

# RESEARCH/INVESTIGACIÓN

## CONFIRMATION OF *MELOIDOGYNE HAPLA* ON STRAWBERRY IN FLORIDA USING MOLECULAR AND MORPHOLOGICAL TECHNIQUES

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### ABSTRACT

Nyoike, T.W., T. Mekete, R. McSorley, E. Weibelzahl-Karigi, and O. E. Liburd. 2012. Identification of the root-knot nematode, *Meloidogyne hapla*, on strawberry in Florida using morphological and molecular methods. *Nematropica* 42:253-259.

Morphological and molecular studies were conducted to identify a root-knot nematode that was found on strawberry roots growing in Marion County, Florida, and on strawberry transplants imported from nurseries in Ontario, Canada. In early spring 2011, strawberry plants growing in the field were observed showing localized stunting and galled roots. Soil samples collected from the field revealed a very high number of *Meloidogyne* infective juveniles (232 J2/100cc soil) in one block but relatively low numbers in two other blocks. Morphological studies based on perineal patterns of root-knot females collected from the field-strawberry roots and from strawberry transplants (received from nurseries in October 2011) were conducted. The perineal patterns indicated that they were *Meloidogyne hapla* Chitwood. Polymerase Chain Reaction (PCR) amplification of the region between COII and 16S rRNA of the mitochondrial DNA produced a single fragment ca 540 bp. Restriction digestion of the amplified PCR products with *DraI* enzyme produced two fragments at 200 and 250 bp indicating that the root-knot nematode in the field and on transplant strawberry plants was *M. hapla*. DNA sequencing of the internal transcribed spacer (ITS) region produced a single fragment ca 990 bp and a BLAST search in the Genbank revealed that the species was 98% identical to other known *M. hapla* sequences in the Genbank. This study ascertains that *M. hapla* recorded on strawberry plants in the field was imported with the transplants from the nurseries in Ontario, Canada. *M. hapla*, is commonly found in cooler climates and high altitudes, and has been reported as a common pest for strawberries in northeastern United States. Implications of importing nematode-infected strawberry transplants are discussed.

*Key words:* Identification; internal transcribed spacer; *Meloidogyne*; mitochondrial DNA; nematode-infected transplants; PCR-RFLP; root-knot nematode; strawberry.

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### RESUMEN

Nyoike, T.W., T. Mekete, R. McSorley, E. Weibelzahl-Karigi, and O. E. Liburd. 2012. Identificación del nematodo nodulador *Meloidogyne hapla*, en fresa en Florida mediante métodos morfológicos y moleculares. *Nematropica* 42:253-259.

Se realizaron estudios morfológicos y moleculares para identificar un nematodo agallador encontrado en raíces de fresa en el condado de Marion, Florida y en trasplantes de fresa importados de viveros de Ontario, Canadá. A principios de la primavera de 2011, se observaron en el campo rodales de plantas de fresa con enanismo y raíces agalladas. Muestras de suelo recolectadas de este campo revelaron un número muy alto de juveniles infectivos de *Meloidogyne* (232 J2/100cc suelo) en uno de los bloques pero relativamente bajo en los otros dos bloques. Se llevaron a cabo estudios morfológicos basados en el patrón perineal de las hembras del nematodo utilizando muestras de raíces de fresa recolectadas en el campo y raíces de trasplantes de fresa (recibidos de viveros en Octubre 2011). El patrón perineal indicó que las hembras eran *Meloidogyne hapla* Chitwood. La amplificación de la región entre COII y el 16S rRNA del DNA mitocondrial mediante la reacción en cadena de la polimerasa produjo un solo fragmento de aprox. 540 bp. La digestión del producto amplificado de la PCR con la enzima *DraI* produjo dos fragmentos a 200 y 250 bp, lo que indicaba que la especie de *Meloidogyne* encontrada en el campo y en los trasplantes era *M. hapla*. La secuenciación del DNA de la región ITS confirmó la identidad de *M. hapla* con un 98% de similitud con otras especies conocidas de *M. hapla* depositadas en el Genbank. Este estudio confirma que *M. hapla* en las plantas de fresa en el campo fue importada con los trasplantes procedentes de Ontario, Canadá. *M. hapla* se encuentra comúnmente en climas más fríos y altitudes altas y se ha citado como

una plaga común de la fresa en el noreste de los Estados Unidos de América. Se discute las implicaciones de importar trasplantes de fresa infestados con el nematodo agallador.

