MELOIDOGYNE SPP. INFECTING ORNAMENTAL PLANTS IN FLORIDA

J. A. Brito[1*], R. Kaur[2], R. Cetintas[3], J. D. Stanley[1], M. L. Mendes[2], T. O. Powers[4], and D. W. Dickson[2]

[1]Division of Plant Industry, Gainesville, FL 32614, USA; [2]Entomology and Nematology Department, University of Florida, Gainesville, FL 32611, USA; [3]Department of Plant Protection, Kahramanmaras Sutcu Imam University, Kahramanmaras, 46060, Turkey; [4]Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583, USA. *Corresponding author: britoj@doacs.state.fl.us

ABSTRACT

Brito, J. A., R. Kaur, R. Cetintas, J. D. Stanley, M. L. Mendes, T. O. Powers, and D. W. Dickson. 2010. *Meloidogyne* spp. infecting ornamental plants in Florida. Nematropica 40:87-103.

A total of 206 root samples were collected from ornamental plants growing in ornamental nurseries and various landscapes in Florida. Isozyme phenotypes, especially esterase (EST) and malate dehydrogenase (MDH) were the main methods used to identify the root-knot nematode species. When needed, the morphology of female perineal patterns, morphometric characters and mitochondrial DNA were used to aid in the identification. Six *Meloidogyne* spp., *M. arenaria*, *M. floridensis*, *M. graminis*, *M. incognita*, *M. javanica* and *M. mayaguensis* were found infecting ornamental plants in Florida. As previously reported EST activity was of highest diagnostic value to identify *Meloidogyne* spp. found in this study; however, MDH was helpful to distinguish *M. mayaguensis* and *M. graminis* from the other root-knot nematode species identified. Five new EST phenotypes were detected associated with 17 unidentified root-knot nematode populations. To our knowledge, this is the first report of ornamental plants in the genera *Dracena* and *Hibiscus*, and *Ligustrum* and *Washingtonia* being host of *M. floridensis* and *M. mayaguensis*, respectively. New plant species host records for *M. mayaguensis* were *Ajuga reptans*, *Amaranthus tricolor*, *Buddleja davidii*, *Caryopteris* × *clandonensis*, *Clerodendrum* × *ugandense*, *Hibiscus grandiflorus*, *Lagerstroemia indica*, *Penta lanceolata*, *Plectranthus scutellarioides*, and *Solandra maxima*.

Key words: Esterase isozyme phenotyping, Florida, malate dehydrogenase isozyme phenotyping, *Meloidogyne* species, ornamental plants, root-knot nematode.

RESUMEN

Brito, J. A., R. Kaur, R. Cetintas, J. D. Stanley, M. L. Mendes, T. O. Powers, and D. W. Dickson. 2010. *Meloidogyne* spp. en plantas ornamentales en Florida. Nematropica 40:87-103.

Se colectaron 206 muestras de raíces de plantas ornamentales cultivadas en viveros y en distintos paisajes en Florida. El principal método para identificar las especies fue el de fenotipo isoenzimático, especialmente de esterasas (EST) y malato deshidrogenasas (MDH). En algunos casos, se complementó la identificación con morfología del patrón perineal de hembras, caracteres morfométricos y ADN mitocondrial. Se encontraron seis especies: *M. arenaria*, *M. floridensis*, *M. graminis*, *M. incognita*, *M. javanica* y *M. mayaguensis* en las plantas ornamentales observadas. La actividad de esterasa fue la de más alta utilidad en el diagnóstico de especies de *Meloidogyne* en este estudio, pero la actividad de malato deshidrogenasa fue útil para distinguir a *M. mayaguensis* y *M. graminis* de otras especies de nematodo agallador. Se detectaron cinco nuevos fenotipos de esterasa asociados con 17 poblaciones de nematodo agallador no identificadas. Hasta donde sabemos, este es el primer registro de *M. floridensis* en plantas de los géneros *Dracena* e *Hibiscus*, y de *M. mayaguensis* en *Ligustrum* y *Washingtonia*. Nuevos registros de especies vegetales para *M. mayaguensis* incluyen *Ajuga reptans*, *Amaranthus tricolor*, *Buddleja davidii*, *Caryopteris* × *clandonensis*, *Clerodendrum* × *ugandense*, *Hibiscus grandiflorus*, *Lagerstroemia indica*, *Penta lanceolata*, *Plectranthus scutellarioides* y *Solandra maxima*.

Palabras clave: especies de *Meloidogyne*, fenotipo isoenzimático de esterasa, fenotipo isoenzimático de malato deshidrogenasa, Florida, nematodo agallador, plantas ornamentales.

INTRODUCTION

In 2007, Florida ranked third among states in the USA in the production and gross sale of nursery plants and ranked first in production of ornamental grasses, woody ornamental plants, preparative nursery materials and palm trees (Anonymous, 2007). Florida led the nation in sales of potted foliage for indoor use and hanging baskets and also was the nation's leader in sales of cut cultivated greens in 2005 (Anonymous, 2008). Many of the ornamental plants currently used for landscaping are susceptible to several pathogens, including root-knot nematodes (*Meloidogyne* spp.) (Barker and Benson, 1977; Benson and Barker, 1985; Sinclair *et al.*, 1987; Martinez *et al.*, 2003).

Up to March 2010, 97 nominal species of *Meloidogyne* have been described. The proper identification of *Meloidogyne* spp. is very important for implementation of plant breeding, nematode management, and particularly for certification and quarantine in regulatory programs. Species identification is primarily based on morphological and morphometrics characters of the males, females and second stage juveniles (Jepson, 1987). Accurate and reliable identification using morphology and morphometrics is a difficult and time consuming task that requires well trained personnel. Morphological characters, especially female perineal patterns, are one of the major characters used to aid in the identification of root-knot nematodes in routine analysis; however, perineal patterns are variable, and may lead to misidentification of aberrant populations and new species. Conversely, biochemical markers such as isozyme phenotypes used in conjunction with morphological and morphometric, allow a more precise and accurate identification of *Meloidogyne* spp.

