

Infection of *Pratylenchus penetrans* by Nematode-pathogenic Fungi

P. TIMPER AND B. B. BRODIE¹

Abstract: Eleven fungal isolates were tested in agar dishes for pathogenicity to *Pratylenchus penetrans*. Of the fungi that produce adhesive conidia, *Hirsutella rhossiliensis* was a virulent pathogen; *Verticillium balanoides*, *Drechmeria coniospora*, and *Nematoctonus* sp. were weak or nonpathogens. The trapping fungi, *Arthrobotrys dactyloides*, *A. oligospora*, *Monacrosporium ellipsosporum*, and *M. cionopagum*, killed most of the *P. penetrans* adults and juveniles added to the fungus cultures. An isolate of *Nematoctonus* that forms adhesive knobs trapped only a small proportion of the nematodes. In 17-cm³ vials, soil moisture influenced survival of *P. penetrans* in the presence of *H. rhossiliensis*; nematode survival decreased with diminishing soil moisture. *Hirsutella rhossiliensis* and *M. ellipsosporum* were equally effective in reducing numbers of *P. penetrans* by 24–25% after 4 days in sand. After 25 days in soil artificially infested with *H. rhossiliensis*, numbers of *P. penetrans* were reduced by 28–53%.

Key words: biological control, *Hirsutella rhossiliensis*, migratory endoparasite, *Monacrosporium ellipsosporum*, nematode, nematophagous fungi, *Pratylenchus penetrans*, root lesion nematode.

Most of the research effort to date on biological control of nematodes has concentrated on sedentary endoparasites such as root-knot and cyst species. The emphasis on these nematodes reflects their importance as agricultural pests. Migratory endoparasites in the genus *Pratylenchus* are also economically important pests of a wide variety of crops (20), yet few studies have examined the impact of antagonists on species in this genus.

In one preliminary study (10), the nematode-trapping fungi *Arthrobotrys superba*, *A. dactyloides*, *A. arthrobotryoides*, and *Dactylella doedycoides* reduced penetration of alfalfa roots by *Pratylenchus penetrans* in sterilized soil. In another preliminary study (15), *A. dactyloides*, *A. arthrobotryoides*, and *Dactylaria thaumesia* slowed the population increase of *P. penetrans* on alfalfa in the laboratory and greenhouse. Of the three fungi tested, *A. dactyloides* was the most effective antagonist. It is clear that more research is needed to ascertain the capacity of nematode antagonists to suppress populations of *Pratylenchus* spp.

The objectives of our study were to identify pathogens of *P. penetrans* from known

antagonists of vermiform stages and to determine their effectiveness in reducing numbers of the nematode in soil. Nematode pathogens of eggs were not selected because, unlike root-knot and cyst species, *Pratylenchus* spp. do not lay their eggs en masse. Eggs laid singly or in small groups are less conducive to spread of pathogenic fungi than are eggs laid en masse; therefore, egg pathogens are not expected to cause significant mortality of *Pratylenchus* spp.

MATERIALS AND METHODS

Nematodes: Juveniles and adults of *P. penetrans* were obtained from alfalfa callus culture (16) using the Baermann pie-pan method (8) 1–3 days before an experiment. The entomopathogenic nematodes *Steinernema glaseri* and *Heterorhabditis bacteriophora* were reared in *Galleria mellonella* (2) and stored after harvesting in distilled water at 23 ± 5 C. Nematodes were extracted from soil by wet sieving through 250- and 38-µm screens followed by centrifugal flotation (7).

Fungi: Eleven fungi (Table 1) were grown on 25% cornmeal agar (1.5% agar) in 9-cm-d petri dishes for 2–4 weeks at 23 ± 5 C before testing for pathogenicity to *P. penetrans*. Soil was infested with fungi by adding dauers of *S. glaseri* infected by a fungal species to soil at a rate of 2/g soil (21). Infected *S. glaseri* were obtained ei-

Received for publication 1 April 1992.

¹ Research Affiliate and Research Plant Pathologist, USDA ARS, U.S. Plant, Soil and Nutrition Laboratory, Tower Rd., Cornell University, Ithaca, NY 14853. The authors thank H. Ferris, B. A. Jaffee, and E. B. Nelson for their helpful comments on the manuscript.

TABLE 1. The ability of various nematode-pathogenic fungi to adhere to and infect *Pratylenchus penetrans*.

