RESEARCH/INVESTIGACIÓN

MELOIDOGYNE SPP. INFECTING ASIAN VEGETABLES IN CENTRAL FLORIDA, USA

H. X. Bui*, M. Gu, G. Riva, and J. A. Desaeger

Entomology and Nematology Department, University of Florida, Gulf Coast Research and Education Center, Wimauma, FL; *Corresponding author: hungbui@ufl.edu

ABSTRACT

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Asian vegetables are important specialty crops in Florida, but no information is available on their host status to plant-parasitic nematodes, in particular to *Meloidogyne* spp. *Meloidogyne* spp. are one of the main soilborne pathogens in Florida and cause considerable damage to many vegetables. We conducted a nematode survey on 10 Asian vegetable farms in Hillsborough County, FL, all owned and operated by Vietnamese growers. A total of 66 root samples representing 20 commonly grown Asian vegetables were collected and evaluated for the presence of *Meloidogyne* spp. *Meloidogyne* females were dissected from roots and collected for molecular identification using primer sets TRNAH/MRH106 and MORF/MTHIS, followed by restricting PCR products using HinfI and MnlI restriction enzymes. Species-specific primers were used to confirm *Meloidogyne* spp. and mtDNA sequencing was used when needed to confirm the identification. We identified four *Meloidogyne* spp., *M. arenaria*, *M. enterolobii*, *M. haplanaria*, and *M. incognita*, infecting Asian vegetables. *Meloidogyne enterolobii* was the most commonly found *Meloidogyne* spp. and accounted for almost 75% of positive samples. Our study revealed that *Meloidogyne* spp. are widespread in Asian vegetable farms and especially *M. enterolobii*. *Meloidogyne enterolobii* is considered one of the most damaging *Meloidogyne* spp., and its high prevalence in Asian vegetable farms is of concern. Asian vegetable growers need to be made aware of this nematode as well of the threat of other *Meloidogyne* spp. to production. Nematode management programs need to be implemented in these Asian vegetable farms to help growers overcome the loss caused by *Meloidogyne* spp. and ensure the sustainability of their farms.

Key words: Asian vegetables, RFLP, species-specific primers, *M. arenaria*, *M. enterolobii*, *M. haplanaria*, *M. incognita*

RESUMEN

Bui, H.X., M. Gu, G. Riva, y J. A. Desaeger. 2022. *Meloidogyne* spp. infectando hortalizas Asiáticas en Florida Central, U.S.A. Nematropica 56-63.

Las hortalizas Asiáticas son cultivos especiales importantes en Florida, pero no hay información disponible sobre su estado como hospederos de nematodos fitoparásitos, en particular a *Meloidogyne* spp. *Meloidogyne* spp. son uno de los patógenos transmitidos por suelo más importantes en Florida y causan daño considerable en muchas hortalizas. Realizamos un estudio de nematodos en 10 fincas de hortalizas Asiáticas en el condado de Hillsborough, FL, todos propiedad y operados por productores vietnamitas. Se tomaron en total 66 muestras de raíces representando 20 hortalizas Asiáticas comúnmente cultivados y se evaluó la presencia de *Meloidogyne* spp. Hembras de *Meloidogyne* se disectaron de las raíces y se colectaron para la identificación molecular con los sets de cebadores TRNAH/MRH106 y MORF/MTHIS,

