# EFFECTS OF SOIL TYPE AND STEAM ON NEMATODE BIOLOGICAL CONTROL POTENTIAL OF THE RHIZOSPHERE COMMUNITY

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## ABSTRACT

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The potential of the rhizosphere community of a sand and a muck soil to provide biological control of *Meloidogyne incognita* on pepper was evaluated in two greenhouse experiments. Steamed or non-steamed soil of each type was placed into pots, planted with pepper (*Capsicum annuum*) seedlings, and inoculated with 2000 eggs of *M. incognita*. A soil type × steam treatment interaction occurred, with root-knot nematodes suppressed in untreated sand, but not in steamed sand and not in any (steamed or untreated) muck soil. A variety of organisms were monitored in both soils including free-living nematodes (bacterivores, fungivores, omnivores, and predators), enchytraeids, Collembola, mites, nematode-trapping fungi, egg-parasitic fungi, *Pasteuria* spp., rhizosphere fungi including *Fusarium* and *Rhizoctonia*, and a variety of rhizosphere bacteria including Gram positive bacteria, fluorescent pseudomonads, and siderophore producers. Determining relative importance of various organisms in biocontrol can be difficult if many different organisms are contributing together to the process. Most of these organisms did not show population patterns consistent with the biological suppression of root-knot nematodes observed in the non-steamed sand. For example, *Pasteuria* and other Gram positive bacteria were more abundant in soils that had been steamed; however, more inoculated root-knot nematodes survived in steamed soils as well. Population trends of predatory nematodes were most consistent with the suppression of root-knot nematodes observed in untreated sand.

*Key words*: enchytraeids, *Meloidogyne incognita*, microarthropods, nematode-antagonistic fungi, *Pasteuria*, predatory nematodes, rhizobacteria, rhizosphere fungi, root-knot nematodes.

## RESUMEN

McSorley, R., K.-H. Wang, N. Kokalis-Burelle, y G. Church. 2006. Efectos del tipo de suelo y el tratamiento con vapor sobre el potencial de la comunidad rizosférica para controlar nematodos. Nematropica 36:197-214.

Se evaluó el potencial de la comunidad rizosférica de un suelo arenoso y de un suelo orgánico para controlar biológicamente *Meloidogyne incognita* en pimiento, en dos experimentos de invernadero. Se colocó suelo de cada tipo, tratado con vapor y no tratado, en macetas con plántulas de pimiento (*Capsicum annuum*) inoculadas con 2000 huevos de *M. incognita*. Se observó una interacción entre el tipo de suelo y el tratamiento con vapor, en donde se suprimieron los nematodos en arena no tratada, pero no en la tratada con vapor ni en el suelo orgánico (tratado o no tratado). Se monitorearon varios organismos en ambos suelos: nematodos de vida libre (bacterívoros, fungívoros, omnívoros y depredadores), enquitreidos, Collembola, ácaros, hongos atrapadores de nematodos, hongos oviparasíticos, *Pasteuria* spp., hongos rizosféricos tales como *Fusarium* y *Rhizoctonia*, y varias bacterias rizosféricas tales como bacterias Gram positivas, pseudomonas fluorescentes, y productoras de sideróforos. Puede ser complicado determinar la importancia relativa de diversos organismos en el control biológico cuando varios organismos contribuyen de manera conjunta al proceso. La mayoría de estos organismos no mostraron patrones de población consistentes con la supresión biológica de

nematodos del nudo radical observada en arena no tratada con vapor. Por ejemplo, se encontraron *Pasteuria* y otras bacterias Gram positivas en mayor abundancia en suelos tratados con vapor; sin embargo, se observó mayor supervivencia de los nematodos inoculados en estos suelos tratados con vapor. Las tendencias de poblaciones de nematodos depredadores fueron las más consistentes con la supresión de nematodos del nudo radical observada en arena no tratada.

*Palabras clave*: enquitreidos, *Meloidogyne incognita*, microartrópodos, hongos antagonistas de nematodos, *Pasteuria*, nematodos depredadores, rizobacterias, hongos rizosféricos, nematodos del nudo radical.

## INTRODUCTION

A great deal of research has been performed on a variety of nematode antagonistic organisms (Stirling, 1991). Some of these antagonists have been quite effective in suppressing populations of plant-parasitic nematodes, including *Pasteuria penetrans* against *Meloidogyne arenaria* (Chen and Dickson, 2004b) and egg-parasitic fungi against *Heterodera avenae* (Kerry *et al.*, 1982; Kerry and Crunp, 1998), *H. glycines* (Chen and Dickson, 2004a), and *H. schachtii* (Chen and Dickson, 2004a; Westphal and Becker, 2001). While results with single antagonists or guilds of closely-related antagonists have been impressive, plant-parasitic nematodes exist in a complex community of organisms, many of which can prey on or parasitize nematodes (Coleman and Crossley, 1996; Dindal, 1990; Stirling, 1991). Thus, some potential for biological control of plant-parasitic nematodes likely exists in most soils, as supported by the widespread distribution of nematode antagonistic fungi (Chen and Dickson, 2004a; Gray, 1987), *Pasteuria* spp. (Chen and Dickson, 1998, 2004b), mites (Walter and Ikonen, 1989), predatory nematodes (Small, 1987), and other organisms (Stirling, 1991).

The concept of nematode suppression resulting from a complex of antagonists rather a single biocontrol organism was confirmed by microcosm studies in which the impact on nematode populations was increased by sequential addition of different predators (Hyvonen and Persson, 1996) or by the effects of plant-growth-promoting rhizobacteria (PGPR) (Kloepper, 1994; Glick, 1995; Cleyet-Marcel *et al.*, 2001). Efforts have been made to stimulate naturally-occurring antagonists in agricultural soils by use of organic agriculture (Yeates *et al.*, 1997), by cover crops or cropping sequences that favored development of nematode antagonistic fungi (Chen and Dickson, 2004a; Wang *et al.*, 2002) or *Pasteuria* (Sikora, 1992), and by the use of organic amendments to stimulate nematode-trapping fungi (Jaffee, 2004; Jaffee and Strong, 2005; Wang *et al.*, 2004a), egg-parasitic fungi (Chen and Dickson, 2004a; Sikora, 1992) or omnivorous and predatory nematodes (Ettema and Bongers, 1993; Wang *et al.*, 2004b) Often, shifts in the microbial ecology of the rhizosphere favor beneficial bacteria such as PGPR that contribute to the suppression of multiple pathogens and increase plant vigor by such mechanisms as the production of antibiotic compounds, antifungal metabolites, and sequestration of iron from the rhizosphere through the release of iron-chelating siderophores (Kloepper and Schroth, 1981; Schippers *et al.*, 1987; Loper, 1988; Paulitz and Loper, 1991; Dwivedi and Johri, 2003).