Species or strain of fungus (ARSEF No.)†	Mode of infection	Adherence or trapping‡	Infection
<i>Hirsutiella rhossiliensis</i> (2006)	Adhesive conidia	+++	+++
<i>Verticillium balanoides</i> (3350)	Adhesive conidia	++	+
<i>V. balanoides</i> C2 (3173)	Adhesive conidia	+	-
<i>Drechmeria coniospora</i> (3346)	Adhesive conidia	++	-
<i>D. coniospora</i> C1 (3354)	Adhesive conidia	++	-
<i>Nematotoxonus</i> sp. (3352)	Adhesive conidia	+	+
<i>Nematotoxonus</i> sp. (3351)	Adhesive knob§	+	+
<i>Monacrosporium cionopagum</i> (3349)	Adhesive knob	+++	+++
<i>M. ellipsosporum</i> (3348)	Adhesive branches	+++	+++
<i>Arthrobotrys dactyloides</i> (3353)	Constricting ring	+++	+++
<i>A. oligospora</i> (3347)	Adhesive network	+++	+++

† USDA-ARS Collection of Entomopathogenic Fungal Cultures, Ithaca, New York. Isolates 2006, 3346, 3347, 3348, 3349, 3350, 3351, and 3353 were supplied by B. A. Jaffee, Nematology Department, University of California, Davis.

‡ The pathogenicity of a fungus was rated as high (+++) if >50%, medium (++) if >5%, and low (+) if <5% of the nematodes had adhering conidia, were trapped, or were infected. Fungus did not infect *P. penetrans* (-).

§ Few adhesive knobs were produced by this isolate.

ther by adding healthy nematodes to a sand culture of the fungus (4) and extracting after 4 days or by adding the nematodes to agar cultures and transferring infected ones with a wire pick to deionized water after 6 hours. Because conidia and trap production often vary among trials, their production was assayed using the entomopathogenic nematode *H. bacteriophora*. This species was used because its acquisition of *Hirsutiella rhossiliensis* conidia is consistently correlated with the amount of conidia in soil (12). Dauers of *H. bacteriophora* were added to three to five replicate containers of soil that were set up at the same time and incubated in the same way as the experimental containers. Dauers were added at a rate of 1000/17-cm³ vial or 3000/150-cm³ container and extracted 1 day later. The mean number of conidia or traps adhering to the second-stage cuticle of 40 *H. bacteriophora* dauers was calculated. This assay yields only a relative estimate and not the actual number of conidia and traps produced in the soil.

Soils: Two soils were used in the experiments. The first was a sand (34% fine, 44% medium, and 22% coarse) with a pH of 7.3 (in water). The second soil was a loamy sand (88.7% sand, 4.6% clay, and 6.6% silt) with a pH of 5.4 (in water). Both soils were moistened and heated to 60 C for 30 min-

utes to destroy most organisms, and were then air dried.

Susceptibility of P. penetrans to nematode-pathogenic fungi: Approximately 200 *P. penetrans* (adults and juveniles) in 0.25 ml water were pipetted onto a single petri dish culture of each of the 11 fungal isolates. Colonies of *Verticillium balanoides* (= *Tolyposcladium balanoides*) and *Drechmeria coniospora* grew slowly. To distribute the conidia that form in these small colonies over the dish, 1 ml of sterilized distilled water was pipetted onto the culture, the water was swirled in the dish to suspend the conidia, and 0.25 ml of the conidia-water suspension was pipetted onto 1.5% water agar. The conidia were then spread over the agar with a sterilized L-shaped glass rod. The dishes were allowed to dry without lids for 1 hour before nematodes were pipetted onto the agar surface. The dishes with nematodes were kept at 23 ± 5 C. Daily for 5 days, 10–20 *P. penetrans* were randomly selected, removed from the culture, and examined at ×500 for adherence of conidia or traps, and fungal infection. The pathogenicity of a fungus was rated as high if >50%, medium if >5%, and low if <5% of the nematodes had adhering conidia, were trapped, or were infected. This subjective rating was used to reflect the inherent inaccuracy of comparing dif-

ferent fungal species growing on agar dishes for pathogenicity to nematodes.

