

Trap Production by Nematophagous Fungi Growing from Parasitized Nematodes

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ABSTRACT

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Nematode-trapping fungi are generally considered facultative parasites that produce traps only under special conditions. However, *Arthrobotrys dactyloides*, *A. oligospora*, *Monacrosporium ellipso sporum*, and *M. cionopagum* produced many traps when growing from parasitized nematodes in saturation extracts of four soils. Thus, conditions that induce traps may normally prevail when these fungi grow from nematodes in soil, and parasitism may be more important to these fungi than has previously been recognized. Whereas *Hirsutella rhossiliensis* (an endoparasitic fungus) produced infective structures only in the atmosphere, the trapping

fungi produced infective structures when submerged and noninfective spores in the atmosphere; therefore, allocation of resources to traps or spores may be affected by soil water. When introduced into untreated loamy sand (8.0% moisture, -25 kPa) in the form of parasitized nematodes, *H. rhossiliensis*, *M. ellipso sporum*, and *A. dactyloides* parasitized significant proportions of assay nematodes (*Heterodera schachtii*); these fungi parasitized fewer nematodes if the soil was disturbed before the assay nematodes were added. In contrast, parasitism of assay nematodes by *A. oligospora* and *M. cionopagum* was not detected in the loamy sand.

Additional keywords: biological control, soil disturbance.

Fungi that are parasites of vermiform, soilborne nematodes are divided into two broad groups: the endoparasitic fungi and the trapping fungi (9). Endoparasitic fungi infect nematodes with spores that adhere to or are ingested by nematodes, whereas trapping fungi infect nematodes after capture with adhesive structures (hyphae, hyphal networks, knobs, or branches) or mechanical structures (constricting or nonconstricting hyphal rings) (2).

Most trapping fungi are considered saprophytes that are also able to capture and parasitize nematodes during brief periods when partially decomposed organic matter is present and when competition among microorganisms is intense; nematodes are thought to be a supplemental rather than a primary substrate (4,5,28). Research has focused on the transition from saprophytic to parasitic behavior, and the circumstances that result in trap formation by fungi growing vegetatively on agar have been well documented. For many species, trap formation is conditioned by the nutrient status of the agar (especially the carbon/nitrogen ratio) (10,26) and by the presence of nematodes (27); traps of these species are said to be induced (20). For fungi that produce infective structures when grown on agar in the absence of nematodes or other specific conditions, as is the case for some trapping fungi and all endoparasites, production of traps or infective spores is said to be spontaneous (3,20). Inferences on the ecology of nematophagous fungi in soil have been based, in part, on whether infective structures form spontaneously or inductively on agar (3,4,18,21).

In contrast to trapping fungi, endoparasitic fungi are considered specialized for parasitism of nematodes (4,20), and the parasitized nematode has recently been recognized as an important form of fungal inoculum in nature. Nematodes parasitized by *Drechmeria coniospora* (19) and *Hirsutella rhossiliensis* Minter & Brady (14,15,31) were used to add these fungi to soil, and sporulation of *H. rhossiliensis* from nematodes was quantified (14,16) and included in an epidemiological model (15). Like other endoparasites, *H. rhossiliensis* appears to have little or no competitive saprophytic ability (17) and typically sporulates (i.e., spontane-

ously produces infective propagules) upon emergence from a parasitized host (14,16) or on agar, regardless of the presence or absence of nematodes. The parasitized nematode has not to our knowledge been studied as a source of fungal inoculum or as a substrate in the life history of trapping fungi.

Soil disturbance reduces the inoculum of *H. rhossiliensis* in soil (25); the phialides of this fungus apparently are essential for adherence of spores to nematodes, and soil disturbance removes spores from phialides. Other nematophagous fungi may respond differently to disturbance. For example, the infective spores of some endoparasitic fungi, such as *D. coniospora* and *Harpoglossum* spp., are borne on short conidiophores and rely on other agents in order to move any distance from the host; detached spores of these fungi readily infect nematodes after dispersal, and their dispersal may be enhanced by soil disturbance. The response of trapping fungi to soil disturbance is unknown. However, soil disturbance may be detrimental if it changes the orientation of traps in soil pores, damages the complex structure of traps, or reduces the adhesiveness of traps.