The relative stability of isozymes phenotypes within *Meloidogyne* spp. (Fargette, 1987a; De Waele and Elsen, 2007;) has made them a useful tool for nematode identification. Among the isozyme systems, esterase (EST) has the highest diagnostic value because the majority of the phenotypes described are nematode-species specific; however, the use of more than one isozyme may be needed as the result of intraspecific variability and differences in migrations obtained from different electrophoresis apparatus and laboratories. Isozyme analysis, specifically EST in combination with malate dehydrogenase (MDH) (Dickson *et al.*, 1970; Esbenshade and Triantaphyllou, 1985), resolved using polyacrylamide gel electrophoresis (PAGE) has proven to be a valuable, fast and reliable method to identify the most common root-knot nematode species collected from different parts of the world (Dickson *et al.*, 1970, 1971; Esbenshade and Triantaphyllou, 1985; Fargette, 1987a; 1987b; Pais and Abrantes, 1989; Carneiro *et al.*, 1996; 2000; Karssen, 2002, Castro *et al.*, 2003; Cofcewicz *et al.*, 2004, 2005; Molinari *et al.*, 2005); however, novel esterase phenotypes have been discovered in root-knot nematode surveys (Esbenshade and Triantaphyllou, 1985; Hernandez *et al.*, 2004; Adam *et al.*, 2005; Molinari *et al.*, 2005). To determine whether theses novel phenotypes represent a new root-knot nematode species, a nematode species already described but with an unknown EST/MDH phenotype or an aberrant pattern; a combination of morphological, morphometric, host range, biochemical and molecular studies are needed. Currently, several DNA-based methods such as restriction fragment length polymorphisms (RFLP), random amplified polymorphic DNA (RAPD), amplification of the ribosomal DNA in the intergenic spacer region (IGS) between the 18S and 5S gene, amplification of the mitochondrial DNA (mtDNA) region between the COII and lRNA genes and the

63 bp repeat region have shown to be useful to aid in the identification of *Meloidogyne* spp. (Powers *et al.*, 1986; 1993; 2005; Blok *et al.*, 1997a; 1997b; 2002; Zijlstra *et al.*, 2000; Randig *et al.*, 2002; Handoo *et al.*, 2004); however, these methods are still expensive to use in large surveys (Powers *et al.*, 2005) as well as in routine analyses in many nematode diagnostic laboratories due to the costs with equipment, reagents and DNA sequencing.

The objectives of the current study were to i) identify the root-knot nematode species found infecting ornamental plant species in Florida, ii) evaluate the usefulness of EST and MDH phenotypes in differentiating *Meloidogyne* spp. for routine diagnostic purposes, and iii) determine the plant host of each of the *Meloidogyne* sp. identified.

MATERIALS AND METHODS

A total of 206 root samples were collected from ornamental plants in 26 counties in Florida for isolation and identification of root-knot nematode species in this study. Species identification was performed using primarily esterase (EST) phenotypes in combination with malate dehydrogenase (MDH) (Esbenshade and Triantaphyllou, 1985); when needed, the morphology of females perineal patterns (Hartman and Sasser, 1985; Rammah and Hirschman, 1988), morphometric characters (Jepson, 1987; Rammah and Hirschman, 1988) and mitochondrial DNA (Powers and Harris, 1993) were used to aid in the identification.

Samples used for this study mainly consisted of roots collected individually from ornamental plants growing in several nurseries, from various landscapes as well as samples submitted to the Nematology Laboratory, Division of Plant Industry, Gainesville, Florida for certification. Each sample was given an accession number, representing the year of collection and the serial number to maintain sample identity. Root samples with limited infection were cut into ca. 2-cm pieces, mixed with pasteurized soil and placed into a clay pot in which a tomato seedling (*Solanum lycopersicum* 'Rutgers') was transplanted. Plants were maintained in a greenhouse at 26 ± 1.8°C until used for nematode identification. The procedure for isozyme extraction from each nematode female was the same as that reported by Brito *et al.*, 2008. At least 26 egg-laying females were dissected directly from each root system and isozyme profiles determined using polyacrylamide gel electrophoresis (PAGE) with two gels run at the same time (Brito *et al.*, 2008). One gel was stained for both MDH and EST activity (Esbenshade and Triantaphyllou, 1985), whereas the second one, was stained only for EST. Extracts from single *M. javanica* females were added separately to individual wells on each gel as standards. The nematodes species with new or unknown EST phenotypes were also stained for two different isozymes; superoxide dismutase (SOD) and glutamic-oxaloacetic transaminase (GOT) activities. Relative mobility of isozymes was calculated and phenotype designations were assigned according to Esbenshade and Triantaphyllou (1985) and Fargette (1987a). There was no EST phenotype described for *M. graminis*. Therefore, the phenotype Mg1 was designated for this nematode species, which represent the first two letters of the *Meloidogyne* sp. followed by the number of major bands of EST activity, as proposed previously (Brito *et al.*, 2008). It is worth mentioning that according to Esbenshade and Triantaphyllou (1985), the phenotype G1 should be assigned to this species; however, that phenotype had already been assigned to *M. graminicola* (Carneiro *et al.*, 2000). Preliminary results of this study have been reported (Brito *et al.*, 2004b).

RESULTS AND DISCUSSION

A total of six root-knot nematode species and six unidentified populations of *Meloidogyne* spp. were found infecting 75 ornamental plant species belonging to 36 botanical families in this study (Tables 1 and 2). Only the major bands of EST and MDH activities were used for phenotype designation and species identification as described in previous studies (Dickson *et al.*, 1970, 1971; Esbenshade and Triantaphyllou, 1985; Fargette, 1987a; Pais and Abrantes, 1989; Carneiro *et al.*, 2000; Karssen, 2002, Castro *et al.*, 2003; Cofcewicz *et al.*, 2005; Molinari *et al.*, 2005). A schematic representation of EST and MDH phenotypes containing relative migration (Rm) values and band numbers detected for each *Meloidogyne* sp. identified in each sample is presented in Figs. 1 and 2.

Esterase phenotypes (EST)

Fourteen distinct EST phenotypes and 26 major bands of EST activities were identified among the populations of *Meloidogyne* spp. examined (Tables 1 and 2; Fig. 1). A total of six root-knot nematode species plus six unidentified populations were found in this study.

The phenotype A2 (Rm: 45.35, 48.83) (Tables 1 and 2; Fig. 1) was detected in 44 populations of *M. arenaria* found infecting several ornamental plants species either alone or in mixed populations with *M. incognita*, *M. javanica* or *M. mayaguensis* (Tables 1 and 2). The A2 phenotype is species-specific for *M. arenaria*, and has been observed in several populations of this nematode in Brazil (Carneiro *et al.*, 2000; Castro *et al.*, 2003; Cofcewicz *et al.*, 2004), Guadalupe, French Guiana and Martinique (Cofcewicz *et al.*, 2005), Portugal (Pais and Abrantes, 1989), West Africa (Fargette *et al.*, 1987b), and the United States (Brito *et al.*, 2008).

