Correlations Between Most Probable Number and Activity of Nematode-Trapping Fungi

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ABSTRACT

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Soil cages were used to determine whether nematode-trapping fungi population density, as measured by most probable number (MPN) procedures, was correlated with the trapping of nematodes. Fungi studied (and trap type) were *Arthrobotrys oligospora* (adhesive networks), *A. eudermata* (adhesive networks), *A. dactyloides* (constricting rings), *Dactylellina ellipsospora* (adhesive knobs), and *D. haptotyla* (adhesive knobs). The fungi were formulated as assimilative hyphae in dried alginate pellets. Pellets were added to field soil, the soil was packed into 80-cm³ cages (PVC pipe, 3.0 cm long and 3.9 cm in diameter), and the cages were

buried in vineyards. After 14 to 61 days, the cages were recovered, and MPN data and trapping activity were determined. For all five fungi, MPN data were correlated with the number of pellets added. Regardless of fungus population density, *A. oligospora* and *A. eudermata* trapped few if any nematodes in soil, and consequently, trapping and fungus population density were not correlated. The correlation between population density and trapping was weak for *A. dactyloides* but relatively strong for *D. ellipsospora* and *D. haptotyla*. High levels of trapping by the latter two fungi required more than 10² fungus propagules per gram of soil.

Additional keywords: *Heterodera schachtii*, plant-parasitic nematodes, *Steinernema glaseri*.

To quantify nematode-trapping fungi in soil, and thereby better understand their ecology and possible use as control agents, researchers have sometimes used a most probable number (MPN) procedure (4). The procedure is time consuming and tedious (22) but also simple. Bait nematodes and aliquots from a soil dilution series are added to replicated, nutrient-poor agar plates, which after incubation are examined microscopically and scored for the presence or absence of nematode-trapping fungi. Published MPN tables or computer programs are then used to convert the qualitative pattern of presence and absence into a quantitative estimate of population density (1,18,27).

Although lacking both the literal and figurative glow of biotechnology—examining organisms on the stage of a light microscope rather than nucleic acids or their products on a gel almost seems antitechnological—this traditional procedure (1) has nevertheless produced useful quantitative data. The MPN procedure has revealed how the population densities of trapping fungi are affected by season (19), soil depth (19), fungivores (8), feces (5), roots (6,20,21), nematicides (24,25), and cover crop amendments (10,11,23–26). Unfortunately, we don't know how these MPN estimates of population density relate to trapping activity in soil. This paper describes that relationship for five nematode-trapping fungi.

MATERIALS AND METHODS

Sites and soils. Experiments were conducted in two mature vineyards located near Lodi, CA, designated site 4 and site 5c in previous reports (8,10). Site 4 soil is a loamy sand, 0.4% organic matter, pH_{calcium chloride} 6.4; and site 5c soil is a sandy loam, 1.0% organic matter, pH_{calcium chloride} 4.8 (8,10). Moisture release data for site 4 soil have been published (8), and data for site 5c were as

follows: at 0, –10, –30, –100, –500, and –1,500 kPa, the percentage of water (grams of water per 100 g of dry soil) was 30.9, 10.9, 8.4, 7.5, 5.3, and 3.7, respectively. Soil was collected 5 to 20 cm deep adjacent to vines, sieved (6-mm openings), and stored less than 14 days at 10°C.

Fungi. Three of the fungi (*Dactylellina ellipsospora*, *Arthrobotrys oligospora*, and *A. eudermata*) were isolated from site 5, which is adjacent to site 5c, and were studied at site 5c (Table 1). These fungi were commonly observed in soil samples from sites 5 and 5c and are therefore considered resident to both. Site 5 was not used because it had recently been fumigated with methyl bromide; by reducing antagonism, fumigation could increase establishment of the added fungi (8,9,13,14,22) and could alter the relationship between fungus population density and trapping. Two nonresident fungi (*D. haptotyla* and *A. dactyloides*), which were isolated from tomato fields in Davis, CA, were studied at site 4 (Table 1), because that soil contained few resident trapping fungi.

Fungi were maintained on quarter-strength cornmeal agar (CMA/4) and were formulated as assimilative hyphae in alginate pellets coated with sand (8). Each dried pellet contained about 0.6 mg of dried hyphae (but no added nutrients), was about 2 mm in diameter, and weighed about 1.9 mg. Pellets were stored in glass vials at –1°C for up to 30 days. At the start of each experiment, pellet viability was tested by placing 50 pellets on CMA/4; after 4 to 7 days at 20°C, pellets were examined at ×100 magnification. Fungi grew from all pellets and produced characteristic conidiophores and conidia.