*Palabras clave:* Identificación; *Meloidogyne*; DNA mitocondrial; trasplantes infestados con nematodos; PCR-RFLP; nematodo agallador; fresa.

## INTRODUCTION

Root-knot nematodes, *Meloidogyne* spp., are known to infect many agricultural crops including strawberry (*Fragaria x ananassa* Duch.). *Meloidogyne incognita*, *M. javanica*, *M. arenaria*, and *M. hapla* are among the most economically important species of root-knot nematodes (Qui *et al.*, 2006). In particular, *M. hapla*, the northern root-knot nematode, is a serious pest of strawberries in the northeastern United States (LaMondia, 2002) and a minor pest in California (Westerdahl, 2009). Other *Meloidogyne* spp. that are potential pests of strawberries include *M. javanica* and *M. incognita* in California (Westerdahl, 2009). However, *M. incognita*, despite being found on all soils in North Carolina, does not cause damage on strawberries (Averre *et al.*, 2011). In Florida, the most economically important nematode damaging strawberries is the sting nematode, *Belonolaimus longicaudatus*, as reported since the 1950s (Noling, 1999). Typical above-ground symptoms for sting nematodes include stunted plants, dormant plants with no new growth, and leaves dying off starting from the older leaves and progressing to the younger leaves (Noling, 1999).

One of the ways in which nematodes can be introduced into a clean field is by using infested planting material. This is a very important source of nematodes especially when dealing with crops that are vegetatively propagated such as strawberries and bananas. The common practice while planting strawberries is to fumigate the soil with fumigants such as methyl bromide [50:50; methyl bromide: chloropicrin] or Telone® products (Noling and Whiden, 2010) for protection against soil-borne diseases as well as nematodes and weeds. This practice is expected to provide season-long control for plants by maintaining nematode populations below damaging levels. However, if endoparasitic nematodes enter the production system via infected transplants, they may not be killed by the fumigant and therefore are a potential risk to the growers. Moreover, endoparasitic nematodes are within the roots and are hard to target with any fumigant without killing the plant.

In spring 2011, localized stunting was observed on beds planted with strawberries in Citra, Marion County, Florida. The strawberry plant roots at those locations contained galls. Around the same time, nematode symptoms associated with root-knot nematode were reported on strawberries in south Florida, and presumed to be caused by *M. hapla* (Noling and Whiden 2010). Generally, *B. longicaudatus* is considered the most damaging nematode to strawberries in the state.

Typically *M. hapla* is found in cooler climates, while *M. incognita*, *M. javanica* and *M. arenaria* are the predominant root-knot nematodes in regions between 30°N and 35°S latitude (Taylor and Sasser, 1978). Since several species of root-knot nematodes occur in Florida, accurate identification of the root-knot nematode on strawberries is critical. To implement an effective management program including crop rotation, choice of cultivar, monitoring pest status and its spread in the field; correct identification of the nematode species is important (Adam *et al.*, 2007; Orui, 1998).

There are several methods available to identify nematodes including polymerase chain reaction (PCR), PCR-RFLP (Harris *et al.*, 1990; Powers and Harris, 1993), isozyme electrophoresis (Esbenshade and Triantaphyllou, 1990), RAPD-PCR (Orui, 1998), specific sequence-characterized-amplified-region (SCAR) –PCR (Fourie *et al.*, 2001), and use of perineal patterns (Hartman and Sasser 1985). The use of perineal patterns to distinguish between *Meloidogyne* spp. needs to be supplemented with other methods of identification due to high interspecies similarities and high intraspecies variation (Hu, 2011). Reliable molecular techniques for nematode diagnostics are well established and can be used to complement morphological studies. DNA can be obtained from a single juvenile or female nematode and amplified through PCR (Harris *et al.*, 1990). The amplified DNA is then digested using restriction enzymes to obtain different fragment sizes using restriction fragment length polymorphism (Harris *et al.*, 1990; Powers *et al.*, 2005). The objective of this study was to conduct both morphological studies and PCR-RFLP to identify the *Meloidogyne* spp. associated with the damage on strawberries grown under Florida conditions.

