

**PATHOGENICITY AND REPRODUCTIVE POTENTIAL OF
MELOIDOGYNE MAYAGUENSIS AND *M. FLORIDENSIS* COMPARED
WITH THREE COMMON *MELOIDOGYNE* SPP.**

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ABSTRACT

Cetintas, R., R. Kaur, J. A. Brito, M. L. Mendes, A. P. Nyczepir, and D. W. Dickson. 2007. Pathogenicity and reproductive potential of *Meloidogyne mayaguensis* and *M. floridensis* compared with three common *Meloidogyne* spp. *Nematopica* 37:21-31.

The pathogenicity and reproductive potential of *Meloidogyne mayaguensis* and *M. floridensis*, two new species recently reported in Florida agriculture, were compared to those of *M. arenaria* race 1, *M. incognita* race 4, and *M. javanica* race 1 on tomato (*Lycopersicon esculentum*) in field microplots. Three trials were conducted, one in fall and two in spring using tomato cvs. Solar Set and Florida 47, respectively. Two levels of each nematode (low = one egg or second-stage juvenile (J2)/100 cm³ of soil; high = three eggs or J2/100 cm³ of soil) were used with nine replicates each. Common vetch (*Vicia sativa*) was used in trial one as a winter cover crop. Nematode densities in the soil, root-galling, eggs per gram fresh root, shoot fresh weight and plant height were recorded. No significant interaction was observed between root-knot nematode species and inoculum levels in trials one or two except for eggs/g of fresh root and J2/100 cm³ of soil at harvest of cv. Solar Set in trial one, fall 2004. All five species of root-knot nematodes induced root-galling and reproduced well on both tomato cultivars, except *M. floridensis*, which produced less galling in all trials. *Meloidogyne mayaguensis* produced the highest percentage of root-galling on cv. Solar Set in the fall trial but not on cv. Florida 47 in spring trials. However, *M. arenaria* showed a higher reproductive potential on cv. Solar Set in the fall trial. Galling on vetch was similar among *M. arenaria*, *M. incognita*, *M. javanica*, and *M. mayaguensis*, but numbers of J2 in soil were lower for *M. floridensis* and *M. javanica* than for *M. arenaria*, *M. incognita*, and *M. mayaguensis*. Although *M. mayaguensis* was observed to induce large galls on tomato, yield reduction occurred in only one of two trials in spring of 2005.

Key words: Common vetch, *Meloidogyne arenaria*, microplot, *M. incognita*, *M. javanica*, pathogenicity, reproduction potential, root-knot nematode, tomato.

RESUMEN

Cetintas, R., R. Kaur, J. A. Brito, M. L. Mendes, A. P. Nyczepir, y D. W. Dickson. 2007. Patogenicidad y potencial reproductivo de *Meloidogyne mayaguensis* y *M. floridensis* comparados con tres especies comunes de *Meloidogyne*. *Nematopica* 37:21-31.

Se comparó la patogenicidad y el potencial reproductivo de *Meloidogyne mayaguensis* y *M. floridensis*, dos especies nuevas recientemente registradas en Florida, con la patogenicidad y potencial reproductivo de *M. arenaria* raza 1, *M. incognita* raza 4, y *M. javanica* raza 1 en tomate (*Lycopersicon esculentum*) en microparcelas. Se condujeron tres experimentos: uno en el otoño y dos en la primavera, utilizando cvs. Solar Set y Florida 47, respectivamente. Se utilizaron dos niveles de inóculo de cada nematodo (bajo = un huevo o juvenil de segundo estadio (J2)/100 cm³ de suelo; alto = tres huevos o J2/100 cm³ de suelo) con nueve repeticiones cada uno. En uno de los experimentos se utilizó *Vicia sativa* como cultivo de cobertura durante el invierno. Se registraron las densidades de población en el suelo, in-

dices de agallamiento, huevos por gramo de raíz fresca, peso fresco de parte aérea y altura de la planta. No se observó ninguna interacción significativa entre la especie de nematodo y el nivel de inóculo en los experimentos uno y dos, excepto entre huevos/g de raíz fresca y J2/100 cm³ de suelo al momento de cosecha del cv. Solar Set en el primer experimento, en otoño de 2004. Todas las especies de nematodos se reprodujeron bien e indujeron agallas en ambos cultivares de tomate, excepto *M. floridensis*, que produjo menos agallas en todos los experimentos. *Meloidogyne mayaguensis* produjo el porcentaje más alto de agallamiento en cv. Solar Set en el experimento del otoño, pero no en Florida 47 en el experimento de primavera. Sin embargo, *M. arenaria* tuvo mayor potencial reproductivo en cv. Solar Set en el otoño. El agallamiento en *V. sativa* fue similar para *M. arenaria*, *M. incognita*, *M. javanica*, y *M. mayaguensis*, pero las densidades de J2 en el suelo fueron más bajas para *M. floridensis* y *M. javanica* que para *M. arenaria*, *M. incognita*, y *M. mayaguensis*. A pesar de que se observó que *M. mayaguensis* induce agallas grandes en tomate, sólo se observó reducción en la producción en uno de los dos experimentos de la primavera, en 2005.