Pellet viability was also tested in site 4 and site 5c soil as follows. Pellets were placed in the bottom of a 6-cm diameter petri dish and covered with 1.5 cm of soil at –20 to –30 kPa. After 7 days at 20°C, the pellets were examined through the bottom of the dish using reflected light and a dissecting microscope. In all cases, hyphae grew from pellets and into the soil and produced characteristic conidiophores and conidia (data not shown).

Assay nematodes. To assay for trapping activity, two nematodes were used: the insect-parasitic nematode *Steinernema glaseri* and the plant-parasitic nematode *Heterodera schachtii*. *S.*

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glaseri was produced on wax moth larvae; infective juveniles were stored for less than 2 months at 10°C (17). To obtain *H. schachtii* second-stage juveniles, cysts of *H. schachtii* (from sugar beet plants grown in a greenhouse) were placed on Baermann funnels, and the collected juveniles were stored at 10°C with aeration for less than 2 days. Because neither assay nematode naturally occurs in the soil at site 4 or site 5c, it was possible to distinguish assay nematodes from resident nematodes when they were extracted from soil and examined.

Soil cages. A soil cage consisted of two segments of PVC pipe (13). Each segment (3.9 cm wide, 3.0 cm high) was covered at one end with nylon mesh (480-µm pore size) and contained 40 cm³ of soil at -20 to -30 kPa; bulk density of the packed soil was about 1.5 g of soil per cm³. When eight or more pellets were added per segment, the pellets were mixed with the soil before the soil was packed into the segment. When fewer than eight pellets were added per segment, the pellets were inserted into the soil after the soil was packed in the segment; the pellets were inserted 1.5 cm deep in the center of the soil mass (one pellet per segment), on a central line 1 cm from the segment wall and 2 cm apart (two pellets per segment), or in a square pattern with each pellet 1 cm from the segment wall (four pellets per segment). Some segments received zero pellets.

Two segments with the same numbers of pellets were attached with pipe-wrap tape, forming a cylindrical cage containing 80 cm³ of soil with mesh covering the outer ends. Cages were stored separately in plastic bags at 10°C until buried in the field, within 6 h of preparation.

Single-fungus experiments. These experiments were started and usually completed in the spring or fall, when soil temperatures were moderate. For each fungus, three similar experiments were

performed. Fungus density was varied by adding different numbers of pellets to each cage. In most experiments, segments contained zero, one, two, four, or eight pellets. However, segments received 0, 4, 8, 16, or 40 pellets in experiments DH1, AO3, and AE3 (Table 2). Experiment DH1 received the larger number of pellets because that was the first experiment performed and the number of pellets needed to obtain high levels of activity was unknown. Data from experiment DH1 indicated that eight pellets per segment produced high levels of trapping, and so fewer pellets were used in subsequent experiments. Experiments AO3 and AE3 received the larger number of pellets because *A. oligospora* and *A. eudermata* trapped few or no nematodes when eight or fewer pellets were added in previous experiments.

Each single-fungus experiment included a total of 20 cages, with four replicate cages for each of the five levels of fungus inoculum. Cages were buried in blocks, each block located between two vines in the vine row. Each cage was oriented vertically (mesh at top and bottom), with 19 cm between the cage top and soil surface, and with 2 to 3 cm between adjacent cages. Monitors (Hobo-Temp Model; Onset Computer Corporation, Pocasset, MA) buried with the cages recorded soil temperature hourly. Degree days were calculated with 10°C as the base. The University of California Integrated Pest Management Program provided data on rainfall, and the growers provided data on irrigation.

Experiments were ended after 15 to 61 days (Table 2). All the cages in each experiment were collected (five cages from one block in experiment DH3 and three cages from one block in experiment AE3 were lost to burrowing animals) and processed at one time. When removed from the soil, cages were sealed in plastic and kept at 10°C. Within 6 h, each cage was separated into its segments. Bottom segments were used to quantify fungus ac-

TABLE 1. Nematode-trapping fungi used in this study

Fungus	ATCC accession number	Type of trap	Conidia per conidiophore	Source site ^a	Test site ^a
<i>Dactylellina haptotyla</i>	204099	Stalked adhesive knobs	2–5	SAFS	4
<i>Arthrobotrys dactyloides</i>	204102	Stalked constricting rings	4–13	SAFS	4
<i>D. ellipsospora</i>	204100	Stalked adhesive knobs	1	5	5c
<i>A. oligospora</i>	MYA-2480	Adhesive networks	>10	5	5c
<i>A. eudermata</i>	MYA-2481	Adhesive networks	1	5	5c

^a SAFS = Sustainable Agriculture Farming Systems Project at the University of California at Davis (11). Sites 4, 5, and 5c are vineyards near Lodi, California.