The primary objective this research was to evaluate and compare the biocontrol potential of two very different soils against root-knot nematodes (*Meloidogyne incognita*). Neither soil had been previously augmented with biological control agents of nematodes nor managed for that purpose.

The approach was to compare steamed and untreated soil of each type which were subsequently inoculated with root-knot nematodes; presumably, suppression would occur in the untreated soils in which many kinds of potential antagonists would be left intact relative to steamed soil. If suppression of root-knot nematodes could be demonstrated in these soils, then additional objectives were to determine which organisms within the soil community were associated with nematode suppression and to observe which organisms were stimulated by the addition of root-knot nematodes to the soil community.

## MATERIALS AND METHODS

Two different soils, a sand and a muck (approximately 100 L each), were collected on 25 Feb. 2004. The sand was collected from the University of Florida, Experimental Designs Field Teaching Laboratory in Gainesville, FL (29°39'N, 82°22'W). The site had been previously planted with various vegetable crops, and contained a three-month old crop of Austrian winter pea (*Pisum arvense* L.) at the time of soil collection. The soil was Millhopper sand (loamy, siliceous, hyperthermic, Grossarenic Paleudult), with 90% sand, 4% silt, and 6% clay, and containing 2.9% soil organic matter. The muck soil was collected from a commercial production site (27°17'N, 81°16'W) near Lake Placid in Highlands County with approximately 50 years history of continuous caladium (*Caladium* × *hortulanum*) tuber production. Caladium tubers had recently been harvested from the site, and the soil was bare at the time of collection. The muck consisted of 47.6% organic matter, and the mineral portion of this soil consisted of 78% sand, 10% silt, and 12% clay.

On the day after collection, soils were transported to the U.S. Horticultural Research Laboratory in Ft. Pierce, FL. Half of the total portion of each soil (ca. 50 L each) was placed in a Casco Cart (Casco Cart Co., Casco, WI) to a depth of approximately 15 cm and steamed to reach an internal soil temperature of 71-82°C for 2 hr using a Lindig rotary lobe blower (Lindig, Inc., Connersville, IN). Two soil probes were inserted into the soil during steaming to measure temperature. Half of each steamed and each untreated soil was used for an experiment conducted at Ft. Pierce and half was used for an experiment conducted at Gainesville.

Experimental protocols were identical at both locations, except as noted. Soil was placed in 11-cm diam plastic pots, each holding 1.0 L of soil. On 27 Feb., a single seedling (5 cm tall) of 'California Wonder' pepper (*Capsicum annuum* L.) was transplanted into each pot. The pepper plants had been germinated and grown in a sterile soil mix (1:4 peat:sand) and maintained in a greenhouse on the University of Florida campus in Gainesville. At three (Ft. Pierce) or five (Gainesville) days after transplanting, half of the pots were inoculated with *Meloidogyne incognita* race 1 and half with water (noninoculated control). Each inoculated pot received 2000 eggs, divided and delivered equally into four 3-cm-deep holes located 1-2 cm from the base of the plant. Nematode inoculum was obtained from the roots of pepper plants maintained in a greenhouse at the University of Florida. Eggs were extracted from pepper roots in a 7.5% Clorox® (The Clorox Company, Oakland, CA) solution (0.45% sodium hypochlorite) (Hussey and Barker, 1973). To avoid potential egg parasites on the inoculum, nematode egg suspensions were surface-sterilized with 3% $H_2O_2$ for 20 min with frequent agitation. Nematode eggs were then rinsed with tap water for 3 min on a 25-um-pore screen. Streptomycim sulfate (1 mg/L) was then

added, and eggs were suspended in the antibiotic overnight. Viability of eggs after surface sterilization was estimated by counting percentage of eggs hatched over 2 to 3 weeks on a Baermann tray (Rodriguez-Kabana and Pope, 1981).

The experimental design was a 2 × 2 × 2 factorial, with 2 soil types (sand, muck) 2 soil treatments (steamed, untreated), and 2 inoculum levels (0, 2000 eggs). The non-inoculated plants were included simply as controls to observe any effects of root-knot nematode inoculation on plant growth. Biological control data were evaluated only in inoculated pots as a 2 × 2 factorial design (2 soil types × 2 soil treatments). The 8 treatment combinations were arranged on greenhouse benches in a randomized complete block design with 4 replications. A triplicate set of pots (to allow for 3 destructive sampling dates needed for analysis of soil microbial communities; see below) was maintained in each replication, for a total of 96 pots in each experiment (Ft. Pierce and Gainesville). Plants were watered as needed and fertilized weekly with 50 ml/plant of 0.54 g/L of 20:20:20 ($N:P_2O_5:K_2O$) fertilizer (Miracle-Gro®, Scotts Miracle-Gro Product, Inc., Marysville, OH). No pesticides were applied to the plants.

On 10-11 May (73-74 days after planting), the experiments were terminated. Plants were cut at the soil line, roots removed from soil, and fresh top and root weights determined. Additional destructive samplings for analysis of soil microbial communities had been conducted at 20 and 48 days after planting (DAP); thus, 32 plants were removed from each experiment on each of the 3 sampling dates.

*Root-knot Nematode Evaluation*

Soil from the pots destructively sampled at 20 and 73 DAP was sampled for evaluation of *M. incognita* J2. Soil from each pot was well-mixed, and a 50-$cm^3$ subsample was removed for extraction by sieving and sugar flotation/centrifugation (Jenkins, 1964). Extracted J2 were counted, and any nematodes with *Pasteuria* spp. or fungal infection were counted.

Following harvest of the plants at 73 DAP, one-half of each root system was examined and the number of egg masses was counted. Nematode eggs were then extracted in a Clorox® solution as described above. Extracted eggs were then placed in a modified Baermann tray (McSorley and Frederick, 2004; Rodriguez-Kabana and Pope, 1981) on two layers of tissue paper (Kimwipes®, Kimberly-Clark Corp., Roswell, GA), and incubated for 7 days at 23°C to obtain hatched J2 for counting.

