Journal of Nematology 34(3):267–272. 2002.

Eggs of *Tylenchulus semipenetrans* Inhibit Growth of *Phytophthora nicotianae* and *Fusarium solani* in vitro[1]

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Abstract: In previous greenhouse and laboratory studies, citrus seedlings infested with the citrus nematode *Tylenchulus semipenetrans* and later inoculated with the fungus *Phytophthora nicotianae* grew larger and contained less fungal protein in root tissues than plants infected by only the fungus, demonstrating antagonism of the nematode to the fungus. In this study, we determined whether eggs of the citrus nematode *T. semipenetrans* and root-knot nematode *Meloidogyne arenaria* affected mycelial growth of *P. nicotianae* and *Fusarium solani* in vitro. Approximately 35,000 live or heat-killed (60 °C, 10 minutes) eggs of each nematode species were surface-sterilized with cupric sulfate, mercuric chloride, and streptomycin sulfate and placed in 5-µl drops onto the center of nutrient agar plates. Nutrient agar plugs from actively growing colonies of *P. nicotianae* or *F. solani* were placed on top of the eggs for 48 hours after which fungal colony growth was determined. Live citrus nematode eggs suppressed mycelial growth of *P. nicotianae* and *F. solani* ($P \leq 0.05$) compared to heat-killed eggs and water controls. Reaction of the fungi to heat-killed eggs was variable. Root-knot nematode eggs had no effect on either *P. nicotianae* or *F. solani* mycelial growth. The experiment demonstrated a species-specific, direct effect of the eggs of the citrus nematode on *P. nicotianae* and *F. solani*.

Key words: antagonistic interaction, citrus, competition, eggs, *Fusarium solani, Meloidogyne arenaria, Phytophthora nicotianae, Tylenchulus semipentrans.*

The citrus nematode, *Tylenchulus semipenetrans* Cobb, is a semi-endoparasite of the cortical cells of citrus fibrous roots. The anterior portion of the adult female extends several cell layers into the cortical parenchyma, while the posterior portion, outside of the root, secretes a gelatinous matrix into which eggs are deposited (Van Gundy, 1958). The eggs with this protective gelatinous matrix are known as an egg mass (Maggenti, 1962) and may contain 75 to >100 eggs (Baines, 1950). The adult female of *T. semipenetrans* is sessile, obtaining its nutrients from specialized transfer cells (6 to 10) called "nurse" cells around the nematode head (Van Gundy, 1958). These "nurse" cells are required for successful reproduction and die upon the female's death.

Tylenchulus semipenetrans reduces the infection of citrus roots by the fungus *P. nicotianae* Dastur Breda de Hann (synonym = *parasitica* Dastur) (El-Borai et al., 2000). Forty-five-day-old sour orange (*Citrus aurantium* L.) seedlings infested by the nematode, and later inoculated with the fungus, contained less fungal protein in the root tissues than plants not infected by the nematode. The fungus reduced stem and root weights in the absence of the nematode but had no effect on stem or root weights in the presence of the nematode. Both organisms feed in the cortex, reduce the mass of the fibrous root system, and are capable of reducing citrus yield (Duncan et al., 1993; Graham and Menge, 1999).

Interactions involving plant-pathogenic nematodes and fungi have been studied extensively and are often synergistic, with the combined effects of the nematode and fungus greater than the sum of the effects of each pathogen alone (Atkinson, 1892; Bergeson, 1972; Carter, 1981; MacGuidwin and Rouse, 1990; Mai and Abawi, 1987; McLean and Lawrence, 1993; Powell, 1971a, 1971b; Powell, 1979; Powell et al., 1971; Powelson and Rowe, 1993; Prot, 1993; Roy et al., 1989; Webster, 1985; Whitney, 1974). Only occasionally has the effect of nematodes on plant-pathogenic fungi been shown to be antagonistic (Gray et al., 1990; Sankaralingam and McGawely, 1994). Orion and Kritzman (1991) and Papert and Kok (1999) reported that the gelatinous matrices of the root-knot nematodes, *M. javanica* and *M. fallax,* provide protection against microbial attack, either due to antibiotic compounds from the matrix or due to associated microorganisms.

