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Susceptibility of a cyst and a root-knot nematode to three nematode-trapping fungi

Bruce A. JAFFEE

Department of Nematology, University of California, Davis, CA 95616-8668, USA.

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Summary – In pots containing silty loam field soil and in Petri dishes containing agar, *Meloidogyne javanica* was more likely to be trapped and colonized than was *Heterodera schachtii* by the nematode-trapping fungi *Arthrobotrys haptotyla* and *A. thaumasia*. The nematodes, however, exhibited equal susceptibility to *Arthrobotrys dactyloides* in soil and nearly equal susceptibility on agar. Because *A. haptotyla* and *A. thaumasia* use adhesive traps but *A. dactyloides* does not, and because other reports have indicated similar susceptibility patterns, cyst nematodes may generally be resistant and root-knot nematodes generally susceptible to fungi with adhesive traps. *A. haptotyla* was noteworthy because it increased to large numbers (>10^4 propagules/g of soil), it substantially (>90 %) suppressed *M. javanica* invasion of roots, and its parasitic activity could be quantified by extraction of trapped nematodes from soil. © Orstom/Elsevier, Paris.

Résumé – ***Sensibilité d'un nématode à kyste et d'un nématode galligène à trois champignons nématophages*** – Il est démontré que deux champignons prédateurs de nématodes, *Arthrobotrys haptotyla* et *A. thaumasia*, ont préférentiellement piégé et colonisé *Meloidogyne javanica* plutôt que *Heterodera schachtii*, tant dans en pots contenant un limon argileux que sur milieu gélosé en boîtes de Petri. Par contre, les deux nématodes ont montré une sensibilité équivalente à celle d'*Arthrobotrys dactyloides* dans le sol et presque équivalente sur gélose. Le fait que *A. haptotyla* et *A. thaumasia* forment des pièges adhésifs tandis que *A. dactyloides* n'en forme pas - d'autres observations indiquant des types de sensibilité identiques - suggère que les nématodes galligènes pourraient être en général sensibles aux champignons formant des pièges adhésifs tandis que les nématodes à kystes y seraient résistants. L'action de *A. haptotyla* doit être notée : il s'est en effet très fortement développé (>10^4 propagules/g de sol), il a inhibé substantiellement (>90 %) l'invasion des racines par *M. javanica*, et il a été possible de quantifier son activité parasitaire par extraction des nématodes piégés dans le sol. © Orstom/Elsevier, Paris.

Keywords: biological control, *Heterodera schachtii*, *Meloidogyne javanica*, nematode-trapping, nematophagous fungi.

Nematode-trapping fungi occur in most soils and are generally thought to trap and parasitize many different nematode species (Duddington, 1957; Gray, 1983; Stirling, 1991). Jaffee *et al.* (1993) therefore assumed equivalent susceptibility of two economically-important species of plant-parasitic nematodes to several nematode-trapping fungi. That assumption was wrong. When challenged with the fungi *Monacrosporium gephyropagum* or *M. ellipsosporum*, three species of root-knot nematode (*Meloidogyne javanica*, *M. incognita*, and *M. chitwoodi*) were much more susceptible than were three isolates of the cyst nematode, *Heterodera schachtii* (Jaffee & Muldoon 1995*a*). This was true in two environments (on agar and in soil) and with two forms of fungus inoculum (fungus-colonized nematodes and hyphae embedded in alginate pellets).

The present paper determines whether the differential susceptibility of *M. javanica* and *H. schachtii* extends to three other nematode-trapping fungi: *Arthrobotrys dactyloides*, *A. haptotyla*, and *A. thaumasia*. These fungi were selected because they are commonly found in the Sustainable Agriculture Farming Project plots at the University of California at Davis (Jaffee *et al.*, 1998). Like *M. gephyropagum* and *M. ellipsosporum*, *A. haptotyla* and *A. thaumasia* produce adhesive traps (stalked adhesive knobs and adhesive networks, respectively). In contrast, *A. dactyloides* produces constricting rings.

Material and methods

NEMATODES AND FUNGI

Second-stage juveniles (J2) of the root-knot nematode, *Meloidogyne javanica*, were obtained from tomato cv. UC 82 grown in sand; roots were gently washed and then aerated in a beaker containing water. *M. javanica* J2 were also obtained from tomatoes grown in nutrient solution (Lambert *et al.*, 1992). Water or nutrient solution was changed daily so that J2 were not more than 24 h old. Nematodes in water or nutrient solution were collected on a sieve and washed onto a Baermann funnel containing tap water; they were collected from the funnel every 40 min. Cysts of *Heterodera schachtii* were obtained from sugar beet cv. SSY1 grown in sand; cysts were incubated on Baermann funnels, and J2 were collected every

40 min. Second-stage juveniles of both nematodes were stored at 10 °C in aerated water for less than 24 h before addition to soil. Infective juveniles of the entomopathogenic nematode *Steinernema glaseri* were obtained from infected *Galleria mellonella* L. (see Kaya & Stock, 1997), and stored for less than 2 months at 10 °C.

Fungi included: *Arthrobotrys dactyloides* Drechsler (ARSEF 5433); *Arthrobotrys haptotyla* (Drechsler) Schenck, Kendrick & Pramer (ARSEF 5435): *Arthrobotrys thaumasia* (Drechsler) Schenck, Kendrick & Pramer (ARSEF 5434); and *Monacrosporium gephyropagum* (Drechsler) Subram (ARSEF 3349). *Arthrobotrys haptotyla* is sometimes called *Monacrosporium haptotylum* (Liu & Zhang, 1994). *M. gephyropagum* is equivalent to *M. cionopagum* (Rubner, 1996); it produces adhesive branches and was included as a standard, because it substantially suppresses *M. javanica* but not *H. schachtii* in loamy sand (Jaffee & Muldoon, 1995*a*).