The EST phenotype Mf3 (Rm: 38.37, 40.69, 44.18) (Brito *et al.*, 2008), which is species- specific for *M. floridensis*, was isolated from three populations found reproducing in *Dracena* sp. and *Hibiscus* sp. (Table 1; Fig. 1). This is the first report of ornamental plants being host for *M. floridensis*. All populations of this nematode species reported in this study were detected singly and not as mixed populations with other *Meloidogyne* spp. Nonetheless, *M. floridensis* has been reported in mixed populations with *M. incognita* and *M. javanica* infecting *Phaseolus* spp. (Brito *et al.*, 2008). It is worth mentioning that *M. floridensis* is known to occur only in Florida (Handoo *et al.*, 2004).

Meloidogyne graminis was the only species infecting nine root samples of *Stenotaphrum secundatum* var. Amerishade and *S. secundatum* and exhibited a single EST band with a very slow migration (Rm: 19.2) (Fig. 1). Perineal patterns of females and morphometrics of J2 were also used to aid the identification of this nematode species (data not shown). Results were similar to those reported previously (Jepson, 1987).

The phenotype I1 (Rm: 39.5), which has been consistently identified from *M. incognita* collected in several regions around the world (Esbenshade and Triantaphyllou, 1985; Pais and Abrantes, 1989; Carneiro *et al.*, 2004; Brito *et al.*, 2008) was observed in 59 populations of *M. incognita* infecting several ornamental plants (Tables 1 and 2; Fig. 1). Nevertheless, four additional populations identified as *M. incognita* exhibited the phenotype I2 (Rm: 39.5, 41.0) (Table 2; Fig. 1). This phenotype shares a common band with phenotype I1 at Rm 39.5 (Fig. 1). Similarly, both phenotypes were detected among populations of *M. incognita* from Brazil (Carneiro *et al.*, 1996, 2000, 2004; Castro *et al.*, 2003; Barbosa *et al.*, 2004; Cofcewicz *et al.*, 2004), Guadalupe, French Guiana and Martin-

Table 1. Isozyme phenotypes of *Meloidogyne* species infecting ornamental plants in Florida.

	Enzyme phenotypes[v]				
Meloidogyne spp.	EST	MDH	Plant hosts	Botanical families	County of origin
M. arenaria	A2	N1	*Alocasia* sp.	Araceae	Alachua, Dade, Hillsborough, Martin, Orange, Palm Beach, Santa Rosa and Volusia
			Buddleia × *weyeriana*	Buddlejaceae	
			Caryopteris × *clandonensis*	Verbenaceae	
			Cypress papyrus	Cyperaceae	
			Euphorbia tirucalli	Euphorbiaceae	
			Gardenia sp.	Rubiaceae	
			Hosta sp.	Agavaceae	
			Hibiscus rosa-sinensis	Malvaceae	
			Rosa sp.	Rosaceae	
			Schefflera actinophylla	Araliaceae	
			S. arboricola	Araliaceae	
			Syagrus romanzoffiana	Arecaceae	
			Zingiber officinale	Zingiberaceae	
M. arenaria	A2	N3	*Allium schoenoprasum* var. *Sibiricum*	Liliaceae	Orange
			Impatiens sp.	Balsaminaceae	
M. floridensis	Mf3	N1	*Dracena* sp.	Ruscaceae	Dade and Lake
			Hibiscus sp.	Malvaceae	
M. graminis	Mg1	N1a	*Stenotaphrum secundatum* var. Amerishade	Poaceae Poaceae	Alachua, Brevard, Hillsborough and Levy

[v]EST = Esterase, MDH = Malate dehydrogenase. Phenotype designation according to Esbenshade and Triantaphyllou (1985) and Brito *et al.*, 2008.
[w]MDH phenotype not resolved.
[x]*Meloidogyne* sp. 1 found in mixed population with *M. mayaguensis*.
[y]New EST phenotypes: designations were assigned using first two letters of the plant host species name from which the root-knot nematode was isolated, followed by number of major isozyme bands (Brito *et al.*, 2008).
[z]*Meloidogyne* sp. 3 and 4 found in mixed population with *M. javanica*.

Table 1. (Continued) Isozyme phenotypes of *Meloidogyne* species infecting ornamental plants in Florida.

Meloidogyne spp.	Enzyme phenotypes[v] EST	MDH	Plant hosts	Botanical families	County of origin
			S. secundatum		
M. incognita	I1	N1	*Brugmansia* sp.	Solanaceae	Alachua, Dade, Flagler, Hardee, Orange, Palm Beach and Volusia
			Buddleja sp.	Buddlejaceae	
			Buxus sp.	Buxaceae	
			Buxus microphylla	Buxaceae	
			Calathea rufibarba	Marantaceae	
			Duranta erecta,	Verbenaceae	
			Epipremnum aureum	Araceae	
			Ficus sp.	Moraceae	
			Gardenia sp.	Rubiaceae	
			Impatiens sp.	Balsaminaceae	
			Ixora spp.	Rubiaceae	
			Ophiopogon sp.	Liliaceae	
			Syagrus romanzoffiana	Arecaceae	
M. javanica	J3	N1	*Amorphophallus* sp.	Araceae	Alachua, Dade, Hillsborough and Palm Beach
			Brugmansia suaveolens	Solanaceae	
			Clerodendrum ugandense	Verbenaceae	
			Lantana montevidensis	Verbenaceae	
			Solanum rantonnetii	Solanaceae	

[v]EST = Esterase, MDH = Malate dehydrogenase. Phenotype designation according to Esbenshade and Triantaphyllou (1985) and Brito *et al.*, 2008.
[w]MDH phenotype not resolved.
[x]*Meloidogyne* sp. 1 found in mixed population with *M. mayaguensis.*
[y]New EST phenotypes: designations were assigned using first two letters of the plant host species name from which the root-knot nematode was isolated, followed by number of major isozyme bands (Brito *et al.*, 2008).
[z]*Meloidogyne* sp. 3 and 4 found in mixed population with *M. javanica.*

Table 1. (Continued) Isozyme phenotypes of *Meloidogyne* species infecting ornamental plants in Florida.