Effects of soil moisture on infection: Two fungi were chosen for further experiments in soil. *Hirsutiella rhossiliensis* was chosen because it was the most pathogenic to *P. penetrans* of the fungi that produce infective conidia, and *Monacrosporium elliposporum* was arbitrarily selected as a representative trapping fungus. To determine the optimal soil moisture for nematode infection, deionized water was added to sand with and without *H. rhossiliensis* to obtain moisture levels between 5 and 10% (= matric potentials between -9 and -4 kPa). Soil moistures lower than 5% were not tested because we believed that nematode movement would have been limited. Sand with and without *H. rhossiliensis* was packed (1.6 g/cm³ bulk density) to the top of separate 17-cm³ vials. The soil-filled vials were incubated at 25 ± 1 C for 9–10 days to allow the fungus to grow out from the infected nematodes before 1233 ± 193 *P. penetrans* in 0.25 ml of deionized water were added to the sand surface. Nematodes were extracted after 4 days, and live and dead *P. penetrans* were counted. In the third trial, the number of *P. penetrans* with emergent *H. rhossiliensis* hyphae was also counted. There were three experimental trials with four vials per combination of fungus treatment and level of soil moisture. Regression was used to determine the relationship of soil moisture to percentage nematode survival.

Effectiveness of H. rhossiliensis and M. elliposporum: The effectiveness of *H. rhossiliensis* and *M. elliposporum* in sand at 6% moisture was compared using methods similar to those in the previous experiment. Because we were unable to obtain infected *S. glaseri* from sand cultures of *M. elliposporum*, fungus-infected *S. glaseri* were hand-picked from agar cultures of the two fungi. The first and third experimental trials had controls (sand without fungal inoculum), but the second trial did not. Each treatment was replicated four times. Differences between the two fungi were assessed

using a one-way analysis of variance (ANOVA).

Survival over 25 days: The effects of *H. rhossiliensis* on survival of *P. penetrans* over 25 days at 21 C were determined in two soils. In the first trial, 80 g of loamy sand with and without the fungus was packed into separate 150-cm³ cups (11% moisture = -13 kPa, 1.5 g/cm³ bulk density). The second and third trials were similar to the first except that 100 g of the sand was packed into 150-cm³ cups (6% moisture = -8 kPa, 1.4 g/cm³ bulk density). Nine days after packing the soil in the cups, 3063 ± 35 adults and juveniles of *P. penetrans* in 0.5 ml deionized water were pipetted onto the soil surface and were extracted 5, 10, 15, and 25 days later. There were five replicate cups for each extraction date. Differences in nematode mortality among the extraction dates and experimental trials were determined with a two-way ANOVA followed by Tukey's procedure (17).

RESULTS

Susceptibility of P. penetrans to nematode-pathogenic fungi: Of the fungi producing adhesive conidia, only *H. rhossiliensis* was highly virulent to *P. penetrans* (Table 1). Conidia of the others adhered to the nematodes but did not infect or infected only a small proportion of the nematodes. Conidia of *Verticillium* and *Drechmeria* spp. adhered only to the lips and the region around the spicules of *P. penetrans*. All trapping fungi tested, except *Nematoctonus* sp., trapped and killed most *P. penetrans* in the petri dishes.

Effects of soil moisture on infection: In the presence of *H. rhossiliensis*, nematode survival decreased with diminishing soil moisture (slope = 5.8; $R^2 = 0.31$; $P < 0.0001$) (Fig. 1). In the absence of the fungus, soil moisture had no effect on nematode survival. In trial 3, the percentage of nematodes with emergent hyphae increased with decreasing soil moisture (1.5, 4.9, and 10.4% at 10, 8, and 6% moisture, respectively). Nematodes with emergent hyphae were not counted in trials 1 and 2.

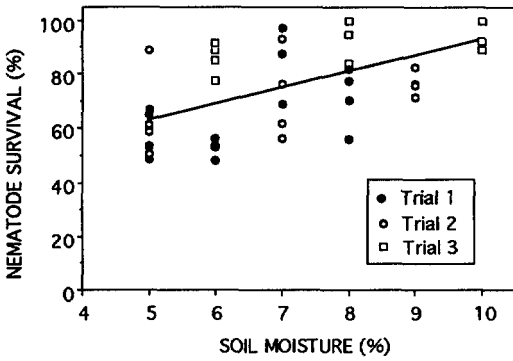


FIG. 1. The influence of soil moisture on survival of *Pratylenchus penetrans* in sand artificially infested with *Hirsutella rhossiliensis*. Percentage survival at each moisture content was determined by dividing the number of live *P. penetrans* extracted from *Hirsutella*-infested sand by the number extracted from uninfested sand and multiplying by 100. Regression: $Y = 34.3 + 5.8x$; $R^2 = 0.31$; $P < 0.0001$. The relative concentration ($\bar{x} \pm SD$) of *Hirsutella* conidia was estimated by bioassay to be 163 ± 46 , 114 ± 30 , and $42 \pm 21/cm^3$ in the first, second, and third trials, respectively.

