BIOLOGICAL EVALUATION AND COMPARISON OF FOUR FLORIDA ISOLATES OF *MELOIDOGYNE FLORIDENSIS*

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ABSTRACT

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A study was conducted to characterize the morphology, enzymatic profile, and host preference of four isolates of the peach root-knot nematode, *Meloidogyne floridensis*. No morphological or biochemical differences were observed among the four isolates. Each isolate showed some mean variability in morphometrics values, but overlapped in their range values ($P > 0.05$). In total, 1,027 females extracted from peach, pepper, tobacco, and tomato did not differ in their isozyme phenotype for esterase and malate dehydrogenase, and matched those reported in the original description. In host differential tests all four isolates of *M. floridensis* exhibited the same reaction as that of *M. incognita* race 2, with pepper, tobacco, tomato and watermelon being susceptible, and cotton and peanut, resistant. In comparative host status studies, both root-knot nematode resistant and susceptible peach cultivars were susceptible to all four isolates. Both resistant and susceptible cultivars of corn, pepper, soybean, and tomato were evaluated. All four isolates of *M. floridensis* reproduced poorly, but were able to overcome the resistance of the *Mi-1 gene* in tomato cv. Crista. Two isolates reproduced poorly, but were not affected by the *N* gene resistance in the pepper cv. Charleston Belle, whereas two isolates reproduced well on this cultivar. Both the root-knot nematode resistant corn cv. Mp 710 and susceptible cv. Dixie 18 were susceptible to all four isolates; whereas, both the resistant soybean cv. Forrest and susceptible cv. S64-J1 were immune to all four isolates of *M. floridensis*.

Key words: host suitability, intraspecific variability, enzymatic profile, *Meloidogyne floridensis*, morphometrics, root-knot nematode.

RESUMEN

Stanley, J. D., J. A. Brito, N. Kokalis-Burelle, J. H. Frank, and D. W. Dickson. 2009. Evaluación biológica y comparación de cuatro aislamientos de *Meloidogyne floridensis* de Florida. Nematropica 39:255-271.

Se condujo un estudio para caracterizar la morfología, el perfil enzimático y las preferencias de hospedantes de cuatro aislamientos del nematodo agallador del duraznero, *Meloidogyne floridensis*. No se observaron diferencias morfológicas o bioquímicas entre los aislamientos. Para cada aislamiento se observó una variación media en los valores morfométricos, pero con rangos superpuestos ($P > 0.05$). No se observaron diferencias en el perfil enzimático para esterasas y malato deshidrogenasas en un total de 1,027 hembras extraídas de duraznero, pimiento, tabaco y tomate, y se observó concordancia con los perfiles publicados en la descripción original. En las pruebas de hospedantes diferenciales, los cuatro aislamientos de *M. floridensis* se comportaron como *M. incognita* raza 2, con pimiento, tabaco, tomate y sandía como susceptibles, y algodón y maní como resistentes. En estudios comparativos, se encontró que variedades de duraznero tanto resistentes como susceptibles a nematodos agalladores se comportaron como susceptibles a los cuatro aislamientos. Se evaluaron variedades resistentes y susceptibles de maíz, pimiento, soya y tomate. Se obtuvo baja reproducción de los cuatro aislamientos de *M. floridensis* en tomate cv. Crista, y se venció la resistencia del gen *Mi-1 gene*. Con dos aislamien-

tos se observó baja reproducción en pimiento cv. Charleston Belle y vencimiento del gen de resistencia *N*, mientras que con los otros dos se observó alta reproducción. Ambos cultivares de maíz, el resistente Mp 710 y el susceptible Dixie 18, fueron susceptibles a los cuatro aislamientos. Las variedades de soya resistente cv. Forrest y susceptible cv. S64-J1 fueron inmunes a los cuatro aislamientos de *M. floridensis*.

Palabras clave: *Meloidogyne floridensis*, morfometría, nematodo agallador, perfil enzimático, susceptibilidad, variabildad intraespecífica.

INTRODUCTION

Meloidogyne floridensis Handoo *et al.*, 2004, also known as the peach root-knot nematode, is a recently described species of root-knot nematode first detected by R. H. Sharpe in 1966 in Gainesville, Florida, USA (Sharpe *et al.*, 1969; Handoo *et al.*, 2004) parasitizing the peach (*Prunus persica* (L.) Batsch) rootstock cvs. Nemaguard and Okinawa. This nematode has also been reported parasitizing Flordaguard, Guardian, and Nemared peach rootstocks, all of which are resistant to (*M. incognita* (Kofoid and White, 1919) Chitwood, 1949) and *M. javanica* (Treub, 1885) Chitwood, 1949) (Sharpe *et al.*, 1969; Sherman *et al.*, 1991; Nyczepir *et al.*, 1998; Brito *et al.*, 2008). *M. floridensis* was mistakenly identified as *M. incognita race* 3 based on host differential tests (Sherman and Lyrene, 1983). Further investigation of this nematode suggested that this was in fact a new *Meloidogyne* species based on host range, morphology, and biochemical and molecular characterization (Nyczepir *et al.*, 1998; Handoo *et al.*, 2004). Eight populations of *M. floridensis* have been identified in six Florida counties, including the population from Gainesville, Fla. recovered from peach and used for the species description (Handoo *et al.*, 2004; Brito *et al.*, 2005; Church, 2005; Brito *et al.*, 2008). While *M. floridensis* was initially thought to be primarily a pathogen of peach, other vegetable crops of economic importance have been reported as hosts including: cucumber (*Cucumis sativus* L.), eggplant (*Solanum melongena* L.), tomato (*Solanum lycopersion* L.), snap bean (*Phaseolus* sp.) and squash (*Cucurbita moschata* Duchesne ex Poir.) (Kokalis-Burelle and Nyczepir, 2004; Brito *et al.*, 2005; Church, 2005). Morphologically *M. floridensis* resembles *M. incognita, M. christiei* Golden and Kaplan, 1986, *M. graminicola* Golden and Birchfield, 1965, and *M. hispanica* Hirschmann, 1986; but in light microscope and SEM observations, it differs from these species either by body length, shape of the head, tail and tail terminus of the J2, body length and shape of spicules in males, and distinctive perineal pattern (Handoo *et al.*, 2004). The unique esterase profile of *M. floridensis,* which is different from all other known root-knot species, has been designated as MF3 (Rm 38.7, 40.69, 44.18) (Carneiro, *et al.*, 2000; Brito *et al.*, 2008). The malate dehydrogenase phenotype has been designated as N1 for *M. floridensis* and is identical to *M. arenaria* (Neal, 1889) Chitwood 1949*, M. incognita*, and *M. javanica* (Esbenshade and Triantaphyllou, 1985). Molecular techniques to unambiguously separate *M. floridensis* from other root-knot nematode species based on IGS rDNA sequence, RAPD-PCR analyses, and high-fidelity PCR-RFLP analyses have been reported (Handoo *et al.*, 2004; Jeyaprakash *et al.*, 2006).