The first three fungi were isolated from the Sustainable Agriculture Farming Project plots at the University of California at Davis (Jaffee *et al.*, 1998). All fungi were maintained on quarter-strength corn meal agar (CMA/4) and were subcultured monthly. Preparation of pelletized hyphae (in alginate, without added nutrients, and with a sand coating) has been described (Lackey *et al.*, 1993; Jaffee & Muldoon, 1995*b*). The dried pellets (about 2 mm diam., 1.6 mg, 0.14 mg hyphae) were stored at 5 °C for 1-14 days before use.

Pot experiment

The silty loam (pH 7.1, organic matter content 1.5 %) used in the pot experiment was collected from the Sustainable Agriculture Farming Systems Project in Yolo County, California (Temple *et al.*, 1994). It was collected from four conventionally-managed plots and was sieved (5 mm) but was not heat treated.

The pot experiment was performed twice (Trials 1 and 2) using soil collected from the same plots but at different times. The soil used in Trial 1 was stored at 10 °C for 60 days and contained (based on wet sieving and centrifugation) 332 bacterivorous, 202 fungivorous, 220 omnivorous, and 380 plant-parasitic nematodes per 100 g of soil (dry weight equivalent). Among the plant-parasitic nematodes were many *Pratylenchus* spp. but no *Meloidogyne* or *Heterodera* spp. The soil in Trial 2 was stored at 10 °C for 120 days; numbers of nematodes were similar to those in Trial 1 but two *Meloidogyne* juveniles per 100 g of soil were detected. Also resident in the soil were relatively small numbers of several nematode-trapping fungi (see Results); by adding pelletized hyphae of certain fungi to soil, I intended to substantially increase the population densities of those fungi above the densities of the resident fungi and to quantify the effect of the added fungi on invasion of roots by *M. javanica* and *H. schachtii*. Heat treatment of the soil would have removed the resident fungi and simplified the experiment but a secondary objective was to determine whether the pelletized fungi could be successfully introduced into field soil.

Before the fungi were added to soil, soil water content was increased to 15 % with distilled water, and the soil was divided into 100 cm^3 lots. Fifty pellets (either control pellets without hyphae or pellets with hyphae of one of the four fungi, stored for 1 day in Trial 1 and 14 days in Trial 2) were mixed into each lot, and the soil was placed in 190-ml polystyrene cups (= pots) and tamped. Because the mass of hyphae added per pot was small (7 mg per pot or about 0.006 %) (Lackey *et al.*, 1993), control pellets containing nonviable hyphae were not included. Pots were covered with aluminum foil to reduce water loss and placed in a clear plastic box with moist paper towels (moisture chamber) at 20 °C in the dark.

After 14 days at 20 °C, 749 ± 28 *M. javanica* or 791 ± 22 *H. schachtii* in 3 ml of 4.5 mM KCl were added to the surface of the soil in each of four replicate pots per treatment; in Trial 2, 709 ± 27 *M. javanica* or 647 ± 17 *H. schachtii* were added. The pots were covered with foil and returned to the incubator. On day 17, covers were removed, six germinated cabbage seeds (*Brassica oleraceae* L.) cv. Grand Slam were planted in each pot, and pots in moisture chambers were placed under fluorescent lights at 20 °C. On day 23, roots were removed from soil, measured, stained, and cleared (Byrd *et al.*, 1983). All the nematodes in roots were counted as *Meloidogyne* sp., *Heterodera* sp., or "others". Because the soil naturally contained few or no *Meloidogyne* spp. and no *Heterodera* spp., and because substantial numbers of these nematodes had been added, I assumed that all *Meloidogyne* sp. and *Heterodera* sp. observed in roots were *M. javanica* and *H. schachtii* originally added on day 14.

Three additional replicate pots per treatment were used to assess fungus population density. On day 14, the soil in each pot was placed in a plastic bag and mixed, 90 g (dry weight equivalent) was placed in a 250-ml flask, and the volume was increased to 200 ml with sterile distilled water. The sample was shaken vigorously for 8 min (this was sufficient to separate all soil particles). A ten-fold dilution series was prepared in sterile-distilled water, and 0.1 ml from each dilution was added in five drops to each of five CMA/4 Petri dishes. Thus, each dish received 45 mg, 4.5 mg, 0.45 mg, 0.045 mg, or 0.0045 mg of soil. Bait nematodes (*S. glaseri*) were added (Jaffee *et al.*, 1998). After 3 weeks at 22 °C, the entire surface of each dish was examined at 50-140× magnification with a dissecting microscope. Dishes were scored for the presence or absence of nematode-trapping fungi by species. Fungi

were identified based on trap, conidiophore, and conidium morphology and with the aid of published keys and original descriptions. A most probable number program (Klee, 1993) was used to estimate the population density of each species.