## MATERIALS AND METHODS

A field study was conducted at the University of Florida, Plant Science Research and Education Center located in Citra, Marion County, Florida, during the 2010/2011 strawberry growing season (October–April). As a standard planting procedure for strawberries, a soil fumigant methyl bromide: chloropicrin (50:50) was injected into tarped planting beds at the rate of 36.5 liters per hectare before transplanting strawberries. Two weeks after soil fumigation, strawberry variety Festival imported from Ontario, Canada as bare-root transplants, was planted on 20 October 2010. Strawberries were planted into three blocks, each 21 by 6.3 m with

6-double rows of strawberries at spacing of 0.35 m by 0.35 m. Growing practices including fertilization, weeding, harvesting, and removal of runners were done according to the standard production practices for north-central Florida (Peres *et al.*, 2010).

**Root-knot sampling and soil extraction:** At the end of the harvest season, on 25 April 2011, six soil samples were collected from each block using a cone-shaped auger (Cole-Parmer, Vernon Hills, IL) and combined into a composite sample per block. Soil samples were stored at 10°C for four days, and nematodes were extracted from a subsample (100 cc) using a centrifugal-flotation method (Jenkins, 1964).

**Root-knot nematode females:** At the end of the harvest season, 24 strawberry plants were gently dug out of the soil leaving the roots system as intact as possible. The plants were cleaned by removing any soil adhering to the roots using tap water, before wrapping them with moist paper towel. Root-knot nematode females were hand-picked from the strawberry roots at 20X under a dissecting microscope (Leica MZ 12 5, Leica Microsystems, Houston, TX) and processed for morphological examination or placed in 1% saline water in a sterile micro-tube and frozen at -6°C until needed. Root-knot nematode females were collected at the end of 2010/2011 strawberry growing season in April; and during the 2011/2012 season, females were recovered from roots upon receiving transplants from strawberry nurseries in October 2011.

**Morphological characterization:** Root-knot nematode females were prepared and perineal patterns studies were conducted as described by Hartman and Sasser (1985).

**Molecular characterization:** DNA was extracted from single females per vial using the DNeasy blood and tissue extraction kit (Qiagen, Santa Clarita, CA) according to the manufacturer's instructions with slight modifications. Briefly, individual female nematodes were hand-picked in 90 µl ATL buffer and incubated overnight at 56°C after adding 20 µl of proteinase K. The females were then vortexed for 15 sec before adding 100 µl Buffer AL and 100 µl ethanol (96%). The mixture was placed in a DNeasy mini spin column and centrifuged at 8000 rpm for 1 min and flow-through sample was discarded. With a new receiving tube, the centrifugation procedure was repeated adding 200 µl Buffer AW1 and 200 µl Buffer AW2 to dry the DNeasy membrane at 14000 rpm for 3 min, discarding the flow-through each time. The DNeasy Mini spin column was placed onto a 1.5 ml microcentrifuge tube and 50 µl Buffer AE was directly pipetted on to the DNeasy membrane to collect the DNA.