The purpose of the present study was to compare the behavior of trapping fungi with that of the endoparasite *H. rhossiliensis* when growing from parasitized nematodes in soil or soil extract. Our hypotheses were that both kinds of fungi would produce infective structures when growing from parasitized nematodes, and that the infective structures would be sensitive to soil disturbance.

MATERIALS AND METHODS

Fungi, nematodes, and soil. The fungi investigated included the endoparasite *H. rhossiliensis* (IMI 265748) and the following trapping fungi: *Arthrobotrys dactyloides* Drechsler (ARSEF 2934), which forms constricting rings; *A. oligospora* Fres. (ARSEF 3347), which forms three-dimensional adhesive networks; *Monacrosporium cionopagum* (Drechsler) Subram. (ARSEF 3349, a gift from J. Gaspard), which forms adhesive branches and two-dimensional adhesive networks; and *M. ellipso sporum* (Grove) Cooke & Dickinson (ARSEF 3348, a gift from J. Gaspard), which forms adhesive knobs. These trapping fungi

were selected because they vary in trap type and appear to vary in dependence on nematodes for nutrition (3,20,21). The fungi were maintained on quarter strength cornmeal agar (CMA/4) (Difco Laboratories, Detroit, MI) and were subcultured monthly. When grown on CMA/4, *H. rhossiliensis*, *M. elliposporum*, and *M. cionopagum* produced infective structures in the absence of nematodes, whereas *A. dactyloides* and *A. oligospora* did not produce infective structures unless nematodes were added to the culture.

The parasitized nematodes were third-stage juveniles (J3) of the entomogenous nematode *Steinernema glaseri* Steiner. This nematode was used because large numbers of uniform juveniles are produced easily (32) and the juveniles are susceptible to *H. rhossiliensis* (31) and trapping fungi (Jaffee, unpublished). In preliminary experiments, a sand culture procedure (14) that consistently provided high numbers of the sugarbeet cyst nematode *Heterodera schachtii* Schmidt and *S. glaseri* (31) parasitized by *H. rhossiliensis* did not provide sufficient numbers of *H. schachtii* or *S. glaseri* parasitized by the trapping fungi. Thus, *S. glaseri* parasitized by each of the five fungi were obtained by a simple agar plate procedure. About 500 *S. glaseri* were added to growing cultures of each fungus on plates of CMA/4 (10 cm in diameter) that had been incubated at 20 C for 4 days (*A. oligospora*), 7 days (*A. dactyloides*, *M. elliposporum*, and *M. cionopagum*), or 14 days (*H. rhossiliensis*). After 1 day at 20 C, almost all of the nematodes were trapped and parasitized (filled with hyphae) by *M. elliposporum*, *M. cionopagum*, and *A. oligospora*. Parasitism required 2 days for *A. dactyloides* and 3 days for *H. rhossiliensis*. Parasitized nematodes were removed individually with a pick and were placed in solutions, on agar, or in soil as indicated for each experiment. The large size of *S. glaseri* facilitated removal from the cultures (small nematodes with traps tended to remain attached to the parent hypha, whereas *S. glaseri* with traps were readily separated from the parent hyphae of the trapping fungi) and also meant that fewer nematodes had to be picked to obtain a desired level of fungal inoculum.

Healthy, second-stage juveniles (J2) of *H. schachtii* were used to assay for fungal parasitism in the soil disturbance experiment. This nematode was susceptible to the five fungi when added to

cultures growing on agar plates (data not shown). Freshly hatched J2 were obtained from cysts from sugarbeets (*Beta vulgaris* L. 'SSNB-2') grown in a greenhouse. Cysts were placed on Baermann funnels, and J2 were collected every 2 h. Suspensions of healthy J2 were aerated at 10 C and were less than 48 h old when used.