AGAR DISH EXPERIMENT

To help determine whether the relative susceptibility of *M. javanica* and *H. schachtii* to the fungi was associated with trapping, susceptibility was compared on agar, where trapping was readily observed. The fungi (minus *M. gephyropagum*) were grown on CMA/4 in 15 × 60 mm plastic Petri dishes at 20 °C. When the agar surface was 75 % colonized, 200 *S. glaseri* in 20 µl distilled water were added to each of three replicate dishes to induce abundant trap formation. Except for *A. thaumasia*, these fungi spontaneously produce traps on agar, but they produce many more traps if nematodes are added. Two to 3 days later, a mixture of *M. javanica* and *H. schachtii* (about 100 of each) in 20 µl distilled water was added to the center of each culture of *A. haptotyla* and *A. dactyloides*. In the case of *A. thaumasia*, the nematodes were not mixed but were added to separate cultures because, once nematodes were entwined and colonized in three-dimensional networks, their species could not be determined. After the water evaporated or was absorbed, the dishes were covered and stored at 20 °C. All nematodes on the dishes were examined periodically with a dissecting microscope at 140× magnification to determine the percentage trapped and the percentage colonized (trapped and filled with hyphae). The agar dish experiment was performed twice (Trials 1 and 2), each time with three replicate plates per treatment.

VIAL EXPERIMENT

Traps of many nematode-trapping fungi do not readily detach from the parent hypha, and the trapped nematode therefore may not be extractable from soil. This is true of *M. gephyropagum* and is disappointing because it limits our ability to quantify parasitism and thus our ability to understand the biology of this biological control agent (Robinson & Jaffee, 1996; Jaffee & Muldoon, 1997). Previous reports (Barron, 1975; Wimble & Young, 1983) and personal preliminary observations, however, indicated that nematodes parasitized by *A. haptotyla* may be extracted from soil because the thin, hyphal segment connecting the adhesive knob to the parent hypha often breaks when soil is mixed. An experiment was conducted to test the following hypothesis: when added to and then extracted from soil infested with *A. haptotyla*, more *M. javanica* than *H. schachtii* will have adherent knobs.

The sandy loam (pH 7.2, organic matter content 1.2 %) used in the vial experiment was collected from a commercial tomato field in Yolo County, California. The sandy loam rather than the silty loam was used for the vial experiment because the sandy loam was easier to handle. The sandy loam was sieved (5 mm), heated to 60 °C for 2 h (to remove resident nematodes and nematophagous fungi), aired on the laboratory bench for 48 h, and stored at 10 °C.

Heat-treated sandy loam (10 % water content) containing pellets was packed into 25 ml vials. There were four pellets per vial, all four of which either contained or did not contain hyphae of *A. haptotyla*. Each vial contained 20 g of soil (dry weight equivalent) in 17.2 cm^3; hence the bulk density was 1.2. A lid with a 2-mm hole for gas exchange covered each vial. Vials were kept in moisture chambers at 20 °C. After 10 days, about 250 J2 (125 *M. javanica* + 125 *H. schachtii*) in 0.5 ml of 4.5 mM KCl were added to the surface of the soil in each vial. After 48 h at 20 °C, the soil was removed from the vial, mixed, and then extracted by wet sieving and centrifugation (Jenkins, 1964). To obtain a high extraction efficiency, soil and nematode suspensions were passed through a sieve (38 µm) after only 10 s of settling; after centrifugation in sucrose, nematodes were collected on a fine sieve (28 µm); and all sieves were washed from underneath to collect nematodes.

In a preliminary experiment, some extracted nematodes were entwined in hyphae of *A. haptotyla* and therefore difficult to observe and count. Suspensions were therefore vigorously shaken before being poured into a counting dish; the shaking freed the nematodes from the hyphae. All nematodes were examined at 140× magnification to determine whether they had adherent knobs; in addition, the number of knobs per nematode was determined on the first ten nematodes encountered in each replicate. Observation of knobs required frequent adjusting of the light source (mirror) and turning of nematodes with a needle. There were six replicate vials per treatment (± *A. haptotyla*), and the experiment was performed twice (Trials 1 and 2).

STATISTICAL ANALYSES

Analysis of variance (SAS release 6.12, SAS Institute Inc., Cary, NC, USA) was used to analyze data from the pot experiment and vial experiment; significance was determined at $P \leq 0.05$. In the pot experiment, Duncan's Multiple Range Test was used to separate the effects of nematode and fungus on suppression. For the agar dish experiment, inferences were based on examination of means and standard errors.

Results

POT EXPERIMENT

The data from Trials 1 and 2 were similar in most but not in all respects, and therefore the data were not combined. Resident nematode-trapping fungi in the silty loam included *A. haptotyla*, *A. thaumasia*, *A. oligospora*, and *M. eudermatum* (Table 1); *M. gephyropagum* in control pots of Trial 1 presumably reflected contamination during quantification of the fungi. When a fungus was added to the soil, the numbers of that fungus detected on day 14 were usually substantial and much larger than those naturally present; this was especially true for *A. haptotyla*, which exceeded 10^4 propagules/g of soil in both trials. *A. dactyloides*, on the other hand, was detected when added but only in relatively small numbers. Both *M. gephyropagum* and *A. thaumasia* were more abundant in Trial 1 than in Trial 2.

In pots receiving pellets without hyphae, 16 % of the *M. javanica* inoculum and 20 % of the *H. schachtii* inoculum penetrated the roots. The pattern of suppression of root penetration was similar in Trials 1 and 2, but *A. thaumasia* was less suppressive in Trial 2 than in Trial 1 (Fig. 1). A factorial analysis indicated a significant interaction between fungus and nematode in both trials. The basis of this statistical interaction was obvious: *A. dactyloides* equally suppressed *M. javanica* and *H. schachtii* whereas the other three fungi suppressed *M. javanica* more than *H. schachtii*. In an analysis by nematode, suppression of *H. schachtii* was greater by *A. dactyloides* than by the other fungi. Suppression of *M. javanica* by *A. haptotyla* and *M. gephyropagum* was substantial (>90 %) and was greater than that by *A. dactyloides* or *A. thaumasia*.