**PCR:** Amplification of the mitochondrial DNA (mtDNA) between cytochrome oxidase subunit II (COII) and the 16S rRNA was achieved by the following primers; RNAF (5'-TACCTTTGACCAATCACGCT-3') and COIIR (5'-GGTCAATGTTTCAGAAATTTGTGG-3') (Powers and Harris, 1993). PCR was carried out

according to the manufacturer's recommendation (DNeasy Blood & Tissue Handbook, Qiagen, Santa Clarita, CA); 25 µl volumes containing 12.5 µl of PCR multiplex (Qiagen, Santa Clarita, CA), 1.5 µl of 10 µM each forward and reverse primers, 4.5 µl deionized water, and 5 µl template DNA. All PCR reactions were run in a thermal Gradient cyclor (PTC-200 Peltier Thermal Cyclor, Watertown, Massachusetts). The PCR conditions were: denaturation at 95°C for 15 min; 40 cycles of 94°C for 30 sec, 62°C for 90 sec, and 72°C for 90 sec; and a final extension at 72°C for 10 min (Joyce *et al.*, 1994). A portion (8 µl) of the amplification product was electrophoresed on a 1.8% agarose gel and stained with ethidium bromide. The sizes of amplified products were determined by comparison with a 1-Kb molecular weight ladder (Invitrogen, Carlsbad, CA).

**Restriction fragment length polymorphism:** Restriction digestion of the PCR products was conducted using *DraI* enzyme (Promega, Madison WI). A total volume of 20 µl including 10 µl of PCR product, 2 µl of *DraI* enzyme, 2 µl of buffer (Promega, Madison, WI) and 6 µl of water was used for the restriction reaction and incubated at 37°C for 4 hrs. The digestion products were separated using 1.8% agarose gel in electrophoresis.

**DNA sequencing:** Additional PCR reactions were carried out to amplify the partial ITS spacer 1, complete sequences of 5.8S and ITS2, and partial 28S rRNA gene region. Forward primer TW81 (GTTCCGTAGGTGAACCTGC) and reverse primer AB28 (ATATGCTTAAGTTCAGCGGT) were used as described by Joyce *et al.* (1994). A 10-µl portion of the DNA suspension from the previous extraction was added to the PCR reaction mixture containing: 12.5 µl PCR supermix (Qiagen, Santa Clarita, CA), 1.5 µl of 10 µM each forward and reverse primers, 4.5 µl deionized water, and 5 µl template DNA. All PCR reactions were run in a thermal Gradient cyclor (PTC-200 Peltier Thermal Cyclor). The PCR conditions were: denaturation at 95°C for 15 min; 35 cycles of 94°C for 1 min, 58°C for 1.5min, and 72°C for 1.5 min; and a final extension at 72°C for 10 min (Joyce *et al.*, 1994). A portion (8 µl) of the amplification product was electrophoresed on a 1.8% agarose gel and stained with ethidium bromide. The sizes of amplified products were determined by comparison with a 1-Kb molecular weight ladder (Invitrogen, Carlsbad, CA). For direct sequencing, PCR products were purified with the QIAquick PCR purification kit (QIAGEN, Santa Clarita, CA) and sequenced at the Interdisciplinary Center for Biotechnology Research (ICBR) at the University of Florida. The primers used for sequencing were the same as those used for amplification. Sequences were edited with BioEdit (BioEdit version 7.0.9, Carlsbad, CA). The sequences were compared with those in Genbank (<http://blast.ncbi.nlm.nih.gov/Blast>) by means of a Basic Local Alignment Search Tool (BLAST) search, and sequences were aligned with

ClustalW2 (CLUSTAL 2.0, Dublin, Ireland).

## RESULTS

*Root-knot nematode symptoms and soil nematode abundance:* Above-ground symptoms associated with nematode infestation were observed on the strawberry plants growing in the field in 2010/2011 growing



Fig. 1a: Strawberry plants A & B showing nematode symptoms (stunted, excessive fibrous root growth and reduced crowns) as compared to healthy plants C & D that were collected from Citra, Marion County, Florida in April 2011.



Fig. 1b. Galled strawberry roots recovered from the bare-root green-top strawberry transplants shipment from Ontario, Canada nurseries in October 2011.