The ability of *M. floridensis* to break resistance in some peach rootstocks makes it an important pathogen which requires

further study. This resistance breaking capability led to the recognition of this previously undescribed species, and caused valid concerns regarding its ability to break resistance in other crops. The impact of *M. floridensis* may increase as growers rely more on the use of root-knot nematode resistance in high-value crops, since fumigants and nematicides are becoming more difficult to utilize.

The objectives of this study were to obtain additional information on this recently described species of root-knot nematode by comparing four isolates of *M. floridensis* from Florida using morphological characters, biochemical analysis, and host-status tests on select root-knot nematode resistant and susceptible plant cultivars.

MATERIALS AND METHODS

Nematode origin

Three of the four isolates of *M. floridensis* used for this research were collected as part of a cooperative root-knot nematode survey conducted throughout the state of Florida by the Florida Department of Agriculture and Consumer Services (FDACS), Division of Plant Industry (DPI), Nematology Section, and the University of Florida Entomology and Nematology Department (Brito *et al.*, 2008). The designation and origin of the four nematode isolates used for this study are as follows: isolate 1 (N03-01894) was obtained from the population used in the species description (Handoo *et al.*, 2004) which was originally found infecting the peach rootstock cv. Nemaguard in Alachua County. Isolate 2 (N03-01582) was collected from tomato in Indian River County; isolate 3 (N04-00503) was collected from tomato in Hendry County; and isolate 4 (N04-00627) was collected from cucumber in a separate field in Hendry County. The numbers assigned to the four *M. floridensis* isolates are DPI, Nematology Section log numbers. The nematode species was identified by morphometrics, perineal patterns, and esterase phenotype. A single egg mass isolate was obtained from each field population and reared on tomato cv. Rutgers in a greenhouse. Eggs were extracted from the root systems using 0.5% NaOCl (Hussey and Barker, 1973; Boneti and Ferraz, 1981).

Comparative morphometrics

Morphometric characters of second stage juveniles (J2), males, and females were examined from each of the four isolates of *M. floridensis* using live specimens narcotized with low heat and mounted in water agar (Esser, 1986). The morphometrics of 20 J2, males, and females were compared to determine inter and intra-isolate variability of these characters. Second-stage juveniles and males were collected from roots placed in a Petri dish with a small amount of water and incubated at room temperature. Females were dissected directly from infected root systems and cut transversely before mounting in water agar. Characters were measured using a compound microscope (Nikon optiphot).

Biochemical analysis

A representative number of females from each *M. floridensis* isolate were subjected to polyacrylamide gel electrophoresis (PAGE) to determine and compare the esterase and malate dehydrogenase phenotypes of each isolate, as well as other *Meloidogyne* species. A replication of individual females was used to represent each *M. floridensis* isolate. Electrophoresis was carried out using a Mini-protean III (BioRad, Hercules, CA) (Brito *et al.*, 2004). At least 247 females representing each isolate were collected from various hosts and analyzed.

Hosts were the root-knot nematode resistant tobacco (*Nicotiana tabacum* L. cv. NC 95), tomato cv. Rutgers), peach cvs. Lovell and Nemaguard), and pepper (*Capsicum annuum* L. cv. California Wonder).

Host differentials

Differential host studies were carried out using cotton (*Gossypium hirsutum* L.cv. Deltapine 16), peanut (*Arachis hypogaea* L. cv. Florunner), pepper cv. California Wonder), tomato cv. Rutgers), watermelon (*Citrullus lanatus* (Thunb.) Matsum. And Nakai cv. Charleston Gray), and the root-knot nematode resistant tobacco cv. NC 95), which is used to differentiate various host races of *M. incognita*. (Taylor and Sasser, 1978). This experiment consisted of five replicates. All plants were grown from seed and germinated in vermiculite in plastic trays. Tobacco seeds were planted approximately 30 days before pepper and the remaining plants 14 days after pepper. This delay allowed for variable germination and growth rates of the various test plants. After all test plants reached a height of between 10 and 15-cm, they were transplanted to 25-cm diam. clay pots containing pasteurized soil (89% sand, 3% silt, 5% clay; pH 6.1, 1.1% organic matter) and allowed to grow for 2 weeks in a greenhouse. The day before inoculation, eggs were extracted as mentioned above and quantified. Plants were set up in a completely randomized design and inoculated with 5,000 eggs/J2 per plant and maintained in a growth room for 60 days with an average temperature of 24°C and a photoperiod of 12 hours. Plants were watered daily and fertilized as needed with a 20-20-20 NPK fertilizer (Peters Professional, Division of United Industries., St. Louis, MO). Insecticides and fungicides were used as needed. After 60 days, plants were removed from pots and root systems washed carefully. Root galling and egg mass indices were determined according to an index scale of 0-5, where 0 = no galls and egg masses (immune); 1 = 1-2 galls and egg masses (resistant); 2 = 3-10 galls and egg masses (resistant); 3 = 11-30 galls and egg masses (susceptible); 4 = 31-100 galls and egg masses (susceptible); and 5 = >100 galls and egg masses per root system (susceptible) (Taylor and Sasser 1978).