Meloidogyne spp.	Enzyme phenotypes[v] EST	MDH	Plant hosts	Botanical families	County of origin
			Tulbaghia violacea	Alliaceae	
M. javanica	J2	N1	*Buddleja davidii*	Buddlejaceae	Alachua and Seminole
			Lagerstroemia indica	Lythraceae	
			Ophiopogan japonicus	Liliaceae	
M. mayaguensis	VS1-S1	N1a	*Ajuga reptan,*	Lamiaceae	Alachua, Citrus, Clay, Collier, Dade, Duval, Flagler, Gilchrist, Hardee, Hillsborough Lake, Nassau, Orange, Palm Beach, Pasco and Putnam
			Brugmansia × *sunray*	Solanaceae	
			Brugmansia sp.	Solanaceae	
			Buddleja davidii	Buddlejaceae	
			Callistemon spp.	Myrtaceae	
			Callistemon citrinus	Myrtaceae	
			C. viminalis	Myrtaceae	
			Clerodendrum ugandense	Verbenaceae	
			Gardenia sp.	Rubiaceae	
			Hibiscus grandiflorus	Malvaceae	
			Lagerstroemia indica	Lythraceae	
			Lantana montevidensis	Verbenaceae	
			Ligustrum sp.	Oleaceae	
			Myrica cerifera	Myricaceae	

[v]EST = Esterase, MDH = Malate dehydrogenase. Phenotype designation according to Esbenshade and Triantaphyllou (1985) and Brito *et al.*, 2008.
[w]MDH phenotype not resolved.
[x]*Meloidogyne* sp. 1 found in mixed population with *M. mayaguensis.*
[y]New EST phenotypes: designations were assigned using first two letters of the plant host species name from which the root-knot nematode was isolated, followed by number of major isozyme bands (Brito *et al.*, 2008).
[z]*Meloidogyne* sp. 3 and 4 found in mixed population with *M. javanica.*

Table 1. (Continued) Isozyme phenotypes of *Meloidogyne* species infecting ornamental plants in Florida.

Meloidogyne spp.	Enzyme phenotypes[v] EST	MDH	Plant hosts	Botanical families	County of origin
			Penta lanceolata	Rubiaceae	
			Plectranthus scutellarioides	Lamiaceae	
			Salix × *sepulcralis*	Salicaceae	
			Solandra maxima	Solanaceae	
			Tecomaria capensis,	Bignoniaceae	
			Tibouchina × *compacta*	Melastomaceae	
			Tibouchina × *elegans*	Melastomaceae	
M. arenaria and M. incognita	A2;I1	N1;N1	*Begonia* sp.	Begoniaceae	Alachua, Marion, Orange, and Volusia
			Gardenia sp.	Rubiaceae	
			Hoya sp.	Asclepiadaceae	
			Ilex crenata	Aquifoliaceae	
			Impatiens sp.	Balsaminaceae	
			Myrica cerifera	Myricaceae	
			Syagrus romanzoffiana	Arecaceae	
M. incognita, M. javanica and M. mayaguensis	A2; I1; VS1-S1	N1; N1 N1a	*Amaranthus tricolor*	Amaranthaceae	Palm Beach
M. arenaria and M. javanica	A2;J3	N1;N1	*Caladium* sp.	Araceae	Highlands and Palm Beach
			Phoenix dactylifera	Arecaceae	
M.arenaria, M. javanica and M. mayaguensis	A2;J3 VS1-S1	N1;N1 N1a	*Washingtonia* sp.	Arecaceae	Pasco

[v]EST = Esterase, MDH = Malate dehydrogenase. Phenotype designation according to Esbenshade and Triantaphyllou (1985) and Brito *et al.*, 2008.
[w]MDH phenotype not resolved.
[x]*Meloidogyne* sp. 1 found in mixed population with *M. mayaguensis.*
[y]New EST phenotypes: designations were assigned using first two letters of the plant host species name from which the root-knot nematode was isolated, followed by number of major isozyme bands (Brito *et al.*, 2008).
[z]*Meloidogyne* sp. 3 and 4 found in mixed population with *M. javanica.*

Table 1. (Continued) Isozyme phenotypes of *Meloidogyne* species infecting ornamental plants in Florida.

	Enzyme phenotypes[v]				
Meloidogyne spp.	EST	MDH	Plant hosts	Botanical families	County of origin
M. arenaria and M. mayaguensis	A2; VS1-S1	N1; N1a	*Clerodendrum ugandense*	Verbenaceae	Dade and Flagler
			Myrica cerifera	Myricaceae	
M. incognita and M. javanica	I1;J3	N1;N1	*Abelia* sp.	Caprifoliaceae	Alachua and Orange
			Justicia brandegeeana	Acanthaceae	
			Petunia sp.	Solanaceae	
			Pseuderanthemum sp.	Acanthaceae	
M. incognita, M. javanica and M. mayaguensis	I1;J3; VS1-S1	N1;N1 N1a	*Caryopteris* × *clandonensis*	Verbenaceae	Alachua
M.incognita and M. mayaguensis	I1; VS1-S1	N1; N1a	*Myrica cerifera*	Myricaceae	Flagler and Madison
			Gardenia jasminoides	Rubiacea	
M. javanica and M. mayaguensis	J3; VS1-S1	N1; N1a	*Brugmansia* sp.	Solanaceae	Alachua, Hardee, Marion and Sarasota
			Hibiscus grandiflorus	Malvaceae	
			Lantana camara	Verbenaceae	
			L. montevidensis	Verbenaceae	
			Washingtonia	Arecaceae	
Meloidogyne sp. 1[x]	Ep2[y]	—	*Myrica cerifera*	Myricaceae	Nassau
Meloidogyne sp. 2	Vo1[y]	N1	*Brugamansia* sp.	Solanaceae	Alachua and Dade
			Myrica cerifera	Myricaceae	

[v]EST = Esterase, MDH = Malate dehydrogenase. Phenotype designation according to Esbenshade and Triantaphyllou (1985) and Brito *et al.*, 2008.
[w]MDH phenotype not resolved.
[x]*Meloidogyne* sp. 1 found in mixed population with *M. mayaguensis.*
[y]New EST phenotypes: designations were assigned using first two letters of the plant host species name from which the root-knot nematode was isolated, followed by number of major isozyme bands (Brito *et al.*, 2008).
[z]*Meloidogyne* sp. 3 and 4 found in mixed population with *M. javanica.*

Table 1. (Continued) Isozyme phenotypes of *Meloidogyne* species infecting ornamental plants in Florida.