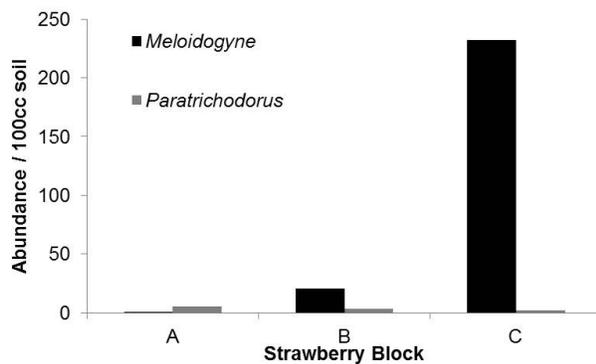


Fig. 2. Abundance (per 100cc soil) of *Meloidogyne* and *Paratrichodorus* spp. from soil samples collected in blocks A, B, and C, planted to strawberries.

season. The plants were stunted, with reduced crowns, and produced small berries (Fig. 1a). Below-ground symptoms included root galling and excessive growth of fibrous roots compared to healthy roots (Fig. 1a). In the 2011/2012 growing season, transplants showing root-knot galling (Fig. 1b) were recovered from bare-root green-top strawberry transplants from the nursery.

The dominant plant-parasitic nematodes recovered from the soil samples were root-knot nematodes (*Meloidogyne* spp.) and stubby-root nematodes (*Paratrichodorus* spp.) (Fig. 2). The highest mean number of root-knot nematode juveniles (232 J2/100 cc soil) was recorded in block C, followed by 21 juveniles in block B and only one J2 in block A. *Paratrichodorus* spp. numbers ranged from 2 to 5 nematodes per 100 cc soil across the blocks.

*Morphological characterization:* Morphological examination of females extracted from strawberry roots revealed a round perineal pattern with fine, undulating longitudinal lines resembling wrinkles. An invaginated line around the vulva as described by Yik and Birchfield (1978) was also observed (Fig. 3). These are typical characteristics of *Meloidogyne hapla* that distinguish it from the other root-knot nematode species.

*DNA extraction and PCR amplification:* DNA was extracted from a single female in the 2010/2011 strawberry growing season and two females (each extracted separately) in the 2011/2012 strawberry transplants shipment. The PCR amplified the mtDNA region, yielding a single PCR product with the length of ca 540 bp for all three samples tested (Fig. 4). Digestion of the PCR product using *DraI* restriction enzyme

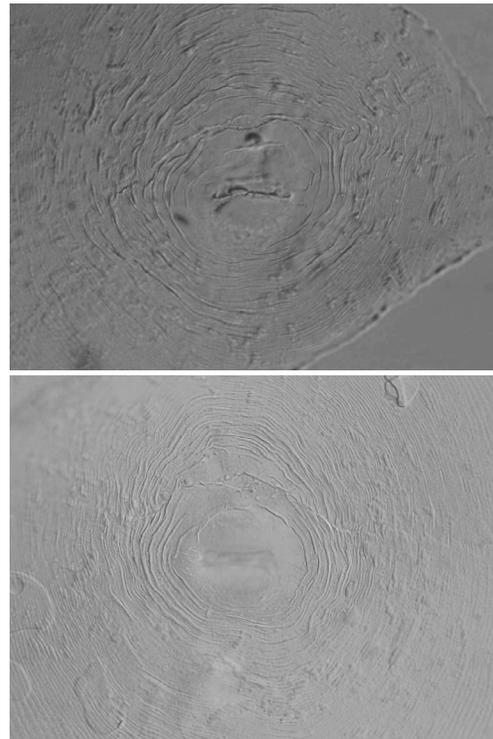


Fig. 3. Female perineal pattern of *Meloidogyne hapla* isolated from field strawberries collected from Citra, Marion County, Florida in April 2011.

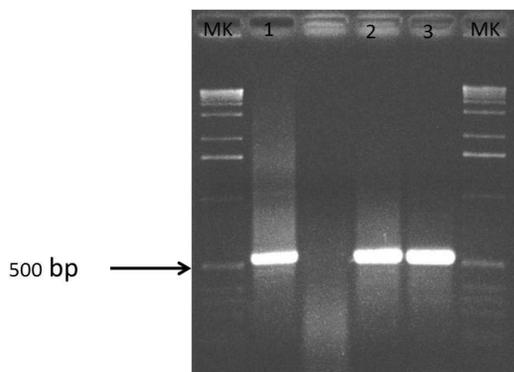


Fig. 4. Amplification products using COII and 1RNA primers of the mtDNA from three *Meloidogyne* spp. females extracted from 2010/2011 and 2011/2012 strawberry plants on 1.8% agarose gel. All samples formed a band at 540 bp. The females are: 1, 2010/2011 from strawberry plants after the growing season; 2 and 3, 2011/2012 from strawberry transplants upon arrival from a nursery in Ontario, Canada, and MK on the first and last loading lines is 1 kb DNA ladder (Invitrogen, Carlsbad, CA).