Four soils were collected (Table 1). Saturation extracts without (NaPO₃)₆ (29) were obtained from these soils, filtered (through a filter with 5 µm pore diameter) to remove nematodes but not bacteria, and stored at 4 C for 24 h before use. Soil M was used for the soil disturbance experiment. The inflection point of the moisture release curve for this soil was -4 kPa and 18% moisture. This soil was selected because it contained low numbers of nematodes (*Criconebella xenoplax* and unidentified bacterial feeders) but no *H. schachtii*. Although the soil was obtained from an orchard with high densities of *H. rhossiliensis*, the soil was collected between the tree rows where the numbers of *H. rhossiliensis* and nematodes were very low (13). The soil was collected in April 1989 and was stored moist at 22 ± 2 C for over 2 yr before use. Long storage at room temperature further reduces the inoculum of *H. rhossiliensis* in this soil (12), and the fungus could not be detected when control vials were assayed (data not shown). Soils B and T were from experimental field plots and were used as sources of soil extract because they contained high levels of *H. schachtii* and the root-knot nematode, *Meloidogyne javanica*, respectively; soil T was of special interest because it contained little organic matter. These soils were stored moist at 10 C for 2-3 months before use. Soil O, located close to our laboratory, was used as a source of soil extract. This soil was stored moist at 22 ± 2 C for 1-2 months before use and contained high numbers of a variety of nematode species (Table 1).

All experiments were conducted twice (trials 1 and 2) at 20 C. Because data were similar for both trials of all experiments, data from trials 1 and 2 were combined for analyses and presentation.

Growth from parasitized nematodes. Five *S. glaseri*, parasitized by one of the five nematophagous fungi, were placed in 1.5 ml of soil extract or 2 mM KCl in a plastic petri dish (35 × 10 mm). Two dishes were prepared for each combination of fungus and solution and were covered and stored in a sealed moisture chamber in the dark. After 2 days, the traps (or spores, for *H. rhossiliensis*) produced from each nematode were counted at 100-140X. Most of the solution was removed from the dish to reduce the depth of field and facilitate quantification. *A. oligospora* produces complex, three-dimensional networks of adhesive loops; the number of adhesive loops per nematode was determined. The number of adhesive branches and the number of two-dimensional networks of *M. cionopagum* were counted.

Nematophagous fungi were observed during growth from soil solution into the atmosphere. Parasitized *S. glaseri* were placed in a small drop (5 µl) of saturation extract from soil M in a plastic petri dish 10 cm in diameter. There were five drops per dish, and each drop contained one parasitized nematode so that each of five replicate dishes contained all five fungi. A moist filter paper was placed under the lid of each dish, and the presence of traps or spores in or out of the drop was recorded after 7 days.

Fungal growth from parasitized *S. glaseri* on blocks of 1.5% water agar was examined. The agar blocks (area of upper surface

TABLE 1. Characteristics of soils

Soil	Source ^a	Texture	pH ^b	Organic matter (%)	Nematodes/100 cm ³ soil ^c
B	Sugarbeet plots	Loam	6.8	1.19	695
M	Mature peach orchard	Loamy sand	4.9	0.24	60
O	Mature almond orchard	Sandy loam	7.4	2.41	1240
T	Tomato plots	Sand	7.9	0.05	4020

^a Soils B, O, and T were from Yolo County, CA, and soil M was from Merced County, CA.

^b Soil was saturated to a paste with distilled water.

^c Nematodes were extracted by wet screening (38 µm pore diameter) and centrifugation in sucrose.

TABLE 2. Numbers of infective structures (traps or adhesive spores) produced by nematophagous fungi growing from parasitized nematodes in 1.5 ml KCl (2 mM) or saturation extracts from four soils (B, M, O, or T)^a

Fungus	Infective structures	Infective structures/parasitized nematode ^b				
		KCL	B	M	O	T
<i>Arthrobotrys dactyloides</i>	Constricting rings	2 ± 1	125 ± 16	90 ± 11	118 ± 11	163 ± 11
<i>Arthrobotrys oligospora</i>	Adhesive, 3-D networks ^c	23 ± 6	58 ± 7	49 ± 6	99 ± 10	158 ± 14
<i>Hirsutiella rhossiliensis</i>	Adhesive spores	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
<i>Monacrosporium cionopagum</i>	Adhesive branches	0 ± 0	150 ± 10	104 ± 12	187 ± 19	228 ± 21
	Adhesive, 2-D networks	0 ± 0	136 ± 17	80 ± 11	109 ± 9	120 ± 13
<i>Monacrosporium elliposporum</i>	Adhesive knobs	10 ± 4	275 ± 22	257 ± 25	229 ± 33	291 ± 28

^a Extracts were filtered (5 µm) and contained no nematodes.

^b After 2 days at 20 C. Each value is the mean ± SE, n = 20.