Suppression of root penetration was either positively correlated with root length or was unrelated to root length; the correlation was never negative, as might occur if the roots were severely diseased by fungal pathogens (data not shown).

AGAR DISH EXPERIMENT

Data from Trials 1 and 2 were similar and therefore combined for analysis. Both nematodes were trapped by the three fungi (Fig. 2A-C). Based on trapping, both nematodes were equally susceptible to *A. dactyloides*, and trapping by this fungus was surprisingly efficient and fast (Fig. 2A). *A. dactyloides* colonized both nematodes but colonized *M. javanica* faster than *H. schachtii* (Fig. 2D). Based on trapping and colonization, *M. javanica* was more susceptible than was *H. schachtii* to *A. haptotyla* (Fig. 2B, E) and *A. thaumasia* (Fig. 2C, F).

VIAL EXPERIMENT

Data from Trials 1 and 2 were similar and therefore combined for analysis. A factorial analysis indicated that extraction efficiency was greater for *H. schachtii* than for *M. javanica* but was unaffected by *A. haptotyla* (Fig. 3A). Both *M. javanica* and *H. schachtii* had adherent knobs of *A. haptotyla* (Fig. 4), but the percentage was greater for *M. javanica* (Fig. 3B). Moreover, among those nematodes with knobs, *M. javanica*

Table 1. *Population densities of* Monacrosporium gephyropagum, Arthrobotrys dactyloides, A. haptotyla, *and* A. thaumasia *in the pot experiment.*

Fungus added	Fungi detected (propagules/g of soil)					
	M. gephyropagum	*A. dactyloides*	*A. haptotyla*	*A. thaumasia*	*A. oligospora*	*M. eudermatum*
Trial 1						
None	6±6	0	4±4	14±9	2±2	2±2
M. gephyropagum	1394±613	0	2±2	3±2	0	0
A. dactyloides	0	74±7	9±2	22±2	3±2	0
A. haptotyla	0	0	11344±4282	0	2	0
A. thaumasia	0	0	5±3	1329±299	0	0
Trial 2						
None	0	0	0	12±7	3±2	2±2
M. gephyropagum	63±20	0	0	8±4	0	0
A. dactyloides	0	54±18	0	9±2	2±2	0
A. haptotyla	0	0	13643±6803	2±2	2±2	0
A. thaumasia	0	0	2±2	301±68	0	0

Values are means±SE of three replicates.

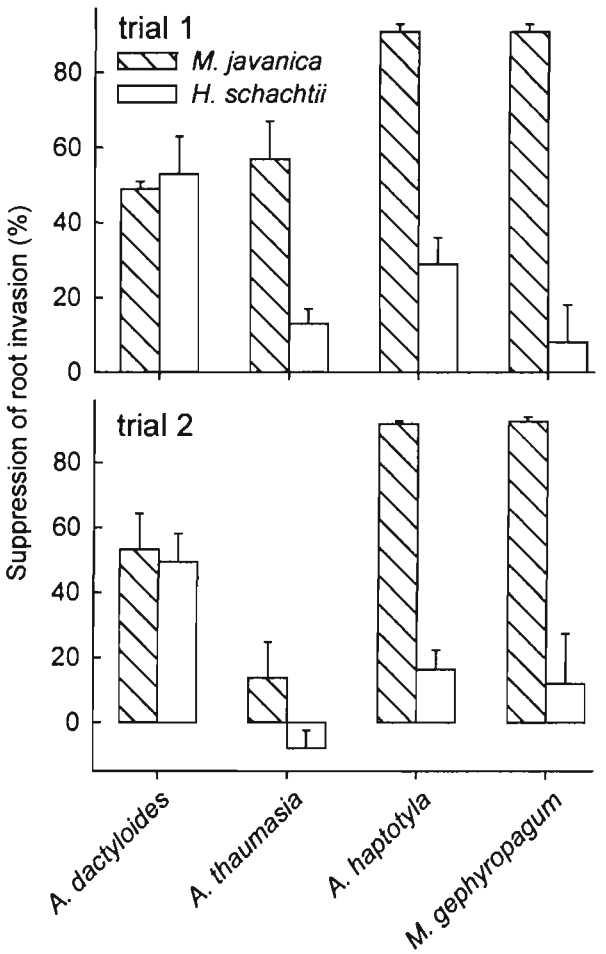

Fig. 1. *Suppression of* Heterodera schachtii *and* Meloidogyne javanica *root invasion by alginate pellets containing hyphae of* Arthrobotrys dactyloides, A. thaumasia, A. haptotyla, *and* Monacrosporium gephyropagum *(pot experiment). Suppression* = (1 − a/b) × 100, *where* a *and* b *refer to the number of nematodes penetrating roots in field soil (silty loam) containing pellets with hyphae and without hyphae, respectively. Values are the means + SE of four replicates.*

had more knobs per nematode than did *H. schachtii* (Fig. 3C). When nematodes were mounted between glass slide and cover slip for observation with the compound microscope, knobs frequently detached from *H. schachtii* but not from *M. javanica*; knobs seemed to adhere more tightly to *M. javanica* than to *H. schachtii* (Fig. 4). Knobs originating from conidia and adhering to nematodes were not observed.