Vegetable and agronomic crop test

The reproduction of four isolates of *M. floridensis* on root-knot nematode resistant and susceptible corn (*Zea mays* L.), pepper, soybean (*Glycine max* (L.) Merr.), and tomato (Table 1.) was compared. This

Table 1. Plant cultivars used for host status studies of four isolates of *Meloidogyne floridensis*.

Host	Susceptible cultivar	Resistant cultivar	Source of resistance	*Meloidogyne* species[z]
Tomato	Talladega	Crista	*Mi*-1 gene	Mi, Mj, Ma
Pepper	Keystone Resistant Giant	Charleston Belle	*N* gene	Mi, Mj, Ma
Corn	Dixie 18	Mp 710	unidentified	Mi, Mj
Soybean	S64-J1	Forrest	*Mir*1 gene	Mi
Peach	Lovell	Nemaguard	unidentified	Mi, Mj

[z] *Meloidogyne* species that select cultivars are resistant to: Mi = *Meloidogyne incognita*, Mj = *Meloidogyne javanica*, Ma = *Meloidogyne arenaria*.

experiment consisted of five replicates. All plants were grown from seed sown in vermiculite and germinated in plastic trays. Seedlings were transplanted to 25-cm diam. clay pots containing pasteurized soil (89% sand, 3% silt, 5% clay; pH 6.1, 1.1% organic matter) approximately two months after seeds were planted. The plants were allowed to grow for two weeks in a greenhouse prior to inoculation to ensure development of a healthy root system. Each plant was inoculated with 5,000 eggs/J2. The experiment was duplicated by running two tests concurrently in separate growth rooms for 60 days with a 12 hour photoperiod and an average temperature of 23°C to ensure that high soil temperatures would not have an effect on resistance (Dropkin, 1969; Ammati *et al.*, 1986; Thies and Fery, 1998). Plants were watered daily and fertilized as needed with a 20-20-20 NPK fertilizer (Peters Professional). After 60 days the plants were removed from the pots and their root systems were thoroughly washed. Root galling and egg mass indices were determined on a 0-5 scale (Taylor and Sasser, 1978). Eggs were extracted (Hussey and Barker, 1973; Boneti and Ferraz, 1981) to determine the reproductive factor (Rf = Pf/Pi) in which Pf = total egg recovery per root system (final population) and Pi = initial inoculum level (initial population) (Oostenbrink, 1966; Sasser *et al.*, 1984). Plants with a Rf ≥ 1 were considered susceptible hosts, 1> Rf > 0.1 were resistant hosts, and a Rf ≤ 0.1 were nonhosts.

Data were subjected to ANOVA using SAS 9.1 (SAS Institute, Cary NC), and means were compared based on Duncan's multiple-range test at $P \leq 0.05$. No interactions were detected between gall, egg mass indices, and reproductive factor based on homogenicity of variance test. The data from both tests were combined.

Prunus test

The reproductive capability of four isolates of *M. floridensis* was compared on the peach rootstocks Nemaguard and Lovell in a greenhouse. The rootstock, Nemaguard, is resistant to *M. incognita* and *M. javanica* (Sharp *et al.*, 1969), whereas Lovell is susceptible (Table 1.). The seeds were soaked in water and stratified. After 2 to 4 months the seedlings were large enough for transplanting into 25-cm diam. clay pots filled with pasteurized soil (89% sand, 3% silt, 5% clay; pH 6.1, 1.1% organic matter) After 1 month they were inoculated with 5,000 eggs/J2 per plant. This experiment consisted of five replicates. Plants were maintained in a greenhouse in a completely randomized design for 115 days with an average temperature of 28°C. This experiment was first conducted in the spring-summer of 2006 and then repeated in the spring-summer of 2007. Gall index, egg mass index and reproductive factor were determined by the same criteria as the previous experiment.

Data were subjected to ANOVA using SAS 9.1 (SAS Institute, Cary NC), and means were compared based on Duncan's multiple-range test at $P \leq 0.05$. No interactions were detected between gall, eggmass indices, and reproductive factor based on homogenicity of variance test. The data from both tests were combined.

RESULTS AND DISCUSSION

Comparative morphometrics

Morphometrics of J2, males, and females of four isolates of *M. floridensis* are reported in Table 2. All four isolates were morphologically similar to each other as well as the original description (Handoo *et al.*, 2004). Some allometric and non-allometric characters differed significantly ($P > 0.05$) among the isolates. However, their

Table 2. Select morphometric characters (mean, standard deviation, and range) of second-stage juveniles, males and females of four isolates of *Meloidogyne floridensis* from Florida[x].