Possible mechanisms by which the citrus nematode suppresses fungal development include direct chemical antagonism by the nematode, nutrient competition, or alteration of the microbial community in the rhizosphere to favor microorganisms antagonistic to *P. nicotianae.* The objective of this study was to consider the first possibility by observing the effect of *T. semipenetrans* eggs on growth of *P. nicotianae* and *Fusarium solani* (Mart.) Sacc in vitro compared to eggs of the root-knot nematode *M. arenaria* (Neal) Chitwood. *Fusarium solani* was included in these experiments because it was the most commonly encountered and abundant fungus in a recent survey of the citrus rhizosphere (El-Borai et al., 2002) and is an opportunistic parasite of citrus fibrous roots (Graham et al., 1985).

Materials and Methods

Bioassays were used to determine the effects of eggs of *T. semipenetrans* and *M. arenaria* race 1 on mycelial growth in vitro of *P. nicotianae* and *F. solani.* Eggs of *T. semipenetrans* were obtained from naturally infected citrus roots from the field. Eggs of *M. arenaria* were from roots of tomato (*Lycopersicon esculentum* Mill. Cv. Rut-

Received for publication 13 August 2001.

[1] This research was supported by the Florida Agricultural Experiment Station and a grant in part from the Florida Citrus Production Research Advisory Council, and approved for publication as Journal Series No. R-08319. This paper is a portion of a PhD dissertation by the first author.

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This paper was edited by Patricia Timper.

gers) grown in steam-pasteurized potting soil. The *M. arenaria* isolate originated from a population in Levy County, Florida.

Eggs, juveniles, and males of both nematodes were scrubbed from root surfaces and collected on nested 74 and 25-µm-pore sieves. Nematodes were separated from soil and plant debris by sucrose centrifugation (Jenkins, 1964) followed by magnesium sulfate fractionation. The magnesium sulfate (225.9 g/liter water) was pipeted beneath the nematode suspension, forming a density gradient, which was then centrifuged 3 minutes at 1,500 rpm (Hendrickx et al., 1976). The aqueous (top) fraction containing the nematodes and eggs was drawn off using a 5-ml pipet and then rinsed repeatedly with tap water over a 20-µm-pore sieve to remove residual $MgSO_4$. To separate eggs from vermiform stages, the suspension was passed repeatedly (15 to 20 times) through a 44-µm-pore sieve, which was rinsed after each pass to remove vermiform stages of *T. semipenetrans* and free-living nematodes. The egg suspension was then concentrated on a 20-µm-pore sieve. The few remaining free-living nematodes in the suspension were removed by handpicking.

Additionally, nematodes and eggs were scrubbed from root surfaces using 5% commercial bleach (NaOCl 0.03%) for 30 seconds followed by water rinsing for 5 minutes to remove residual NaOCl (Hussey and Barker, 1973; McClure et al., 1973). The same sucrose centrifugation, magnesium-sulfate fractionation, and sieving procedures were used as described previously. Bleach was used to remove residue of the matrix from eggs.

Eggs in all experiments were surface-sterilized in a laminar flow hood as follows: Nematode eggs were back-washed from 20-µm-pore sieves into 12-ml sterile disposable plastic tubes. The egg suspension was allowed to settle for 1 hour, and volume was reduced to 0.5 ml per tube using a sterilized 5-ml pipet. The eggs were then treated with cupric sulfate (0.1%) for 30 minutes, mercuric chloride (0.025%) for 10 minutes, and then streptomycin sulfate (0.2%) for 24 hours. After each sterilant, the eggs were rinsed seven times with 1-Liter exchanges of sterile-distilled water on an autoclaved 25-µm-pore sieve.

A single nematode species was used in each of 10 experiments. Three treatments were used in eight of the experiments: live surface-sterilized eggs, heat-killed surface-sterilized eggs, and a water control. Egg suspensions in the 12-ml sterile disposable plastic tubes were placed in a water bath (60 °C for 10 minutes) to obtain heat-killed eggs. Eggs of *T. semipenetrans,* extracted without bleach, were tested twice against each fungus (four experiments), and eggs extracted with bleach were tested a single time against each of the fungi (two experiments). Eggs of *M. arenaria* extracted with or without bleach were tested a single time against each fungus (four experiments). Due to an insufficient number of eggs, heat-killed eggs were omitted from the two experiments in which *M. arenaria* eggs were extracted without bleach.