Discussion

M. javanica J2 appear to be substantially more susceptible than *H. schachtii* J2 to certain nematode-trapping fungi, including *M. ellipsosporum*, *M. gephyropagum*, *A. haptotyla*, and *A. thaumasia*. The significance of this finding for biological control research is clear: *M. javanica* may be a good target but *H. schachtii* is definitely a poor target for these natural enemies. Moreover, susceptibility should be quantified (preferably in soil) rather than assumed. For example, Cooke (1962*a*, *b*) found that organic amendments to soil stimulated both nematodes and nematode-trapping fungi but concluded that the data did not indicate density-dependent, predator-prey dynamics; however, neither the identity of the nematodes nor their susceptibility to the fungi was determined.

Although not extensive, data from other studies (in soil, on agar, or in water) are consistent with the idea that J2 of cyst nematodes are generally less susceptible than those of root-knot nematodes to fungi with adhesive traps. In soil, three populations of *H. schachtii* were less susceptible than three species of root-knot nematode (*M. chitwoodi*, *M. incognita*, and *M. javanica*) to *M. ellipsosporum* and *M. gephyropagum* (Jaffee & Muldoon, 1995*a*). On agar, two species of cyst nematode (*Globodera pallida* and *G. rostochiensis*) were less susceptible than a root-knot nematode (*M. hapla*) and a lesion nematode (*Pratylenchus penetrans*) to *Arthrobotrys oligospora* (Den Belder & Jansen, 1994); *G. rostochiensis* was not trapped by the adhesive knobs of *Dactylella lysipaga* but root-knot nematodes (*M. hapla*, *M. incognita*, and *M. javanica*) and several other nematode species were (Wimble & Young, 1983); and *G. rostochiensis* was relatively resistant to the adhesive knobs of *A. dasguptae* (Boag *et al.*, 1988). In water, *H. schachtii* was resistant to *A. haptotyla* and other nematode-trapping fungi (Dowe, 1966).

An exception is the report by Velvis and Kamp (1995) indicating that *A. haptotyla* parasitized *G. pallida* in soil; that study focused on *Hirsutella rhossiliensis*, however, and did not provide data for *A. haptotyla*. In another study (Duponnois *et al.*, 1996), the proportion of nematodes captured in adhesive traps of *A. oligospora* and *A. conoides* on agar varied greatly among *Meloidogyne* spp., with few J2 of *M. javanica* being trapped; unfortunately, the data lack a treatment in which the susceptibility of *M. javanica* equals or exceeds that of the other *Meloidogyne* spp., suggesting the possibility that the *M. javanica* J2 were not vigorous at the outset and therefore did not induce or move into traps.

Researchers have unsuccessfully attempted to use various nematode-trapping fungi to control cyst nematodes (Hutchinson & Mai, 1954; Duddington *et al.*, 1956; Hams & Wilkin, 1961). Although the disappointing results could reflect low nematode susceptibility, attempts to control root-knot nematode have also been disappointing (*e.g.*, Mankau, 1961*a*, *b*).

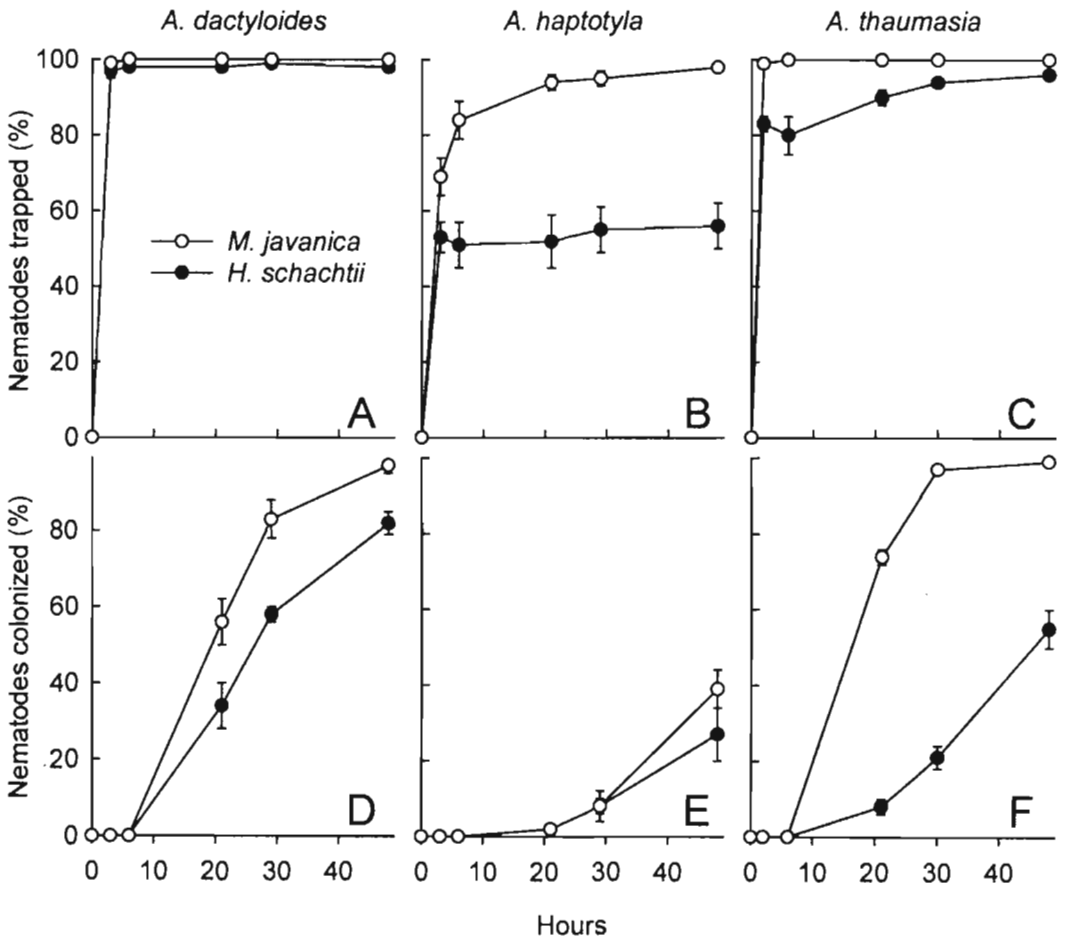

Fig. 2. *Susceptibility of* Heterodera schachtii *and* Meloidogyne javanica *to* Arthrobotrys dactyloides, A. haptotyla, *and* A. thaumasia *on agar (Petri dish experiment). A-C: Percentage of nematodes trapped; D-F: Percentage of nematodes colonized (filled with hyphae). Values are means + SE of six replicate dishes.*