Character	Nematode isolates[y]			
	1	2	3	4
	Second stage juveniles (J2)			
Body length	384.0 ± 14.9 a[z] (348-482)	371.7 ± 14.8 b (338-393)	387.0 ± 18.2 a (352-417)	370 ± 15.0 b (335-392)
Body width	14.9 ± 0.4 a (14.0-16.0)	14.7 ± 0.43 b (13.7-15.6)	14.5 ± 0.5 b (13.7-14.8)	14.5 ± 0.4 b (13.2-15.2)
Stylet length	10.9 ± 0.1 b (10.0-10.5)	10.1 ± 0.4 b (9.8-11.3)	10.2 ± 0.3 b (9.8-10.8)	10.7 ± 0.3 a (10.2-11.4)
DGO, from stylet base	2.9 ± 2.0 b (2.5-3.0)	3.2 ± 0.3 a (2.9-3.9)	3.3 ± 0.3 a (2.9-3.9)	3.2 ± 0.4 a (2.6-3.9)
Center median bulb to anterior end	52.0 ± 2.1 b (48.0-55.0)	54.5 ± 2.4 a (50.0-59.0)	55.1 ± 2.9 a (48.2-61.2)	55.7 ± 2.2 a (51.4-59.7)
Excretory pore to anterior end	80.9 ± 3.8 b (74.5-86.0)	79.5 ± 2.5 b (74.0-85.0)	83.7 ± 3.8 a (76.4-89.1)	82.1 ± 3.4 b (75.4-92.2)
Tail length	41.1 ± 2.8 a (34.0-45.0)	44.0 ± 2.3 a (39.0-48.0)	43.3 ± 3.1 a (38.2-48.0)	43.4 ± 2.4 a (38.2-48.0)
Base of esophageal gland to anterior end	118.0 ± 9.8 a (96.0-139.0)	113.0 ± 6.7 a (103-129)	116.0 ± 8.9 a (102-132)	118 ± 7.3 a (103-131)
Hyaline tail terminus length	10.1 ± 1.1 ab (8.5-12.0)	8.6 ± 1.1 c (5.9-9.8)	8.5 ± 1.4 bc (5.8-10.7)	10.8 ± 0.9 a (8.8-11.8)
a	26.0 ± 1.2 a (23.0-28.0)	25.0 ± 0.8 a (24.0-26.0)	26.7 ± 1.2 a (24.5-29.0)	26.0 ± 1.2 a (23.0-28.0)
b	3.8 ± 0.2 a (3.5-4.1)	4.8 ± 0.2 ab (4.4-5.2)	4.9 ± 0.5 ab (4.2-5.6)	4.8 ± 0.2 b (4.3-5.0)
b'	3.3 ± 0.3 ab (2.7-4.0)	3.3 ± 0.2 ab (2.8-3.7)	3.4 ± 0.3 a (3.0-4.0)	3.1 ± 0.2 b (2.6-3.6)
c	9.3 ± 0.6 a (8.1-11.2)	8.5 ± 0.4 c (8.0-9.3)	8.9 ± 0.6 b (7.7-10.2)	8.5 ± 0.33 c (7.8-9.1)

[x]Measurements (µm) were taken using 20 specimens from each isolate.

[y]Origin of *M. floridensis* isolates are as follows: 1-(N03-01894) was obtained from the population used in the species description (Handoo *et al.*, 2004), 2 was collected from tomato in Indian River County, Fla, 3 was collected from tomato in Hendry County, Fla; and isolate 4 was collected from cucumber in a separate field in Hendry County.

[z]Means in same row followed by the same letter are not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

Table 2. (Continued) Select morphometric characters (mean, standard deviation, and range) of second-stage juveniles, males and females of four isolates of *Meloidogyne floridensis* from Florida[x].

Character	Nematode isolates[y]			
	1	2	3	4
	Males			
Body length	1,514 ± 326 a (793-2,038)	1,477.8 ± 255.6 a (993-1,875)	1,547 ± 206.5 a (1,072-1,867)	1,203 ± 297.6 b (838-1,847)
Body width	33.2 ± 3.9 b (23.5-41.2	34.2 ± 3.5 a (28.4-41.0)	35.7 ± 2.7 a (28.4-39.2)	32.9 ± 2.9 b (27.4-39.2)
Stylet length	21.2 ± 1.7 b (18.0-24.0)	21.4 ± 1.7 ab (17.6-24.5)	21.9 ± 0.7 ab (20.6-22.8)	22.1 ± 1.2 a (20.6-24.5)
Stylet knob width	5.1 ± 0.4 b (4.4-6.0)	5.8 ± 0.3 a (4.9-6.3)	5.3 ± 0.3 b (4.9-5.7)	5.1 ± 0.5 b (4.4-5.9)
Stylet knob height	2.9 ± 0.3 b (2.3-3.4)	3.1 ± 0.2 a (2.9-3.4)	3.1 ± 0.2 a (2.9-3.4)	3.1 ± 0.2 a (2.7-3.4)
DGO	3.2 ± 0.5 ab (2.4-4.4)	2.81 ± 0.3 c (2.4-3.4)	0.36 ± 0.5 a (2.5-4.4)	3.0 ± 0.5 bc (2.5-4.4)
Excretory pore to anterior end	162.8 ± 30.8 ab (105-209)	151 ± 20.2 b (119-183)	175.6 ± 19.9 a (132-212)	155 ± 25.8 b (122-226)
Center median bulb to anterior end	90.1 ± 9.2 a (73.5-111)	89.8 ± 9.3 a (68.6-106)	91.2 ± 7.1 a (71.3-102)	91.6 ± 6.9 a (81.3-112)
Tail length	11.4 ± 1.7 b (8.8-15.0)	13.1 ± 2.0 a (9.8-18.6)	13.2 ± 1.3 a (10.8-15.6)	10.1 ± 1.6 c (7.8-13.7)
Spicule length	30.7 ± 2.6 a (26.4-34.3)	28.4 ± 2.8 b (21.5-34.3)	30.7 ± 2.5 a (25.4-35.3)	30.0 ± 1.9 ab (26.5-33.3)
Gubernaculum length	8.6 ± 0.9 a (6.9-9.8)	7.6 ± 1.1 b (5.8-9.3)	7.7 ± 1.0 b (5.9-9.8)	7.8 ± 1.0 b (5.9-9.8)
a	44.7 ± 7.8 a (26.9-58.7)	43.2 ± 6.4 a (30.7-56.3)	43.9 ± 5.8 a (34.7-52.4)	36.5 ± 7.2 a (27.9-54)
b	12.8 ± 2.2 a (8.5-16.3)	12.3 ± 2.2 a (8.4-16.4)	13.5 ± 2.1 a (9.7-17.9)	9.07 ± 2.0 b (6.6-14.1)

[x]Measurements (µm) were taken using 20 specimens from each isolate.