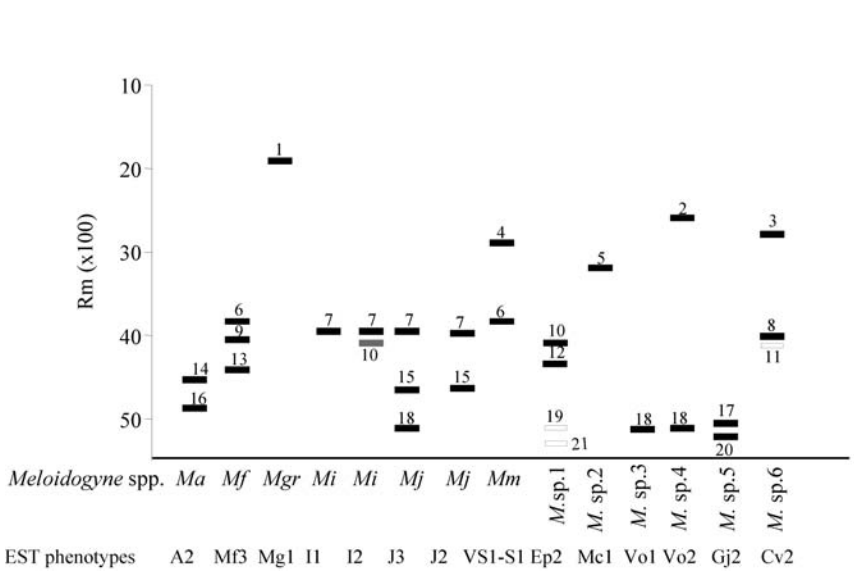
	Enzyme phenotypes[v]				
Meloidogyne spp.	EST	MDH	Plant hosts	Botanical families	County of origin
			Podocarpus sp.	Podocarpaceae	Brevard and Hillsborough
Meloidogyne sp. 3[z]	Vo1[y]	N1	*Vibernun odoratissimum*	Caprifoliaceae	
			V. suspensum	Caprifoliaceae	
Meloidogyne sp.4	Vo2	N1	*Vibernun odoratissimum*	Caprifoliaceae	Brevard and Hillsborough
			Vibernun suspensum	Caprifoliaceae	
Meloidogyne sp. 5	Gj2[y]	N1a	*Gardenia jasminoides* and unidentified ornamental plant	Rubiaceae	Hardee and Highlands
Meloidogyne sp. 6	Cv2[y]	N1	*Buxus microphylla*	Buxaceae	Alachua
			Callistemon viminalis	Myrtaceae	
			Gardenia sp.	Rubiaceae	
			Plectranthus scutellariodes	Lamiaceae	
			Liriope muscari	Liliaceae	

[v]EST = Esterase, MDH = Malate dehydrogenase. Phenotype designation according to Esbenshade and Triantaphyllou (1985) and Brito *et al.*, 2008.
[w]MDH phenotype not resolved.
[x]*Meloidogyne* sp. 1 found in mixed population with *M. mayaguensis*.
[y]New EST phenotypes: designations were assigned using first two letters of the plant host species name from which the root-knot nematode was isolated, followed by number of major isozyme bands (Brito *et al.*, 2008).
[z]*Meloidogyne* sp. 3 and 4 found in mixed population with *M. javanica*.

Table 2. *Meloidogyne* species found infecting unidentified ornamental plants in Florida along with their isozyme phenotypes and county of origin.

	Enzyme phenotypes[y]			
Meloidogyne spp.[x]	EST	MDH	Number of Samples	County of origin
Ma	A2	N1	2	Dade and Palm Beach
Mi	I1	N1	7	Calhoun, Dade, Hillsborough, Jackson, Manatee and Palm Beach
Mi	I1	N2a	1	Palm Beach
Mi	I2	N1	2	Dade and Hendry
Mj	J3	N1	6	Gadsden, Hillsborough, Lake, Lee and Orange
Mm	VS1-S1	N1a	5	Dade, Hardee, Hendry, Palm Beach and St. Johns
Ma and *Mi*	A2; I1	N1; N1	1	Palm Beach
Ma and *Mm*	A2; VS1-S1	N1; N1a	1	Broward
Ma, Mi and *Mm*	A2; I1; VS1-S1	N1; N1; N1a	1	Volusia
Ma, Mi, Mj and *Mm*	A2; I1; J3; VS1-S1	N3; N1; N1 N1a	1	Lake
Mi and *Mj*	I2; J3	N1; N1	2	Alachua and Orange
Mj and *Mm*	J3; VS1-S1	N1; N1a	1	Collier

[x] *Meloidogyne* spp.: *Ma* = *M. arenaria*, *Mi* = *M. incognita*, *Mj* = *M. javanica*, *Mm* = *M. mayaguensis*.
[y] Est = Esterase, MDH = Malate dehydrogenase. Phenotype designation according to Esbenshade and Triantaphyllou (1985).



Band no.	Rm (x100)[z]
1	19.2
2	26
3	28
4	29.06
5	32
6	38.37
7	39.5
8	40.2
9	40.69
10	41
11	41.3
12	43.7
13	44.18
14	45.35
15	46.5
16	48.83
17	50.6
18	51.16
19	51.3
20	52.2
21	54.1

Fig. 1. Schematic representation of esterase phenotypes of *Meloidogyne* spp. infecting ornamentals in Florida. A2 = *M. arenaria*, Mf3 = *M. floridensis*, Mg1 = *M. graminis*, I1 and I2 = *M. incognita*, J3 and J2 = *M. javanica*, VS1-S1 = *M. mayaguensis*, Ep2 = *M.* sp.1, Mc1 = *M.* sp.2, Vo1 = *M.* sp.3, Vo2 = *M.* sp.4, Gj2 = *M.* sp.5 and Cv2 = *M.* sp.6. [z]Rm = Relative mobility

ique (Cofcewicz *et al.*, 2005), Portugal (Pais and Abrantes, 1989) and United States (Brito *et al.*, 2008). Phenotypes I1 and I2 were never found together in the same sample in this study; however the detection of both phenotypes among populations of *M. incognita* could be associated with some intraspecific variability.

The species-specific phenotype J3 appeared in 36 populations identified as *M. javanica* (Tables 1 and 2; Fig. 1). These populations occurred singly or mixed with *M. arenaria, M. incognita* and *M. mayaguensis* (Tables 1 and 2). These results were consistent with those in previous studies (Esbenshade and Triantaphyllou, 1985; Pais and Abrantes, 1989; Tomaszewski *et al.*, 1994; Carneiro *et al.*, 1996, 2000, 2004; Castro *et al.*, 2003; Cofcewicz *et al.*, 2004, 2005; Molinari *et al.*, 2005; Brito *et al.*, 2008). Three populations of *M. javanica* infecting *Buddleja davidii* and *Ophiopogon japonicus* exhibited the J2 phenotype (Table 1; Fig. 1). Likewise, this phenotype was also reported from populations of *M. javanica* infecting *Abelmoschus esculentus* (Oliveira *et al.*, 2007) and *Musa* sp. (Cofcewicz *et al.*, 2004) in Brazil, and also *Musa* sp. from Guadalupe, French Guiana and Martinique (Cofcewicz *et al.*, 2005).

All 72 nematode populations identified as *M. mayaguensis* exhibited two major bands of EST activity (Rm 29.06; 38.37), consistent with the VS1-S1 phenotype (Fig. 1). At times, each of these bands resolved into two minor bands. Morphology of perineal patterns and morphometrics of selected characters were similar to those from the species description (Rammanh and Hisrchmann, 1988) and other isolates of *M. mayaguensis* found in Florida (Brito *et al.*, 2004a). Furthermore, analysis of the mtDNA region between COII and lrRNA genes was also used to compare with the EST phenotype. Results were in agreement with those reported previously (Brito *et al.*, 2004a).