^c For *A. oligospora*, each network consists of one or more hyphal loops, and the total number of loops is indicated.

= 1 cm²) were cut from agar in sterile dishes or from agar in dishes inoculated with 50–100 healthy *S. glaseri* 3 days earlier; none of the blocks contained nematodes when transferred to sterile, 1.5% water agar in petri dishes 10 cm in diameter. One parasitized nematode was placed on the surface of each block. Each dish contained five blocks (one block for each fungus), and there were five replicate dishes per type of block (\pm exposure to nematodes). Blocks were examined for traps or spores after 2 days.

Effect of soil disturbance on parasitism of nematodes. Thirty *S. glaseri*, parasitized with one of the nematophagous fungi, were mixed into untreated, loamy sand (soil M). The loamy sand was

placed into 25-ml vials with holes in the bottom, and the vials were covered with lids (25); the dry weight, volume, bulk density, and water content of the soil in each vial was 26.1 g, 17 cm³, 1.54 g/cm³, and 8.0% (-25 kPa), respectively. Control vials received no parasitized nematodes. The vials (12 per fungus and control) were placed in a moist chamber for 21 days before the soil from half of the vials was disturbed. A 21-day incubation period was used to allow sufficient time for the fungi to utilize the host completely; resources remaining in the host would mask the effect of disturbance on infective propagules. Because *S. glaseri* is a large nematode, 2–3 wk at 20 C were required for total conversion of assimilate into structures external to the host. To

