. Some of these studies, however, were not done in field soil but in agar cultures (Galper et al., 1995), in fresh animal feces (Wolstrup et al., 1996), or in sterilized soil (Jansson, 1982). I had considered that the key element of agar cultures, fresh feces, and sterile soil might be a simplified microbial community—one that permitted *A. oli-*

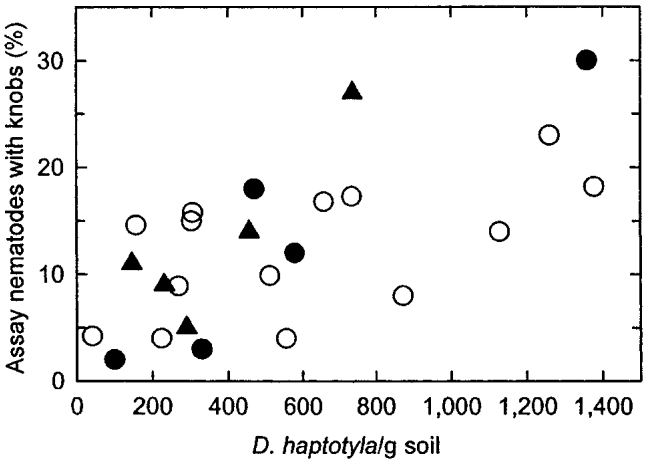

FIG. 5. Correlations between the population density of *Dactylellina haptotyla* and the percentage of assay nematodes with adherent knobs. Values are the means of six replicate cages. Experiment 4a (●, r = 0.93, P = 0.02), experiment 4b (▲, r = 0.88, P = 0.05). Previously published data (○) are included from experiments involving *D. haptotyla* and grape leaf amendments in site 4 soil (Jaffee, 2002). According to correlation analysis for all data in Fig. 5 (n = 24), r = 0.66 and $P < 0.01$.

gospora to grow and persist. I further expected that if *A. oligospora* grew and persisted in field soil, it would also trap nematodes in that more complex environment. This report and previous ones (Jaffee, 2002, 2003) indicate otherwise.

A few studies reporting nematode suppression by *A. oligospora* did use nonsterile soil. Bouwman et al. (1994, 1996) found that numbers of bacterivorous nematodes were reduced in the presence of *A. oligospora,* but *A. oligospora* population density was not quantified and the soil was initially sterilized. Moreover, numbers of bacterivorous nematodes were measured over many weeks, and such long-term changes in resident nematodes, as opposed to the short-term changes exhibited by assay nematodes, can reflect many factors influencing nematode birth and death rates. Cooke (1963b) even reported that *A. oligospora* enhanced rather than suppressed numbers of resident nematodes in soil microcosms. On the other hand, Koppenhöfer et al. (1996) did use field soil and short-term assays for trapping and did infer that *A. oligospora* trapped substantial numbers of nematodes. Reconciling these discrepancies requires better data on trap production and longevity in soil, i.e., a better understanding of *A. oligospora* ecology.

Unlike *A. oligospora, D. haptotyla* consistently trapped assay nematodes in field soil. While the fungi were studied independently here, the data were similar when these fungi were studied together in site 5 soil without organic amendment: *A. oligospora* trapped few *S. glaseri* and *D. haptotyla* trapped many (Jaffee, 2003). In the current study, *D. haptotyla* trapped fewer than 35% of the assay nematodes, but the assay nematode was *H. schachtii,* which is not very susceptible to fungi with adhesive traps like those of *D. haptotyla. Dactylellina haptotyla* would probably have trapped many more assay nematodes had *Meloidogyne* spp. or *S. glaseri* been used (Jaffee, 1998, 2000, 2003; Jaffee and Muldoon, 1995a).

The two fungi also responded differently to the organic amendments. *Arthrobotrys oligospora* population density was consistently enhanced by the larger quantity of alfalfa leaves, and it seems possible that larger quantities of amendment could have resulted in even larger numbers of *A. oligospora. Dactylellina haptotyla* population density and trapping, however, were most enhanced by the smaller quantity and least enhanced by the larger quantity of alfalfa leaves. The biology underlying these results is unknown, but perhaps the larger quantity of alfalfa produced fungicidal compounds or enhanced fungivores so that the positive effects of the amendment on *D. haptotyla* were balanced by the negative ones. Although the responses of the trapping fungi also could be mediated through the resident nematodes (Boogert et al., 1994), resident bacterivorous and fungivorous nematodes did not respond differently to the two levels of alfalfa amendment in site 4 soil. But like *D. haptotyla,* resident omnivorous nematodes were more stimulated by the smaller than the larger quantity of alfalfa leaves. It's unclear why omnivorous nematodes and *D. haptotyla* would respond similarly to alfalfa amendments.

If one accepts that knob-forming fungi like *D. haptotyla* depend not on saprophytism but on parasitism of nematodes for carbon, energy, and nutrients (Cooke, 1963a; Jansson and Nordbring-Hertz, 1980), one could speculate that the smaller quantity of alfalfa stimulated bacteria, which in turn stimulated bacterivorous nematodes, which finally supported a numerical response by *D. haptotyla* (Jaffee and Muldoon, 1995b). This explanation would agree with that presented by Linford and colleagues (Linford et al., 1938; Linford and Yap, 1939), who also suggested that *D. ellipsospora* was the most important trapping fungus in Hawaiian pineapple soils. Like *D. haptotyla, D. ellipsospora* produces adhesive knobs and can trap large proportions of assay nematodes in site 5 soil (Jaffee, 2003). To provide evidence that this explanation is valid, data are needed that show how the resident nematodes support a numerical response by *D. haptotyla* (Boogert et al., 1994).

Unfortunately, the organic amendments frequently reduced the extraction efficiency of assay nematodes. In experiments with *D. haptotyla,* it seems unlikely that this reduction resulted from increased trapping because most assay nematodes with attached *D. haptotyla* knobs are readily extracted from soil (Jaffee, 2003). In experiments with *A. oligospora,* controls without the fungus indicated that *A. oligospora* was not responsible for reduced extraction efficiency. I suspect that the assay nematodes became enmeshed with nondecomposed leaf fragments and were lost during extraction, but predation by omnivorous nematodes or by an undetected

natural enemy is also possible. Toxicity seems an unlikely explanation because the assay nematodes were in the soil for too little time to decompose. Regardless of the cause, the reduction in extraction efficiency by unknown mechanisms is undesirable because it complicates the estimation of trapping.

Concluding with some positive statement about the potential for biological control is a cliché to be avoided, especially for studies like this one, which although done in field soil and partially in the field was still quite removed from farming reality. But the study does reveal some interesting and useful questions. Why did *A. oligospora* trap so few nematodes in these experiments? Is the increase in *D. haptotyla* number and activity following organic amendment based on *D. haptotyla* saprophytism or on its numerical response to increases in bacterivorous nematodes? Can *D. haptotyla* and similar fungi protect seedling crops from plant-parasitic nematodes?

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