Effectiveness of H. rhossiliensis and M. ellipso sporum: Hirsutella rhossiliensis and M. ellipso sporum were equally effective in reducing numbers of *P. penetrans* compared with the no fungus control ($P = 0.02$). After 4 days in sand, 923 ± 77 ($\bar{x} \pm SE$), 936 ± 72 , and 1229 ± 70 live *P. penetrans* were recovered from vials containing *H. rhossiliensis*, *M. ellipso sporum*, and the control, respectively.

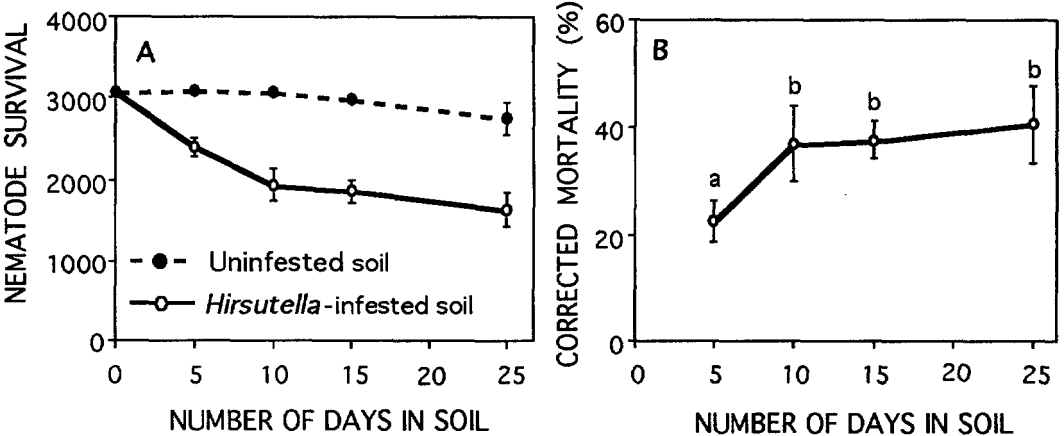


FIG. 2. Effects of *Hirsutella rhossiliensis* on *Pratylenchus penetrans* over 25 days in soil. A) Nematode survival in *Hirsutella*-infested and uninfested soil. B) Nematode mortality in *Hirsutella*-infested soil that was corrected by the mortality in the uninfested soil (1). Percentages with the same letter are not significantly different ($P > 0.05$). The relative concentration of *Hirsutella* conidia was estimated by bioassay to be $102 \pm 13/cm^3$ ($\bar{x} \pm SD$). The data points are the means ($\pm SE$) of three experimental trials.

Although they were not counted, fungus-infected *P. penetrans* were observed in sand containing *H. rhossiliensis* and *M. ellipso sporum*. The relative concentration of conidia and knobs was $174/cm^3$ and $203/cm^3$ of sand, respectively.

Survival over 25 days: Nematode survival decreased over 25 days in the *Hirsutella*-infested soil but was unchanged in the uninfested soil (Fig. 2A). Survival of *P. penetrans* in the *Hirsutella*-infested soil, when corrected for the survival in the uninfested soil (1), shows that most nematodes died between days 0 and 10 ($P \leq 0.05$); thereafter, their mortality did not significantly increase (Fig. 2B). After 25 days in *Hirsutella*-infested soil, numbers of *P. penetrans* were reduced by 28–53%.

DISCUSSION

Other nematode-pathogenic fungi have been tested for pathogenicity to *Pratylenchus* spp. in agar dishes. An isolate of *H. rhossiliensis* (= *heteroderae*) from Germany, and the trapping fungi *Dactylella lysipaga* (adhesive knobs) and *Monacrosporium salinum* (adhesive network) infected *Pratylenchus* spp. (14,18,23). An isolate of *D. conio spora* (CBS 615.82) was similar to our isolates of this fungus in that the conidia ad-

hered to the head and tail of *P. penetrans* and *P. coffeae* but did not infect the nematodes (6).

Isolates of fungi can differ in their pathogenicity and virulence (3,11,14). Some nematode-pathogenic fungi may be adapted to infecting only certain nematode species. Both isolates of *D. coniospora* (ARSEF 3346 and 3354) and *T. balanoides* (ARSEF 3350) were found infecting unidentified bacterivorous nematodes, and *T. balanoides* C2 (ARSEF 3173) was found infecting *Aphelenchoides* sp. Consequently, these fungi may not be adapted to infecting *Pratylenchus* species. Other isolates of these fungi may be more virulent towards *P. penetrans*.