In all of the above experiments, approximately 35,000 eggs in a 5-µl water droplet were deposited centrally on the surface of nutrient agar (Sigma Chemical Company, St. Louis, MO) in 100 × 15-mm petri plates. Nutrient agar plugs (5-mm-diam.) cut with a cork borer from margins of actively growing colonies of either *P. nicotianae* or *F. solani* were placed on the agar surface over the eggs. Control plates received 5 µl sterile-distilled water in place of nematode eggs. Plates were stacked randomly and stored in the dark at room temperature (25 ± 1 °C). Each treatment was replicated 15 times (one plate per replicate). After 48 hours, *P. nicotianae* colony growth from the edge of the agar plug was measured. A circular template with eight equidistant lines radiating from the center, made from an inverted petri plate, was placed beneath the bioassay dish. A dissecting microscope and ocular micrometer were used to measure the farthest growth of mycelia along each line, and the eight measurements were averaged.

An additional experiment was conducted using live eggs of *T. semipenetrans* extracted without bleach and a water control treatment, except that the agar plug with *P. nicotianae* was placed adjacent to the drop of egg suspension or water. The spatial pattern of mycelial growth was observed after 48 hours.

To verify the effectiveness of the treatments, live and heat-killed eggs of *T. semipenetrans* and *M. arenaria* were observed for hatching for 1 month. Approximately 1,000 eggs from each treatment were maintained in 3 ml sterile distilled water on a 60 × 15-mm petri plate and examined weekly. Live, surface-sterilized eggs were also plated on nutrient agar and examined after 1 and 2 weeks for development of microorganisms.

To determine the effects of the sterilization chemicals on fungal growth, a series of dilutions (water control, 10^0, 10^{-2}, 10^{-3}, and 10^{-4}) were made of the original concentrations of each compound used to surface-sterilize the eggs. Five µl from each dilution was pipeted onto the center of the nutrient agar, and a plug of *P. nicotianae* was placed on the drop. After 48 hours, *P. nicotianae* colony growth was measured as described. Eight plates were used with each dilution of each chemical sterilant.

Data were analyzed by Analysis of Variance (ANOVA), and mean separation was determined with Duncan's multiple-range test ($P \leq 0.05$).

Results

Live eggs of *T. semipenetrans* reduced growth of *P. nicotianae* by 74% to 94% ($P \leq 0.05$) compared to water controls (Fig. 1A). The effect was apparent when the agar plug with the fungus was placed either on top of eggs or adjacent to them (Fig. 2A, B). Response of the