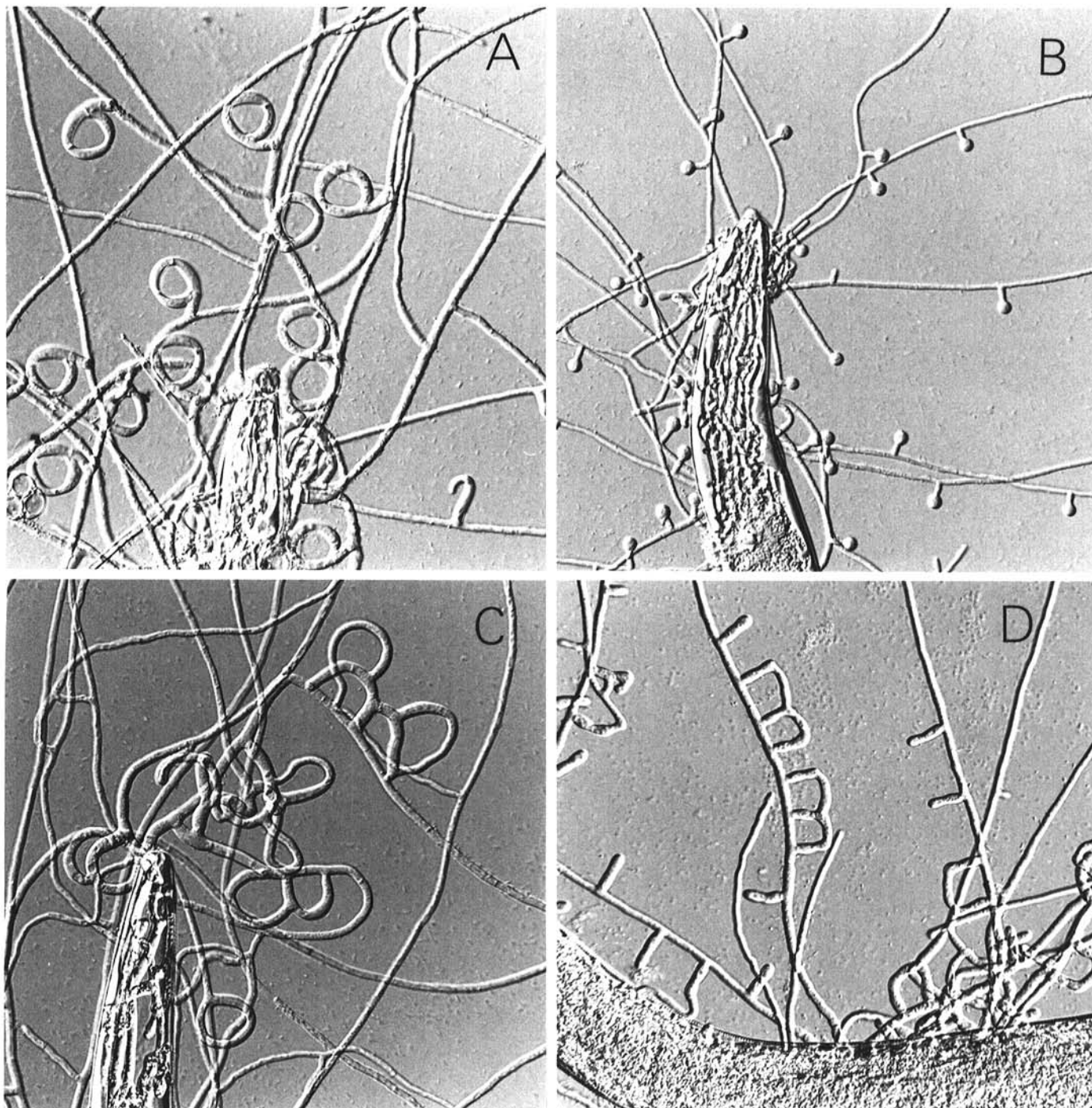


Fig. 1. Micrographs of trapping fungi growing from parasitized nematodes (*Steinernema glaseri*) in soil extract. The extract contained no other nematodes. A portion of the parasitized nematode is present in each micrograph. Magnification for all micrographs is 240 \times . **A**, Constricting rings produced by *Arthrobotrys dactyloides*; **B**, Adhesive knobs produced by *Monacrosporium ellipso sporum*; **C**, Adhesive, three-dimensional networks produced by *A. oligospora* (the networks appear to be two-dimensional because they have been flattened by the coverslip); **D**, Adhesive branches and two-dimensional networks produced by *M. cionopagum*.

disturb soil, soil from a vial was placed in a plastic bag, shaken for 5 s, and packed to the original volume in the same vial. Each vial was then inoculated with 118 ± 12 ($\bar{x} \pm SD$) (trial 1) or 107 ± 20 (trial 2) assay nematodes (healthy J2 of *H. schachtii*) in 0.5 ml 2 mM KCl. After 66 h, the nematodes were extracted from each vial by wet screening (28 μ m pore diameter) and centrifugation in water followed by sucrose (23). Material collected on a 28- μ m-pore sieve after sucrose centrifugation was washed into a counting dish and examined at 70–140 \times . All of the assay nematodes recovered from each sample were counted, and those without spores of *H. rhossiliensis* or traps of the four trapping fungi were considered healthy. Spores or traps of other fungi were not observed.

RESULTS

Growth from parasitized nematodes. After 2 days, all of the trapping fungi produced abundant traps when growing from parasitized nematodes in 1.5 ml soil extract (Table 2 and Fig. 1). Trap number tended to be highest in extract from soil T and lowest in extract from soil M. All of the trapping fungi except *M. cionopagum* produced traps when growing from parasitized nematodes in 2 mM KCl, but trap number was generally greater in soil extract than in KCl. *H. rhossiliensis* grew slowly from parasitized nematodes submerged in solution and produced no phialides or spores (Table 2). The trapping fungi also failed to produce spores in the solutions.

When growing from parasitized nematodes in 5- μ l drops of saturation extract from soil M, all of the trapping fungi produced traps in the drop but produced conidiophores and spores in the atmosphere surrounding the drop (Table 3). *H. rhossiliensis* grew vegetatively in the drop but produced phialides and spores after hyphae grew into the atmosphere.

After 2 days, *A. dactyloides*, *M. elliposporum*, and *M. cionopagum* produced traps when growing from parasitized nematodes on agar not exposed to healthy nematodes but produced more traps on agar exposed to healthy nematodes (Table 3). *A. oligospora* produced no traps on agar unless the agar had been exposed to nematodes. *H. rhossiliensis* produced many spores regardless of the agar type. Whereas all the spores of *H. rhossiliensis* were produced in the air, most of the traps formed by the trapping fungi were in the agar; the remainder formed on the surface of the agar.