Clearly, factors other than nematode susceptibility can limit the activity of these fungi.

The relative susceptibility of cyst and root-knot nematode J2, if truly general, would fit a broader pattern: J2 of cyst nematodes seem tougher and better adapted to persist than those of root-knot nematodes. Thus, cyst nematode J2 are relatively large and robust (Fig. 4), they move through root cells rather than between them, and they tend to persist in eggs unless conditions are suitable for invasion of roots. Indeed, the terms 'r' and 'K' strategists have been applied to the root-knot and potato cyst nematode, respectively (Trudgill *et al.*, 1992).

This congruence between general biological characteristics and susceptibility to certain nematode-trapping fungi, however, is imperfect. A variety of other fungi attack eggs and females and, to the best of my knowledge, differences in susceptibility between these stages of root-knot and cyst nematodes have not been reported. Moreover, differences in J2 susceptibility were negligible with *A. dactyloides* in the current study and with *H. rhossiliensis* in an earlier study (Tedford *et al.*, 1992).

Why cyst and root-knot nematodes differ in susceptibility to certain nematode-trapping fungi is not clear, but differential adhesion seems to be important (Jaffee & Muldoon, 1995*a*). The current data support the idea that adhesion is involved because the one fungus without adhesive traps, *A. dactyloides*, captured cyst and root-knot nematodes equally well.

A. dactyloides has attracted attention in part because its method of obtaining food is dramatic - rings rapidly constricting around nematodes usually impress the nonscientist and occasionally impress the veteran

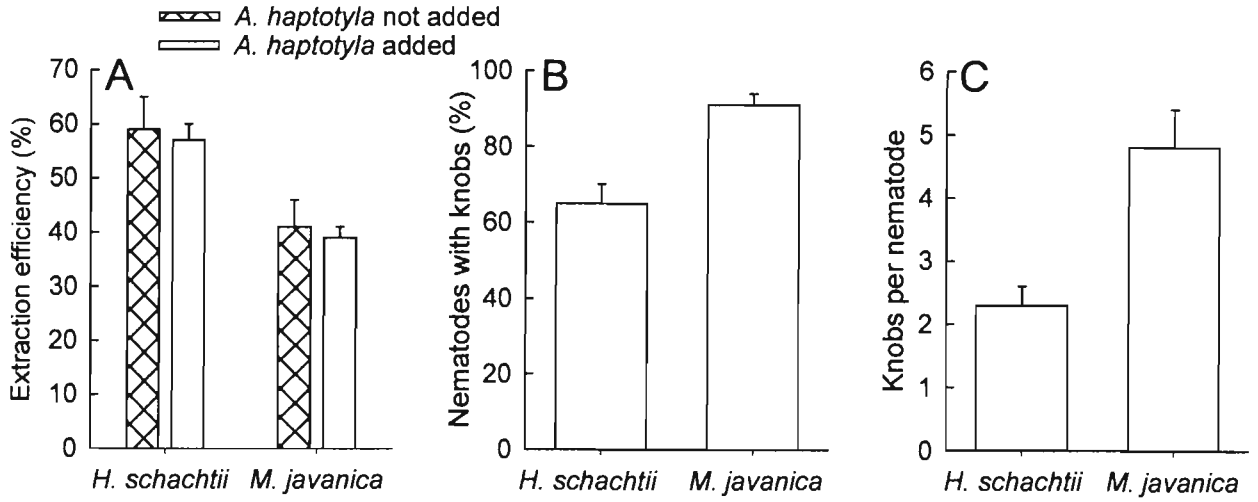

Fig. 3. Acquisition of Arthrobotrys haptotyla *adhesive knobs by* Heterodera schachtii *and* Meloidogyne javanica *in heat-treated sandy loam. The knobs adhere to nematodes and usually detach from the parent hypha when nematodes are extracted from soil by wet screening and centrifugation. A: Extraction efficiency = (number of cyst or root-knot nematodes recovered per vial/number added per vial) × 100; B: Percentage of extracted nematodes with adherent knobs; C: Number of adherent knobs per nematode (among nematodes with at least one knob). Values are means + SE of twelve replicate vials.*