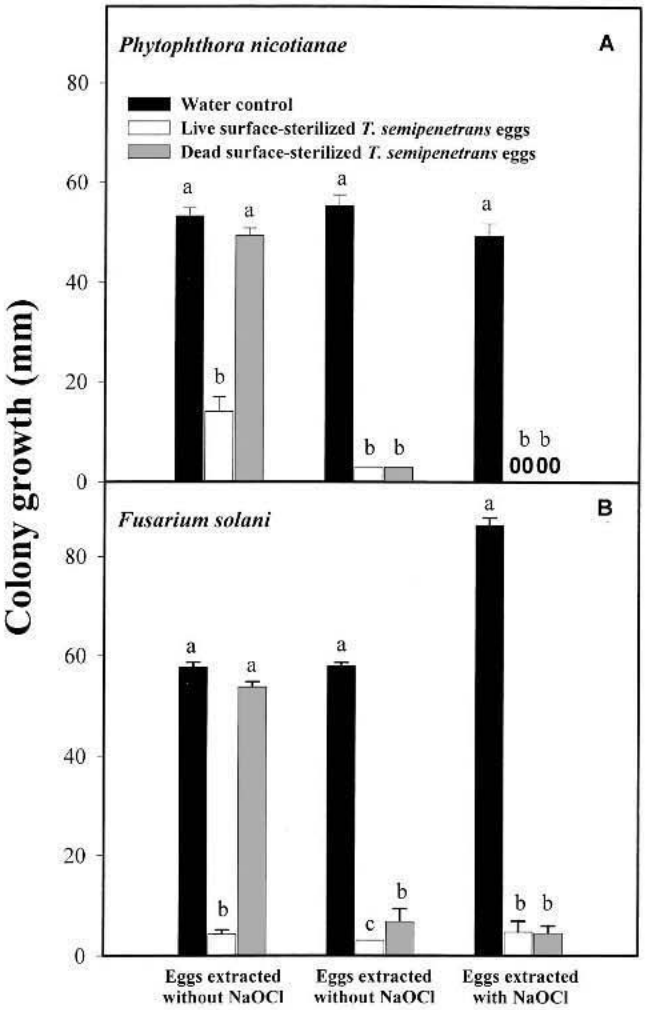

FIG. 1. Mean (± SEM) colony growth of *Phytophthora nicotianae* (A) and *Fusarium solani* (B) after 48 hours on nutrient agar with or without eggs of *Tylenchulus semipenetrans*. For each fungus species, nematode eggs were extracted without bleach in two experiments and with bleach in a single experiment. Agar plugs (5-mm-diam.) from cultures of *P. nicotianae* or *F. solani* were placed on top of 5 µl water with or without ca. 35,000 nematode eggs. Bars with a common letter within each experiment are not different according to Duncan's multiple-range test at ($P \leq 0.05$).

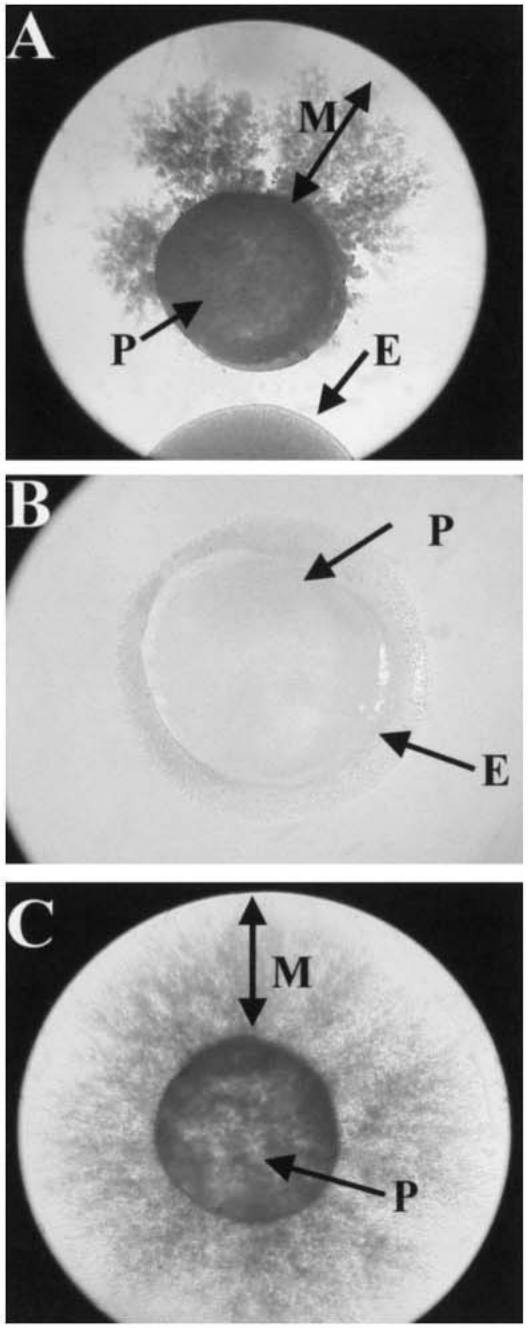

FIG. 2. Effect of *Tylenchulus semipenetrans* eggs on *Phytophthora nicotianae* mycelial growth in vitro after 48 hours. A) Fungal plug adjacent to live surface-sterilized *T. semipenetrans* eggs. B) Fungal plug on the top of live surface-sterilized *T. semipenetrans* eggs. C) Water control (*P. nicotianae* plug only). E = *T. semipenetrans* live eggs, P = *P. nicotianae* plug, M = *P. nicotianae* mycelial growth. *P. nicotianae* plug= 5-mm diam.

fungus to heat-killed eggs of *T. semipenetrans* was variable (Fig. 1A). In one experiment the fungus grew normally in the presence of heat-killed eggs, and in others the inhibitory effects of live or heat-killed eggs were indistinguishable. Eggs of *T. semipenetrans* extracted with bleach also inhibited growth of *P. nicotianae* (Fig. 1A). Differences in growth of *F. solani* in the presence or absence of live or heat-killed *T. semipenetrans* eggs was similar to that of *P. nicotianae* (Fig. 1B). With bleach extraction, both live and heat-killed eggs inhibited *F. solani* ($P \leq 0.05$) colony growth after 48 hours by 94% compared to water controls (Fig. 1B); however, after 72 hours (data not shown), heat-killed eggs lost some of their inhibitory activity (55% inhibition) compared to live eggs (79% inhibition; $P \leq 0.05$).