[y]Origin of *M. floridensis* isolates are as follows: 1-(N03-01894) was obtained from the population used in the species description (Handoo *et al.*, 2004), 2 was collected from tomato in Indian River County, Fla, 3 was collected from tomato in Hendry County, Fla; and isolate 4 was collected from cucumber in a separate field in Hendry County.

[z]Means in same row followed by the same letter are not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

Table 2. (Continued) Select morphometric characters (mean, standard deviation, and range) of second-stage juveniles, males and females of four isolates of *Meloidogyne floridensis* from Florida[x].

	Nematode isolates[y]			
Character	1	2	3	4
c	132.4 ± 29.4 a (72.0-174)	114.2 ± 24.2 b (84.1-179)	120 ± 15.9 ab (89.3-153)	118.8 ± 21.9 ab (75.2-154)
			Females	
Stylet length	14.1 ± 0.9 ab (12.7-16.6)	14.3 ± 0.7 ab (13.0-15.6)	13.8 ± 1.3 b (10.8-15.7)	14.7 ± 0.7 a (13.5-16.1)
DGO	3.1 ± 0.4 c (2.5-3.9)	4.6 ± 0.7 a (3.9-5.9)	3.8 ± 0.5 b (2.9-4.7)	3.9 ± 0.16 b (3.5-4.4)
Vulval slit length	25.6 ± 3.1 a (21.6-31.3)	22.8 ± 1.6 b (21.0-25.9)	22.5 ± 1.8 b (19.6-25.5)	23.4 ± 1.3 b (21.5-26.4)

[x]Measurements (µm) were taken using 20 specimens from each isolate.
[y]Origin of *M. floridensis* isolates are as follows: 1-(N03-01894) was obtained from the population used in the species description (Handoo *et al.*, 2004), 2 was collected from tomato in Indian River County, Fla, 3 was collected from tomato in Hendry County, Fla; and isolate 4 was collected from cucumber in a separate field in Hendry County.
[z]Means in same row followed by the same letter are not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

range values overlapped. These significant differences were expected because each isolate originated from a single egg mass, which caused less variability in the examined characters. The differences for the non-allometric characters, such as stylet length were < 1 µm and difficult to quantify using a light microscope. In comparison to the original description of *M. floridensis,* the measurements of certain morphological characters of the four isolates in this study appeared to be greater, but in fact are due to the slight difference between measurements taken from narcotized specimens and those that have been fixed and mounted in glycerin, as was done in the species description of *M. floridensis*. The difference in measurements due to fixation shrinkage in certain *Pratylenchus* species was estimated to be 5 to 7% (Saha and Kahn, 1989). When taking this into consideration the values of the fresh specimens overlapped the range of those in the original description. As previously mentioned, the statistical differences in individual characters do not outweigh the fact that the ranges overlap. The morphological comparisons of four isolates of *M. floridensis* and comparison to the original description of this species do not show evidence of differences among and between isolates.

Biochemical analysis

The isozyme phenotype identified from all females from four *M. floridensis* isolates remained constant regardless of host and matched the designation of MF3 (Rm 38.7, 40.69, 44.18) for *M. floridensis* for esterase (Brito *et al.*, 2008) and the designation of

N1 for malate dehydrogenase (Esbenshade and Triantaphyllou, 1985) (Fig. 1). These results also indicate that *M. floridensis* does not demonstrate esterase polymorphism based on select hosts as was reported for *M. konaensis* Eisenback *et al.*, 1994 (Sipes *et al.*, 2005).

Host differentials

Based on the egg mass ratings, tomato, pepper, tobacco, and watermelon are susceptible to all four isolates of *M. floridensis*, whereas peanut and cotton are resistant (Table 3). However, there was low reproduction on cotton for isolates 2 and 3 (egg mass index 1.6-1.8). The results obtained in this study indicate that tobacco and pepper are susceptible hosts to *M. floridensis*. This designation does not agree with the original description for this species. The only susceptible host differentials reported in the original description were tomato and watermelon (Handoo *et al.*, 2004). All four isolates reproduced on pepper and tobacco as well as tomato and watermelon (Table 3). To confirm these findings three additional steps were taken. First, the reproductive factor (Rf) for pepper and tobacco was determined (Table 3); second, 52 females were extracted from both tobacco and pepper root systems and subjected to PAGE to confirm their identity (Fig. 1), and third, pepper and tobacco were re-evaluated for their suitability to all four *M. floridensis* isolates and were determined to be susceptible to based on egg mass index (Table 4). The designation of the pepper cv. California Wonder and the root-knot nematode resistant tobacco cv. NC 95 as being susceptible to *M. floridensis* differs from other data reported in the literature indicating these cultivars as resistant (Handoo *et al.*, 2004; Kokalis-Burelle and Nyczepir, 2004). The discrepancies between our results and those reported in