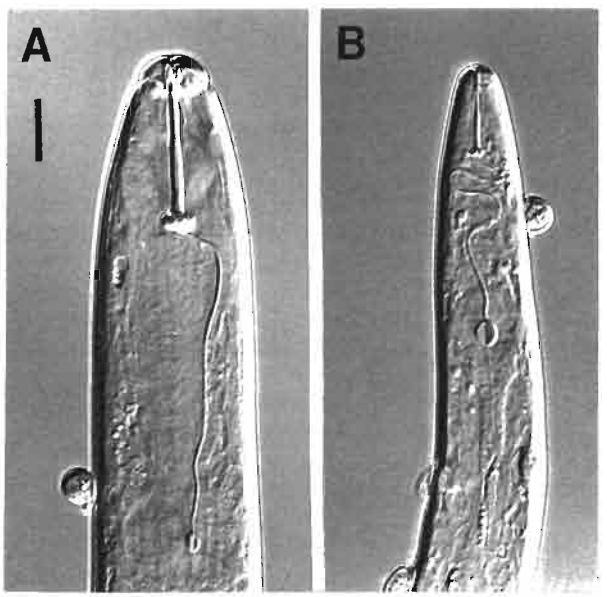

Fig. 4. Light micrographs of detached adhesive knobs of Arthrobotrys haptotyla. *A:* Heterodera schachtii; *B:* Meloidogyne javanica. *To reduce depth of field and thereby improve the differential interference contrast image (Barron, 1980, 1986), slides were allowed to dry; nematodes therefore were compressed. (Magnification is equivalent in A and B; scale bar = 10 µm).*

nematologist. Many researchers, however, consider *A. dactyloides* interesting but unimportant, because attempts to use it and other trapping fungi as control agents have not been successful (but see Stirling and Smith, 1998). While use of these fungi as control agents does not seem to be easy, discussions of their potential importance or lack thereof are premature given that so little is known about their biology in soil. As a control agent, *A. dactyloides* has some desirable qualities: it attacks both cyst and root-knot nematodes, and if formulated in alginate, it can "establish" in soil, at least for short times.

Like *A. dactyloides*, *A. haptotyla* warrants additional research. First, *A. haptotyla* grows rapidly in shake culture and, when added to soil as pelletized hyphae, substantially suppressed root penetration by *M. javanica* in the current study and in a field microplot experiment (B. Jaffee, unpubl.). Second, it resides in local soils and therefore appears adapted to local conditions. Third, *A. haptotyla* increases to large numbers when added to soil. Finally, because adhesive knobs detach from parent hyphae (Barron, 1975; Wimble & Young, 1983), nematodes parasitized by *A. haptotyla* can be extracted from soil, and so parasitism can be quantified.

Quantifying parasitism (or predation, antibiosis, etc.) is important in biological control and in ecological research in general. Without direct evidence of interaction between control agent and target, researchers should remain uncertain about how biological control does or does not occur. Uncertainty is standard with most nematode-trapping fungi, because extraction of trapped nematodes from soil is often

inefficient; parasitism is therefore inferred by a reduction in nematode extraction (Jansson, 1982), a reduction in nematode root-penetration (*e.g.*, Jaffee & Muldoon, 1997), or both (Robinson & Jaffee, 1996).

Uncertainty about parasitism motivated the agar dish and vial experiments in the present study. If the susceptibility patterns obtained in the dish, vial, and pot experiments did not match, the pattern in the pot experiment may not have involved trapping and parasitism at all. Because the patterns were similar on agar and in soil and because trapping was directly assessed on agar, the pattern from soil apparently reflected trapping. In the case of *A. haptotyla*, of course, direct observation confirmed that differences in root invasion were associated with differences in how the nematodes interacted with the traps.

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References

BARRON, G.L. (1975). Detachable adhesive knobs in *Dactylaria*. *Trans. Br. Mycol. Soc.*, 65: 311-312.

BARRON, G.L. (1980). Fungal parasites of rotifers: *Harposporium*. *Can. J. Bot.*, 58: 2193-2199.

BARRON, G.L. (1986). A new *Harposporium* parasitic in bdelloid rotifers. *Can. J. Bot.* 64: 2379-2382.

BOAG, B., ROBERTSON, W.M. & AINSWORTH, L.F. (1988). Observations on the specificity of the nematophagous fungus *Arthrobotrys dasguptae* (Shome & Shome) to plant-parasitic nematodes. *Nematologica*, 34: 238-245.

BYRD, D.W. JR., KIRKPATRICK, T. & BARKER, K.R. (1983). An improved technique for clearing and staining plant tissues for detection of nematodes. *J. Nematol.*, 15: 142-143.

COOKE, R.C. (1962*a*). The ecology of nematode-trapping fungi in soil. *Ann. appl. Biol.*, 50: 507-513.

COOKE, R.C. (1962*b*). Behavior of nematode-trapping fungi during decomposition of organic matter in soil. *Trans. Br. Myc. Soc.*, 45: 314-320.

DEN BELDER, E. & JANSEN, E. (1994). Capture of plant-parasitic nematodes by an adhesive hyphae forming isolate of *Arthrobotrys oligospora* and some other nematode-trapping fungi. *Nematologica*, 40: 423-437.

DOWE, A. (1966). Untersuchungen zur Frage der Wirtsspezifität nematodenfangender Pilze (Hyphomycetes). *Wiss. Z. Univ. Rostock*, 15: 261-264.

DUDDINGTON, C.L., JONES, F.G.W. & WILLIAMS, T.D. (1956). An experiment on the effect of a predacious fungus upon the soil population of potato root eelworm, *Heterodera rostochiensis* Woll. *Nematologica*, 1: 341-343.

DUDDINGTON, C.L. (1957). *The friendly fungi*. London, UK, Faber & Faber, 188 p.

DUPONNOIS, R., MATEILLE, T., SENE, V., SAWADOGO, A. & FARGETTE, M. (1996). Effects of different West African species and strains of *Arthrobotrys* nematophagous fungi on *Meloidogyne* species. *Entomophaga*, 41: 475-483.

GRAY, N.F. (1983). The ecology of nematophagous fungi: *Panagrellus redivivus* as the target organism. *Pl. & Soil*, 73: 293-297.