*Free-living Nematodes*

At planting and at the final harvest of both experiments, free-living nematodes were extracted from 50-$cm^3$ soil samples that were sieved and placed on Baermann trays as described above. This Baermann extraction method typically recovers more omnivorous and predatory nematodes than does centrifugation (McSorley and Frederick, 2004). Extracted nematodes were classified into trophic groups (Yeates *et al.*, 1993) and counted. Omnivorous and predatory nematodes were further identified to genus since they are potential biocontrol agents for root-knot nematodes.

*Other Soil Invertebrates*

Immediately prior to planting, 4 replicate samples were removed from each of the 4 soils (2 soil types × 2 soil treatments) for the extraction of microarthropods. Microarthropods were extracted from 100-$cm^3$ samples placed on Berlese funnel traps (Edwards, 1991). Each sample was incubated beneath a 60-watt light bulb for

3 days, and extracted specimens were collected in 70% ethanol in a beaker placed beneath the funnel. Microarthropods were also evaluated at harvest in the experiment at Gainesville but not at Ft. Pierce. In addition, enchytraeids were counted in the centrifuged samples used for extraction of root-knot J2 because these worms did not pass through the Baermann filters easily.

*Nematode-antagonistic Fungi*

Soil collected at harvest was stored at 10°C and then assayed for nematode-antagonistic fungi on 26 May (Gainesville experiment) or 4 June (Ft. Pierce experiment). Ten g of soil from each sample were suspended in 20 ml sterile distilled water, and diluted two times with 10-fold dilutions to obtain 0.500, 0.050, and 0.005 g soil/ml. A 100-ml aliquot of each dilution was plated on ¼-strength cornmeal agar (CMA/4) in a 6-cm-diam Petri dish containing 100 ppm streptomycin (Jaffee and Muldoon, 1995; Wang *et al.*, 2004a). Each dilution was replicated 5 times, and the Petri dishes were stored at 22°C for 3 weeks. The entire surface of the agar in each dish was then examined with an inverted microscope. The presence or absence of nematode-antagonistic fungi was determined, and fungi were identified according to a key to the nematode-destroying fungi (Cooke and Godfrey, 1964). The number of fungal propagules was estimated by a most probable number program (Woomer *et al.*, 1990).

*Egg-parasitic Fungi*

Alginate screens embedded with eggs of *M. incognita* were prepared as described by Rodriguez-Kabana *et al.* (1994). Screens were inserted into pots (one per pot) on 4 March and on 4 May into an opening made with a spatula in the soil at ½ the distance between the base of the plant and the edge of the pot. Alginate screens were removed from pots 48 hr after insertion, rinsed with sterile deionized water, and placed in screw-topped tubes with sterile water for shipping. In the laboratory, screens were examined for presence of egg-parasitic fungi (Rodriguez-Kabana *et al.*, 1994).

*Microbial Communities*

Following destructive sampling of one set of pots in each experiment at 20, 48, and 73 DAP, one-half of each root system was removed and analyzed for rhizosphere bacteria and fungi by the following procedure. A one-gram sample representing all parts of the root system was pulverized in 5 mL of sterile phosphate buffer using a Kleco Tissue Pulverizer (Garcia Manufacturing, Visalia, CA). Cylinders were shaken for 5 seconds and the resulting suspension was serially diluted to $10^{-5}$, vortexing well before each dilution.

Two dilutions were then plated onto each of the following media at 50 uL per plate using a Spiral Plater (Spiral Systems, Cincinnati, OH): 10% Tryptic soy agar (TSA) for total culturable bacteria; Ohio State medium (OSM) for total culturable fungi (Schmitthenner and Williams, 1958); Chrome Azurol S medium (CAS) for siderophore-producing bacteria (Schwyn and Neilands, 1986); Richard's medium for *Rhizoctonia* species (Martinson and Baker, 1962); Komada's medium for *Fusarium oxysporoum* (Komada, 1975); and S1 medium for fluorescent pseudomonads (Gould *et al.*, 1985). Samples were then heated to 80°C for 20 min and plated on 10% TSA to isolate gram-positive, spore-forming bacteria. All plates were incubated at 25°C in the dark (2-5 days), and then counted to determine number of colony forming units (cfu)/g root.

Analyses for free-living nematodes, other soil invertebrates, and nematode-

antagonistic fungi were performed at the University of Florida in Gainesville, FL. Analyses for egg-parasitic fungi and microbial communities were performed at the U.S. Horticultural Research Laboratory in Ft. Pierce, FL. Samples collected at one experimental location but analyzed at the other location were shipped overnight and were available for analysis on the day following collection.

All data were examined by analysis of variance (ANOVA) for a 2 × 2 factorial design (soil type × steam treatment) or a 2 × 2 × 2 factorial design (when inoculation with *M. incognita* was included), using MSTAT-C software (Freed *et al.*, 1991). Nematode data were transformed by $\log_{10}(x+1)$ prior to ANOVA, but untransformed arithmetic means are reported.

## RESULTS

### *Initial Soil Conditions*

At the beginning of the experiments, most of the soil organisms recovered were more abundant ($P < 0.05$) in untreated soil than in steamed soil (Table 1). Abundances of most of these organisms in sand and muck soil were similar, except for *Eudorylaimus*, *Meloidogyne*, *Mononchus*, and *Paratrichodorus*, for which significant ($P < 0.01$) soil type × treatment interactions occurred. *Eudorylaimus* was more abundant ($P < 0.01$) in untreated sand than in untreated muck, and *Mononchus* followed a similar trend. *Meloidogyne* occurred only in untreated muck, while *Paratrichodorus* occurred only in untreated sand.

Table 1. Effect of soil treatment (steamed, untreated) on invertebrates recovered from soil samples at beginning of experiments, Feb. 2004.

| | Numbers per sample[z] | | | |
|---|---|---|---|---|
| | Muck | | Sand | |
| Organism measured | Steamed | Untreated | Steamed | Untreated |
| Mites | 0.5* | 1.8 | 0.0* | 3.2 |
| Total arthropods | 0.5* | 2.5 | 0.2* | 4.2 |
| Bacterivorous nematodes | 0.2** | 85.0 | 0.0** | 170.5 |
| Fungivorous nematodes | 2.2** | 49.0 | 0.0** | 57.0 |
| *Aporcelaimellus* | 0.0** | 1.0 | 0.0** | 3.0 |
| *Eudorylaimus* | 0.2** | 12.2 | 0.0** | 51.0 |
| *Ironus* | 0.0 | 0.0 | 0.0 | 1.8 |
| *Meloidogyne* | 0.0** | 15.5 | 0.0 | 0.0 |
| *Mononchus* | 0.0* | 1.0 | 0.0** | 5.2 |
| *Paratrichodorus* | 0.0 | 0.0 | 0.0** | 1.2 |

[z]Data are means of 4 replications. Numbers per 100 $cm^3$ soil for mites and total arthropods, numbers per 50 $cm^3$ soil for other organisms.