Effect of soil disturbance on parasitism of nematodes. *A. dactyloides* and *H. rhossiliensis* reduced the number of healthy assay nematodes recovered relative to the control only if the soil was undisturbed (Fig. 2A). *M. elliposporum* reduced recovery of healthy nematodes in disturbed and undisturbed soil, but the reduction was greater in undisturbed soil. In contrast, the number of healthy assay nematodes recovered from vials receiving *A. oligospora* or *M. cionopagum* was similar to that from the control, whether or not the soil was disturbed (Fig. 2A); the recovery of 30–40% of the nematodes added in these treatments is typical for the recovery when the same soil has been heated to 60 C to eliminate or suppress naturally present enemies of nematodes.

Nematodes with traps (or spores in the case of *H. rhossiliensis*) were recovered from vials inoculated with *A. dactyloides*, *M. elliposporum*, and *H. rhossiliensis* but not from vials inoculated with *A. oligospora* or *M. cionopagum* (Fig. 2B). Nearly 90% of the assay nematodes recovered from undisturbed vials inoculated with *H. rhossiliensis* had at least one spore; of those with spores, most were filled with hyphae. The number of assay nematodes with spores of *H. rhossiliensis* was dramatically reduced from undisturbed to disturbed soil (Fig. 2B). Constricting rings were present on 30% of the assay nematodes recovered from undisturbed vials inoculated with *A. dactyloides* (Fig. 2B); few constricting rings were present on nematodes extracted from disturbed soil. Most nematodes with constricting rings were colonized. Whereas the spores and constricting rings on extracted nematodes had detached from the parent hypha, knobs of *M. elliposporum* remained attached. Thus, nematodes with adhesive knobs attached to the cuticle (60% of the nematodes recovered) were usually surrounded by a mass of hyphae and knobs. Although these nematodes were scored as trapped, most appeared vigorous and healthy and were not visibly infected when examined at 400 \times . Soil disturbance tended to reduce the number of recovered assay nematodes trapped by *M. elliposporum*, but this reduction was much less than that for *A. dactyloides* or *H. rhossiliensis* (Fig. 2B). Although trapping by *M. cionopagum* was not detected, adhesive branches typical of this fungus were observed in the extracts of the appropriate samples.

DISCUSSION

Our data suggest that nematode-trapping fungi may typically produce traps when growing from parasitized nematodes in soil. Therefore, the dependence on specialized conditions for trap induction on agar may not be important in soil. One could argue that our results are consistent with results from agar culture in that the traps may have been induced by compounds derived from the host nematode (*S. glaseri*) or from healthy nematodes in the soils used as sources of soil extract. However, induction is not ecologically important if traps are always induced in the normal environment. Even without reference to our observations, the perceived need and importance of external sources of carbon and nitrogen, healthy nematodes, and intense microbial competition should be questioned because the parasitized nematode is a rich source of carbon and nitrogen, and healthy nematodes and microbial competition occur virtually all the time in every soil. In this respect, we agree with Balan and Lechevalier (1) who suggested, based on limited observations of *A. dactyloides*, that trap formation may be unusual on agar plates but typical in soil.

Our results are to some extent inconsistent with those of Jansson and Nordbring-Hertz (20,21), who expanded on the work of Cooke (3) and divided fungal parasites of vermiform nematodes into three groups. Fungi in group 1 produce adhesive three-dimensional networks inductively; these fungi grow quickly and are considered relatively inefficient parasites and efficient saprophytes (but see Cooke [3] regarding saprophytic ability). Fungi in group

TABLE 3. Trap and spore production by nematophagous fungi growing from parasitized nematodes in 5- μ l drops of soil extract or on water agar blocks previously exposed or not exposed to healthy nematodes

Fungus	In 5- μ l drops ^a				On agar ^b	
	Inside drop		Outside drop		Not exposed to nemas	Exposed to nemas
	Traps	Spores	Traps	Spores	Traps ^c	Traps ^c
<i>Arthrobotrys dactyloides</i>	+	—	—	+	24 \pm 5	45 \pm 8
<i>Arthrobotrys oligospora</i>	+	—	—	+	0 \pm 0	32 \pm 6
<i>Hirsutella rhossiliensis</i>	—	—	—	+	112 \pm 9	111 \pm 15
<i>Monacrosporium cionopagum</i>	+	—	—	+	14 \pm 3	138 \pm 10
<i>Monacrosporium elliposporum</i>	+	—	—	+	36 \pm 8	108 \pm 10

^a After 7 days at 20 C. Extracts were filtered (5 μ m) and contained no nematodes. Ten nematodes per fungus were examined.