Our results, showing that high soil moisture limits infection of nematodes by *H. rhossiliensis*, confirm those of Tedford et al. (19). Two probable explanations are offered for this phenomenon. First, nematode movement is optimal at soil moistures near the inflection point of the moisture release curve of a given soil (22). Soil moistures above and below the inflection point would reduce nematode encounters with adhesive conidia by decreasing nematode movement. Second, *H. rhossiliensis*, when submerged in water, produces emergent hyphae from infected nematodes but does not sporulate (5); presumably the fungus behaves similarly in soil pores filled with water.

Although the bioassay used to estimate the number of conidia and knobs per volume of sand (12) indicated similar concentrations of *H. rhossiliensis* and *M. ellipsosporum*, more knobs than conidia may have been present. We observed fewer *H. bacteriophora* extracted from sand containing *M. ellipsosporum* than from sand containing *H. rhossiliensis*. This observation may be due to many of the nematodes remaining entangled in adhesive knobs and hyphae of *M. ellipsosporum*. Nematodes entangled in hyphae may be difficult to extract because they are dragged down by soil particles in the final step of the centrifugal flotation.

The lack of significant *P. penetrans* mortality after day 10 in soil infested with *Hir-*

sutella can be explained by reduced nematode movement. Between day 0 and 10, *P. penetrans* may be actively searching for a host and thereby encountering *Hirsutella* conidia. At some time around day 10, the nematodes may stop moving, presumably to conserve energy, and therefore do not encounter the fungus. It is unlikely that the amount of fungal inoculum substantially decreased in the soil over the 25 days because the half life of *H. rhossiliensis* conidia is 6–7 weeks (4), and the nematodes that became infected between day 0 and 10 were generating additional conidia.

The percentage of *P. penetrans* acquiring *Hirsutella* conidia in soil (inferred from nematode mortality) was lower than the percentage of *H. bacteriophora* acquiring conidia in the conidia assay. After 5 days in *Hirsutella*-infested soil, $23 \pm 7\%$ ($\bar{x} \pm SD$) of *P. penetrans* acquired conidia, whereas after 1 day in infested soil, $51 \pm 17\%$ of *H. bacteriophora* acquired conidia. Two possible explanations are that *P. penetrans* is not as susceptible as *H. bacteriophora* to conidial adhesion or that *P. penetrans* is less active than *H. bacteriophora* and, consequently, encounters fewer conidia. Timper et al. (21) suggested that variation among species of entomopathogenic nematodes in motility and susceptibility to *H. rhossiliensis* was partly responsible for observed differences in mortality in soil infested with the fungus. *Pratylenchus penetrans* and *H. bacteriophora* have not been quantitatively compared with respect to conidial adhesion or motility. However, it is clear from observations of the two nematodes in water that *H. bacteriophora* is more active than *P. penetrans*. Therefore, the low percentage of *P. penetrans* acquiring *Hirsutella* conidia compared with *H. bacteriophora* is, at least, partly due to the comparative sluggishness of *P. penetrans*.

At the end of the growing season for annual crops, many vermiform *Pratylenchus* spp. leave the roots and enter the soil, where they remain until a new crop is planted in the fall or spring. During the growing season of corn and potato, ca. 20% of *P. scribneri* were outside the roots,

whereas ca. 50% were outside the roots at harvest (9). In a study with *P. penetrans* (13), Olthof detected 80–92% of the nematodes outside the roots of various potato cultivars at harvest. During their soil phase, the nematodes are susceptible to infection by fungal pathogens as long as the soil temperature and moisture permit movement. Our results indicate that even when the soil conditions are favorable for nematode movement, mortality of *P. penetrans* from a pathogenic fungus was only moderate. The maximum suppression of *P. penetrans* achieved with *H. rhossiliensis* was 53% after 25 days.

Because only moderate mortality occurred in soil and many nematodes are protected within the roots, a biological control agent alone may not maintain populations of *Pratylenchus* spp. below crop damaging levels. However, biological control, when integrated with other nematode management strategies such as rotation with nonhost plants or plant resistance, may suppress populations of *Pratylenchus* spp. to nondamaging levels.