seguido de la digestión de los productos de PCR con las enzimas de restricción HinfI y MnlI. Se utilizaron cebadores específicos de especie para confirmar *Meloidogyne* spp. y se utilizó la secuenciación de mtDNA cuando fue necesario para confirmar la identificación. Identificamos cuatro *Meloidogyne* spp., *M. arenaria*, *M. enterolobii*, *M. haplanaria*, y *M. incognita*, infectando hortalizas Asiáticas. *Meloidogyne enterolobii* fue la *Meloidogyne* spp. más común y representó casi el 75% de las muestras positivas. Nuestro estudió mostró que *Meloidogyne* spp. están muy distribuidas en fincas de hortalizas Asiáticas y especialmente M. enterolobii. *Meloidogyne enterolobii* es considerada una de las *Meloidogyne* spp. mas dañinas y su alta prevalencia en fincas de hortalizas Asiáticas es de preocupación. Los productores de hortalizas Asiáticas deben ser concientes de este nematodo y de la amenaza de otras *Meloidogyne* spp. a la producción. Es necesario implementar programas de manejo de nematodos en estas fincas de hortalizas Asiáticas para ayudar a los productores a superar las pérdidas causadas por *Meloidogyne* spp. y asegurar la sostenibilidad de sus fincas.

Palabras clave: Vegetales Asiáticos, RFLP, imprimadores específicos de especie, *M. arenaria*, *M. enterolobii*, *M. haplanaria*, *M. incognita*

INTRODUCTION

Plant-parasitic nematodes are a costly burden for agricultural production with an estimated $125 billion loss annually (Bernard *et al*., 2017). They are often called the invisible enemy, as the damage they cause is often not recognized or mistaken for disease or nutrient problems (Bernard *et al*., 2017). *Meloidogyne* spp. (root-knot nematode) are the most well-known group of plant-parasitic nematodes globally and are extremely common in Florida's warm and sandy soils (Crow, 2020). They are one of the main reasons for the use of soil fumigants in much of Florida's high-value crop production systems (Noling, 1996). Many reports have confirmed the host status of ornamental plants, turfgrass, tomato, and other vegetables to various *Meloidogyne* spp. in Florida (Brito *et al*., 2007; 2008; 2010; Baidoo *et al*., 2016; Joseph *et al*., 2016; Crow, 2020). However, the host status of Asian vegetables to *Meloidogyne* spp. is not well-documented.

Currently, Asian vegetables are grown on more than 4,000 ha in Florida for local consumption and export to northern states, especially in the winter (Gu *et al*., 2021a). With the increasing demand for ethnic Asian vegetables in the U.S and the subtropical climate in Florida, many Asian vegetables can be grown year round. As such, they provide a continuous food source for plant-parasitic nematodes, especially for the many different *Meloidogyne* spp. that thrive in Florida's warm and sandy soils. In this study, we surveyed 10 Asian vegetable farms in Hillsborough County, FL for the presence of plant-parasitic nematodes, in particular *Meloidogyne* spp.

MATERIALS AND METHODS

Sample collection

Root and soil samples were collected from 10 Asian vegetable farms, all owned and operated by Vietnamese growers in Wimauma, Hillsborough County, FL. Farm size ranged from 2 to 52 ha and samples were collected between June 2020 and June 2021. A total of 66 root samples were collected from 20 commonly grown Asian vegetable crops (Table 1). Samples were collected with a shovel and stored at 4°C prior to collecting *Meloidogyne* spp. females for molecular identification.

Molecular identification of Meloidogyne *spp.*

DNA was extracted from a single female picked from infected roots using the NaOH digestion method (Hübschen *et al*., 2004). There were 3-5 females evaluated per sample. *Meloidogyne* spp. were identified using primer sets TRNAH/MRH106 or MORF/MTHIS, followed by restricting PCR products using HinfI and MnlI restriction enzymes (Table 2). The PCR was performed using the Eppendorf Mastercycler® Pro Thermal Cyclers (Eppendorf, Enfield, CT) in a 25 µl reaction volume consisting of 12.5 µl of 2× Apex™ Taq Red DNA Polymerase Master Mix (Genesee Scientific, San Diego, CA), 1 µl of DNA

Table 1. Asian vegetables grown in the surveyed farms in Wimauma, Hillsborough County, Florida.