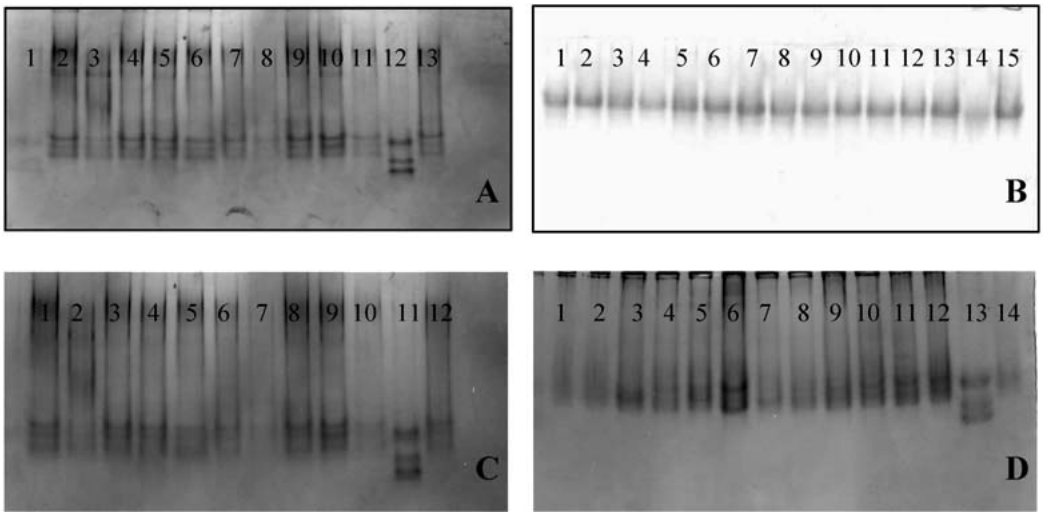

Fig. 1. A: Esterase enzyme phenotype MF3 of 11 single females of *Meloidogyne floridensis*, lanes 1 and 12 single females of *M. javanica* control. B: Malate dehydrogenase phenotype N1 of 11 single females of *M. floridensis*, lanes 1 and 14 single females of *M. javanica* control, also designated as N1. C: Esterase enzyme phenotype MF3 of 11 single females of *M. floridensis* collected from pepper, lane11 single female of *M. javanica* control. D: Esterase enzyme phenotype MF3 of 13 single females of *M. floridensis* collected from tobacco, lane 13 single female of *M. javanica* control.

Table 3. Root galling and egg mass rating of four isolates of *Meloidogyne floridensis* on six differential host plant species[v].

	Nematode isolate[w]											
	1			2			3			4		
Plant	Gall[y] index	Egg mass[x] index	Rf[y]	Gall index	Egg mass index	Rf	Gall index	Egg mass index	Rf	Gall index	Egg mass index	Rf
Tomato	3.8	4.4	na[z]	4.2	4.2	na	4.0	4.0	na	4.6	4.6	na
Cotton	0	0	na	1.4	1.6	na	1.8	1.8	na	0	0	na
Peanut	0	0	na	0	0	na	0	0	na	0	0	na
Watermelon	3.8	3.8	na	4.6	4.0	na	4.4	4.0	na	4.0	4.4	na
Pepper	2.0	3.2	5.2	0.6	2.6	3.3	1.4	2.4	1.2	1.0	2.6	1.0
Tobacco	3.8	4.0	20	4.0	4.0	20	3.6	3.8	13	2.2	2.2	1.0

[v]Plant cultivars designated by Taylor and Sasser, 1978 for the differential host test include tobacco (*Nicotiana tabacum* cv. NC 95), cotton (*Gossypium hirsutum* cv. Deltapine 61), pepper (*Capsicum annuum* cv. California Wonder), watermelon (*Citrullus lanatus* cv. Charleston Gray), peanut (*Arachis hypogaea* cv. Florunner), tomato (*Solanum esculentum* cv. Rutgers).

[w]Origin of *M. floridensis* isolates are as follows: 1-(N03-01894) was obtained from the population used in the species description (Handoo *et al.*, 2004), 2 was collected from tomato in Indian River County, Fla, 3 was collected from tomato in Hendry County, Fla; and isolate 4 was collected from cucumber in a separate field in Hendry County.

[x]Galling and egg mass index = 0-5 scale where 0 = no galls or egg masses, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, 5 = > 100 (Taylor and Sasser, 1978).

[y]Reproduction factor (Rf) calculated for pepper and tobacco only, Rf = final population (pf)/initial population (pi). Plants with a Rf ≥ 1 are considered good hosts, 1 > Rf > 0.1 poor hosts, and Rf < 0.1 non hosts (Oostenbrink, 1966; Sasser *et al.*, 1984).

[z]Rf not assessed for these plants.

Table 4. Root galling and egg mass ratings of four isolates of Meloidogyne floridensis on pepper, tobacco, and tomato[x].

	Nematode isolate[y]							
	1		2		3		4	
Plant	Gall[z] index	Egg mass[z] index	Gall index	Egg mass index	Gall index	Egg mass index	Gall index	Egg mass index
Pepper	2.0	3.2	1.0	2.8	1.4	3.2	1.0	2.7
Tobacco	4.0	4.0	4.5	4.4	3.6	3.8	4.8	4.0
Tomato (control)	4.0	4.4	4.5	4.2	4.0	4.0	4.0	4.5

[x]Selected plant cultivars designated by Taylor and Sasser, 1978 for the differential host test including tomato (*Solanum esculentum* cv. Rutgers), tobacco (*Nicotiana tabacum* cv. NC 95), and pepper (*Capsicum annuum* cv. California Wonder).

[y]Origin of *M. floridensis* isolates are as follows: 1-(N03-01894) was obtained from the population used in the species description (Handoo *et al.*, 2004), 2 was collected from tomato in Indian River County, Fla, 3 was collected from tomato in Hendry County, Fla; and isolate 4 was collected from cucumber in a separate field in Hendry County.

[z]Galling and egg mass index = 0-5 scale where 0 = no galls or egg masses, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, 5 = > 100 (Taylor and Sasser, 1978).

the literature may be attributed to the differences in inoculum preparation and inoculum level. In our test the standard 0.5% NaOCl (Hussey and Barker, 1973; Boneti and Ferraz, 1981) was used for egg extraction as well as the standard inoculum level of 5,000 eggs/J2 per plant (Hartman and Sasser, 1985).