Palabras clave: *Meloidogyne arenaria*, microparcela, *M. incognita*, *M. javanica*, patogenicidad, potencial reproductivo, nematodo del nudo radical, tomate, *Vicia sativa*.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is the most important vegetable produced in Florida with 0.6 billion kg picked for fresh market on 170,000 ha in 2004-05 (Anonymous, 2006). The farm level value exceeds \$662 million (Anonymous, 2006). In the United States, nearly the entire fresh market tomato crop is grown in Florida from December through May. The total cost of producing and harvesting tomatoes in Florida varies among different growing regions in the state. Pest and pathogen management is a large part of these costs. Root-knot nematodes (*Meloidogyne* spp.) are considered major soilborne pathogens of tomato worldwide, but are especially problematic in Florida (Ornat and Verdejo-Lucas, 1999; Sorribas and Verdejo-Lucas, 1994). The root-knot nematode species that infect tomato in Florida include *M. arenaria*, *M. floridensis*, *M. incognita*, *M. javanica* and *M. mayaguensis* (Brito *et al.*, 2004b; Brito *et al.*, 2005; Church, 2005). However, nothing is known about the pathogenicity or reproductive potential of *M. floridensis* and *M. mayaguensis* on this crop. *Meloidogyne mayaguensis* and *M. floridensis* are of concern because of

their ability to overcome the *Mi-1* resistance gene in tomato (Fargette *et al.*, 1996; Guimarães *et al.*, 2003; Rodríguez *et al.*, 2003), and resistance to root-knot nematode in peach (Handoo *et al.*, 2004), respectively. The objective of this study was to compare the pathogenicity and reproductive potential of *M. mayaguensis* and *M. floridensis* to those of *M. arenaria*, *M. incognita* and *M. javanica* on tomato.

MATERIALS AND METHODS

Microplot studies were conducted at the University of Florida Plant Science Research and Education Unit, Marion County, FL, USA. Tomato cv. Solar Set and cv. Florida 47 were used. Both are commonly grown commercially, with cv. Solar Set being better suited for late-summer plantings and cv. Florida 47 better suited for late-winter plantings.

Nematode Source

The original host and origin of each *Meloidogyne* isolate was as follows: *M. arenaria* race I from peanut (*Arachis hypogaea*), Levy County, FL; *M. incognita*, and *M. javanica* from tobacco (*Nicotiana tabacum*), north

Florida; *M. floridensis* from peach (*Prunus persica*), Alachua County, FL; and *M. mayaguensis* from an unidentified ornamental plant from Broward County, FL (Brito *et al.*, 2003). All nematode isolates were derived from single egg mass culture and reared on tomato cv. Rutgers placed in separate greenhouses. Species identification was confirmed by subjecting at least 26 single females of each nematode isolate to polyacrylamide gel electrophoresis (Esbenshade and Triantaphyllou, 1985) using a BioRad mini-PROTEIN III unit (BioRad, Philadelphia, PA). Two females of *M. javanica* per gel were used as standards. Electrophoresis was carried out in a refrigerated discontinuous buffer system with 8% acrylamide running gel, pH 8.8, and 4% acrylamide stacking gel, pH 6.8 (BioRad). Following electrophoresis, the gels were removed and placed in an enzyme reaction mixture to determine esterase and malate dehydrogenase activity (Esbenshade and Triantaphyllou, 1985).

Microplot Trial One

A 30-m wide \times 185-m long weed fallowed field located at the Plant Science Research and Extension Center, Citra, FL was chosen for the microplot study. In mid-May 2004 the herbicide paraquat was

applied broadcast, and 3 weeks later the site was disked and planted with Argentine bahiagrass (*Paspalum notatum* Flugge) at 35 kg seed/ha. The bahiagrass was mowed weekly at ca. 10 cm height throughout the summer and fall months. The bahiagrass served to suppress weed growth and provide a wind break in the alleyways separating the rows of microplots. Microplots were cropped to tomato cultivars and common vetch as summarized in Table 1.

In mid-June 2004 the site was divided into five 6-m wide \times 20-m long strips. Twelve soil cores were collected in a zig-zag manner from each of the five strips with a cone-shaped 2.5-cm-diam. \times 15-cm deep sampling tube. The soil from each strip was mixed thoroughly and a 100 cm³ subsample was used for extracting nematodes by centrifugal-flotation (Jenkins, 1964). No root-knot nematodes were found in the soil from the microplot site. The soil at the site was classified as Arredondo fine sand (92.7% sand, 3.9% silt, 3.4% clay and <1% organic matter; pH 7.4).