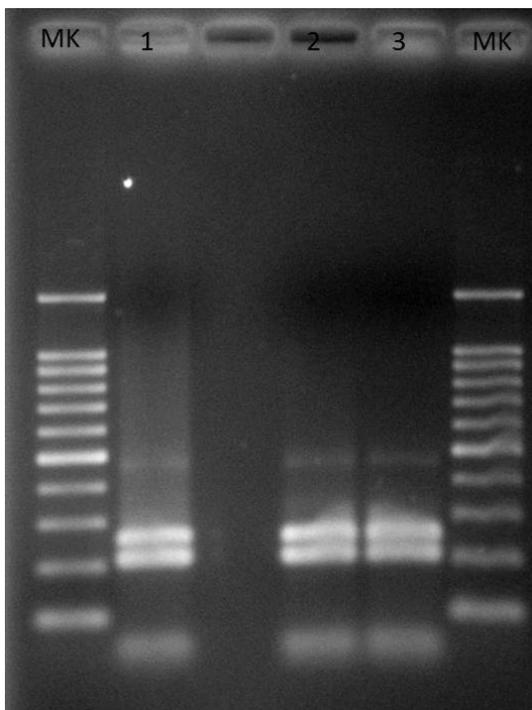


Fig. 5. Restriction fragment patterns of the PCR-amplified products of the region between COII and 1RNA of the mtDNA after digestion with endonuclease *DraI* on 1.8% agarose gel. The samples formed two bands at 200 and 250 bp. The samples are: 1, 2010/2011 from strawberry plants after the growing season; 2 and 3, 2011/2012 from strawberry transplants from a nursery in Ontario, Canada, and MK on the first and the last loading lines is 1 kb DNA ladder (Invitrogen, Carlsbad, CA).

generated RFLP fragments at 200 and 250 bp for all the samples (Fig. 5).

PCR with primers TW81 and AB28 yielded a single fragment of ca 990 for the tested isolates. A BLAST search of Genbank revealed that our *M. hapla* sequences were identical to those of *M. hapla* from Netherlands (NCBI accession # AY281854), California (NCBI accession # AY268108), Australia (NCBI accession # AF516722), and others, with maximum identity up to 98%.

## DISCUSSION

During the 2010/2011 growing season, several strawberry plants were observed to be stunted and with low yield despite similar management practices across the blocks. The unevenly distributed nematode infestation across the blocks suggests the possibility that nematode-infected transplants were only in a few of the boxes received from nurseries. The block with the highest number of juveniles in the soil had significantly lower yield than the block with the lowest number of nematodes (Nyoike, unpublished data).

PCR-RFLP analysis identified the nematode as *M. hapla*. PCR amplification resulted with a band formation at 540 bp and two bands after endonuclease digestion at 200 and 250 bp. Orui (1998) reported similar results using *DraI* for discrimination among 10 different species of *Meloidogyne*. When suspecting more than one *Meloidogyne* species to be present or due to intraspecific variations within one species, other endonucleases should be used to confirm the results of the species identity (Orui, 1998). Our results were further confirmed with DNA sequencing that yielded up to 98% identity when our samples were compared with known gene sequences in the Genbank.

