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Colonization of Greenhouse Nematode Cultures by Nematophagous Mites and Fungi

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Abstract: Unproductive >7-year-old greenhouse cultures of citrus nematode (*Tylenchulus semipenetrans*) had a well-developed soil invertebrate fauna that included nematophagous mite species characteristic of Florida citrus groves. Nematophagous mite densities in box cultures were 285 ± 42 mites/liter, 2.5 to 25 times higher than densities in citrus nematode-infested groves. Vigorous root-knot nematode (*Meloidogyne incognita*) cultures grown in steam-pasteurized soil had few nematophagous mites until more than 3 months after inoculation. Mite species diversity had a significant ($P < 0.0001$) positive linear relationship with culture age that explained about one-half the variance in species number. Nematophagous mite densities rose and then fell with culture age. In root-knot cultures >3-months-old, mite densities often exceeded 1,000 mites/liter. Twelve species of nematophagous fungi also were isolated from greenhouse nematode cultures.

Key words: biological control, culture, fungus, mite, nematode, nematophagous fungus, *Meloidogyne incognita*, *Tylenchulus semipenetrans*.

Monoxenic culture techniques have been developed for a relatively limited number of plant-parasitic nematodes, and these techniques are often labor-intensive or do not yield large numbers of nematodes (8). Thus, it is often necessary to culture plant-parasitic nematodes on the roots of potted plants (greenhouse cultures). Population densities within greenhouse cultures are dependent upon suitable edaphic conditions and the availability of roots; however, densities can be unexpectedly low, and occasionally cultures fail. Nematophagous mites and fungi are common in soils, and should not be present initially in cultures established in steam-pasteurized potting soils, but greenhouse cultures of plant-parasitic nematodes would be rich resources for any antagonists that were able to invade them.

Nematophagous mites are capable of suppressing populations of plant-parasitic nematodes in pot experiments (3,11,12). Walter and Ikonen (13) found that two species of *Protogamasellus* could consume an average of 0.82 μg (dry weight) of nematodes per day. Long-term cultures of the citrus nematode, *Tylenchulus semipenetrans* (Cobb), often yield population densities far lower than anticipated (Kaplan, unpubl.). Because nematophagous mite species were associated with citrus roots in a survey of Florida citrus groves (14), we decided to examine the arthropod community in greenhouse nematode cultures. The purpose of this paper is to report on antagonists that have colonized greenhouse cultures of the root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood, and of the citrus nematode.

MATERIALS AND METHODS

Nematode cultures: In June 1983, five wooden slat boxes 45 cm wide, 57 cm long, and 25 cm deep were filled to 18-cm depth (46,170 cm^3) with 50% Astatula sand (hyperthermic, uncoated; typic, quartzipsamments), 25% peat moss, and 25% vermiculite infested with *T. semipenetrans* from a variety of sources, and planted with 4–12 rough lemon (*Citrus limon* L.) seedlings in a greenhouse at the U.S. Horticultural Research Laboratory (USHRL) in Orlando, Florida. The cooling and heating regime in the greenhouse maintained a soil temperature of 25 C ± 5 C with air temperatures of 25 ± 5 C, and the boxes were watered daily by an automated system.

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In September 1985, existing cultures of *M. incognita* race 1 from the University of Florida, Gainesville (UFG), were transferred to the USHRL. Cultures were initiated at USHRL monthly with eggs and second-stage juveniles (J2) extracted from 2–3 month-old UFG cultures with 0.53% sodium hypochlorite (4). Eggs were placed on Baermann pans at 25 C, from which approximately 10,000 J2 and eggs were used to infest pots containing 1-month-old pepper (*Capsicum annuum* L.) cv. California Wonder or eggplant (*Solanum melongena* L.) cv. Black Beauty. Plants were potted in 15-cm-d clay pots filled with steam-pasteurized (60 minutes at 66 C) Astatula sand. Young plants and established cultures were maintained in a greenhouse during the colder months. During the summer, cultures were maintained in a screen house and watered daily. Cultures were identified by the month in which they were infested, i.e., cultures infested in June were subsequently referred to as "June series" cultures.