The field was then divided into 10 strips, each 1.2-m wide \times 35-m long with 1.8 m wide alleyways separating each strip. Glyphosate was applied as a spray over each strip to kill the bahiagrass. Six weeks later (18 August 2004) holes measuring 50-cm-diam. \times 43-cm deep were dug, each cen-

Table 1. Dates of transplanting tomato cultivars and seeding common vetch in microplot trials one and two.

| Microplots | Transplanting/seeding dates [†] | Harvest dates |
|-----------------------|------------------------------------------|---------------|
| Trial one | | |
| Tomato cv. Solar Set | 8/24/04 | 11/16/04 |
| Common vetch | 11/23/04 | 3/25/05 |
| Tomato cv. Florida 47 | 4/22/05 | 6/27/05 |
| Trial two | | |
| Tomato cv. Florida 47 | 4/22/05 | 6/27/05 |

[†]Tomato cultivars were transplanted and common vetch was seeded in the microplots.

tered on 1.8 m spacing in each of the 10 strips. Round plastic pots, 47-cm-diam. \times 50-cm deep were inserted in each hole with 7 cm remaining above ground level. Each pot had five 20-cm diam holes drilled in the bottom for drainage. The previously removed soil was screened to remove all grass root debris, back-filled into each pot, and compacted to remove any airpockets. A broadcast application of a 6-17-16 N-P-K with micronutrients was applied to each plot to give 56 kg of N/ha. The fertilizer was raked into the soil surface and water was applied by overhead sprinklers. The microplots were arranged in a randomized complete block design with nine replications.

The treatments consisted of five nematodes species—*M. arenaria*, *M. floridensis*, *M. incognita*, *M. javanica* and *M. mayaguensis*; two inoculum levels—low (1 egg or J2/100 cm³ of soil) and high (3 eggs or J2/100 cm³ of soil), and a nontreated control. The inoculum was prepared by extracting nematode eggs from tomato roots using 0.5% NaOCl (Hussey and Barker, 1973) as modified by Boneti and Ferraz (1981). Before adding the nematode inoculant, 15 uniformly spaced holes, five each with depths of 7, 14 and 21 cm, were made by pressing a template into soil. The inoculum suspension was then sprinkled uniformly over the soil in 1,500 ml of water. The holes were filled by raking and leveling the top soil surface. Three seedlings of tomato cv. Solar Set were transplanted in a triangle with 20 cm spacing between plants. The plots were watered twice daily via a single twin-wall drip tape (Chapin, Watertown, NY) placed in the center of microplots. The tape had emitters spaced 30 cm apart and a flow rate of 1.9 l/min/30.5m. The plots were fertilized weekly with a 1 liter mixture containing 12 g of 20-20-20 NPK (Peters Professional, Division of United Industries Corp., St. Louis, MO) and hand weeded as needed. Insecticides and fungicides were

applied once or twice weekly as recommended for tomato (Olson *et al.*, 2004). The plants were staked and string tied 1 month after transplanting. The experiment was repeated the following spring.

Plant heights were recorded on 15 November and 1 day later plants were dug and shoot fresh weights were determined. Each root system was rated for percentage of root-galling based on a 0 to 10 scale where 0 = 0% of root system galled, 1 = 10%, 2 = 20%, 3 = 30%, 4 = 40%, 5 = 50%, 6 = 60%, 7 = 70%, 8 = 80%, 9 = 90% and 10 = 100% of root system galled (Zeck, 1971). Following the determination of gall rating, each root system was washed, blotted dry, and weighed. Roots were cut into approximately 2.5 cm pieces and thoroughly mixed. A 50 g subsample of roots was taken and used for egg extraction using 1% NaOCl (Hussey and Barker, 1973) as modified by Boneti and Ferraz (1981). Five cores of soil were taken from each microplot as previously described. The soil was mixed thoroughly and a 100 cm³ subsample was used for extracting nematodes by centrifugal-flotation (Jenkins, 1964). Nematodes were observed and counted using an inverted microscope. Fruit was not harvested due to the poor quality resulting from damage caused by excessive rainfall and winds from two hurricanes that hit the area in August and September, 2004.

A winter cover crop of common vetch (*Vicia sativa* L.) was seeded at 6 g/m² into each microplot on 23 November 2004 and harvested 25 March 2005. Before harvest, each plot was visually rated for plant top growth using a 0 to 10 scale, with 0 = 0, 1 = 10, 2 = 20, 3 = 30, 4 = 40, 5 = 50, 6 = 60, 7 = 70, 8 = 80, 9 = 90 and 10 = 100% of microplots covered with vetch. Plants were cut at ground level and shoot fresh weight was recorded as stated above. Shoots were dried for 1 week at 60°C and dry weight was recorded. Root systems were dug and

washed carefully, stained with food coloring (Thies *et al.*, 2002), and root-galling and egg mass indices (Taylor and Sasser, 1978) were determined. Soil samples were taken and the number of J2/100 cm³ of soil recorded as described above.

Tomato cv. Florida 47 seedlings were transplanted into microplots 22 April 2005, as explained above. On 27 June 2005 the plants were cut at ground level to determine fresh shoot weight, and roots were dug to determine percentage galling and egg masses, as stated above. The number of J2/100 cm³ of soil was also determined at harvest, and tomato yields were recorded.

Microplot Trial Two

A second set of microplots was installed 18 April 2005 and tomato cv. Florida 47 was transplanted into each microplot on 22 April 2005 (Table 1). Microplot site preparation, size and installation and experimental methodology were the same as those described for trial one. On 27 June 2005 tomato plants were dug, plant height, shoot fresh weight, fruit yield, gall and egg mass indices, eggs/g fresh root, and J2/100 cm³ of soil at harvest were determined as described above.