Both samples collected at the end of 2010/2011 growing season and at the beginning of 2011/2012 (soon after importing the transplants) tested positive for *M. hapla*. This study confirms that *M. hapla* was imported with the transplants from Ontario, Canada. There have been previous reports of *M. hapla* imported into Florida from the northern areas but no confirmation studies of the species had been performed (Howard *et al.*, 1985; Noling and Whiden, 2010). At the end of the 2010 strawberry growing season, nematode symptoms associated with root-knot nematode (*M. hapla*) infestation were reported on strawberries but no species confirmation was done (Noling and Whiden, 2010). Strawberry plants were observed to be stunted, low yielding, with shortened growth life and galled roots. Similarly, in North Carolina, *M. hapla* has also been introduced to the state through transplants (Averre *et al.*, 2011). The current study confirms the presence of *M. hapla* on transplants in Florida by both molecular and morphological methods. Morphological and molecular studies have also been used to characterize a population of *M. hapla* found damaging on coffee in Hawaii (Handoo *et al.* 2005). Their study demonstrates

that various morphological characters of second-stage juveniles, males, and females can be combined with molecular studies to compare different *M. hapla* populations.

The occurrence of *M. hapla* on transplants is an indication that stricter import sanitation rules should be put in place, but it is worth mentioning that *M. hapla* is not considered a “quarantine pest” (Noling and Whiden, 2010). In Florida, growers are expected to buy strawberry transplants only from certified nurseries. The strawberry transplants used in this study were obtained from a certified nursery in Canada.

*Meloidogyne hapla* is more prevalent in colder latitudes and in high elevations of the tropics (Powers and Harris, 1993). This makes the survival of *M. hapla* through hot summer months in Florida on cultivated and non-cultivated host crops questionable. However, during this past summer (2011), *M. hapla* was able to survive in the field on peanut for a period of 4 months (D. W. Dickson, personal comm.). At this point we can speculate that if the nematode establishes in Florida, it may infect the same hosts as other *M. hapla* populations. Moreover, *M. hapla* is also known to have a wide host-range affecting more than 550 crop and weed species (Jepson, 1987). Currently, *Meloidogyne* spp. are not the most damaging nematodes on strawberries in Florida, but this could potentially change if more nematode-infected transplants are imported into the state. It is therefore important to put stricter regulations on clean strawberry transplants from the nurseries to ensure that growers are safe from such sources of nematode inoculum.

Introduction of endoparasitic nematodes such as root-knot nematode with the planting materials can be quite problematic because soil fumigation is only carried out prior to planting. Furthermore, endoparasitic nematodes are hard to target with any contact nematicide. Strawberry season in Florida runs from October to April, and after final harvest growers wait about 4-5 months before planting the next crop. Currently, quite a number of growers in the state are exploring the use of 2-yr-old plastic mulch. In this approach, strawberry planting beds are irrigated intermittently throughout summer. In such a case, survival of nematodes under these conditions in the soil is unknown. Until further studies are conducted on the survivability over two or more years, it remains unclear whether this nematode can survive under Florida conditions. Alternatively, growers may plant a cover crop and use a susceptible host such as a leguminous crop, which could only lead to an increase in pest numbers if the nematode is able to survive during the summer season.