Vegetable type	Common name	Scientific name
Fruit	Bitter gourd	*Momordica charantia*
	Luffa	*Luffa cylindrica*
	Okra	*Abelmoschus esculentus*
	Vietnamese eggplant	*Solanum macrocarpon*
	Vietnamese muskmelon (Indian cream cobra melon)	*Cucumis melo*
Leafy	Amaranth	*Amaranthus* sp.
	Malabar spinach	*Basella alba*
	Parsley	*Petroselinum crispum*
	Perilla	*Perrila frutescens* var. *crispa*
	Savory	*Satureja* sp.
	Sweet potato	*Ipomoea batatas*
	Thai Basil	*Ocimum basilicum*
	Tong Ho	*Glebionis coronaria*
	Tossa Jute	*Corchorus olitorius*
	Vietnamese Coriander	*Persicaria odorata*
	Water spinach	*Ipomoea aquatica*
Leafy and Tuber	Cassava	*Manihot esculenta*
Rhizome	Galangal	*Alpinia galanga/Alpinia officinarum*
	Ginger	*Zingiber officinale*
Stem	Indian Taro	*Colocasia gigantean*

Table 2. The primers used to identify *Meloidogyne* spp. associated with Asian vegetables in Wimauma, Hillsborough County, Florida.

Primer code	Primer sequence (5′–3′)	Reference
TRNAH	TGAATTTTTTATTGTGATTAA	Stanton *et al*., 1997; Pagan *et al*., 2015
MRH106	AATTTCTAAAGACTTTTCTTAGT	
MORF	ATC GGGGTTTAATAATGGG	
MTHIS	AAATTCAATTGAAATTAATAGC	
C2F3	GGTCAATGTTCAGAAATTTGTGG	Powers and Harris, 1993
1108	TACCTTTGACCAATCACGCT	
D2A	ACAAGTACCGTGAGGGAAAGT	Ye *et al*., 2015
D3B	TGCGAAGGAACCAGCTACTA	
Me-F (*M. enterolobii*)	AACTTTTGTGAAAGTGCCGCTG	Long *et al*., 2006
Me-R (*M. enterolobii*)	TCAGTTCAGGCAGGATCAACC	
Far (*M. arenaria*)	TCGGCGATAGAGGTAAATGAC	Adam *et al*., 2007
Rar (*M. arenaria*)	TCGGCGATAGACACTACAAACT	
Inc-K14-F (*M. incognita*)	CCCGCTACACCCTCAACTTC	Randig *et al*., 2002
Inc-K14-R (*M. incognita*)	GGGATGTGTAAATGCTCCTG	
Mi-F (*M. incognita*)	GTGAGGATTCAGCTCCCCAG	Adams *et al*., 2007
Mi-R (*M. incognita*)	ACGAGGAACATACTTCTCCGTCC	

extract, 0.5 µl of 10 µM each primer, and 10.5 µl of sterile water. The amplicons were separated by gel electrophoresis using a 1.2% Apex general purpose agarose gel (Genesee Scientific) that had been stained by GelRed® Nucleic Acid Stain (Biotium Inc., Hayward, CA), and visualized under UV light using the Gel DocTM EZ Imager (Bio-Rad Laboratories, Life Science Group, Hercules, CA). To confirm the *Meloidogyne* spp. identity, species-specific primers were used with some modification (Table 3). When needed, the mtDNA COXII region DNA was amplified using the primer

Table 3. PCR amplification proceedures for specific primers used to identify *Meloidogyne* spp. associated with Asian vegetables in Wimauma, Hillsborough County, Florida.