*, **Indicate differences between corresponding means for steamed and untreated at $P < 0.05$ and $P < 0.01$, respectively.

*Plant Growth*

In the Gainesville experiment, inoculation with *M. incognita* did not affect plant growth ($P > 0.10$). Plants grown in sand had heavier tops and roots than those grown in muck (Table 2). At Ft. Pierce, top weight and root weight were reduced ($P < 0.05$) by inoculation with *M. incognita* (Table 3). As in the Gainesville test, top and root weights were greater ($P < 0.05$) in sand than in muck soil. However at Ft. Pierce, plants grown in steamed soil were heavier ($P < 0.01$) than those grown in untreated soil.

*Root-knot Nematodes*

At 20 DAP (data not shown), more *M. incognita* J2 were recovered ($P < 0.01$) from steamed soil than from untreated soil (14.0 vs 4.6/50 $cm^3$ soil in the Gainesville trial; 63.1 vs 36.1/50 $cm^3$ soil at Ft. Pierce). In the Ft. Pierce trial, more J2 were recovered ($P < 0.05$) from muck (75.6/50 $cm^3$) than from sand (23.6/50 $cm^3$). More nematodes with *Pasteuria* attached were recovered ($P < 0.10$) from sand than from muck in both experiments (2.8/50 $cm^3$ sand, 0.4/50 $cm^3$ muck at Gainesville; 8.4/50 $cm^3$ sand, 1.8/50 $cm^3$ muck at Ft. Pierce). The percentage of nematodes with *Pasteuria* was higher ($P < 0.05$) in sand than in muck in both experiments as well (28.9% in sand vs. 3.0% in muck at Ft. Pierce; 37.0% in sand vs. 3.3% in muck at Gainesville). At Ft. Pierce, although more nematodes with *Pasteuria* were recovered from steamed sand than from untreated sand, the percentage of J2 with *Pasteuria* was similar ($P > 0.10$) in both soil treatments (33.1% in steamed vs. 24.6% in untreated).

Table 2. Effect of soil type (sand, muck) and treatment (steamed, untreated) on pepper plants at Gainesville location, at termination of experiment (73 days after planting).

| Soil type | Soil treatment | | |
|---|---|---|---|
| | Steamed | Untreated | Mean |
| | Top fresh weight (g) | | |
| Muck | 20* | 30 | 25 b[z] |
| Sand | 33 | 32 | 32 a |
| Mean | 27 | 31 | |
| | Root fresh weight (g) | | |
| Muck | 19 | 33 | 26 b |
| Sand | 61 | 54 | 57 a |
| Mean | 40 | 43 | |

[z]Data are pooled across plants inoculated with root-knot nematodes and non-inoculated plants. No effect (at $P < 0.05$) of nematode inoculation on any plant measurements at this site. For a given plant measurement, means in columns followed by different letters differ ($P < 0.05$) for soil type effect by analysis of variance.

*Indicates difference between steamed and untreated at $P < 0.05$.

At harvest, number of egg masses per root system was greater ($P < 0.05$) in plants grown in steamed sand than in plants grown in untreated sand at Gainesville, but did not differ in steamed and untreated muck (Table 4). At both locations, interesting interactions ($P < 0.05$) occurred for the number of *M. incognita* J2 recovered from roots. At Gainesville, the numbers of *M. incognita* J2 hatched per gram of root were greatly reduced ($P < 0.01$) in untreated compared to steamed sand, but were unaffected in muck. At Ft. Pierce, J2 numbers were also decreased in untreated sand, but actually increased in untreated muck (Table 4). At both locations, numbers of J2 recovered from roots were higher in muck than in sand ($P < 0.05$).

At the end of the experiments in May, root-knot nematode J2 in soil were more abundant ($P < 0.01$) in steamed sand than in untreated sand in both experiments, but no differences occurred between steamed muck and untreated muck (Table 5). This

Table 3. Effect of soil type (sand, muck), treatment (steamed, untreated), and nematode inoculation on pepper plants at Ft. Pierce, at termination of experiment (73 days after planting).

| | Inoculated[y] | | | Noninoculated | | |
|---|---|---|---|---|---|---|
| Soil type | Steamed | Untreated | Mean | Steamed | Untreated | Mean |
| | | | Top fresh weight (g) | | | |
| Muck | 32 | 24 | 28 b[z] | 31 | 33 | 32 b |
| Sand | 45 | 24 | 35 a | 54 | 33 | 43 a |
| Mean | 38** | 24 | 31 B | 42** | 33 | 38 A |
| | | | Root fresh weight (g) | | | |
| Muck | 69 | 41 | 55 b | 62 | 77 | 70 b |
| Sand | 169 | 59 | 114 a | 253 | 64 | 159 a |
| Mean | 119** | 50 | 85 B | 158** | 71 | 114 A |

[y]Inoculated with 2000 eggs of *Meloidogyne incognita* per plant.
[z]For a given plant measurement, means in columns followed by different letters differ ($P < 0.05$) for soil type effect by analysis of variance. Overall means for inoculated and noninoculated plants followed by different capital letters are different ($P < 0.05$) according to analysis of variance.
**Indicates difference between steamed and untreated at $P < 0.01$.