Eggs of *M. arenaria*, extracted with or without bleach, had no effect on *P. nicotianae* mycelial growth (Fig. 3A). Eggs extracted without bleach inhibited ($P \leq 0.05$) growth of *F. solani* by 64% compared to water controls; however, eggs extracted with bleach did not affect mycelial growth (Fig. 3B).

Sixty-five percent of surface-sterilized *T. semipenetrans* eggs hatched compared to 75% of eggs rinsed with water only. Seventy-five percent of surface-sterilized *M. arenaria* eggs hatched compared to 90% of eggs rinsed with water only. No juveniles hatched from heat-killed

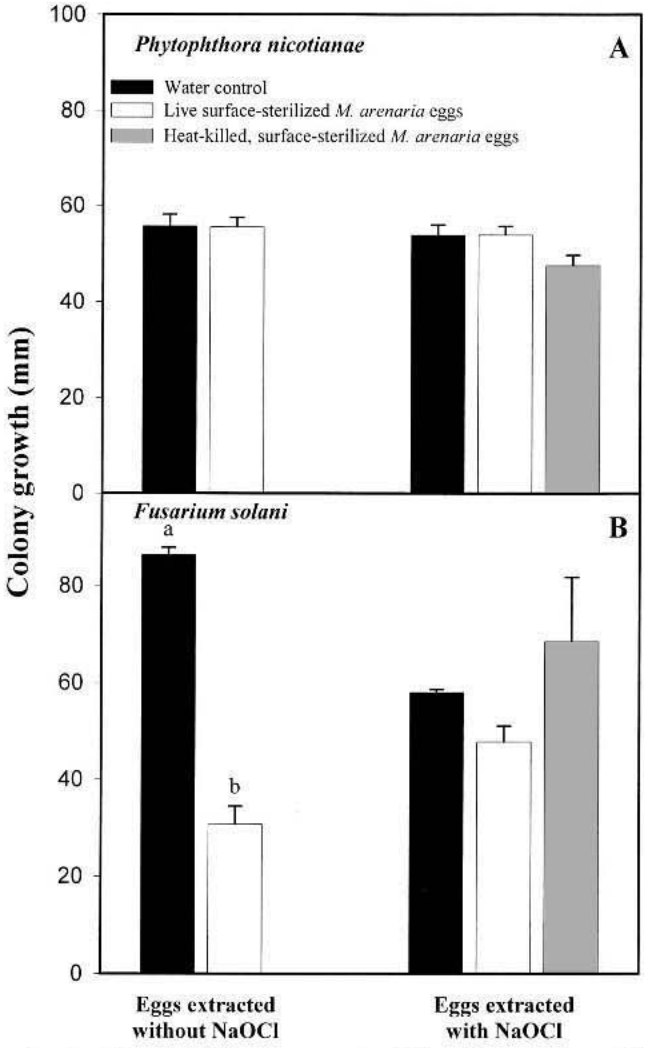

FIG. 3. Mean (± SEM) colony growth of *Phytophthora nicotianae* (A) and *Fusarium solani* (B) after 48 hours on nutrient agar with or without eggs of *Meloidogyne arenaria*. For each fungus species, nematode eggs were extracted without or with bleach. Agar plugs (5-mm-diam.) from cultures of *P. nicotianae* or *F. solani* were placed on top of 5 µl water with or without ca. 35,000 nematode eggs. Bars with a common letter within each experiment are not different according to Duncan's multiple-range test at ($P \leq 0.05$).

eggs in any experiment. No fungal or bacterial colonies were observed when eggs were plated on nutrient agar or on any of the bioassay plates.