TABLE 2. Experiments conducted in this study

Experiment ^a	Days in field	Soil temperature (°C) ^b	Degree days ^c	Days with rain or irrigation ^d	Water potential at harvest (-kPa) ^e
DH1	18	17, 24, 20	181	0	100
DH2	25	14, 22, 18	209	2	18
DH3	61	14, 26, 20	588	4	<1,500
AD1	21	18, 25, 21	237	0	<1,500
AD2	24	14, 23, 19	204	0	30
AD3	42	14, 23, 19	385	1	28
DE1	17	20, 28, 24	236	1	18
DE2	48	20, 28, 24	680	4	10
DE3	31	22, 30, 26	497	4	30
AO1	17	20, 25, 23	220	1	22
AO2	27	14, 23, 19	239	1	52
AO3	15	21, 28, 24	216	0	100
AE1	21	19, 26, 22	253	2	26
AE2	32	13, 26, 20	308	1	52
AE3	15	21, 29, 24	214	0	200
Comparative 1	14	19, 26, 23	179	0	370
Comparative 2	15	19, 25, 22	173	1	22

^a In all but the last two experiments, the fungi were studied individually. In the single-fungus experiments, the letters indicate the fungus studied (AD = *Arthrobotrys dactyloides*; AE = *A. eudermata*; AO = *A. oligospora*; DE = *Dactylellina ellipsospora*; and DH = *D. haptotyla*) and the number (1, 2, and 3) indicates the experiment. In single-fungus experiments, segments received 0, 1, 2, 4, or 8 pellets, except segments in DH1, AO3, and AE3, which received 0, 4, 8, 16, or 40 pellets. In the comparative experiments, the five fungi were studied together, with 0 or 4 pellets added per segment.

^b Soil temperature (minimum, maximum, and mean) at 20 cm depth.

^c Accumulated degree days, base 10°C, at 20 cm depth.

^d Number of days with more than 5 mm of rain or irrigation.

^e Moisture release curves (from 0 to -1,500 kPa) were used to convert water content to water potential.

tivity by adding and then recovering and examining assay nematodes. Because the overall objective is to control plant-parasitic nematodes, a plant-parasitic nematode like *H. schachtii* was the preferred assay nematode for all fungi. However, *H. schachtii* is not readily trapped by adhesive networks and other adhesive structures (7), and so *S. glaseri*, which is captured in all kinds of traps (12), was used to assay trapping by *A. oligospora* and *A. eudermata*. *S. glaseri* was also used for *D. ellipsospora* to enable comparison with *A. oligospora* and *A. eudermata* (all three fungi were tested in site 5c soil). *H. schachtii* was used to assay for trapping by *A. dactyloides*. *H. schachtii* was also used for *D. haptotyla*, even though this fungus produces adhesive traps, because large percentages of *S. glaseri* are trapped by this fungus, whether the fungus population density is small or large (9).

Assay nematodes (either 1,500 *H. schachtii* or 75 *S. glaseri*) in 0.5 ml of 4 mM KCl (1.0 ml if the soil seemed dry) were added to the exposed soil surface of each segment, and segments were individually sealed in plastic bags and kept in a plastic container at 20°C in the dark. Fewer *S. glaseri* were added than *H. schachtii* because *S. glaseri* is much larger and therefore has a greater extraction efficiency and is easier to find in an extracted sample (9). After 2.8 days, the soil in each segment was placed in a 1-liter beaker with sufficient water to form a slurry. The slurry was mixed with a rubber spatula. After the plastic bag and segment were rinsed with water and the water was added to the 1-liter beaker, the soil in the beaker was suspended by rapidly filling the beaker with water. The soil suspension was then poured onto a sieve (38- μ m pore openings). The material collected on the sieve was centrifuged in water to reduce organic debris and then centrifuged in sucrose to collect nematodes (16). For segments receiving *H. schachtii*, the nematode suspension was reduced to 10 ml after 2 h, and a 1-ml sample was examined with a dissecting microscope at $\times 70$ to $\times 140$ magnification. The following were counted: *H. schachtii* with and without adherent traps; resident nematodes by trophic group (28); and enchytraeids. Segments receiving *S. glaseri* were processed in the same way, except that all the *S. glaseri* in each sample were examined.