All four isolates of *M. floridensis* fit the same differential host status profile as that of *M. incognita* race 2. Susceptible plants were tobacco, tomato, pepper, and watermelon and those considered as resistant were cotton and peanut. These data are supported by, and consistent with, the results determined by the reproductive factor and esterase and malate dehydrogenase isozyme phenotypes.

Vegetable and agronomic crop test

The tomato cv. Talladega was susceptible to all four isolates of *M. floridensis* whereas the root-knot nematode resistant tomato cv. Crista was resistant (Table 5). The root-knot nematode susceptible pepper cv. Keystone Resistant Giant was determined to be susceptible to isolates 1, 2, and 3; and resistant to isolate 4, whereas the root-knot nematode resistant bell pepper cv. Charleston Belle was susceptible to isolates 2 and 3 and resistant to 1 and 4. Both the root-knot nematode susceptible corn cv. Dixie 18 and the resistant Mp-710 were determined to be susceptible to all four isolates. However, isolate 4 had statistically higher reproduction on corn cv. Dixie 18. There was no detectable reproduction by any of the four nematode isolates on either the root-knot nematode susceptible (S64-J1) or resistant (Forrest) soybean cultivars, indicating that soybean is immune to *M. floridensis*.

These results indicate that, based on egg mass indices and reproductive factor (pf/pi), all four isolates of *M. floridensis* reproduced poorly, but were able to overcome the resistance of the *Mi-1*gene in cv. Crista tomato. Isolates 1 and 4 reproduced poorly, but were able to overcome the *N* gene resistance in the pepper cv. Charleston Belle; whereas, isolates 2 and 3 reproduced well. All four isolates of *M. floridensis* overcame the unidentified source of resistance to the two major root-knot nematode species, *M. incognita* and *M. javanica* in the corn cv. Mp-710; however, isolate 4 had a higher reproduction factor than isolates 1, 2, and 3.

The differences observed among these four isolates of *M. floridensis* may indicate variability among isolates with regard to their ability to overcome resistance, as well as general host preference. This indicates the need for further studies to determine if different host races exist.

Prunus test

In both peach tests, the root-knot nematode susceptible cv. Lovell and the resistant cv. Nemaguard were susceptible to all four isolates of *M. floridensis* (Table 6). While galling and egg mass indices, and reproductive factors were somewhat higher on Lovell than Nemaguard, those parameters were high enough on Nemaguard to consider it susceptible to all four nematode isolates. All four *M. floridensis* isolates were able to break the unidentified source of resistance to *M. incognita* and *M. javanica* in Nemaguard as was reported in the original description of *M. floridensis* (Handoo *et al.*, 2004), demonstrating that *M. floridensis* has the ability to infect and reproduce on both root-knot nematode resistant and susceptible peach cultivars.

In summary, the four isolates of *M. floridensis* included in this study were found to be within the morphological parameters established for the original description of the species, with no evidence of differences

Table 5. Select agronomic and vegetable genotypes test: Reproduction and gall indices of four isolates of *Meloidogyne floridensis* on select agronomic and vegetable genotypes[t].

	Nematode isolate[u]											
	1			2			3			4		
Plant	Gall[v] index	Egg mass[v] index	Rf[w]	Gall index	Egg mass index	Rf	Gall index	Egg mass index	Rf	Gall index	Egg mass index	Rf
	Tomato Cultivar											
Talladega	3.7 A[x]a[y]	3.7 Aa	3.9 Ba	4.3 Aa	3.9 Aa	5.5 Aa	4.0 Aa	3.7 Aa	3.9 Ba	3.6 Aa	3.7 Aa	2.9 Bb
Crista	2.0 Cb	2.1 Abc	0.5 Acd	3.1 Ab	2.4 Abc	0.5 Acd	2.6 Bb	2.2 Ab	0.6 Ad	2.2 BCb	2.1 Abc	0.2 Bcd
	Pepper Cultivar											
Keystone Resistant Giant Charleston	2.0 Ac	2.7 ABb	1.3 Ac	2.2 Ac	2.9 Ab	1.5 Ac	2.0 Abc	2.4 Bb	1.3 Ac	1.0 Bc	1.7 Cb	0.5 Bc
Bell	1.5 ABc	1.9 Ac	0.4 Bcd	1.9 Ac	2.0 Ac	1.1 Acd	1.0 Cd	2.1 Ab	1.2 Ac	1.3 BCc	1.4 Bc	0.3 Bcd

[t]Means are an average of combined duplicate tests based on the homogenicity of variance test.

[u]Origin of *M. floridensis* isolates are as follows: 1-(N03-01894) was obtained from the population used in the species description (Handoo *et al.*, 2004), 2 was collected from tomato in Indian River County, Fla, 3 was collected from tomato in Hendry County, Fla; and isolate 4 was collected from cucumber in a separate field in Hendry County.

[v]Galling and egg mass index = 0-5 scale where 0 = no galls or egg masses, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, 5 = > 100 (Taylor and Sasser, 1978).

[w]Reproduction factor (Rf) = final population (pf)/initial population (pi). Plants with a Rf ≥ 1 are considered good hosts, 1 > Rf > 0.1 poor hosts, and Rf < 0.1 non hosts (Oostenbrink, 1966; Sasser *et al.*, 1984).

[x]Means in the same row followed by the same upper case letter are not significantly ($P \leq 0.05$) different based on Duncan's multiple-range test and are to be compared horizontally across isolates within a cultivar and within the corresponding index (gall, egg mass, and Rf).

[y]Means in the same column followed by the same lower case letter are not significantly ($P \leq 0.05$) different based on Duncan's multiple-range test and are to be compared vertically, within an isolate and within the corresponding index (gall, egg mass, and Rf).

[z]There was no galling, egg mass production, or final population (pf) detected on either soybean cultivar from any of the *M. floridensis* isolates, indicating that soybean is a nonhost.