LITERATURE CITED

- Abbott, W. S. 1925. A method for computing the effectiveness of an insecticide. *Journal of Economic Entomology* 18:265–267.
- Dutky, S. R., J. V. Thompson, and G. E. Cantwell. 1964. A technique for the mass propagation of the DD-136 nematode. *Journal of Insect Pathology* 6:417–422.
- Irving, F., and B. R. Kerry. 1986. Variation between strains of the nematophagous fungus, *Verticillium chlamydosporium* Goddard. II. Factors affecting parasitism of cyst nematode eggs. *Nematologica* 32:474–485.
- Jaffee, B. A., A. E. Muldoon, R. Phillips, and M. Mangel. 1990. Rates of spore transmission, mortality, and production for the nematophagous fungus *Hirsutella rhossiliensis*. *Phytopathology* 80:1083–1088.
- Jaffee, B. A., A. E. Muldoon, and E. C. Tedford. 1992. Trap production by nematophagous fungi growing from parasitized nematodes. *Phytopathology* 82:615–620.
- Jansson, H.-B., C. Dackman, and B. M. Zuckerman. 1987. Adhesion and infection of plant parasitic nematodes by the fungus *Drechmeria coniospora*. *Nematologica* 33:480–487.
- Jenkins, W. R. 1964. A rapid centrifugal-floitation technique for separating nematodes from soil. *Plant Disease Reporter* 48:692.
- Kable, P. F., and W. F. Mai. 1968. Influence of soil moisture on *Pratylenchus penetrans*. *Nematologica* 14:101–122.
- MacGuidwin, A. E. 1989. Distribution of *Pratylenchus scribneri* between root and soil habitats. *Journal of Nematology* 21:409–415.
- Mai, W. F., J. R. Bloom, and T. A. Chen, eds. 1977. Biology and ecology of the plant-parasitic nematode *Pratylenchus penetrans*. Pennsylvania State University Bulletin No. 815, University Park.
- McCoy, C. W. 1990. Entomogenous fungi as microbial pesticides. Pp. 139–159 in R. R. Baker and P. E. Dunn, eds. *New directions in biological control: Alternatives for suppressing agricultural pests and diseases*. New York: Alan R. Liss.
- McInnis, T. M., and B. A. Jaffee. 1989. An assay for *Hirsutella rhossiliensis* spores and the importance of phialides for nematode inoculation. *Journal of Nematology* 21:229–234.
- Olthof, T. H. 1986. Reaction of six *Solanum tuberosum* cultivars to *Pratylenchus penetrans*. *Journal of Nematology* 18:54–58.
- Peloille, M., J.-P. Frankowski, and J.-C. Cayrol. Étude de quatre souches de *Monacrosporium* en vue de leur utilisation en lutte biologique contre les nématodes phytophages. *Acta Oecologica, Applicata* 5:245–257.
- Rao, G. V. and R. B. Malek. 1973. Effects of nematode-trapping fungi on populations of *Pratylenchus penetrans*. Abstracts from the Second International Congress of Plant Pathology, St. Paul, MN. No. 0556.
- Riedel, R. M., and J. G. Foster. 1970. Monoxenic culture of *Ditylenchus dipsaci* and *Pratylenchus penetrans* with modified Krusberg's and White's media. *Plant Disease Reporter* 54:251–254.
- Steel, R. G. D., and J. H. Torrie. 1980. Principles and procedures of statistics: A biometrical approach. New York: McGraw-Hill.
- Sturhan, D., and R. Schneider. 1980. *Hirsutella heteroderae*, ein neuer nematodenparasitärer Pilz. *Phytopathologische Zeitschrift* 99:105–115.
- Tedford, E. C., B. A. Jaffee, and A. E. Muldoon. 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.
- Thames, W. H. 1982. The genus *Pratylenchus*. Pp. 108–126 in R. D. Riggs, ed. *Nematology in the southern region of the United States*. Southern Cooperative Series Bulletin 276, Fayetteville, AR.
- Timper, P., H. K. Kaya, and B. A. Jaffee. 1991. Survival of entomogenous nematodes in soil infested with the nematode-parasitic fungus *Hirsutella rhossiliensis* (Deuteromycotina: Hyphomycetes). *Biological Control* 1:42–50.
- Wallace, H. R. 1963. *The biology of plant-parasitic nematodes*. London: Edward Arnold.
- Wimble, D. B. and T. W. K. Young. 1983. Capture of nematodes by adhesive knobs in *Dactylella lysipaga*. *Microbios* 36:33–39.