Trapping was quantified in two ways. For *D. haptotyla*, *A. dactyloides*, and *D. ellipsospora*, the percentage of trapped nematodes was equal to $A/B \times 100$, where *A* is the number of assay nematodes recovered with at least one trap and *B* is the total number of assay nematodes recovered from the sample. For *A. oligospora* and *A. eudermata*, the percentage of trapped nematodes was equal to $[1 - (U/C)] \times 100$, where *U* is the number of untrapped assay nematodes recovered from the sample and *C* is the mean number of untrapped assay nematodes recovered from samples that received no pellets; a different *C* value was determined for each experiment. The first equation was used for *D. haptotyla*, *A. dactyloides*, and *D. ellipsospora* because most assay nematodes trapped with these fungi can be extracted from soil. The second equation was used for *A. oligospora* and *A. eudermata* because nematodes trapped in adhesive networks usually cannot be extracted from soil (15).

To quantify the population density of nematode-trapping fungi, the soil from each top segment was removed and mixed in a plastic bag. A subsample (20 g of moist soil) was added to a sterile 150-ml flask, and the volume was increased to 50 ml with sterile distilled water. The suspension was vigorously shaken on a wrist-action shaker for 8 min, and a 10-fold dilution series was prepared. From the original suspension and three additional dilutions, one 0.1-ml aliquot was added to each of five CMA/4 plates so that each plate received about 37, 3.7, 0.37, or 0.037 mg of soil (dry weight equivalent when soil moisture was 8%). Each plate then received about 1,000 bait nematodes (*S. glaseri*). After 7, 14, and 21 days, the plates were examined at $\times 20$ to $\times 70$ magnification for nematode-trapping fungi, which were identified to species based on trap, conidiophore, and conidium morphology (3). The qualitative assessment (presence or absence on each replicate

plate) was converted into an estimate of population density with an MPN program (1,18). The remaining soil from each top segment was used to determine water content.

Comparative experiments. In the experiments with single fungi, the relationship between population density (as determined by the MPN procedure) and trapping differed greatly depending on the fungus. These experiments, however, were not designed to permit direct comparison between the different fungi. The fungi were therefore directly compared in two additional experiments. Cages were packed with site 5c soil at -25 kPa and four or zero pellets per segment. Cages were buried at site 5c and recovered after 14 days in the first experiment and after 15 days in the second. In each experiment, there were five replicate cages for each of the five fungi and five replicates of a control containing no fungus, for a total of 30 cages. Fungus population density and trapping were quantified as described before except that the assay nematode was always *S. glaseri*.

Statistical analysis. This study used correlation rather than regression because a less powerful analysis seemed more appropriate, given the likelihood that estimates of population density and trapping activity would be greatly affected by the experimental conditions. In particular, the percentage of assay nematodes trapped may change with soil porosity, soil moisture, and assay nematode (7,8), reducing the predictive and descriptive powers of regression equations. Of course, correlations may also lack generality, i.e., MPN data and trapping may be correlated in some soils but not in others.

Data from single-fungus experiments were analyzed to determine whether fungus population density or trapping was correlated with the number of pellets added and, more importantly, whether trapping was correlated with fungus population density. A combined analysis including all three experiments for each fungus was considered superior to three separate analyses for each fungus. A combined analysis, however, could reveal a correlation based solely on differences between the three experiments. For example, a particular fungus might exhibit high trapping and high population density in one experiment, moderate trapping and moderate population density in a second experiment, and low trapping and low population density in a third experiment. If combined, these data would indicate a positive correlation between trapping and population density when no such relationship was evident within any one experiment. To guard against this kind of correlation (i.e., to provide a more conservative correlation analysis), partial Spearman correlation analysis was used for each fungus, with experiment as a categorical variable within each analysis. Because partial Spearman correlation analysis is based on a relative ranking of the data within each experiment, transformations are unnecessary and correlations reflect variation in data within each experiment. For comparisons of trapping and fungus population density, a first analysis included all samples and a second analysis excluded samples lacking the fungus (numerous samples without fungus and with no trapping could increase the chances of finding a positive relationship between fungus density and activity). Data from the comparative experiment were not statistically analyzed; inferences were based on examination of means and variances.