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#### LITERATURE CITED

- Adam, M. A. M., M. S. Phillips, and V. C. Blok. 2007. Molecular diagnostic key for identification of juveniles of seven and economically important species of root-knot nematode (*Meloidogyne* spp). *Plant Pathology* 56:190-197.
- Averre, C. W., W. O. Cline, R. K. Jones, and R. D. Milholland. 2011. Diagnosis of strawberry diseases. North Carolina Cooperative Extension Service, North Carolina State University, Raleigh, NC <http://www.ces.ncsu.edu/depts/hort/consumer/agpubs/ag-386.pdf>. Accessed December 2011.
- Esbenshade, P. R., and A. C. Triantaphyllou, 1990. Isozyme phenotypes for the identification of *Meloidogyne* species. *Journal of Nematology* 22:10-15.
- Fourie, H., C. Zijlstra, and A. H. McDonald. 2001. Identification of root-knot nematode species occurring in South Africa using the SCAR-PCR technique. *Nematology* 37:675-680.
- Handoo, Z, A., A. M. Skantar, L. K. Carta, and D. P. Schmitt. 2005. Morphological and molecular evaluation of a *Meloidogyne hapla* population damaging coffee (*Coffea arabica*) in Maui, Hawaii. *Journal of Nematology* 37:136-145.
- 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 pattern-morphology. Pp. 69-77 in Barker K. R., C. C. Carter, and J. N. Sasser, eds. *An advanced treatise on Meloidogyne*. Volume II: Methodology, eds. NC State University Graphics, Raleigh, NC.
- Howard, C. M., A. J. Overman, J. F. Price, and E. A. Albrechts. 1985. Diseases, nematodes, mites, and insects affecting strawberries in Florida. Bulletin 857. Agricultural Experiment Stations, Institute of Food and Agricultural Sciences (IFAS), University of Florida, Gainesville, FL.
- Hu, M. X., K. Zhuo, and J. L. Liao. 2011. Multiplex PCR for the simultaneous identification and detection of *Meloidogyne incognita*, *M. enterolobii*, and *M. javanica* using DNA extracted directly from individual galls. *Phytopathology* 101:1270-1277.
- Jenkins, W. R. 1964. A rapid centrifugal-floatation technique for separating nematodes from soil. *Plant Disease Reporter* 48:692.
- Jepson, S. B. 1987. Identification of root-knot nematodes (*Meloidogyne* species). CAB International, Wallingford, UK.
- Joyce, S.A., A. P. Reid, F. Driver, and J. Curran. 1994. Application of polymerase chain reaction (PCR)

- methods to the identification of entomopathogenic nematodes. Pp.178-187 in A.M. Burnell, R.U. Ehlers, and J.P. Masson, eds. Genetics of entomopathogenic nematode-bacterium complexes. Proceedings of symposium and workshop, St. Patrick's College, Maynooth, Co, Kildare, Ireland. Luxembourg, DGXII, European commission .
- LaMondia, J. A. 2002. Seasonal populations of *Pratylenchus penetrans* and *Meloidogyne hapla* in strawberry roots. *Journal of Nematology* 34:409-413.
- Noling, J. W. 1999. Nematode management in strawberries. ENY-031. IFAS extension, University of Florida, Gainesville, FL <http://edis.ifas.ufl.edu/pdf/NG/NG03100.pdf> Accessed on December 14 2011.
- Noling, J.W., and A. Whidden. 2010. International issues and growing concerns for new nematode problems in the Florida strawberry industry. *Berry/Vegetable Times*, June 2010, UF IFAS Extension, University of Florida, Gainesville, FL. <http://strawberry.ifas.ufl.edu/BerryTimes/BVT0610.pdf>.
- Orui, Y. 1998. Identification of Japanese species of genus *Meloidogyne* (Nematoda: Meloidogynidae) by PCR-RFLP analysis. *Applied Entomology and Zoology* 33:43-51.
- Peres, N. A., J. F. Price, W. M. Stall, C. K. Chandler, B. M. Santos, S. M. Olson, S. A. Smith, and E. H. Simonne. 2010. Strawberry production in Florida. Pp. 263-272 in S. M. Olson and B. Santos, eds. *Vegetable production handbook for Florida 2010-2011*. UF IFAS Extension, University of Florida, Gainesville, FL.
- Powers, T. O., and T. S. Harris. 1993. A polymerase chain reaction method 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 nematode survey. *Journal of Nematology* 37:226-235.
- Qui, J. J., B. B. Westerdahl, C. Anderson, and V. M. Williamson. 2006. Sensitive PCR detection of *Meloidogyne arenaria*, *M. incognita*, and *M. javanica* extracted from soil. *Journal of Nematology* 38:434-441.
- Taylor, A. L., and J. N. Sasser. 1978. *Biology, identification and control of root-knot nematodes (Meloidogyne species)*. North Carolina State University Graphics, Raleigh, NC.
- Westerdahl, B. B. 2009. *Nematodes. UC IPM Pest management guidelines: Strawberry*. University of California, Davis, CA. <http://www.ipm.ucdavis.edu/PMG/r734200111.html> Accessed January 2012.

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