Compared to water controls, the original concentrations of mercuric chloride (0.025%), streptomycin sulfate (0.2%), and the mixture of all sterilants inhibited *P. nicotianae* mycelial growth by 100%, 66%, and 100%, respectively (Table 1). The original concentration of cupric sulfate did not affect mycelial growth. None of the dilutions of either the mixture or any individual sterilant affected growth of *P. nicotianae*.

TABLE 1. Effect of cupric sulfate, mercuric chloride, and streptomycin sulfate on *Phytophthora nicotianae* mycelial growth after 48 hours in vitro.

	Phytophthora nicotianae colony growth (mm)			
Dilutions	Cupric sulfate	Mercuric chloride	Streptomycin sulfate	Cupric sulfate Mercuric chloride Streptomycin sulfate
Water control	27.8 a	27.8 a	27.8 a	22.4 a
10^{0}	29.1 a	00.0 b	9.4 b	00.0 b
10^{-2}	29.8 a	25.9 a	28.1 a	23.1 a
10^{-3}	30.8 a	27.8 a	26.2 a	23.1 a
10^{-4}	33.8 a	29.1 a	28.7 a	23.4 a

Numbers are the means of eight replications for each dilution. Numbers in a column with a common letter are not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

DISCUSSION

The results of these experiments demonstrated a direct, species-specific effect of citrus nematode eggs on *P. nicotianae* and *F. solani* mycelial growth. Although the results using heat-killed eggs were inconsistent, live eggs of *T. semipenetrans* always inhibited mycelial growth of both fungi, in contrast to live eggs of *M. arenaria*. It is unlikely that the disinfectants used to surface-sterilize the eggs in this experiment caused the inhibition. The seven, 1-liter exchanges of water used to rinse eggs almost certainly diluted the original concentration of sterilants more than 100-fold, which was shown to eliminate any effect on growth of *P. nicotianae*. Moreover, eggs of both nematode species were disinfested in the same manner. Our inability to isolate bacterial or fungal colonies from surface-disinfested eggs is further evidence that chemicals emanating from eggs, and not sterilants or associated microorganisms, were responsible for the observed inhibition.

The possibility that *T. semipenetrans* produces antifungal chemicals is supported by other research (El Borai et al., 2000, 2002). *Tylenchulus semipenetrans* was shown to reduce the amount of *P. nicotianae* in citrus roots, and concomitant infection by both organisms increased the growth of citrus seedlings compared to seedlings infected by *P. nicotianae* alone (El-Borai et al., 2000). Infection of citrus roots by *T. semipenetrans* in the field was also shown to alter the community of microorganisms in the rhizoshpere (El-Borai et al., 2002). Bacteria such as *Burkholderia cepacia, Bacillus megaterium,* and others were isolated in higher numbers from fibrous root segments containing egg masses of *T. semipentrans,* compared to non-infected root segments. While these bacteria were found to increase the growth of citrus seedlings infected by *P. nicotianae,* they did not suppress the amount of the fungus in the plant roots. Thus, while certain rhizosphere microrganisms associated with *T. semipenetrans* may increase plant tolerance to infection by *P. nicotianae,* they were not found to directly inhibit the fungus.

The gelatinous matrix of root-knot nematodes has been shown to protect eggs against microbial attack (Orion and Kritzman, 1991; Papert and Kok, 1999). The nature of this protection is unknown. Our data appear to be the first to demonstrate inhibition of fun-

gal mycelial growth by nematode eggs. Inhibition of *P. nicotianae* and *F. solani* by *T. semipenetrans* eggs, with or without bleach extraction, suggests that the effect is likely due to chemicals secreted by eggs rather than chemicals contained in the gelatinous matrix, which is dissolved and removed by bleach. Conversely, *M. arenaria* eggs extracted without bleach did inhibit *F. solani* mycelial growth, suggesting the residual gelatinous matrix may contain constitutive compounds that inhibit the growth of certain fungi. Differential effects of root-knot and citrus nematode eggs on plant-pathogenic fungi may result from adaptation to different parasitic behaviors. Because the female root-knot nematode remains sessile inside the developing root tissue for most of its life (Bird, 1962), this species may experience less selection pressure to protect its feeding site compared to the semi-endoparasitic *T. semipenetrans* that infects and remains exposed on the surface of fully developed roots (Cohn, 1965). The narrow host range of *T. semipenetrans*, compared to *M. arenaria*, may exert additional pressure for defense of the feeding site.