RESULTS

Soil temperature and water. Although soil temperatures were sometimes greater than 27°C and sometimes less than 15°C, mean soil temperatures were usually between 18 and 24°C; degree day accumulation ranged from 173 to 680 (Table 2). At harvest, soil water potentials were usually moderate (between -20 and -50 kPa), but the soils were sometimes relatively wet (experiments DH2, DE1, and DE2) and sometimes dry (experiments DH3 and AD1).

Number of pellets added, fungus population density, and recovery of assay nematodes from soil (single-fungus experiments). For all five fungi, fungus population density as deter-

mined by the MPN procedure was positively correlated with the number of pellets added per segment (Fig. 1; Table 3); the correlations remained significant ($P < 0.01$) even when data with zero pellets per segment were excluded (data not shown). The experimental design did not permit statistical comparison among fungi,

but *A. dactyloides* apparently produced fewer propagules per pellet than did the other fungi (Fig. 1).
Addition of *D. haptotyla*, *A. dactyloides*, or *D. ellipsospora* pellets reduced the number of assay nematodes recovered from soil (Fig. 2; Table 4); this reduction was greatest with *A.*

Fig. 1. Population densities of five nematode-trapping fungi as a function of fungus inoculum added to soil (single-fungus experiments). Values are the means $\pm$ standard error of four replicate cages. Each experiment with each fungus was performed three times; data from experiments 1, 2, and 3 are represented by circles, squares, and triangles, respectively.

TABLE 3. Fungus population density (as determined by most probable number procedures) versus the number of pellets added: partial Spearman correlation statistics

Fungus	<i>n</i>	Coefficient	<i>P</i>
<i>Dactylellina haptotyla</i>	55	0.90	<0.01
<i>Arthrobotrys dactyloides</i>	60	0.76	<0.01
<i>D. ellipsospora</i>	60	0.79	<0.01
<i>A. oligospora</i>	60	0.89	<0.01
<i>A. eudermata</i>	57	0.84	<0.01

Fig. 2. Recovery of assay nematodes from soil cages (expressed as a percentage of the number of assay nematodes added) as a function of fungus inoculum added to soil (single-fungus experiments). Values are the means $\pm$ standard error of four replicate cages. Each experiment with each fungus was performed three times; data from experiments 1, 2, and 3 are represented by circles, squares, and triangles, respectively.

TABLE 4. Percentage of assay nematodes recovered versus the number of pellets added: partial Spearman correlation statistics

Fungus	<i>n</i>	Coefficient	<i>P</i>
<i>Dactylellina haptotyla</i>	55	-0.60	<0.01
<i>Arthrobotrys dactyloides</i>	60	-0.66	<0.01
<i>D. ellipsospora</i>	60	-0.43	<0.01
<i>A. oligospora</i>	60	0.14	0.30
<i>A. eudermata</i>	57	-0.01	0.96

dactyloides. For *D. haptotyla*, the negative relationship remained significant even when the data from segments with 16 and 40 pellets were excluded (correlation coefficient = -0.46 ; $P < 0.01$). In contrast, there was no relationship between the number of assay nematodes recovered and the number of pellets added for *A. oligospora* or *A. eudermata* (Fig. 2; Table 4).

Fungus population density and trapping of nematodes (single-fungus experiments). The percentage of assay nematodes with adherent knobs of *D. haptotyla*, constricting rings of *A. dactyloides*, or adherent knobs of *D. ellipso-spora* was positively correlated with the population density of those fungi (Table 5; Fig. 3). The data for *A. dactyloides*, however, differed from those for *D. haptotyla* and *D. ellipso-spora* in several ways. When fungus population density was zero (based on MPN), *D. haptotyla* or *D. ellipso-spora* trapped no nematodes but *A. dactyloides* sometimes trapped many nematodes. Trapping by *A. dactyloides* also was substantial at much smaller fungus population densities than was trapping by *D. haptotyla* or *D. ellipso-spora*. Finally, the relationship between trapping and fungus population density was significant for *D. haptotyla* and *D. ellipso-spora*, but not for *A. dactyloides*, when observations with zero population density were excluded (Table 5).