Table 4. Effect of soil type (sand, muck) and treatment (steamed, untreated) on *Meloidogyne incognita* recovered from roots of pepper plants at termination of two experiments (73 days after planting).

| | Egg masses per root system | | | Nematodes per g of root[x] | | |
|---|---|---|---|---|---|---|
| Soil type | Steamed | Untreated | Mean | Steamed | Untreated | Mean |
| | | | Gainesville experiment | | | |
| Muck | 169 | 217 | 193 | 894 | 410 | 652 a[y] |
| Sand | 488* | 173 | 331 | 855** | 62 | 458 b |
| Mean | 329 | 195 | | 875** | 236 | |
| | | | Ft. Pierce experiment | | | |
| Muck | 177 | 155 | 166 | 321* | 690 | 506 A |
| Sand | 365 | 152 | 258 | 322@ | 90 | 206 B |
| Mean | 271 @[z] | 154 | | 321 | 390 | |

[x]J2 hatched from eggs extracted per g fresh root weight.
[y]For a given experiment, data in column followed by different letters indicate that main effect means for soil type are different (a, b at $P < 0.05$; A, B at $P < 0.01$) by analysis of variance. No letters indicate that main effect means for soil type do not differ at $P < 0.10$.
[z]@, *, **Indicate differences between corresponding means for steamed and untreated at $P < 0.10$, $P < 0.05$; and $P < 0.01$, respectively.

soil type by treatment interaction ($P < 0.01$) was similar to that observed for nematodes recovered from roots (Table 4). More nematodes with *Pasteuria* were recovered from steamed sand than from untreated sand (Table 5) and the percentage of J2 with *Pasteuria* was greater ($P < 0.05$) in untreated sand (13.6%) than in steamed sand (6.8%) at Ft. Pierce, but not at Gainesville (3.7% in steamed vs. 1.0% in untreated sand). A greater percentage of J2 with *Pasteuria* were recovered from sand than muck ($P < 0.05$) at both sites (15.2% in sand vs. 5.2% in muck at Ft. Pierce; 4.6% in sand vs 0.1% in muck at Gainesville).

*Free-living Nematodes*

At the end of the experiments, several different types of nematodes were more abundant ($P < 0.10$) in untreated soil than in steamed soil (Tables 6 and 7). Total bacterivores, while decreased initially by steam treatment (Table 1), were more abundant in steamed soil at the end of the Ft. Pierce experiment (Table 6). Fungivores were more abundant ($P < 0.01$) in untreated muck than in steamed muck at Gainesville, but were not affected in sand (Table 6).

Trends in numbers of predatory and omnivorous nematodes were particularly interesting. The most common omnivore genera (*Eudorylaimus*, *Aporcelaimellus Mesodorylaimus*) were absent or rare in steamed soil, but their abundances in sand and muck were similar (Tables 6 and 7). *Mononchus* emerged as the dominant predatory nematode at Ft. Pierce, while *Ironus* was the main predatory nematode recovered in the Gainesville experiment (Table 7). In either case, however, the dominant predator was absent from steamed soil, and more abundant ($P < 0.05$) in sand than in muck (Table 7).

Table 5. Effect of soil type (sand, muck) and treatment (steamed, untreated) on *Meloidogyne incognita* recovered from soil samples at termination of two experiments (73 days after planting).

| | Nematodes per 50 cm$^3$ soil | | | Nematodes with *Pasteuria* per 50 cm$^3$ soil[y] | | |
|---|---|---|---|---|---|---|
| Soil type | Steamed | Untreated | Mean | Steamed | Untreated | Mean |
| | | | Gainesville experiment | | | |
| Muck | 266 | 243 | 254 a[z] | 0.0 | 0.5 | 0.2 B |
| Sand | 427** | 11 | 219 b | 29.0** | 0.2 | 14.6 A |
| Mean | 346** | 127 | | 14.5** | 0.4 | |
| | | | Ft. Pierce experiment | | | |
| Muck | 268 | 444 | 356 | 11.2 | 17.8 | 14.5 |
| Sand | 692** | 22 | 357 | 72.8* | 5.0 | 38.9 |
| Mean | 480 | 233 | | 42.0 | 11.4 | |

[y]Number of *M. incognita* J2 with *Pasteuria* attached.

[z]For a given experiment, data in columns followed by different letters indicate that main effect means for soil type are different (a, b at $P < 0.05$; A, B at $P < 0.01$) by analysis of variance. No letters indicate that main effect means for soil type do not differ at $P < 0.10$.

*, **Indicate differences between corresponding means for steamed and untreated at $P < 0.05$ and $P < 0.01$, respectively.

Table 6. Effect of soil type (sand, muck) and treatment (steamed, untreated) on nematodes and enchytraeids recovered from soil samples at Gainesville and Ft. Pierce, at termination of experiments (73 days after planting).

| | Nematodes per 50 $cm^3$ soil | | | | | |
|---|---|---|---|---|---|---|
| | Gainesville | | | Ft. Pierce | | |
| Soil type | Steamed | Untreated | Mean | Steamed | Untreated | Mean |
| | | | Total bacterivores | | | |
| Muck | 0.5 | 250.8 | 125.6 | 379.0 | 193.5 | 286.2 |
| Sand | 574.5 | 164.8 | 369.6 | 1392.8 | 152.5 | 772.6 |
| Mean | 287.5 | 207.8 | | 885.9* | 173.0 | |
| | | | Total fungivores | | | |
| Muck | 5.2** | 478.0 | 241.6 a[y] | 6.0 | 23.8 | 14.9 |
| Sand | 13.8 | 34.0 | 23.9 b | 6.2 | 49.0 | 27.6 |
| Mean | 9.5** | 256.0 | | 6.1* | 36.4 | |
| | | | Total omnivores and predators | | | |
| Muck | 0.0 | 1.5 | 0.8 B | 0.0 | 50.0 | 25.0 |
| Sand | 0.0** | 5.6 | 2.9 A | 0.5 | 36.0 | 18.2 |
| Mean | 0.0** | 3.6 | | 0.2** | 43.0 | |
| | | | *Eudorylaimus* | | | |
| Muck | 0.0 | 0.8 | 0.4 | 0.0 | 14.2 | 7.1 |
| Sand | 0.0 | 1.0 | 0.5 | 0.5 | 16.8 | 8.6 |
| Mean | 0.0 | 0.9 | | 0.2** | 15.5 | |
| | | | *Aporcelaimellus* | | | |
| Muck | 0.0 | 0.2 | 0.1 | 0.0 | 2.2 | 1.1 |
| Sand | 0.0 | 1.0 | 0.5 | 0.0 | 1.0 | 0.5 |
| Mean | 0.0 @[z] | 0.6 | | 0.0 @ | 1.6 | |
| | | | Total enchytraeids | | | |
| Muck | 0.8 | 0.2 | 0.5 B | 0.0 | 0.8 | 0.4 b |
| Sand | 0.2** | 49.8 | 25.0 A | 0.0* | 13.2 | 6.6 a |
| Mean | 0.5 | 25.0 | | 0.0* | 7.0 | |

[y]For a given organism, data in columns followed by different letters indicate that main effect means for soil type are different (a, b at $P < 0.05$; A, B at $P < 0.01$) by analysis of variance. No letters indicate that main effect means for soil type do not differ at $P < 0.10$.