Statistical Analysis

Statistical analyses were performed using the linear model procedure of SAS (9.1 SAS Institute, Cary, NC). No significant interactions were observed between root-knot nematode species and inoculum levels in either trial, except for eggs/g fresh root and J2/100 cm³ of soil from trial one, therefore, the data were combined for statistical analyses. Data on root-galling, egg mass, eggs/g fresh root, and J2/100 cm³ of soil were transformed [$\log_{10}(x + 1)$] before analysis to normalize variance and only nontransformed means are

reported in tables. Means of root-galling, egg masses, eggs/g fresh root, J2/100 cm³ of soil, and tomato plant growth were separated by Waller-Duncan multiple range test at $P \leq 0.05$.

RESULTS

Microplots Trial One

There were no significant interactions between nematode species and inoculum levels for root-galling, plant height, and shoot fresh weight ($P \leq 0.05$) (Table 2), thus these data were combined. *Meloidogyne mayaguensis* produced a greater percentage of galls (97%) on cv. Solar Set than any other root-knot nematode species tested, whereas, *M. floridensis* produced the lowest (13%) ($P \leq 0.05$). The next highest percentage was *M. arenaria* at 79%. Root galling did not differ between *M. incognita* and *M. javanica* (Table 2). The galls induced by *M. mayaguensis* often formed a large coalesced gall mass on primary roots (Fig. 1A, B) and large bead-like galls on secondary roots (Fig. 1C).

Plant heights of tomato cv. Solar Set did not differ between the low and high levels of inoculum, but did differ among the nematode species (Table 2). Plants infected with *M. incognita* and *M. mayaguensis* were the shortest ($P \leq 0.05$) as compared to plants infected with the other root-knot nematode species and noninfected plants. Differences in shoot fresh weights were observed among all the root-knot nematode species ($P \leq 0.05$) (Table 2).

Interactions between nematode species and inoculum levels were observed for eggs/g fresh root and J2/100 cm³ of soil at harvest on cv. Solar Set ($P \leq 0.05$) (Table 3). *Meloidogyne arenaria* produced the highest number of eggs at both inoculum levels with *M. floridensis* and *M. javanica* producing the fewest ($P \leq 0.05$). *Meloidogyne*

Table 2. Effect of five *Meloidogyne* species on root-galling, plant height, and shoot fresh weight of tomato cv. Solar Set grown in microplot trial one fall 2004.

| Treatments | Root galling (%) ^a | Plant height (cm) | Shoot fresh weight (kg) |
|------------------------------|-------------------------------|-------------------|-------------------------|
| <i>M. arenaria</i> | 79 b ^{*,z} | 46 d ^c | 1.2 a ^c |
| <i>M. floridensis</i> | 13 d | 48 b | 0.9 b |
| <i>M. incognita</i> | 72 c | 44 f | 0.6 e |
| <i>M. javanica</i> | 72 c | 47 c | 0.9 b |
| <i>M. mayaguensis</i> | 97 a | 45 e | 0.7 d |
| Control | 0 e | 49 a | 0.8 c |
| | <i>P</i> -value | <i>P</i> -value | <i>P</i> -value |
| Treatments | <0.0001 | <0.0001 | <0.0001 |
| Inoculum levels | 3.202 | 0.061 | 3.231 |
| Treatments × inoculum levels | 3.201 | 0.056 | 2.311 |

^aGall rate: 0-10 scale; where 0 = no galling, 1 = 10%, ... 10 = 100% of root system galled (Zeck, 1971).

^bData were transformed with $[\log_{10}(x + 1)]$ before analysis and nontransformed data are presented in table.

^cMeans within columns with the same letter are not significantly different according to Waller Duncan's multiple-range test ($P \leq 0.05$).

floridensis, *M. incognita* and *M. javanica* produced more eggs at the high inoculum level than at the low level ($P \leq 0.05$) (Table 3), whereas, *M. mayaguensis* and *M. arenaria* produced similar number of eggs/g of root regardless of inoculum level.

The number of J2 recovered from each microplot was relatively low for each nematode species, however, there were greater numbers in plots infested with *M. arenaria* than those infested by other species of root-knot nematodes at both inoculum levels ($P \leq 0.05$) (Table 3). Nematode densities in the microplots infested with *M. floridensis*, *M. javanica* and *M. mayaguensis* at harvest were not influenced by inoculum level (Table 3).

There were no interactions between the nematode inoculum levels and nematode species for any measured parameters on common vetch in winter 2004 in trial one (Table 4). Common vetch was susceptible to all five species of root-knot nematodes and differences in plant responses were observed among the nematode spe-

cies. *Meloidogyne floridensis* continued to produce fewer galls and egg masses than the other four root-knot nematode species ($P > 0.05$). There were greater numbers of J2 of *M. incognita* and fewer numbers of J2 of *M. floridensis* and *M. javanica* at vetch harvest ($P < 0.05$). *Meloidogyne arenaria* had a greater impact on vetch plant growth than the other four nematode species (Table 4).