Whereas trapped assay nematodes were frequently recovered from soil receiving *D. haptotyla*, *A. dactyloides*, or *D. ellipso-spora*, they were rarely recovered from soil receiving *A. oligospora* or *A. eudermata*. This was expected because *A. oligospora* and *A. eudermata* produce adhesive networks, and nematodes caught in adhesive networks are seldom extracted from soil. For these fungi, the number of assay nematodes recovered should decrease as trapping increases. But trapping, which was based on an equation that combined recovery and direct observation of trapped nematodes, was not correlated with the population density of *A. oligospora* or *A. eudermata* (Fig. 3; Table 5). The negative values for trapping (a biological impossibility) and the substantial variability in data for *A. oligospora* and *A. eudermata* reflect the substantial but normal variability in extraction of nematodes, as discussed previously and as evidenced when *A. oligospora* and *A. eudermata* population densities were zero (Fig. 3).

Comparative experiments. When all five fungi were studied simultaneously (four pellets per segment in site 5c soil), the data were largely consistent with the single-fungus experiments: *D. haptotyla* and *D. ellipso-spora* produced large numbers of propagules and trapped nearly all of the assay nematodes; and *A. oligospora* and *A. eudermata* produced moderate to large numbers of propagules and trapped few assay nematodes (Fig. 4). Although *A. dactyloides* trapped substantial numbers of *H. schachtii* in site 4 soil, it failed to trap any *S. glaseri* in site 5c soil, even though similar numbers of propagules were detected in both soils. Nematode suspensions extracted from treatments receiving *A. dactyloides* pellets contained hyphal fragments with constricting rings.

Effects on resident nematodes and enchytraeids (single-fungus experiments). Pellets usually had little effect on resident nematodes (Table 6). They usually enhanced enchytraeids, but the increase was small.

Fig. 3. Percentage of assay nematodes trapped as a function of trapping fungus population density (single-fungus experiments). The percentage of assay nematodes trapped was based on direct observation of trapped nematodes (for *Dactylellina haptotyla*, *Arthrobotrys dactyloides*, and *D. ellipso-spora*) or on reduced recovery of assay nematodes plus direct observation of trapped nematodes (for *A. oligospora* and *A. eudermata*). Each value represents data from one cage. Each experiment was performed three times; data from experiments 1, 2, and 3 are represented by circles, squares, and triangles, respectively.

TABLE 5. Percentage of assay nematodes trapped versus fungus population density (as determined by most probable number procedure): partial Spearman correlation statistics

Fungus	Including zero values for fungus population density			Excluding zero values for fungus population density		
	<i>n</i>	Coefficient	<i>P</i>	<i>n</i>	Coefficient	<i>P</i>
<i>Dactylellina haptotyla</i>	55	0.85	<0.01	44	0.55	<0.01
<i>Arthrobotrys dactyloides</i>	60	0.71	<0.01	34	0.31	0.08
<i>D. ellipso-spora</i>	60	0.79	<0.01	48	0.49	<0.01
<i>A. oligospora</i>	60	-0.06	0.65	50	0.12	0.42
<i>A. eudermata</i>	57	-0.02	0.86	48	-0.05	0.77

DISCUSSION

At the outset of this study, I was concerned that the results were predictable and therefore would be trivial: why wouldn't trapping of nematodes be correlated with any reasonable estimate of fungus population density or biomass? I proceeded anyway because data showing a relationship between population density and trapping were lacking and because our understanding of what these fungi do in soil is poor. As it turned out, the results were not so predictable. The correlation between MPN data and trapping was strong for two fungi that form adhesive knobs (*D. haptotyla* and *D. ellipsospora*), weaker and inconsistent for a fungus that forms constricting rings (*A. dactyloides*), and nonexistent for two fungi that form adhesive networks (*A. oligospora* and *A. eudermata*). In the latter cases, there was no correlation because the fungi trapped few or no nematodes even when fungus population density exceeded 10^3 propagules per gram of soil.

Although not correlated with trapping, the MPN data for *A. oligospora* and *A. eudermata* are still valuable because they show that the failure to trap did not result from a failure to establish. The lack of trapping also cannot be attributed to host status of the assay nematode (both fungi rapidly trap and consume *S. glaseri* on agar media) or to the use of site 5c soil (both fungi were residents of that soil and were isolated from an adjacent part of the vineyard). Moreover, preliminary data indicate that *A. oligospora* fails to trap *S. glaseri* in site 4 soil and fails to trap *Meloidogyne javanica*, a root-knot nematode, in either soil (B. A. Jaffee, unpublished data). The failure to trap might be explained by the absence of organic inputs; Cooke (2) suggested that *A. oligospora*, *A. eudermata*, and other network-forming fungi may only trap nematodes during certain phases of saprophytic competition when organic matter is added to soil. But *A. oligospora* also failed to

trap assay nematodes when site 5 soil was amended with alfalfa or grape leaves, even though these amendments increased *A. oligospora* population density (B. A. Jaffee, unpublished data).