Table 5. (Continued) Select agronomic and vegetable genotypes test: Reproduction and gall indices of four isolates of *Meloidogyne floridensis* on select agronomic and vegetable genotypes[t].

	Nematode isolate[u]											
	1			2			3			4		
Plant	Gall[v] index	Egg mass[v] index	Rf[w]	Gall index	Egg mass index	Rf	Gall index	Egg mass index	Rf	Gall index	Egg mass index	Rf
	Corn Cultivar											
Dixie 18	0.2 Bc	0.3 Bc	1.8 Bb	2.0 Ac	2.3 Ac	2.8 ABb	1.7 Ac	2.0 Ab	2.3 ABb	1.6 Ac	1.8 Ac	4.6 Aa
MP 710	0.0 Bd	0.0 Bd	1.3 Bc	0.8 Ad	1.3 Ad	1.5 Bc	0.1 Be	0.1 Bc	1.2 Bc	0.9 Ad	1.3 Ad	3.1 Ab
	Soybean Cultivar											
S64-J1	No reproduction[z]											
Forrest	No reproduction											

[t]Means are an average of combined duplicate tests based on the homogenicity of variance test.
[u]Origin of *M. floridensis* isolates are as follows: 1-(N03-01894) was obtained from the population used in the species description (Handoo *et al.*, 2004), 2 was collected from tomato in Indian River County, Fla, 3 was collected from tomato in Hendry County, Fla; and isolate 4 was collected from cucumber in a separate field in Hendry County.
[v]Galling and egg mass index = 0-5 scale where 0 = no galls or egg masses, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, 5 = > 100 (Taylor and Sasser, 1978).
[w]Reproduction factor (Rf) = final population (pf)/initial population (pi). Plants with a Rf ≥ 1 are considered good hosts, 1 > Rf > 0.1 poor hosts, and Rf < 0.1 non hosts (Oostenbrink, 1966; Sasser *et al.*, 1984).
[x]Means in the same row followed by the same upper case letter are not significantly ($P \leq 0.05$) different based on Duncan's multiple-range test and are to be compared horizontally across isolates within a cultivar and within the corresponding index (gall, egg mass, and Rf).
[y]Means in the same column followed by the same lower case letter are not significantly ($P \leq 0.05$) different based on Duncan's multiple-range test and are to be compared vertically, within an isolate and within the corresponding index (gall, egg mass, and Rf).
[z]There was no galling, egg mass production, or final population (pf) detected on either soybean cultivar from any of the *M. floridensis* isolates, indicating that soybean is a nonhost.

Table 6. Reproduction and gall indices of four isolates of *Meloidogyne floridensis* on the root-knot nematode resistant peach cultivar Nemaguard and the susceptible cultivar Lovell[u].

	Nematode isolate[v]											
	1			2			3			4		
Plant	Gall[w] index	Egg mass[w] index	Rf[x]	Gall index	Egg mass index	Rf	Gall index	Egg mass index	Rf	Gall index	Egg mass index	Rf
Lovell	3.9 A[y]a[z]	3.4 Aa	2.2 Aa	4.0 Aa	3.6 Aa	2.2 Aa	3.4 Aa	3.3 Aa	2.0 Aa	4.0 Aa	3.4 Aa	2.2 Aa
Nemaguard	3.2 Bb	3.0 Bb	1.7 Ab	3.9 Aa	3.3 Aa	1.7 Ab	3.2 Ba	3.0 Ba	1.6 Ab	3.7 ABa	3.0 Bb	1.7 Ab

[u]Means are an average of combined duplicate tests based on the homogenicity of variance test.
[v]Origin of *M. floridensis* isolates are as follows: 1-(N03-01894) was obtained from the population used in the species description (Handoo *et al.*, 2004), 2 was collected from tomato in Indian River County, Fla, 3 was collected from tomato in Hendry County, Fla; and isolate 4 was collected from cucumber in a separate field in Hendry County.
[w]Galling and egg mass index = 0-5 scale where 0 = no galls or egg masses, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, 5 = >100 (Taylor and Sasser, 1978).
[x]Reproduction factor (Rf) = final population (pf)/initial population (pi). Plants with a Rf ≥ 1 are considered good hosts, 1 > Rf > 0.1 poor hosts, and Rf < 0.1 non hosts (Oostenbrink, 1966; Sasser *et al.*, 1984).
[y]Means in the same row followed by the same upper case letter are not significantly ($P \leq 0.05$) different based on Duncan's multiple-range test and are to be compared horizontally across isolates within a cultivar and within the corresponding index (gall, egg mass, and Rf).
[z]Means in the same column followed by the same lower case letter are not significantly ($P \leq 0.05$) different based on Duncan's multiple-range test and are to be compared vertically, within an isolate and within the corresponding index (gall, egg mass, and Rf).

among and between isolates. Furthermore, *M. floridensis* does not demonstrate esterase polymorphism based on select hosts as seen with some *Meloidogyne* spp. Tobacco and pepper were found to be susceptible hosts for *M. floridensis*. All isolates studied overcame *Mi-1* and *N* gene resistance in tomato and pepper, respectively and unknown resistance gene (s) in corn and peach genotypes.

The results of this study demonstrate the ability of *M. floridensis* to overcome the resistance of several genes in different root-knot nematode resistant plant cultivars. These data combined with those previously published and cited provide sufficient evidence that this nematode presents a valid concern to peach growers providing peach root-stock to the peach industry as well as any growers that may rely on the use of root-knot nematode resistant cultivars as effective nematicides become less available. Further studies are needed to determine the ability of this nematode to overcome root-knot nematode resistance conferred by other crops. Emphasis should be placed on attaining more information on the management of *M. floridensis* by the use of the Myrobalan plum root-stock and the M*a* gene which has demonstrated resistance to this nematode (Handoo *et al.*, 2004).

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