Arthropod and nematode sampling: Beginning in July 1989, nematode cultures were sampled for arthropods with a 2.1-cm-d cork borer. Six evenly spaced cores were taken to 8.0-cm depth from box cultures, and three equidistant cores to 7.0-cm depth from pot cultures. The holes were refilled with the appropriate potting soil. The cores were bulked, and arthropods were extracted with Tullgren funnels (14). Box cultures were sampled for arthropods in July, October, and December 1989 and January, February, and March 1990. On 19 July, three uninfected pepper plants, and equal numbers of *M. incognita* cultures on pepper (2 or 3 cultures) and eggplant (2 or 3 cultures) were arbitrarily chosen at 41 (June series), 72 (May series), 103 (April series), and 134 (March series) days after being infested with root-knot nematodes. Six samples ($n = 6$) were collected from each nematode culture for arthropod analysis, and four samples ($n = 4$) were collected from each nematode culture to estimate nematode population densities.

Over the next 20 weeks, the May and June series were periodically sampled for arthropods. Other samples were taken as needed to obtain live animals or inoculum for sampling nematophagous fungi. All mite species except *Neocunaxoides andrei* Baker & Hoffman and *Proctolaelaps* sp. detected in nematode cultures were placed in small arenas (13) containing 500 burrowing nematodes, *Radopholus citrophilus* Huettel, Dickson, & Kaplan, reared on carrot disk culture (7). Voucher specimens of mites were deposited at the Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, Florida, and with the Canadian National Collection, Biosystematics Canada, Ottawa, Ontario.

Root-knot nematode populations in pot cultures were estimated on 19 July 1989 from 3 equidistant 2.1-cm-d cores taken to 7-cm depth. The three cores from each pot were bulked, roots were removed with a 6-mm-pore sieve, and a 30-cm^3 subsample was processed with a sieving-centrifugation technique (6). Approximately 30 cm of root segments from each sample were inspected for galls at ×40 magnification for an index of root-knot nematode infection (galls per cm root). Statistical analyses for mite and nematode data were performed using the GLM procedure of SAS (9).

Nematophagous fungi: Cores containing soil and roots from box and pot cultures were taken for extraction of nematophagous fungi by a sprinkle–bait plate method (14) from 0.15 g of soil shaken from roots or small nematode-infected root segments placed on a thin layer of water agar (WA) (4 ml of 1% agar) in 9-cm-d petri dishes. Following the addition of 5,000 burrowing nematodes derived from carrot disk culture (7) to each dish, they were incubated at 25 C. In addition, four samples of 5,000 root-knot nematode eggs and J2 prepared as for infestation of new nematode cultures were plated on WA dishes and incubated at 25 C for 5–7 days.

Results

Nematophagous fungi: The root-knot nematode pot cultures contained 12 spe-

cies of nematophagous fungi, including *Arthrobotrys dactyloides* Drechsler, *A. musiformis* Drechsler, *A. oligospora* Fresenius, *Catenaria anguillulae* Sorokine, *Dactylella cionapaga* Drechsler, *D. ellipsospora* Grove, *Dactylaria eudermata* Drechsler, *Haptoglossa heterospora* Drechsler, *Myzocytium* sp., *Paecilomyces lilacinus* (Thom.) Samson, *Rhizophidium* sp., and *Verticillium chlamydosporium* Goddard. Five species of nematode-trapping fungi (all of the above except *D. eudermata*) and the egg parasite *V. chlamydosporium* were recovered from the four samples of root-knot nematode inoculum. *Arthrobotrys dactyloides, C. anguilullae, D. cionapaga, D. ellipsospora,* and an unidentified Oomycete were isolated from citrus nematode box cultures.

Nematophagous mites: Twelve species of nematophagous mites were isolated from greenhouse nematode cultures (Table 1), and all except two very rare species, *Neocunaxoides andrei* and *Proctolaelaps* sp., were established in laboratory cultures on nematode prey. Citrus nematode box cultures averaged 285 ± 42 mites/liter of soil (ca. 13,160 mites/box). Mite densities were stable in the six samples taken over the 8-month sampling period ($P < 0.20$). *Protogamasellus* sp., *Protogamasellus mica* (Athias-Henriot), and *Stratiolaelaps miles* (Berlese) represented 96.5% of the nematophagous mites collected in citrus nematode cultures.

In contrast, both series of root-knot nematode pot cultures were dominated by the mites *Lasioseius subterraneus* Chant, *Geolaelaps* sp., *Coleoscirus simplex* (Ewing), *Gamasellodes bicolor* (Berlese), *G. rectiventris* Lindquist, and *Protogamasellus mica* (Athias-Henriot), whose combined totals represented 97.3 and 79.2% of the nematophagous mites in the May and June series, respectively. Only *P. mica* and *Neoscirula* sp. were relatively abundant in both citrus and root-knot nematode cultures.