[z]@, *, **Indicate differences between corresponding means for steamed and untreated at $P < 0.10$, $P < 0.05$ and $P < 0.01$, respectively.

Table 7. Effect of soil type (sand, muck) and treatment (steamed, untreated) on selected nematode genera recovered from soil samples at Gainesville and Ft. Pierce, at termination of experiments (73 days after planting).

| Soil type | Nematodes per 50 cm$^3$ soil | | |
|---|---|---|---|
| | Steamed | Untreated | Mean |
| | *Ironus*, Gainesville | | |
| Muck | 0 | 0.2 | 0.1 b[z] |
| Sand | 0* | 3.8 | 1.9 a |
| Mean | 0** | 2.0 | |
| | *Mesodorylaimus*, Ft. Pierce | | |
| Muck | 0 | 30.2 | 15.1 |
| Sand | 0 | 9.2 | 4.6 |
| Mean | 0** | 19.8 | |
| | *Mononchus*, Ft. Pierce | | |
| Muck | 0 | 1.5 | 0.8 B |
| Sand | 0** | 8.8 | 4.4 A |
| Mean | 0** | 5.1 | |

[z]For a given nematode, data in columns followed by different letters indicate that main effects for soil type are different (a, b at $P < 0.05$; A, B at $P < 0.01$) by analysis of variance. No letters indicate that main effect means for soil type do not differ at $P < 0.10$.
*, **Indicate differences between corresponding means for steamed and untreated at $P < 0.05$ and $P < 0.01$, respectively.

Table 8. Effect of soil type (sand, muck) and treatment (steamed, untreated) on arthropods recovered from soil Berlese samples at Gainesville, at termination of experiment (73 days after planting).

| Soil type | Arthropods per 100 cm$^3$ soil | | |
|---|---|---|---|
| | Steamed | Untreated | Mean |
| | Mites | | |
| Muck | 12.5 | 20.2 | 16.4 A[z] |
| Sand | 2.0 | 3.8 | 2.9 B |
| Mean | 7.2 | 12.0 | |
| | Collembola | | |
| Muck | 0* | 27.8 | 13.9 |
| Sand | 0 | 5.5 | 2.8 |
| Mean | 0* | 16.6 | |
| | Total arthropods | | |
| Muck | 13.5** | 47.2 | 30.4 A |
| Sand | 2.5 | 10.8 | 6.6 B |
| Mean | 8.0** | 29.0 | |

[z]For a given organism, data in columns followed by different letters indicate that main effect means for soil type are different ($P < 0.01$) by analysis of variance. No letters indicate that main effect means for soil type do not differ at $P < 0.10$.
*, **Indicate differences between corresponding means for steamed and untreated at $P < 0.05$ and $P < 0.01$, respectively.

### *Other Soil Invertebrates*

Microarthropods were collected from soil at the end of the experiment in Gainesville (Table 8). Mites were unaffected by steam treatment but were more common ($P < 0.01$) in muck than in sand. Collembola were absent from steamed samples but were unaffected by soil type. Enchytraeids showed a soil type × treatment interaction ($P < 0.05$) in both experiments. Numbers of enchytraeids were greater in sand than in muck, and greatest in untreated sand (Table 6).

### *Nematode-antagonistic Fungi*

At Gainesville, more nematodes with fungi were recovered from untreated muck (8.6% with fungi) than from steamed muck (0% with fungi), but the reverse effect was observed in sand (2.5% with fungi in steamed sand, 0% in untreated sand). Several nematode-trapping fungi were isolated from soil, including *Arthrobotrys oligospora*, *A. dactyloides*, and *Monacrosporium ellipsoporium*, which were isolated in low numbers (2.0 cfu/g soil) from untreated soil collected at the end of the

experiment in Ft. Pierce. In untreated soil in the Gainesville experiment, *A. oligospora* was more abundant ($P < 0.05$) in sand (50.5 cfu/g soil) than in muck (zero cfu recovered).

*Egg-parasitic Fungi*

The number of parasitized eggs in alginate screens did not differ between sand and muck, or between steamed and unsteamed soil in either experiment at either of the two sampling times when this technique was used (data not shown). The only significant ($P < 0.05$) difference observed regarding the effects of treatments on nematode eggs using the alginate technique was a decrease in the number of viable eggs in sand (35.6/sample) compared to the muck soil (40.4/sample), at 6 DAP in the Ft. Pierce test.

*Microbial Communities*

Gram-positive bacterial populations were higher in sand than in muck at all sampling times at both locations (Table 9). Regardless of soil type, steaming increased

Table 9. Effect of soil type (sand, muck) and treatment (steamed, untreated) on populations of several types of soilborne bacteria isolated on selective media.

| | Colony forming units[y] | | | | | |
|---|---|---|---|---|---|---|
| | Ft. Pierce | | | Gainesville | | |
| Treatment | 20 DAP[z] | 48 DAP | 73 DAP | 20 DAP | 48 DAP | 73 DAP |
| | | | Gram + bacteria | | | |
| Sand | 5.44 a | 5.48 a | 5.61 a | 5.27 a | 5.48 a | 5.41 a |
| Muck | 4.52 b | 4.56 b | 4.94 b | 4.55 b | 4.68 b | 3.92 b |
| Steam | 5.27 a | 5.20 a | 5.49 a | 5.19 a | 5.30 a | 4.33 a |
| No steam | 4.63 b | 4.78 b | 5.07 b | 4.55 b | 4.86 b | 4.86 a |
| | | | Flourescent pseudomonads | | | |
| Sand | 4.89 a | 5.87 a | 5.79 a | 4.99 a | 5.42 a | 5.80 a |
| Muck | 4.38 b | 4.18 b | 3.28 b | 4.45 b | 4.60 b | 4.95 b |
| Steam | 4.65 a | 4.45 b | 3.32 b | 4.85 a | 4.99 a | 5.22 a |
| No steam | 4.57 a | 5.51 a | 5.75 a | 4.57 a | 5.03 a | 5.53 a |
| | | | Siderophore producers | | | |
| Sand | 5.28 a | 6.38 a | 6.62 a | 5.34 a | 5.98 a | 6.28 a |
| Muck | 5.11 a | 5.31 a | 6.19 a | 5.52 a | 5.25 a | 6.37 a |
| Steam | 5.44 a | 5.29 a | 6.81 a | 5.77 a | 5.56 a | 6.36 a |
| No steam | 4.95 b | 6.40 a | 6.00 a | 5.13 b | 5.67 a | 6.29 a |