In the repeat trial on tomato following vetch in summer 2005, *M. mayaguensis* (94%), *M. incognita* (88%), *M. javanica* (87%), and *M. arenaria* (75%) induced a similar amount of root-galling and egg masses (Table 5), whereas *M. floridensis* induced the lowest amount of root-galling (56%) ($P < 0.05$). There were no differences in plant heights when grown in nematode-infested and noninfested microplots (Table 5). All nematodes reduced shoot fresh weight and fruit yield when compared to the nontreated control, but among the five nematode species, only *M. mayaguensis* infested plots produced a

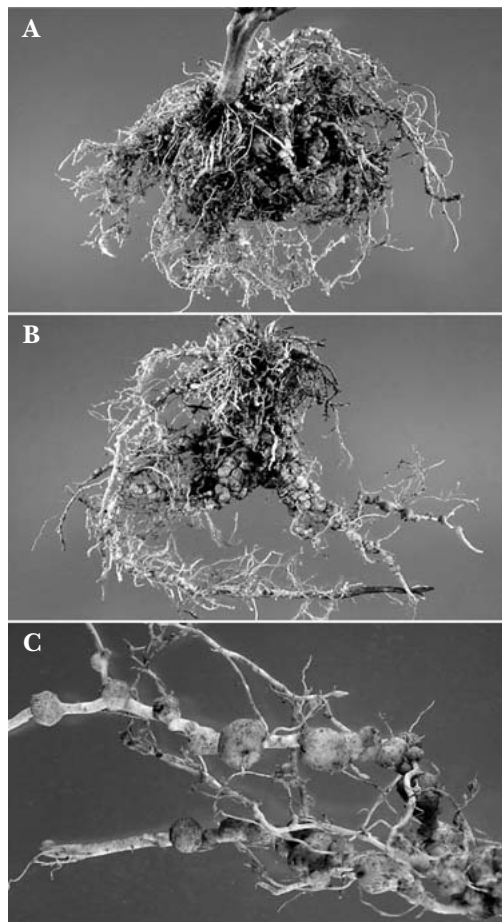


Fig. 1. Root systems of tomato cv. Solar Set infected with *Meloidogyne mayaguensis*. A) and B) root system showing large coalesced galls. C) A close up of secondary roots showing large bead-shaped galls.

lower fruit yield among the five nematode species ($P > 0.05$) (Table 5).

Microplot Trial Two

In microplot trial two during spring of 2005, there were no differences in root galling, egg masses, eggs or J2 among the five nematode species except that *M. floridensis* induced less root-galling ($P > 0.05$) (Table 6). All nematode species reduced plant heights and fruit yields compared with the

nontreated plants with *M. javanica* causing the greatest reduction in plant height among the nematode species ($P > 0.05$).

DISCUSSION

Meloidogyne mayaguensis induced more root-galling on tomato cv. Solar Set than any other root-knot nematode species evaluated, however, when the experiment was repeated with cv. Florida 47 there were no differences in number of galls produced among the five species, except for *M. floridensis*, which produced fewer galls than the other nematode species in both the trials. Visual observations revealed that the galls induced by *M. mayaguensis* were larger on tomato, regardless of cultivar, than those produced by all other nematode species tested; however, this galling effect was not observed on vetch. Also, root galls induced by *M. mayaguensis* frequently coalesced with each other. In Senegal, *M. mayaguensis* has been found to cause severe root-galling on tomato under field conditions and it is common in vegetable producing areas (Mateille *et al.*, 1995). Likewise, *M. mayaguensis* has been found to cause severe root-galling in vegetables in Venezuela (R. Crozzoli pers. comm.) and on root-knot nematode resistant bell pepper and tomato in Brazil (Carneiro *et al.*, 2006). Although *M. mayaguensis* has been reported to reproduce well on tomato cultivars carrying the *Mi-1* resistance gene, such as cv. Sanibel in the USA (Brito *et al.*, 2004a), cv. Rossol in Ivory Coast and Burkina Faso (Fargette *et al.*, 1996), cv. Guadajira in Cuba (Rodriguez *et al.*, 2003), cvs. Andrea and Débora (Carneiro *et al.*, 2006) and cv. Viradoro tomato in Brazil (Guimarães *et al.*, 2003), there is no information available on the effect of this nematode on pathogenicity and reproductive potential relative to other major root-knot nematode species. In addition to tomato,

Table 3. Effect of two inoculum levels on reproduction of five *Meloidogyne* species in microplot trial one, planted with tomato cv. Solar Set in fall 2004.

| Parameter | Inoculum ^y | <i>M. arenaria</i> | <i>M. floridensis</i> | <i>M. incognita</i> | <i>M. javanica</i> | <i>M. mayaguensis</i> |
|--------------------------------|-----------------------|--------------------|-----------------------|---------------------|--------------------|-----------------------|
| Eggs/g fresh root ^z | Low | 2430 aA | 51 bC | 747 bBC | 374 bC | 1500 aB |
| | High | 3187 aA | 234 aC | 1530 aB | 795 aBC | 1702 aB |
| J2/100 cm ³ soil | Low | 126 bA | 4 aC | 56 bB | 37 aB | 32 aB |
| | High | 936 aA | 5 aD | 202 aB | 25 aD | 79 aC |

^yInoculum levels: low (1 egg or J2/100 cm³ of soil) and high (3 eggs or J2/100 cm³ of soil).