HAMS, A.F. & WILKIN, G.D. (1961). Observations on the use of predacious fungi for the control of *Heterodera* spp. *Ann. appl. Biol.*, 49: 515-523.

HUTCHINSON, S.A. & MAI, W.F. (1954). A study of the efficiency of the catching organs of *Dactylaria eudermata* (Drechs.) in relation to *Heterodera rostochiensis* (Wr.) in soil. *Pl. Dis. Reptr*, 38: 185-186.

JAFFEE, B.A., FERRIS, H. & SCOW, K.M. (1998). Nematode-trapping fungi in organic and conventional cropping systems. *Phytopathology*, 88: 344-350.

JAFFEE, B.A. & MULDOON, A.E. (1995*a*). Susceptibility of root-knot and cyst nematodes to the nematode-trapping fungi *Monacrosporium ellipsosporum* and *M. cionopagum*. *Soil Biol Biochem.*, 27: 1083-1090.

JAFFEE, B.A. & MULDOON, A.E. (1995*b*). Numerical responses of the nematophagous fungi *Hirsutella rhossiliensis*, *Monacrosporium cionopagum*, and *M. ellipsosporum*. *Mycologia*, 87 : 643-650.

JAFFEE, B.A. & MULDOON, A.E. (1997). Suppression of the root-knot nematode *Meloidogyne javanica* by alginate pellets containing the nematophagous fungi *Hirsutella rhossiliensis*, *Monacrosporium cionopagum* and *M. ellipsosporum*. *Biocontr. Sci. Technol.*, 7: 203-217.

JAFFEE, B.A., TEDFORD, E.C. & MULDOON, A.E. (1993). Tests for density-dependent parasitism of nematodes by nematode-trapping and endoparasitic fungi. *Biol. Contr.*, 3: 329-336.

JANSSON, H.-B. (1982). Predacity by nematophagous fungi and its relation to the attraction of nematodes. *Microb. Ecol.*, 8: 233-240.

JENKINS, W.R. (1964). A rapid centrifugal-flotation technique for separating nematodes from soil. *Pl. Dis. Reptr*, 48: 692.

KAYA, H.K. & STOCK, S.P. (1997). Techniques in insect nematology. *In :* Lacey, L. (Ed.). *Manual of techniques in insect pathology*. San Diego, CA, USA, Academic Press: 281-324.

KLEE, A. (1993). A computer program for the determination of most probable number and its confidence limits. *J. microb. Methods*, 18: 91-98.

LACKEY, B.A., MULDOON, A.E. & JAFFEE, B.A. (1993). Alginate pellet formulation of *Hirsutella rhossiliensis* for biological control of plant-parasitic nematodes. *Biol. Contr.*, 3: 155-160.

LAMBERT, K.N., TEDFORD, E.C., CASWELL, E.P. & WILLIAMSON, V.M. (1992). A system for continuous production of root-knot nematode juveniles in hydroponic culture. *Phytopathology*, 82: 512-515.

LIU, X.Z. & ZHANG, K.Q. (1994). Nematode-trapping species of *Monacrosporium* with special reference to two new species. *Mycol. Res.*, 98: 862-868.

MANKAU, R. (1961*a*). An attempt to control root-knot nematode with *Dactylaria thaumasia* and *Arthrobotrys arthrobotryoides*. *Pl. Dis. Reptr*, 45: 164-166.

MANKAU, R. (1961*b*). The use of nematode-trapping fungi to control root-knot nematodes. *Nematologica*, 6: 326-332.

ROBINSON, A.F. & JAFFEE, B.A. (1996). Repulsion of *Meloidogyne incognita* by alginate pellets containing hyphae of *Monacrosporium cionopagum*, *M. ellipsosporum*, or *Hirsutella rhossiliensis*. *J. Nematol.*, 28: 133-147.

RUBNER, A. (1996). Revision of predacious hyphomycetes in the *Dactylella-Monacrosporium* complex. *Stud. Mycol.*, 39: 1-134.

STIRLING, G.R. (1991). *Biological control of plant parasitic nematodes*. Wallingford, UK, CAB International, 282 p.

STIRLING, G.R. & SMITH, L.J. (1998). Field tests of formulated products containing either *Verticillium chlamydosporium* or *Arthrobotrys dactyloides* for biological control of root-knot nematodes. *Biol. Contr.*, 11: 231-239.

TEDFORD, E.C., JAFFEE, B.A. & MULDOON, A.E. (1992). Effect of soil moisture and texture on transmission of the nematophagous fungus *Hirsutella rhossiliensis* to cyst and root-knot nematodes. *Phytopathology*, 82: 1002-1007.

TEMPLE, S.R., FRIEDMAN, D.B., SOMASCO, O., FERRIS, H., SCOW, K.M. & KLONSKY, K. (1994). An interdisciplinary, experiment station-based participatory comparison of alternative crop management systems for California's Sacramento Valley. *Am. J. alternat. Agric.*, 9: 64-71.

TRUDGILL, D.L., KERRY, B.R. & PHILLIPS, M.S. (1992). Seminar: Integrated control of nematodes (with particular reference to cyst and root knot nematodes). *Nematologica*, 38: 482-487.

VELVIS, H. & KAMP, P. (1995). Infection of second stage juveniles of potato cyst nematodes by the nematophagous fungus *Hirsutella rhossiliensis* in Dutch potato fields. *Nematologica*, 41: 617-627.

WIMBLE, D. B. & YOUNG, T. W. K. (1983). Capture of nematodes by adhesive knobs in *Dactylella lysipaga*. *Microbios*, 36: 33-39.