[y]Data are $\log_{10}$ of number of colony forming units per g root. For each pair of means, data in columns followed by different letters indicate that means are different ($P < 0.01$) by analysis of variance and LSD test.
[z]Days after planting (DAP) = time of sample collection.

the number of Gram-positive bacteria compared to the non-steamed soil at all sampling times in the Ft. Pierce trial and at two of the three sampling times in the Gainesville trial (Table 9). Populations of fluorescent pseudomonads were higher in sand than in muck at all samplings in both trials. The effect of steaming on fluorescent pseudomonads was mixed in the Ft. Pierce trial, with increases in populations in steamed soil later in the season. There was no effect of steaming on fluorescent pseudomonads in the Gainesville trial. Populations of siderophore-producing bacteria did not differ between sand and muck at any sampling time in either trial. However, at the first sampling time in both tests, steaming increased the number of these organisms.

Populations of *Rhizoctonia* and *Fusarium* did not differ between sand and muck early in the season in either test. However, late in the season, *Rhizoctonia* and *Fusarium* populations in muck were higher ($P < 0.05$) than those in sand at both locations (data not shown). Steaming did not affect *Rhizoctonia* populations in either soil at any sampling time but did reduce numbers of *Fusarium* propagules late in the season in the Gainesville trial ($\log_{10}$ of cfu = 2.97 in steamed vs. 3.74 in non-steamed soil). The number of fungal colonies isolated on non-selective growing media was higher in the muck at the mid- and late-season sampling times at both locations (data not shown). Steam had little effect on total numbers of culturable fungi, but did increase their numbers in the mid-season sample in the Gainesville trial ($\log_{10}$ of cfu = 3.88 in steamed vs. 3.11 in non-steamed soil).

*Response of Organisms to Inoculation*

Numbers of soil invertebrates did not differ ($P > 0.10$) between pots inoculated with *M. incognita* and those that were not inoculated (data not shown). However, the percentage of J2 in soil that were parasitized by *Pasteuria* were greater ($P < 0.05$) in inoculated pots (2.3%) than in noninoculated pots (0.5%) in May at Gainesville. Similarly, the percentage of J2 parasitized by fungi were somewhat greater ($P < 0.10$) in inoculated pots (3.3%) than in noninoculated pots (0.5%). Neither percentage of infection by *Pasteuria* (7.5% in inoculated, 10.8% in noninoculated) nor by fungi (12.6% in inoculated, highly variable; 0% in noninoculated) were affected by inoculation in the experiment at Ft. Pierce.

## DISCUSSION

Examination of root-knot nematode levels in roots and soil at the end of both experiments revealed strong soil type × treatment interactions, with nematodes reduced in untreated sand compared to steamed sand, but with no effect in muck soil. The suppression of final root-knot nematode populations in untreated sand indicates that a strong potential for biocontrol existed in this soil. However, no evidence for suppression of root-knot nematodes was observed in the muck soil.

It is interesting to speculate which organisms may have contributed to the suppression of root-knot nematodes in the untreated sand. Most of the potential antagonists were not stimulated by inoculation of soil with root-knot nematodes, with the exception of *Pasteuria* and nematode antagonistic fungi. It was thought that the inoculation of pots with 2000 nematode eggs would provide a food source for eventual population increase of nematode antagonists, since *Pasteuria*, nematode-trapping fungi, mites, or omnivorous nematodes have increased in cultures or other systems when nematodes were present as a food source (Chen and Dickson, 2004b; Jaffee and Strong, 2005; Sikora, 1992; Walter *et al.*, 1993; Wang *et al.*, 2004b).

*Pasteuria* was clearly stimulated by the added root-knot nematodes, and parasitism of J2 in sand was much greater than in muck. Thus, suppression of root-knot nematodes in sand could be due in part to *Pasteuria*. However, *Pasteuria* is known to be tolerant of high temperatures (Chen *et al.*, 1995; Chen and Dickson, 2004b) and can survive steaming (Williams *et al.*, 1989), consistent with the data in the current experiment in which percentages of J2 with *Pasteuria* were similar in untreated and steamed soil. Therefore the great difference in nematode suppression in untreated and steamed sand could not be explained by differences in the level of infection by *Pasteuria*.

*Arthrobotrys oligospora* was the most abundant nematode-trapping fungus quantified from soil in the Gainesville experiment, and was more common in sand than in muck. This fungus is a generalist that attacks a wide range of nematodes (Jaffee and Muldoon, 1995; Wang *et al.*, 2004a), and so its impact in biocontrol here cannot be ruled out. Although sporadic in its occurrence, maximum numbers were obtained in one experiment in the untreated sand, where nematode suppression also occurred. A wide range of fungal antagonists can attack plant-parasitic nematodes (Stirling, 1991), and unidentified fungi parasitizing J2 were also observed in these experiments. However, parasitism of J2 by fungi was greater in steamed sand than in untreated sand, the reverse of what was observed with nematode suppression, so data on fungal infection of nematodes are inconsistent with the suppression observed.

A variety of invertebrates were reduced to near zero levels initially in steamed soil, but numbers of many of these increased in untreated soil over the course of the experiments. Omnivorous nematodes (*Eudorylaimus*, *Mesodorylaimus*, *Aporcelaimellus*) showed similar patterns, remaining significantly higher in untreated than in steamed soil at the conclusion of the experiments. The same pattern occurred in both sand and muck soils. Therefore it is difficult to attribute the suppression of root-knot nematodes in sand to omnivorous nematodes because they showed the same response in muck where no biocontrol occurred.