^zInteractions between nematode species and inoculum levels were observed for eggs/g fresh root and J2/100 cm³ of soil at harvest ($P < 0.0001$). Data are means of nine replications. Means within each column (lower case) and within each row (upper case) with same letter are not significantly different according to Waller Duncan's multiple-range test ($P \leq 0.05$).

M. mayaguensis had also been reported infecting lines and cultivars of common bean (*Phaseolus vulgaris*), cowpea (*Vigna unguiculata*), mung bean (*P. aureus*), lima bean (*P.*

lunatus), and jack bean (*Canavalia ensiformis*) from Venezuela (Crozzoli *et al.*, 2006) and several vegetables from six botanical families in Cuba (Rodriguez *et al.*, 2003).

Table 4. Effect of *Meloidogyne* species on disease incidence, second-stage juveniles/100 cm³ of soil, and plant growth parameters on common vetch (*Vicia sativa*) following tomato cv. Solar Set in microplot trial one, winter 2004.

| Treatments | Gall index ^w | Egg mass index ^w | J2/100 cm ³ soil | Plant top growth rating ^x | Shoot fresh weight (g) | Shoot dry weight (g) |
|------------------------------|-------------------------|-----------------------------|-----------------------------|--------------------------------------|------------------------|----------------------|
| <i>M. arenaria</i> | 4.9 a ^{yz} | 4.8 a ^{yz} | 163 b ^{yz} | 3.1 e ^z | 320 c ^z | 71 e ^z |
| <i>M. floridensis</i> | 3.2 b | 2.7 c | 18 c | 7.2 b | 566 a | 117 ab |
| <i>M. incognita</i> | 4.8 a | 4.6 ab | 245 a | 4.9 d | 408 b | 90 d |
| <i>M. javanica</i> | 4.7 a | 4.2 b | 24 c | 5.8 cd | 430 b | 97 cd |
| <i>M. mayaguensis</i> | 4.4 a | 4.8 a | 165 b | 6.1 c | 482 b | 105 bc |
| Control | 0.0 c | 0.0 d | 0 c | 8.7 a | 585 a | 125 a |
| | <i>P</i> value | <i>P</i> value | <i>P</i> value | <i>P</i> value | <i>P</i> value | <i>P</i> value |
| Treatments | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.0001 | <0.0001 |
| Inoculum levels | 0.125 | 0.608 | 0.093 | 0.521 | 0.569 | 0.360 |
| Treatments × inoculum levels | 0.940 | 0.951 | 0.230 | 0.320 | 0.698 | 0.276 |

^wGall and egg mass indices: 0-5 scale; where 0 = no galls or egg masses, 1 = 1-2 galls or egg masses, 2 = 3-10 galls or egg masses, 3 = 11-30 galls or egg masses; 4 = 31-100 galls or egg masses, and 5 = >100 galls or egg mass per root system (Taylor and Sasser, 1978).

^xPlant-top growth rating: 0-10 scale; where 0 = no plant growth and 10 = 100% of microplot covered, maximum plant growth.

^yData were transformed [$\log_{10}(x + 1)$] before analysis and nontransformed data are shown in table.

^zMeans within column with same letter are not significantly different according to Waller Duncan's multiple-range test ($P \leq 0.05$).

Table 5. Effect of five *Meloidogyne* species on root galling, egg masses, eggs/g fresh root, second-stage juveniles, and growth parameters of tomato cv. Florida 47 grown in microplot trial one following winter cover crop of common vetch, summer 2005.

| Treatments | Root galling (%) ^w | Egg mass index ^x | Eggs/g fresh root | J2/100 cm ³ soil | Plant height (cm) | Shoot fresh weight (g) | Fruit yield (kg/microplot) |
|------------------------------|-------------------------------|-----------------------------|----------------------|-----------------------------|-------------------|------------------------|----------------------------|
| <i>M. arenaria</i> | 75 a ^{v,z} | 4.9 a ^{v,z} | 511 a ^{v,z} | 251 a ^{v,z} | 45 a ^z | 479 b ^r | 1.5 b ^r |
| <i>M. floridensis</i> | 56 b | 4.6 b | 480 a | 298 a | 53 a | 382 c | 1.5 b |
| <i>M. incognita</i> | 88 a | 4.8 ab | 491 a | 319 a | 49 a | 449 bc | 1.4 b |
| <i>M. javanica</i> | 87 a | 4.7 ab | 424 a | 229 a | 44 a | 463 bc | 1.4 b |
| <i>M. mayaguensis</i> | 94 a | 4.7 ab | 450 a | 274 a | 45 a | 485 b | 0.9 c |
| Control | 0 c | 0.0 c | 0 b | 0 b | 48 a | 765 a | 2.6 a |
| | <i>P</i> -value | <i>P</i> -value | <i>P</i> -value | <i>P</i> -value | <i>P</i> -value | <i>P</i> -value | <i>P</i> -value |
| Treatments | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| Inoculum levels | 0.570 | 1.000 | 0.663 | 0.174 | 0.654 | 0.595 | 0.336 |
| Treatments × inoculum levels | 0.582 | 0.177 | 0.995 | 0.631 | 0.865 | 0.073 | 0.535 |

^wGall rate: 0-10 scale, where 0 = no galling, 1 = 10%, ... 10 = 100% of root system galled (Zeck, 1971).