Nematode fungal interactions are often synergistic. There are fewer examples of antagonistic interactions between plant-parasitic nematodes and plant-pathogenic fungi. Gray et al. (1990) found that survival of alfalfa seedlings was lower following inoculation with *P. megasperma* f. sp. *medicaginis* than following inoculation with both *M. hapla* and *P. megasperma* f. sp. *medicaginis*. *Meloidogyne incognita* and *Pythium graminicola* were found to be mutually inhibitory on sugarcane (Valle-Lamboy and Ayala, 1980). Presence of the nematode interfered with the development of the fungus in the roots, and plants grew larger when both pathogens were together than in the presence of either alone. *Meloidogyne incognita* was found to protect *Phaseolus vulgaris* roots from infection by the fungus *Rhizoctonia solani* (Costa Manso and Huang, 1986). Sankaralingam and McGawely (1994) also reported an antagonistic interaction between *Rotylenchulus reniformis* and *R. solani* on cotton. The combined effect of the nematode and the fungus was antagonistic, with respect to cotton seedling blight. Because *T. semipenetrans* and *R. reniformis* are both sedentary semi-endoparasites with similar life histories, it would be worthwhile to test *R. reniformis* eggs for activity against plant-pathogenic fungi.

Tylenchulus semipenetrans eggs appear to have an antagonistic effect on *P. nicotianae* mycelial growth in vitro. These results may explain the *T. semipenetrans*-mediated suppression of root infection by *P. nicotianae* that resulted in increased citrus seedling growth (El-Borai et al., 2000). Identification and characterization of anti-fungal compounds secreted from eggs of *T. semipenetrans* are warranted.

Literature Cited

Atkinson, G. F. 1892. Some diseases of cotton. Bulletin of Alabama Agricultural Experiment Station 41:61–65.

Baines, R. C. 1950. Nematodes on citrus. California Agriculture 4:7.

Bergeson, G. B. 1972. Concepts of nematode-fungus association in plant disease complexes. A review. Experimental Parasitology 32:301–314.

Bird, A. F. 1962. The inducement of giant cells by *Meloidogyne javanica* relative to roots. Nematologica 8:1–10.

Carter, W. W. 1981. The effect of *Meloidogyne incognita* and tissue wounding on severity of seedling disease of cotton caused by *Rhizoctonia solani*. Journal of Nematology 13:374–376.

Cohn, E. 1965. On the feeding and histopathology of the citrus nematode. Nematologica 11:594–600.

Costa Manso, E. S. B. G., and C. S. Huang. 1986. Interacao entre *Meloidogyne incognita* (Race 4) eiso ladoes de *Rhizocotonia solani*, em *Phaseolus vulgaris*. ev. Rico 23. Fitopathologia Brasileria 11:857–864.

Duncan, L. W., J. H. Graham, and L. W. Timmer. 1993. Seasonal patterns associated with *Tylenchulus semipenetrans* and *Phytophthora nicotianae* in the citrus rhizosphere. Phytopathology 83:573–581.

El-Borai, K. F., L. W. Duncan, and J. H. Graham. 2000. *Tylenchulus semipenetrans* suppresses infection of citrus fibrous roots by the fungus *Phytophthora nicotianae* (= *Parasitica*). Journal of Nematology 32:427–428 (Abstr.).

El-Borai, F. E., L. W. Duncan, J. H. Graham, and E. Dickstein. 2002. *Tylenchulus semipenetrans* alters the microbial community in the citrus rhizosphere. Journal of Nematology, in press.

Graham, J. H., R. H. Brlansky, L. W. Timmer, R. F. Lee, L. J. Marais, and J. S. Bender. 1985. Comparison of citrus tree declines with necrosis of major roots and their association with *Fusarium solani*. Plant Disease 69:1055–1058.

Graham, J. H., and J. A. Menge. 1999. Root diseases. Pp. 126–135 *in* L. W. Timmer and L. W. Duncan, eds. Citrus health management. St. Paul: American Phytopathological Society.

Gray, F. A., G. D. Griffin, D. A. Johnson, J. W. Eckert, and J. E. Kazimir. 1990. Interrelationships between *Meloidogyne hapla* and *Phytophthora megasperma* f. sp. *Medicaginis* in seedlings damping-off and root infection of alfalfa. Phytopathology 80:228–232.