In July 1989, nematophagous mite densities in root-knot nematode pot cultures were variable (Table 2). Uninfested potted plants had nearly undetectable densities of mites, and even 41 days after nematode infestation, few pots had been colonized by mites. Numbers of root-knot nematode J2 and root-knot galls per cm root were not significantly correlated with nematophagous mite density. Mite densities were highest in the 72-day-old cultures, when large numbers of *Meloidogyne* J2 were present in the soil, but only five of the six replicate cultures were colonized by predatory mites. In the older cultures (103 and 134 days after infestation), high densities of a collembolan *Proisotoma* nr. *sepulchris* Folsom (31,600 ± 12,200 and 10,200 ± 2,100 per liter of soil, respectively) were associated with decreased numbers of both nematophagous mites and nematodes in all pots. Gut contents of 60 *P.* nr. *sepulchris*

TABLE 1. Occurrence and densities of mites in greenhouse nematode cultures.

Mite species	Citrus nematode cultures	Root-knot nematode cultures	
		June series	May series
Stratiolaelaps miles	39.3 (7.7)	0	0
Protogamasellus sp.	99.8 (20.5)	0	0.4 (0.4)
Protogamasellus mica	136.3 (25.3)	65.3 (44.0)	17.1 (15.2)
Rhodacarus denticulatus	6.2 (5.3)	0	0
Neoscirula sp.	12.4 (3.1)	10.7 (9.3)	4.6 (2.4)
Proctolaelaps sp.	0.2 (0.2)	0	0
Coleoscirus simplex	0.2 (0.2)	41.3 (11.5)	113.3 (28.0)
Lasioseius subterraneus	0.2 (0.2)	144.0 (52.0)	419.9 (100.0)
Geolaelaps sp	0	32.0 (8.9)	172.0 (46.0)
Gamasellodes bicolor	0	12.0 (5.6)	126.6 (55.7)
Gamasellodes rectiventris	0	60.0 (44.0)	3.9 (2.9)
Neocunaxoides andrei	0	0	1.2 (0.8)
Total	285.1 (42.2)	365.2 (97.3)	859.8 (167.8)

Values are means of 6 replications. Values in parentheses are standard errors.

TABLE 2. Percentage of root-knot nematode pot cultures colonized by mites and numbers of mites, second-stage juvenile nematodes (J2), and root galls in cultures of different ages (days since nematode infestation).

Age of culture (days)	Cultures colonized by mites (%)	Number of mites per liter soil	Root-knot J2 per cm^3 soil	Galls per cm root
0	33	10 (10)	0	0
41	33	18 (11)	64 (15)	1.49 (0.31)
72	67	233 (92)	111 (41)	6.14 (1.34)
103	83	133 (75)	87 (8)	3.91 (0.33)
134	67	25 (14)	56 (12)	4.51 (0.98)

Values are means (n = 6 for mites; n = 4 for nematodes). Values in parentheses are standard errors.

from the 103-day-old cultures were observed under a compound microscope, but only decaying roots and associated microflora could be identified. In the laboratory, these springtails fed on vermiform nematodes.

Over a 20-week period, there were significant changes in mite numbers with increasing culture age ($P < 0.0001$ and $P < 0.001$ for the May and June series, respectively) (Fig. 1). Both series fit quadratic models; peak population densities were achieved only 3–5 months after inoculation with root-knot nematodes (May-series day 142 = 1,436.4 ± 420.1 per liter of soil, June-series day 105 = 778.1 ± 437.4 per liter of soil). All pots were colonized by mites in both series. Arthropods other than nematophagous mites were rare in these cultures, and the high densities of collembola observed in July did not recur. There were positive linear relationships between species of nematophagous mite species per pot culture and culture age (Fig. 2) in both the May ($R^2 = 0.66$, $P < 0.0001$) and June series ($R^2 = 0.33$, $P < 0.0004$), and for the pooled collections on all dates ($R^2 = 0.49$, $P < 0.0001$).