^xEgg mass index : 0-5 scale; where 0 = egg mass, 1 = 1-2 egg masses, 2 = 3-10 egg masses, 3 = 11-30 egg masses, 4 = 31-100 egg masses, 5 = >100 egg masses (Taylor and Sasser, 1978).

^yData were transformed [$\log_{10}(x + 1)$] before analysis and nontransformed data are shown in table.

^zMeans within column with same letter are not significantly different according to Waller Duncan's multiple-range test ($P \leq 0.05$).

Some variability was observed among the nematode species on the two tomato cultivars used in this study. The reproductive capability on cv. Solar Set varied among the root-knot nematode species evaluated, however on cv. Florida 47, these nematodes reproduced similarly. Plants of cv. Solar Set did not develop well in trial one, which may have been due to the hurricanes that hit Florida in fall 2004, leading to poor fruit set. On the other hand, plant height and fruit yield obtained from cv. Florida 47 in summer 2005 from trial one following vetch, and trial two were more consistent with each other. The control plots consistently produced the highest yield of tomato regardless of the trial.

Results of this study indicate that *M. mayaguensis* and the other common

root-knot nematode species were pathogenic to tomato cv. Florida 47 and cv. Solar Set, whereas *M. floridensis* was only pathogenic on tomato cv. Florida 47. Two explanations might be due to cultivar differences or low inoculum viability used to infest the soil in trial one. Low inoculum viability was ruled out as a possible explanation because the same *M. floridensis* inoculum used in the microplot experiment was also used to inoculate four plants of tomato cv. Solar Set growing in clay pots (15-cm-diam.) as a test to confirm viability under greenhouse conditions. All tomato root systems were heavily galled, thus substantiating good inoculum viability and root infection at harvest. It is possible that the size of the pots helped to concentrate the inoculum around the tomato root-sys-

Table 6. Effect of five *Meloidogyne* species on disease incidence, second-stage juveniles (J2)/100 cm³ of soil, and growth parameters of tomato cv. Florida 47 grown in microplot trial two, summer 2005.

| Treatments | Root galling (%) ^w | Egg mass index ^x | Eggs/g fresh root | J2/100 cm ³ soil | Plant height (cm) | Shoot fresh weight (g) | Fruit yield (kg/microplot) |
|-----------------------------|-------------------------------|-----------------------------|---------------------|-----------------------------|-------------------|------------------------|----------------------------|
| <i>M. arenaria</i> | 82 a ^{y,z} | 4.8 a ^{yz} | 520 a ^{yz} | 351 a ^{yz} | 49 b ^r | 525 b ^r | 1.5 b ^r |
| <i>M. floridensis</i> | 39 b | 4.7 a | 528 a | 320 a | 50 b | 465 b | 1.3 bc |
| <i>M. incognita</i> | 88 a | 4.7 a | 531 a | 398 a | 49 b | 469 b | 1.1 c |
| <i>M. javanica</i> | 88 a | 4.7 a | 525 a | 254 a | 44 c | 423 b | 1.2 c |
| <i>M. mayaguensis</i> | 94 a | 4.8 a | 416 a | 357 a | 48 b | 463 b | 1.4 bc |
| Control | 0 c | 0.0 b | 0 b | 0 b | 56 a | 839 a | 2.7 a |
| | <i>P</i> -value | <i>P</i> -value | <i>P</i> -value | <i>P</i> -value | <i>P</i> -value | <i>P</i> -value | <i>P</i> -value |
| Treatments | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| Inoculum levels | 0.881 | 0.853 | 0.743 | 0.189 | 0.086 | 0.715 | 0.074 |
| Treatments × inoculum level | 0.557 | 0.627 | 0.581 | 0.691 | 0.238 | 0.287 | 0.241 |

^wGall rate: 0-10 scale, where 0 = no galling, 1 = 10, ... 10 = 100% root system galled (Zeck, 1971).

^xEgg mass index: 0-5 scale; where 0 = no egg mass, 1 = 1-2 egg masses, 2 = 3-10 egg masses, 3 = 11-30 egg masses; 4 = 31-100 egg masses, and 5 = >100 egg masses per root system (Taylor and Sasser, 1978).

^yData were transformed [$\log_{10}(x + 1)$] before analysis and nontransformed data are shown in table.

^zMeans within column with same letter are not significantly different according to Waller Duncan's multiple-range test ($P \leq 0.05$).

tems and produced higher infection rates than that occurred in microplots, or it might be due to the differences in environmental conditions between the microplots and test pots.