^b After 2 days at 20 C. Each value is the mean \pm SE per nematode, n = 10.

^c For *H. rhossiliensis*, the number of adhesive spores is indicated.

3 are endoparasites. These fungi grow slowly, spontaneously produce infective spores, and are considered efficient parasites but poor saprophytes. Fungi in group 2, considered intermediate to those in group 1 and 3, include those that produce constricting rings, adhesive knobs, and adhesive branches. In the present study, the rapid formation of traps by *A. oligospora* (group 1) and by *A. dactyloides*, *M. elliposporum*, and *M. cionopagum* (group 2) when growing from nematodes in soil extracts (containing no nematodes and obtained from soils unamended with organic matter) suggests that these fungi may be more similar to endoparasites (i.e., may be more dependent on parasitism) than previously recognized. To strengthen this argument, other fungi that do not exhibit spontaneous trap formation on agar should be examined, and additional quantitative data on parasitic and saprophytic activity in soil are needed. Our data supported pre-

vious studies (18,20) in that *A. oligospora*, *M. cionopagum*, and *A. dactyloides* were inefficient parasites compared to *H. rhossiliensis* in our soil experiment.

Before concluding that *A. oligospora* and other trapping fungi are parasitically less efficient than the endoparasitic fungi, we should recognize that the abiotic environment may affect allocation of resources and, thus, the production of infective structures. The energy and material obtained from a captured resource, such as a nematode, is limited and must be partitioned among hyphae, traps, and spores (for trapping fungi) and hyphae and spores (for *H. rhossiliensis*). In our petri dish studies, *H. rhossiliensis* produced infective structures only in the atmosphere. Furthermore, we have found that acquisition of *H. rhossiliensis* spores by nematodes in soil is suppressed at high water potentials (*unpublished*). Therefore, we used a low water potential in the soil disturbance experiment. However, the petri dish studies suggest that the trapping fungi may respond quite differently to water than does *H. rhossiliensis* and may produce more spores than traps when soil is dry. These spores may eventually germinate to produce traps (6), but in the short term, trap production may be decreased in drier soils. Others have also observed trap formation in liquid environments (7,8), and the effect of soil water on trap formation and sporulation by trapping fungi deserves study.

Trap number tended to be highest in the extract from the soil containing the highest nematode density (soil T) and lowest in the extract from the soil containing the lowest nematode density (soil M). Although the differences in trap induction may be attributable to nematode density, the soils differed in other properties. For example, the low pH of soil M may have affected trap production in extracts and in soil.

In our assessment of parasitism in the soil disturbance experiment, a low recovery of healthy nematodes could have reflected a high proportion of recovered nematodes bearing spores or traps or a low recovery of assay nematodes. Because traps of many trapping fungi do not detach readily from the parent hypha, we expected trapping to be manifested as reduced recovery of assay nematodes; this was not observed for *H. rhossiliensis*, because its spores readily detach from the phialides, and there was insufficient time for nematodes with spores to be degraded by the fungus. In contrast to our expectations, a significant proportion of nematodes trapped by *A. dactyloides* and *M. elliposporum* were recovered.

Like the spores of *H. rhossiliensis*, the traps of trapping fungi are borne on hyphae that radiate into the environment surrounding the parasitized host (i.e., the fungus expends energy in actively dispersing infective propagules). Like *H. rhossiliensis* and certain mycorrhizal fungi (22,25), at least two trapping fungi (*A. dactyloides* and *M. elliposporum*) were sensitive to soil disturbance in the present study. *M. elliposporum* was less sensitive than was *H. rhossiliensis* or *A. dactyloides*. The reason for this is unknown, but the adhesive knobs of *M. elliposporum* are structurally simple and may be especially sturdy, or new knobs may have been produced from spores that germinated after the soil was disturbed (6,24).

The short interval between soil disturbance and assay may have limited the potential production of new traps, and the difference in the number of healthy nematodes recovered from disturbed vs. undisturbed soil may have been less if this interval was lengthened. Thus, disturbance does not necessarily eradicate these fungi, but may at least temporarily reduce inoculum and parasitism. Eradication may result if the fungus has converted all captured resource into traps, and no reserves (such as those in spores or assimilative hyphae) remain for new growth. The main point is that infective propagules represent important investments that may be lost when soil is disturbed. It follows that cultivation of soil may reduce inoculum of these fungi, and estimates of fungal density in soil may be low if based on soil samples that are mixed or otherwise disturbed.