Different dominant predatory nematodes emerged at Gainesville (*Ironus*) and Ft. Pierce (*Mononchus*) but both followed similar patterns. In each case, a soil type × treatment interaction occurred, with each of these nematodes more common in untreated sand than in steamed sand, but unaffected in muck. This is consistent with the pattern of root-knot nematode suppression observed. Both *Ironus* and *Mononchus* feed on a variety of prey and can build up their population levels on nematode food sources (Grootaert and Maertens, 1976; Hunt, 1977; Yeates *et al.*, 1993), although success with predatory nematodes as biocontrol agents has generally been limited (Stirling, 1991). But according to the data from the current experiments, the possibility that these predatory nematodes were responsible for the observed decline in root-knot nematodes cannot be ruled out. Numbers of these predators were similar in pots inoculated with root-knot nematodes and noninoculated pots, indicating that the presence of root-knot nematodes did not significantly stimulate predator numbers. However, predatory nematodes are generalists in their food habits and typically feed on a variety of nematode genera (Grootaert and Maertens, 1976; Hunt, 1977; Small, 1987; Yeates *et al.*, 1993). Bacterivorous nematode numbers of 150/50 $cm^3$ soil would amount to about 3000 bacterivores per pot, more than the 2000 root-knot nematode eggs that were added per pot. Thus, even in noninoculated pots, a sub-

stantial food resource was available for predators. In inoculated pots, it is unclear to what degree predators were maintained on root-knot J2, bacterivores, or other nematodes. Nevertheless, the greatest suppression of root-knot nematodes occurred in untreated sand, where predatory nematodes were most abundant as well.

Enchytraeids showed a pattern of abundance that was similar to that observed with predatory nematodes. Their numbers were greater in untreated sand than in steamed sand, consistent with reduction in root-knot nematodes. Enchytraeids are considered to be fungivores (Coleman and Crossley, 1996; Dindal, 1990; Hyvonen and Persson, 1996) and had no impact on nematode populations (Huhta *et al.*, 1998), although accidental ingestion of nematodes is possible (Stirling, 1991). It is unclear why enchytraeids showed the pattern of abundance observed here.

Microarthropod population levels were monitored in one experiment (Gainesville). Mite populations recovered in steamed soils to levels similar to those in untreated pots, so their occurrence is not consistent with the reduction of root-knot nematodes in untreated sand, even though mites are known to be efficient predators of root-knot and other nematodes (Walter *et al.*, 1993). Collembola are primarily fungivores (Coleman and Crossley, 1996), but feed on nematodes in some cases (Gilmore and Potter, 1993; Huhta *et al.*, 1998; Hyvonen and Persson, 1996; Walter and Ikonen, 1989). They were quite common in untreated muck, but root-knot nematode suppression occurred in sand, not in muck. While patterns of microarthropod (mite and Collembola) occurrence were not consistent with root-knot nematode reductions, it should be recognized that microarthropods vary in their efficiency as predators of nematodes (Walter *et al.*, 1993; Walter and Ikonen, 1989). Even oribatid mites, which are typically fungivores, may occasionally prey on nematodes (Hyvonen and Persson, 1996; Walter and Ikonen, 1989). We made no attempt to identify these organisms below order level, so it is possible that the genera or species of microarthropods present in untreated sand could be more efficient predators of root-knot nematodes, even if total numbers (at order level) showed no association with root-knot nematode suppression.

Numbers of egg-parasitic fungi recovered were low and not affected by the treatments, so it seems unlikely that they contributed to the substantial reductions of root-knot nematodes in the untreated sand. Gram-positive bacteria were increased by steaming, opposite to what might be expected of potential biocontrol organisms in the untreated sand. Siderophore-producing bacteria were generally unaffected by treatment, but fluorescent pseudomonads were consistenly more abundant in sand than in muck. On two sampling dates in the Ft. Pierce test, they were more common in the untreated sand, but showed no such response in the Gainesville test. Nematodes may be affected by PGPR (Kloepper, 1994), but the only group that showed any increased abundance in untreated sand in the current study was the fluorescent pseudomonads, although their responses were inconsistent.

Attempts were made to correlate numbers of potential antagonists with numbers of root-knot nematodes in soil at the end of the experiments, using only samples from nematode-inoculated sand, since reduction of root-knot nematodes was observed only in sand. The logarithm of the number of root-knot juveniles in soil was correlated ($P < 0.05$) negatively and individually with the logarithms of numbers of enchytraeids (r = -0.858), total omnivorous nematodes (r = -0.881), or *Ironus* (r = -0.830) at Gainesville; and with

total omnivores (r = -0.917) or *Mononchus* (r = -0.911) at Ft. Pierce. Numbers of other organisms were not negatively correlated ($P > 0.05$) with root-knot nematode numbers at either site. These negative correlations are consistent with the treatment effects observed with *Ironus, Mononchus*, and enchytraeids, all of which had their greatest numbers in untreated sand. Total omnivores, however, showed similar patterns in both sand and muck, and were reduced to near zero levels by steaming in both soil types.

Overall, substantial suppression of root-knot nematodes in untreated sand was observed, but a range of organisms could be responsible. Population trends of predatory nematodes were most consistent with the observed suppression, but it is possible that this was coincidental or that other organisms contributed as well. Such associations should be explored further to clarify relative importance of different organisms in biocontrol. Building of microcosms by adding combinations of different organisms is one approach for separating the relative contributions of several components (Huhta *et al.*, 1998; Hyvonen and Persson, 1996; Jaffee, 2004). In the current study, no direct observations of feeding on nematodes were made, except for parasitism of J2 by *Pasteuria* or fungi. Despite examining a wide range of potential antagonists in the soil communities in the present study, these may represent only a fraction of the vast array of potential nematode antagonists that exist in soil. For example, tardigrades (Hyvonen and Persson, 1996) or amoebae (Yeates and Foissner, 1995) might potentially impact nematodes, but these were not assessed here.

Despite a substantial reduction of root-knot nematode populations in untreated sand, little benefit to plant growth was obtained. Plants consistently grew better in steamed soil, possibly implying that other organisms such as fungal or bacterial plant pathogens affected plant performance more than nematodes, although no disease problems were observed. Clearly, the practical implications of nematode biocontrol must be assessed at the field level (Sikora, 1992; Stirling, 1991). However, using biocontrol to demonstrate improved plant performance in these greenhouse experiments was not a primary goal of the current study. The main point was to demonstrate suppression of root-knot nematodes and to provide some clues and evidence about which organisms may or may not play important roles in this suppression. While a number of different organisms were examined, it is impossible to evaluate every potential biocontrol organism present in soil. Nevertheless, observed nematode suppression likely results from the combined efforts of many potential antagonists, even those that remained undetected by the methods used.

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