Hendrickx, G. J., W. A. Coolen, and R. Moermans. 1976. Influence de la nature et de la densité des liquides de séparation sur les nematodes pendant le procéss de centrifugation-flotation. Nematologia Mediterranea 4:31–40.

Hussey, R. S., and K. R. Barker. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Disease Report 57:1025–1028.

Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Reporter 48:492.

MacGuidwin, A. E., and D. I. Rouse. 1990. Role of *Pratylenchus penetrans* in the potato early dying disease of russet Burbank Potato. Phytopathology 80:1077–1082.

Maggenti, A. R. 1962. Production of gelatinous matrix and taxonomic signification in *Tylenchulus* (Nematoda-Tylenchulinae). Proceedings of the Helminthological Society of Washington 29:139.

Mai, W. F., and G. S. Abawi. 1987. Interactions among root-knot nematodes and *Fusarium* wilt fungi on host plants. Annual Review of Phytopathology 25:317–338.

McClure, M. A., T. H. Kruk, and I. Misaghi. 1973. A method for obtaining quantities of clean *Meloidogyne* eggs. Journal of Nematology 5:230.

McLean, K. S., and G. W. Lawrence. 1993. Interrelationship of *Heterodera glycines* and *Fusarium solani* in sudden death syndrome. Journal of Nematology 25:434–439.

Orion, D., and G. Kritzman. 1991. Antimicrobial activity of *Meloidogyne javanica* gelatinous matrix. Revue de Nématologie 14:481–484.

Papert, A., and C. J. Kok. 1999. Microbiology of egg masses of the root knot nematode *Meloidogyne fallax*. Edinburgh, Switzerland: International Congress of Phytopathology.

Powell, N. T. 1971a. Interaction between nematodes and fungi in disease complexes. Annual Review of Phytopathology 9:253–274.

Powell, N. T. 1971b. Interaction of plant-parasitic nematodes with other disease-causing agents. Pp. 119–136 *in* B. M. Zuckerman, W. F. Mai, and R. A. Rohde, eds. Plant-parasitic nematodes, vol. 2. New York: Academic Press.

Powell, N. T. 1979. Internal synergism among organisms inducing

disease. Pp. 113–133 *in* J. G. Horsfall and E. B. Cowling, eds. Plant disease, an advanced treatise, vol. 4, New York: Academic Press.

Powell, N. T., P. L. Melendez, and C. K. Batten. 1971. Disease complexes in tobacco involving *Meloidogyne incognita* and certain soilborne fungi. Phytopathology 61:1332–1337.

Powelson, M. L., and R. C. Rowe. 1993. Biology and management of early dying of potato. Annual Review of Phytopathology 31:111–126.

Prot, J. 1993. Biochemical and genetic basis of fungus-nematode interactions. Pp. 273–285 *in* M. V. Khan, ed. Nematode interactions. New York: Chapman and Hall.

Roy, K. W., G. W. Lawrence, H. H. Hodges, K. S. McLean, and J. F. Killebrew. 1989. Sudden death syndrome of soybean: *Fusarium solani* as incitant and relation of *Heterodera glycines* to disease severity. Phytopathology 79:191–197.

Sankaralingam, A., and E. C. McGawley. 1994. Interrelationships of *Rotylenchulus reniformis* with *Rhizoctonia solani* on cotton. Journal of Nematology 26:475–485.

Valle-Lamboy, S., and A. Ayala. 1980. Pathogenicity of *Meloidogyne incognita* and *Pratylenchus zeae,* and their association with *Pythium graminicola* on roots of sugarcane in Puerto Rico. Journal of Agriculture 64:338–347.

Van Gundy, S. D. 1958. The life history of the citrus nematode *Tylenchulus semipenetrans* Cobb. Nematologica 3:283–294.

Webster, J. M. 1985. Interaction of *Meloidogyne* with fungi on crop plants. Pp. 183–192 *in* J. N. Sasser and C. C. Carter, eds. An advance treatise on *Meloidogyne,* vol. 1. Raleigh: North Carolina State University Graphics.

Whitney, E. D. 1974. Synergistic effect of *Pythium ultimum* and the additive effect of *P. aphanidermatum* with *Heterodera schachtii* on sugarbeet. Phytopathology 64:380–383.