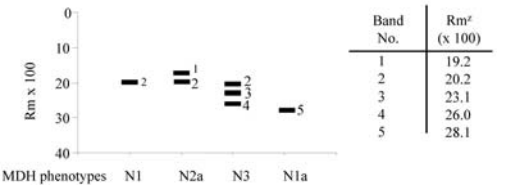


Fig. 2. Schematic representation of malate dehydrogenase phenotypes of *Meloidogyne* spp. populations found infecting ornamental plants in Florida. N1 = *M. arenaria*, *M. floridensis*, *M. incognita*, *M. javanica*, *M.* sp.3, *M.* sp.4, and *M.* sp.6; N2a = *M. incognita*; N3 = *M. arenaria*, N1a = *M. graminis*, *M. mayaguensis*, *M.* sp.2 and *M.* sp.5. Phenotypes designation according to Esbenshade and Triantaphyllou (1985). [z]Rm= Relative mobility.

The EST phenotype, VS1-S1 proved to be of high diagnostic value to distinguish *M. mayaguensis* from all other root-knot nematode species identified in this study, particularly *M. incognita*. It is most likely that *M. mayaguensis* has been erroneously identified as *M. incognita* in the past in Florida due to some similarity of the perineal patterns of these two species (Brito *et al.*, 2004a, 2008). In North America, this nematode is known to occur only in Florida (Brito *et al.*, 2004a, b). *Meloidogyne mayaguensis* was identified from root samples of several ornamental plant species belonging to 16 botanical families (Table 1). To our knowledge *Ajuga reptans*, *Amaranthus tricolor*, *Buddleja davidii*, *Caryopteris* × *clandonensis*, *Clerodendrum ugandense*, *Hibiscus grandiflorus*, *Lagerstroemia indica*, *Penta lanceolata*, *Plectranthus scutellarioides*, and *Solandra maxima* are new host records for *M. mayaguensis*.

It is worth mentioning that the phenotype VS1-S1 also has been reported for another root-knot nematode species, *M. enterolobii* from China (Yang and Eisenback, 1983; Esbenshade and Triantaphyllou, 1985). These two root-knot nematode species share not only biochemical and morphological characteristics, but they also have identical sequences of the mtDNA region between COII and lrRNA genes (Xu *et al.*, 2004). Furthermore, sequence data obtained from studies based on COI, ITS, and IGS showed that the Swiss *M. enterolobii* populations, and two isolates of *M. mayaguensis*, each from Brazil and the USA showed 100% similarity (Kiewnick *et al.*, 2007; 2008). Currently, *M. mayguensis* is being synonymized with *M. enterolobii* (Gerrit Karssen, personal communication).

Six unique EST profiles were detected from 19 unidentified populations of *Meloidogyne* spp. infecting different ornamental plants in this study. These populations were named *Meloidogyne* sp. 1 to 6. Two populations of *Meloidogyne* sp. 1 exhibited two EST major bands Rm: 41.0, 43.70) (Table 1; Fig. 1) similar to a phenotype (Ep2) already described for a root-knot nematode infecting originally *Eclipta prostrata* in Florida (Brito *et al.*, 2008), whereas five new EST phenotypes (Mc1, Cv2, Gj2, Vo1, and Vo2) were described from the remaining 17 unidentified populations (Table 1). Phenotype designations used to assign these new phenotypes were the same as previously reported (Esbenshade and Triantaphyllou, 1985; Brito *et al.*, 2008). A phenotype designated as Mc1 with one major band of activity at Rm: 32.0 was detected in three populations of *Meloidogyne* sp. 2 infecting initially *Myrica cerifera* (Table 1; Fig. 1). In a differential host test (Hartman and Sasser, 1985) single egg mass isolates obtained from *Meloidogyne* sp. 2 reproduced on tobacco 'NC95', cotton 'DPL 16', watermelon 'Charleston Grey', pepper 'California Wonder' and tomato 'Rutgers', but not on peanut 'Florunner'. Furthermore, the isolates also reproduced well on potato but did not reproduce on corn or wheat (data not shown).

The phenotype Vo1, with one major band of activity (Rm 51.16) (Table 1; Fig. 1) and Vo2, with two major bands (26.0; 51.16)

(Table 1; Fig. 1) were observed in mixture population with *Meloidogyne* sp. 3 and *Meloidogyne* 4 infecting *Vibernum odoratissimum*. These two phenotypes share a major band at Rm 51.16 and remained stable when nematode isolates were inoculated and reared on tomato 'Rutgers'. Furthermore, nematode isolates with phenotypes Vo1 or Vo2 showed the same host reactions when inoculated on differential host plant cultivars (Hartman and Sasser, 1985); both isolates reproduced on all plant cultivars except peanut 'Florunner' and pepper 'California Wonder'. These nematode isolates showed the same MDH (NI) (Fig. 2), glutamic-oxaloacetic transaminase (NIa) and superoxide dismutase (N2b) phenotypes (Esbenshade and Triantaphyllou, 1985) regardless of the EST phenotype.

Another phenotype, Gj2 with two major bands (Rm 50.6; 52.2) (Table 1; Fig. 1) was detected in two nematode populations designated as *Meloidogyne* sp. 5, which were found infecting *Gardenia jasminoides* and an unidentified ornamental plant in Hardee and Highlands Counties, respectively. Isolates of these nematodes had the same host reactions as those of *M. javanica* race 1 and *M. arenaria* race 2 (Hartman and Sasser, 1985). *Meloidogyne* sp. 6, initially found reproducing on *Callistemon viminalis* in Alachua County, exhibited the phenotype Cv2 and had three bands (Rm 28; 40.2) (Table 1; Fig. 1). Isolates obtained from the field population showed the same differential host reaction as that of *M. incognita* race 2. Currently, single egg masses obtained from all unidentified root-nematode species are being reared on tomato 'Rutgers' and will be used for further investigation as an attempt to identify each nematode species.