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LITERATURE CITED

- Anonymous. 2006. The National Agricultural Statistics Service. Released 9 January 2006, Agricultural Statistics Board, U.S. Department of Agriculture. Accessed on 5 February 2006 at <http://usda.mannlib.cornell.edu/reports/nassr/fruit/pvg-bb/2006/vege0116.pdf>.
- Bonetí, J. I. S., and S. Ferraz. 1981. Modificação do método de Hussey & Barker para extração de ovos de *Meloidogyne exigua* de raízes de cafeeiro. Fitopatologia Brasileira 6:553.
- Brito, J. A., T. O. Powers, P. G. Mullin, R. N. Inserra, and D. W. Dickson. 2003. Morphological and molecular characterization of *Meloidogyne mayaguensis* from Florida. Journal of Nematology 35:327-328.
- Brito, J. A., J. D. Stanley, R. Cetintas, M. Di Vito, J. A. Thies, and D. W. Dickson. 2004a. Reproduction of *Meloidogyne mayaguensis* from Florida on root-knot nematode resistant tomato and pepper. Journal of Nematology 36:308.
- Brito, J. A., J. D. Stanley, R. Cetintas, T. O. Powers, R. N. Inserra, E. J. McAvoy, M. L. Mendes, W. T. Crow, and D. W. Dickson. 2004b. Identification and host preference of *Meloidogyne mayaguensis*, and other root-knot nematodes from Florida, and their susceptibility to *Pasteuria penetrans*. Journal of Nematology 36:308-309.
- Brito, J. A., J. D. Stanley, R. Cetintas, J. Hamill, and D. W. Dickson. 2005. A new root-knot nematode infecting vegetables in Florida. Journal of Nematology 37:359.
- Carneiro, R. M. D., M. R. A. Almeida, R. S. Braga, C. A. Almeida, and R. Gioria. 2006. Primeiro registro de *Meloidogyne mayaguensis* parasitando

- plantas de tomate e pimentaõ resistentes à meloidoginose no estado d e São Paulo. *Nematologia Brasileira* 30:81-86.
- Church G. T. 2005. First report of the root-knot nematode *Meloidogyne floridensis* on tomato (*Lycopersicon esculentum*) in Florida. *Plant Disease* 89:527.
- Crozzoli, R., G. Perichi, D. Perez, and M. Espinoza. 2006. Response of some legumes to Venezuelan population of the root-knot nematode *Meloidogyne mayaguensis*. *Nematropica* 36:120.
- Esbenshade, P. R. and A. C. Triantaphyllou. 1985. Electrophoretic methods for the study of root-knot nematode enzymes. Pp. 115-123 in K. R. Barker, C. C. Carter, and J. N. Sasser, eds. An advanced treatise on *Meloidogyne*. North Carolina State University Graphics, Raleigh.
- Fargette, M., M. S. Phillips, V. C. Blok, R. Waugh, and D. L. Trudgill. 1996. A RFLP study of relationships between species, isolates and resistance-breaking lines of tropical species of *Meloidogyne*. *Fundamental and Applied Nematology* 19:193-200.
- Guimarães, L. M. P., R. M. Moura, and E. M. R. Pedrosa. 2003. Parasitismo de *Meloidogyne mayaguensis* em diferentes espécies botânicas. *Nematologia Brasileira* 27:139-145.
- Handoo, Z. A., A. P. Nyczepir, D. Esmenjaud, J. G. van der Beek, P. Castagnone-Sereno, L. K. Carta, A. M. Skantar, and J. A. Higgins. 2004. Morphological, molecular and differential-host characterization of *Meloidogyne floridensis* n. sp. (Nematoda: Meloidogynidae), a root-knot nematode parasitizing peach in Florida. *Journal of Nematology* 36:20-35.
- Hussey, R. S., and K. R. Barker. 1973. A comparison of methods on collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Disease Reporter* 57:1025-1028.
- Jenkins, W. R. 1964. A rapid centrifugal flotation technique for separating nematodes from soil. *Plant Disease Reporter* 48:62.
- Mateille, T., M. T. Diop, R. Duponnois, and J. Thioulouse. 1995. Influence of environmental factors on the distribution of nematode populations parasitizing vegetables in Senegal. *Nematologica* 41:320.
- Olson S. M., D. Maynard, G. Hochmuth, C. Vavrina, W. Stall, T. Kucharek, S. Webb, T. Taylor, S. Smith, and E. Simonne. 2004. Tomato production in Florida. Pp. 301-316 in *Vegetable production Guide for Florida*. University of Florida IFAS Extension, Gainesville.
- Ornat, C., and S. Verdejo-Lucas. 1999. Distribución y densidad de población de *Meloidogyne* spp. en cultivos hortícolas de la comarca de El Maresme (Barcelona). *Investigación agraria. Producción y Protección Vegetales* 14:191-201.
- Rodríguez, M. G., L. Sánchez, and J. Rowe. 2003. Host status of agriculturally important plant families of the root-knot nematode *Meloidogyne mayaguensis*. *Nematropica* 33:125-130.
- Sorribas, F. J., and S. Verdejo-Lucas. 1994. Survey of *Meloidogyne* spp. in tomato production fields of Baix Llobregat County, Spain. *Journal of Nematology* 26:731-736.
- Taylor, A. L., and J. N. Sasser. 1978. Biology, identification and control of root-knot nematodes (*Meloidogyne* species). North Carolina State University Graphics, Raleigh.
- Thies J. A., S. B. Merrill, and E. L. Corley. 2002. Red food coloring stain: New, safer procedures for staining nematodes in roots and egg masses on root surfaces. *Journal of Nematology* 34:179-181.
- Zeck W. M. 1971. A rating scheme for field evaluation of root knot nematode infestations. *Pflanzenschutz-Nachrichten* 24:141-144.

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