DISCUSSION

Citrus nematode J2 are about the same size as root-knot nematode J2, i.e., 0.1144

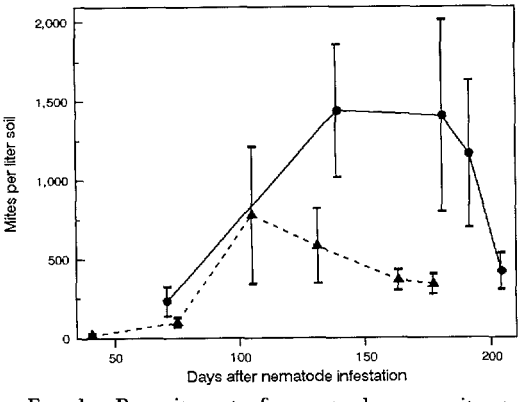

FIG. 1. Recruitment of nematophagous mites to greenhouse cultures of root-knot nematode (*Meloidogyne incognita*) over time. Solid line indicates cultures infested with root-knot nematodes in May, and dashed line indicates cultures infested in June 1989. Six cultures were sampled on each date. May = −283.77 + 5.53 (DPI) − 0.02 (DPI)^2; $R^2 = 0.66$; $P < 0.0001$. June = −66.1 + 1.78 (DPI) − 0.01 (DPI)^2; $R^2 = 0.56$; $P < 0.0001$. DPI = days postinfestation.

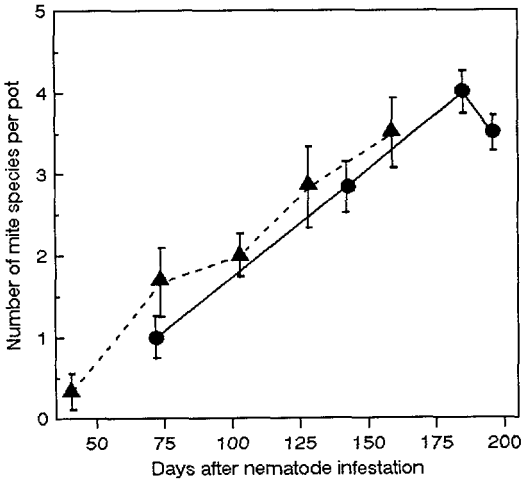

FIG. 2. Recruitment of nematophagous mite species to greenhouse cultures of root-knot nematode (*Meloidogyne incognita*) over time. Solid line indicates cultures infested with root-knot nematodes in May, and dashed line indicates cultures infested with root-knot nematodes in June 1989. Six cultures were sampled on each date. May = −2.13 + 0.05 (DPI); $R^2 = 0.687$; $P < 0.0001$. June = −0.96 + 0.04 (DPI); $R^2 = 0.571$; $P < 0.0001$. DPI = days postinfestation.

μg wet weight (10) ([0.1144 × 0.25 μg dry]/wet weight = 0.0285 μg dry weight per citrus nematode J2). If the *Protogamasellus* mites in the citrus nematode cultures fed only on citrus nematode J2, they could account for ca. 29 citrus nematodes/mite/day ([0.82 μg nematode dry weight/day consumed by each mite] ÷ [0.0285 μg dry weight/nematode]). At a mean density of 236.1 mites per liter of soil × 46.2 liters of soil per box × 29 citrus nematodes/day, ca. 316,000 citrus nematode J2 would be consumed per box each day. Because these mites are general predators, and free-living nematodes and arthropods are also abundant in these boxes, it is unlikely that only citrus nematode J2 are eaten. Collembolans are also known to feed opportunistically on plant-parasitic nematodes (2). However, if actual consumption is only a fraction of the above number, a large number of citrus nematodes would be lost from each box culture to *Protogamasellus* mites alone.

In general, our root-knot nematode cultures do not seem to be greatly affected by the antagonists present. Most pot cultures are used in experiments or sacrificed to obtain inoculum for new cultures before mite populations reach high levels. In the cultures that were allowed to survive for 6 months (May and June series), nematophagous mite populations did rise to potentially damaging levels. *Lasioseius* mites can consume about 3.0 μg (dry weight) of nematodes per day (15) or about 105 root-knot J2/day/mite. That is, *L. subterraneus* alone could be consuming an average of 22,680 (June series) or 66,134 (May series) root-knot juveniles per pot per day in our cultures. Other species of *Lasioseius* have been reported to be aggressive nematophages (1,3,5,11,15), and may represent useful natural enemies of plant-parasitic nematodes.

Our greenhouse nematode cultures resemble experiments with natural enemy treatments (including uncolonized controls) randomly applied by the surrounding environment. This could be a serious problem if, for example, antagonists in nematode inoculum were not equally distributed among treatments in field or greenhouse experiments. The brief exposure to bleach during extraction of root-knot eggs did not preclude fungal antagonists from being introduced into new cultures. We suspect that our cultures are not unusual, and that most eventually become contaminated with nematode fungal and arthropod antagonists.

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