Malate dehydrogenase phenotypes

Five bands of MDH activity and four MDH phenotypes were observed among the populations of *Meloidogyne* spp. in this study (Tables 1 and 2; Fig. 2). The phenotype N1 (Rm: 20.2) was detected in all populations of *M. arenaria*, *M. floridensis*, *M. incognita*, *M. javanica*, *Meloidogyne* sp. 3, *Meloidogyne* sp. 4 and *Meloidogyne* sp. 6 (Tables 1 and 2; Fig. 2), except one population of *M incognita*, which exhibited a unique phenotype (N2a) with two major bands of activity at Rm: 20.2: 23.1 (Table 2; Fig. 2) and two populations of *M. arenaria*, which showed the N3 phenotype (Rm: 20.0:23.1:26.0) with three bands of MDH activity (Tables 1; Fig. 2). The N1 phenotype has been commonly associated with the three major species of *Meloidogyne* collected in other regions of the world (Esbenshade and Triantaphyllou, 1985; Pais and Abrantes, 1989; Carneiro *et al.*, 2004, Brito *et al.,* 2008). Nonetheless, the phenotype N3 has been detected in some populations of *M. arenaria* in the United States (Brito *et al.*, 2008) and also from other geographical regions (Esbenshade and Triantaphyllou, 1985; Pais and Abrantes, 1989; Cofcewicz *et al.*, 2005). To our knowledge, this is the first report of the phenotype N2a identified from *M. incognita*, which could be associated with some variability among populations of this nematode species. This phenotype remained stable when a nematode isolate was inoculated and reared on tomato 'Rutgers'.

The phenotype N1a, which showed one very strong band (Rm: 28.1) of MDH activity was detected in all the populations of *M. graminis*, *M. mayaguensis*, *Meloidogyne* sp. 2 and *Meloidogyne* sp. 5 (Tables 1 and 2; Fig. 2). An identical phenotype has been reported from populations of *M. chitwoodi*, *M. enterolobii*, *M. naasi*, *M. oryzae*, *M. plantani* (Esbenshade and Triantaphyllou, 1985), and *M. partityla* and *M. graminicola* (Brito *et al.*, 2006). Therefore it is of restricted diagnostic value to differentiate

these *Meloidogyne* spp.; however, the N1a phenotype could aid in the discrimination of these root-knot nematode species from *M. arenaria*, *M. floridensis*, *M. incognita* and *M. javanica*.

The results obtained in this study, in combination with those already reported in other studies, clearly show the usefulness of isozymes as a valuable tool for identification of *Meloidogyne* spp. in a large number of samples. The EST and MDH phenotypes were useful to detect mixed populations of *Meloidogyne* spp. as well as to determine new host records for root-knot species; however, EST had a higher diagnostic value than MDH in the discrimination of the *Meloidogyne* spp. found infecting ornamental plants in Florida.

ACKNOWLEDGEMENTS

This research was supported by the Tropical and Subtropical Agriculture Research grant (T-STAR) #2005-34135-15895, Cooperative State Research, Education and Extension Services (CSREES), United States Department of Agriculture, USA. The authors thank Dr. Richard E. Weaver, Jr., Botany Section, Division of Plant Industry, Gainesville, Florida, for identification of weed species and also Ana L. Ochoa, Christine A. Zamora, Charles L. Spriggs, Ping Qiao, Matthew W. Brodie and Sol F. Locker for their support during the field work.

LITERATURE CITED

Adam, M. A. M., M. S. Phillps, and V. C. Block. 2005. Identification *Meloidogyne* spp. from northeast Libya and comparison of their inter- and intro-specific genetic variation using RAPDs. Nematology 7:599-609.

Anonymous. 2007. The National Agricultural Statistics Service. Released 9 September 2007, Agricultural Statistics Board, U.S. Department of Agriculture. http://usda.mannlib.cornell.edu/usda/current/NursProd/NursProd-09-26-2007.pdf. Accessed April 2010.

Anonymous. 2008. Florida Department of Agriculture and Consumer Services. Overview of Florida Agriculture. http://www.florida-agriculture.com/agfacts.htm. Accessed April 2010.

Barbosa, D. H. S. G., H. D. Vieira, R. M. Souza, and C. P. Silva. 2004. Survey of root-knot nematode (*Meloidogyne* spp.) in coffee plantations in state of Rio de Janeiro, Brazil. Nematologia Brasileira 28:43-47.

Barker, K. R., and D. M. Benson. 1977. Japanese hollies: intolerant hosts of *Meloidogyne arenaria* in microplots. Journal of Nematology 9:330-334.

Benson, D. M., and K. R. Barker. 1985. Nematodes - a threat to ornamental plants in the nursery and landscape. Plant Disease 69:97-100.

Brito, J. A., R. Kaur, R. Cetintas, J. D. Stanley, M. L. Mendes, E. J. McAvoy, T. O Powers, and D. W. Dickson. 2008. Identification and isozyme characterization of *Meloidogyne* spp. infecting horticultural and agronomic crops, and weed plants in Florida. Nematology 10:757-766.

Brito, J. A., R. Kaur, D. W. Dickson, J. R. Rich, and L. A. Halsey. 2006. The pecan root-knot nematode, *Meloidogyne partityla* Kleynhans, 1986. Nematology Circular No. 222, Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, Florida.

Brito, J. A., T. O. Powers, P. G., Mullin, R. N., Inserra, and D. W. Dickson. 2004a. Morphological and molecular characterization of *Meloidogyne mayaguensis* isolates from Florida. Journal of Nematology 36:232-240.

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.

Block, V. C., M. S. Phillips, J. W. McNicol, and M. Fargette. 1997a. Genetic variation in tropical *Meloidogyne* spp. as shown by RAPD-PCR. Fundamental and Applied Nematology 20:127-133.

Block, V. C., M. S. Phillips, and M. Fargette. 1997b. Comparison of sequences from the ribosomal DNA intergenic region of *Meloidoygne mayaguensis* and other major root-knot nematodes. Journal of Nematology 29:16-22.

Blok, V. C., J. Wishart, M. Fargette, K. Berthier, and M. S. Phillips. 2002. Mitochondrial DNA differences distinguishing *Meloidogyne mayaguensis* from the major species of tropical root-knot nematodes. Nematology 4:773-781.

Carneiro, R. M. D. G., M. R. A. Almeida, and R. G. Carneiro. 1996. Enzyme phenotypes of Brazilian populations of *Meloidogyne* spp. Fundamental and Applied Nematology 19:555-560.

Carneiro, R. M. D. G., M. S. Tigano, O. Randig, M. R. A. Almeida, and J. L. Sarah. 2004. Identification and genetic diversity of *Meloidogyne* spp. (Tylenchida: Meloidogynidae) on coffee from Brazil, Central America and Hawaii. Nematology 6:287-298.

Carneiro, R. M. D. G., M. R. A, Almeida, and P. Quénéhervé. 2000. Enzyme phenotypes of *Meloidogyne* spp. populations. Nematology 2:645-654.

Castro, J. M. C., R. D. Lima, and Carneiro, R. M. D. G. 2003. Variabilidade isoenzimática de populações de *Meloidogyne* spp. provenientes de regiões Brasileiras produtoras de soja. Nematologia Brasileira 27:1-12.