Much of the previous work on trapping fungi has examined behavior on agar or in soil amended with organic matter. These fungi, however, are common inhabitants of mineral soils, and

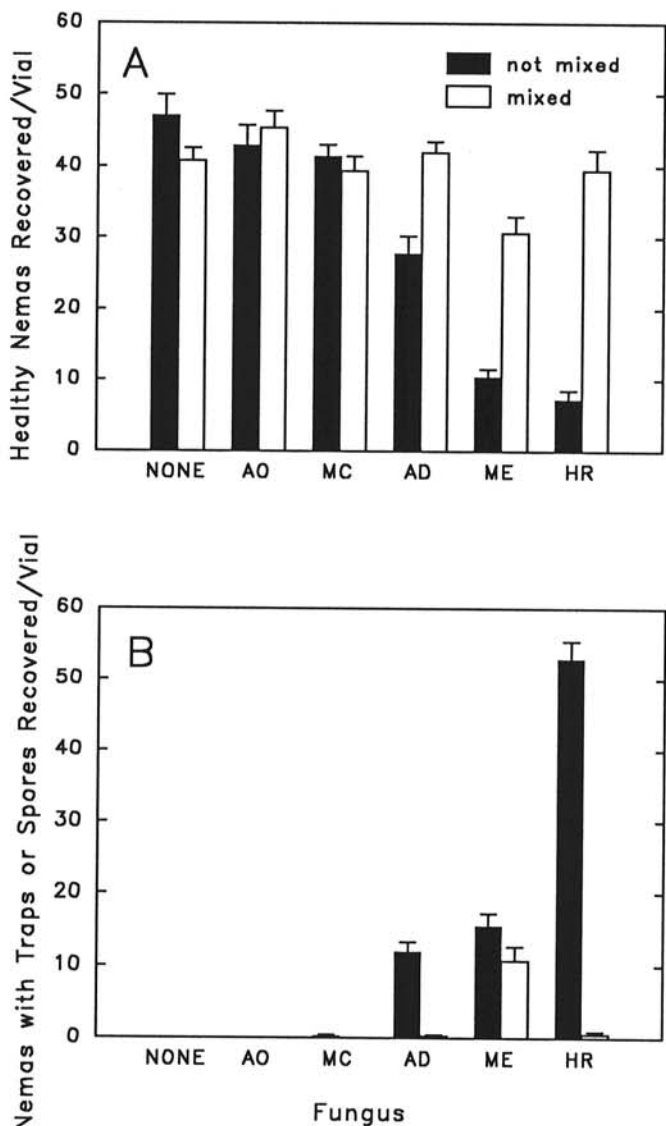


Fig. 2. Parasitism of assay nematodes (juveniles of *Heterodera schachtii*) in mixed or unmixed soil previously inoculated with no fungus (NONE), *Arthrobotrys oligospora* (AO), *Monacrosporium cionopagum* (MC), *A. dactyloides* (AD), *M. elliposporum* (ME), or *Hirsutella rhossiliensis* (HR). Fungi were added to soil in vials in the form of parasitized nematodes (30 *Steinernema glaseri* per vial). After 21 days at 20 C, soil was mixed or left undisturbed and inoculated with 107–118 healthy assay nematodes per vial. Assay nematodes were recovered after 66 h at 20 C. **A**, Number of healthy assay nematodes (i.e., nematodes without spores or traps) recovered per vial. **B**, Number of parasitized nematodes recovered per vial; parasitized nematodes were those with traps of AO, MC, AD, or ME, or spores of HR in appropriate vials. Each value is the mean \pm SE of 12 replicate vials.

behavior in unamended mineral soil also requires attention (30). A key question is "Do trapping fungi respond to nematode density?"—i.e., "Is parasitism density-dependent?" (4,5,9,15,30). Our observations indicate that trapping fungi are similar to *H. rhossiliensis* in that they are primed for parasitism when they grow from a parasitized nematode. We therefore hypothesize that, like *H. rhossiliensis* (15), at least some trapping fungi will exhibit density-dependent parasitism in mineral soil.

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