The value of the MPN procedure would of course increase if we knew its detection efficiency. To calculate detection efficiency, we must know the numbers of specific kinds of propagules present in soil and the kinds of propagules detected; neither was known in this study. The soil probably contained several kinds of propagules because all five fungi rapidly convert the material in the pellets into new hyphae, conidiophores, and conidia. According to Eren and Pramer (4), the MPN procedure detected 15% of *A. conoides* conidia added to soil, but the authors provided no methods and no data other than that. Furthermore, one can easily imagine how conidia that are added to soil might provide detection efficiencies much greater than or much less than conidia or other propagules that are produced in situ.

MPN detection efficiency remains largely unknown, but MPN data were strongly correlated with the number of pellets added to soil. This correlation again indicates that the technique can provide rational and reproducible data. The correlation may not otherwise seem noteworthy (how much one finds should be related to how much one adds) until one considers that, regardless of inoculum quantity, fungus population density will likely return to some low level, dictated by the biotic and abiotic environment of the cages in the field. Apparently, the experiments did not continue long enough for that to happen.

The relationship between *A. dactyloides* trapping and population density requires more study. With site 4 soil and *H. schachtii* as the assay nematode, trapping by *A. dactyloides* was correlated with population density, but the fungus sometimes trapped many nematodes even when the MPN procedure detected no fungus, suggesting that the MPN procedure underestimated *A. dactyloides* population density. With site 5c soil and *S. glaseri* as the assay nematode, there was no correlation because *A. dactyloides* trapped no nematodes. These differences could reflect change in soil environment or change in assay nematode, but the latter seems unlikely because the fungus traps and consumes both nematodes on agar media.

Concerning biological control of plant-parasitic nematodes, the data for *D. haptotyla* and *D. ellipsospora* are both discouraging and encouraging. The data are discouraging because substantial trapping occurred only when fungus numbers exceeded those normally attained by resident nematode-trapping fungi (10,11,19, 20,22–26). The inference is that resident populations are unlikely to suppress plant-parasitic nematodes, although the relationship between population density and trapping could differ for added fungi versus resident fungi. On the other hand, the data are encouraging both because the MPN procedure seems useful for predicting trapping by these two fungi and because large numbers and activities were obtained by adding fungus inoculum to field soil in a near field environment. Field soil is generally considered resistant to the introduction of nematode-trapping and other fungi (8,14,22), but establishing large numbers and high levels of activity is clearly possible.

Fig. 4. Relationship between trapping and fungus population density (comparative experiments): *Arthrobotrys dactyloides*, ▲; *Dactylellina haptotyla*, ○; *D. ellipsospora*, ●; *A. oligospora*, ■; and *A. eudermata*, □. Values are the means $\pm$ standard error of five replicate cages. The same symbols are used for both experiments because the results were similar.

TABLE 6. Relationship between the number of pellets added to soil and the population densities of resident nematodes and enchytraeids in single-fungus experiments

Fungus	Mean population density per section (40 cm ³ or 60 g of soil) ^a					
	Nematodes by trophic group					Enchytraeids
	Bacterivores	Fungivores	Omnivores	Plant parasites	Predators	
<i>Dactylellina haptotyla</i>	298 (196, 285)	47 (69, 35)	170 (142, 201)	30	4	10 (3, 12)
<i>Arthrobotrys dactyloides</i>	121	70	56	58	4	5 (2, 7)
<i>D. ellipsospora</i>	94	46 (68, 29)	10	59	64	7 (5, 11)
<i>A. oligospora</i>	86 (64, 82)	40 (32, 43)	7	24	21	7
<i>A. eudermata</i>	82	43	7	33	30	11 (6, 22)

^a The first value is the mean population density over all levels of pellets and over three experiments for each fungus. If the correlation between the number of pellets and nematode or enchytraeid population density is significant ($P \leq 0.05$), mean values for 0 pellets followed by 8 pellets are shown in parentheses.

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