Cofcewicz, E. T., R. M. D. G. Carneiro, P. Castagnone-Sereno, and P. Quénéhervé. 2004. Enzyme phenotypes and genetic diversity of root-knot nematodes parasitizing *Musa* in Brazil. Nematology 6:85-95.

Cofcewicz, E. T., R. M. D. G. Carneiro, O. Randig, C. Chabrier, and P. Quénéhervé. 2005. Diversity of *Meloidogyne* spp. on *Musa* in Martinique, Guadeloupe, and French Guiana. Journal of Nematology 37:313-322.

De Waele, D., and A. Elsen, 2007. Challenges in tropical plant nematology. Annual Review of Phytopathology 45:457-485

Dickson, D. W., J. N. Sasser, and D. Huisingh. 1970. Comparative disc-electrophoretic protein analysis of selected *Meloidogyne*, *Ditylenchus*, *Heterodera*, and *Aphelenchus* spp. Journal of Nematology 2:286-293.

Dickson, D. W., D. Huisingh, and J. N. Sasser, 1971. Dehydrogenases, acid, alkaline phosphatases and esterases for chemotaxonomy of selected *Meloidogyne*, *Ditylenchus*, *Heterodera*, and *Aphelenchus* spp. Journal of Nematology 3:1-16.

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*. Raleigh, NC: North Carolina State University Graphics.

Fargette, M. 1987a. Use of the esterase phenotypes in the taxonomy of the genus *Meloidogyne*. 1. Stability of the esterase phenotype. Revue De Nématologie 10:39-43.

Fargette, M. 1987b. Use of the esterase phenotypes in the taxonomy of the genus *Meloidogyne*. 2. Esterase phenotypes observed in West African populations and their characterization. Revue De Nématologie 10:45-56.

Harris, T. S., L. J. Sandall, and T. O. Powers. 1990. Identification of single *Meloidogyne* juveniles by polymerase chain reaction amplification of mitochondrial DNA. Journal of Nematology 22:518-524.

Hartman, K. M., and J. N. Sasser. 1985. Identification of *Meloidogyne* species on the basis of differential host test and perineal patterns morphology. Pp. 69-77 *in* K. P. Barker, C. C. C. Carter, and J. N. Sasser, Eds. An Advanced treatise on *Meloidogyne*. Vol. II: Methodology. Raleigh, NC, USA, North Carolina State University Graphics.

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.

Hernandez, A., M. Fargette, and J. L. Sarah. 2004. Characterization of *Meloidogyne* spp. (Tylenchida: Meloidogynidae) from coffee plantations in Central America and Brazil. Nematology 6:193-204.

Jepson S. B. 1987. Identification of root-knot nematodes. Wallingford, United Kingdom, CABI, 247 pp.

Karssen, G. 2002. The plant-parasitic nematode genus *Meloidogyne* Göldi, 1982 (Tylenchida) in Europe. Leiden, The Netherlands, Brill Academic, 157 pp.

Kiewnick, S., R. Eder, I. Roth, M. Oggenfuss, B. Frey, and J. E. Frey. 2007. Occurrence of root-knot nematodes in Switzerland. Journal of Nematology 39:88. (Abstract).

Kiewnick, S., G. Karssen, J. A. Brito, M. Oggenfuss, and J. E. Frey. 2008. Occurrence of *M. enterobolii* in Switzerland. Journal of Plant Disease and Protection 115:134.

Martinez, A., J. W-.Woodward, and M. Pearce. 2003. Diseases of Leyland cypress in the landscape. http:// www.ces.uga. edu/pubcd/B1229.htm. Accessed April 2010.

Molinari, S., F. Lamberti, R. Crozzoli, S. B. Sharma, and L. Sánchez Portales. 2005. Isozyme patterns of exotic *Meloidogyne* spp. populations. Nematologia Mediterranea 33:61-65.

Oliveira, R. D. L., M. B. Silva, N. D. C. Aguiar, F. L. K. Bérgamo, A. S. V. Costa, and L. Prezotti. 2007. The influence of parasitic nematodes on okra crop in eastern Minas Gerais State, Brazil. Horticultura Brasileira 25:88-93.

Pais, C. A., and I. M. de. O. Abrantes. 1989. Esterase and malate dehydrogenase phenotypes in Portuguese populations of *Meloidogyne* species. Journal of Nematology 21:342-346.

Powers, T. O., and T. S. Harris. 1993. A polymerase chain reaction for identification of five major *Meloidogyne* species. Journal of Nematology 25:1-6.

Powers, T. O., P. G., Mullin, T. S. Harris, L. A. Sutton, and R. S. Higgins, 2005. Incorporating molecular identification of *Meloidogyne* spp. into a large-scale regional survey. Journal of Nematology 37:226-235.

Powers, T. O, E. G. Platzer, and B. C. Hyman. 1986. Species-specific restriction site polymorphism in root-knot nematode mitochondrial DNA. Journal of Nematology 18: 288-293.

Rammah, A., and H. Hirschmann. 1988. *Meloidogyne mayaguensis* n. sp. (Meloidogynidae), a root-knot nematode from Puerto Rico. Journal of Nematology 20:58-69.

Randig, O., M. Bongiovanni, R. M. D. G. Carneiro, and P. Castagnone-Sereno. 2002. Genetic diversity of root-knot nematodes from Brazil and development of SCAR markers specific for the coffee-damaging species**.** Genome 45:862-870.

Sinclair, W. A.*,* H. H. Lyon, and W. T. Johnson. 1987. Diseases of trees and shrubs. Ithaca, N.Y. Comstock Publishing Associates, Cornell University Press.

Tomaszewski, E. K., M. A. M. Khalil, A. A. El-Deeb, T. O. Powers, and J. L. Starr. 1994. *Meloidogyne javanica* parasitic on peanuts. Journal of Nematology 26:436-441.

Xu, J., P. Liu, Q.Memg, and H. Long. 2004. Characterization of *Meloidogyne* species from China using isozymes phenotypes, and amplified mitochondrial DNA restriction fragment length polymorphism. European Journal of Plant Pathology 110:309-315.

Yang, G., and J. D. Eisenback. 1983. *Meloidogyne enterolobii* n. sp. (Meloidogynidae), a root-knot nematode parasitize pacara earpod tree in China, Journal of Nematology 15:381-391.

Zijlstra, C., T. H. M. Donkers-Venne, and M. Fargette. 2000. Identification of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* using sequence characterized amplified regions (SCAR) based PCR assays. Nematology 2:847-853.

Received: *Accepted for publication:*

1/IV/2010 *13/V/2010*

Recibido: *Aceptado para publicación*