Primer	Initial degeneration	Response parameter (40 cycle)			Final extension
		Degeneration	Annealing	Extension	
TRNAH/MRH106	95°C, 15 min	94°C, 60 s	50°C, 60 s	68°C, 60 s	68°C, 10 min
MORF/MTHIS	95°C, 15 min	94°C, 60 s	50°C, 60 s	68°C, 60 s	68°C, 10 min
C2F3/1108	95°C, 15min	94°C, 45 s	55°C, 45 s	68°C, 60 s	68°C, 10 min
D2A/D3B	95°C, 15min	94°C, 45 s	55°C, 45 s	68°C, 60 s	68°C, 10 min
Me-F/Me-R	95°C, 15 min	94°C, 30 s	68°C, 30 s	72°C, 60 s	72°C, 10 min
Far/Rar	95°C, 15 min	94°C, 30 s	54°C, 30 s	72°C, 60 s	72°C, 10 min
Inc-K14-F/Inc-K14-R	95°C, 15 min	94°C, 30 s	55°C, 45 s	72°C, 60 s	72°C, 10 min
Mi-F/Mi-R	95°C, 15 min	94°C, 30 s	62°C, 30 s	72°C, 60 s	72°C, 10 min

sets C2F3/1108 and D2A/D3B (Table 2). Sequencing results were submitted to the National Center for Biotechnology Information (NCBI) website (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to search for sequence similarity (Altschul *et al*., 1990).

RESULTS AND DISCUSSION

Out of the 66 root samples, 62 samples had characteristic symptoms caused by *Meloidogyne* spp. Gall size varied from small (Thai basil, ginger, jute) to large (sweet potato, water spinach, luffa, Vietnamese muskmelon, Vietnamese eggplant) (Fig. 1). Across the Asian vegetables sampled, four *Meloidogyne* spp. were identified: *M. arenaria*, *M. enterolobii, M. haplanaria* and *M. incognita* (Table 4). Among these four identified *Meloidogyne* spp., *M. enterolobii* was found in 74% of the samples, *M. arenaria* and *M. incognita* were each found in 10% of the samples, and *M. haplanaria* was found in 6% of the samples. Thai basil and luffa had high incidence of infection with five *Meloidogyne* spp. infected root samples out of eight root samples and three *Meloidogyne* spp. infected samples out of five root samples, respectively.

Several *Meloidogyne* spp. infecting ornamental, horticultural, agronomic crops, turfgrass and weed plants have been reported in Florida (Brito *et al*., 2007, 2008, 2010; Baidoo *et al*., 2016; Joseph *et al*., 2016; Crow, 2020). Although in these reports some *Meloidogyne* spp. infecting Asian vegetables such as okra (*M. mayaguensis* aka *M. enterolobii*), sweet basil (*M. mayaguensis* aka *M. enterolobii*, *M. arenaria*), and ginger (*M. arenaria*) were identified (Brito *et al*., 2007, 2008, 2010), none of these crops came from actual commercial Asian vegetable farms. Also, this is the first report of various *Meloidogyne* spp. infecting Asian vegetables such as water spinach, luffa, Malabar spinach, perilla, jute, and amaranth.

In our survey, the very wide host range of *M. enterolobii* was confirmed, which included eight different host plants belonging to seven botanical families. *Meloidogyne incognita* and *M. arenaria* were found on five and four host plants belonging to five and three botanical families, respectively. *Meloidogyne haplanaria* was found on two host plants of two botanical families (Table 5). Many farms were infected with mixed populations of two to three *Meloidogyne* spp. (Table 4).

Meloidogyne enterolobii was first described in China in 1983 (Yang and Eisenback, 1983). Since its discovery, *M. enterolobii* has rapidly become the most widespread and destructive among *Meloidogyne* spp. worldwide due to its high pathogenicity and the ability to break down *Meloidogyne* spp. resistance of crops including soybean, sweetpotato, and tomato (Castagnone-Sereno, 2012; Philbrick *et al*., 2020; Collett *et al*., 2021). It is considered one of the most damaging *Meloidogyne* spp. and a major emerging threat to agriculture in the southeastern U.S. While it was first reported in the U.S. from Florida in 2004 (Brito *et al*., 2004), it now has been found in several other southeastern states (Ruter *et al*., 2019; Schwarz *et al*., 2020; Gu *et al*., 2021b; FIND*Me*, 2022).

The high prevalence of *M. enterolobii* in our limited survey is of concern. Many of the surveyed farms were established at least 10-30 years ago and it is impossible to tell how long *M. enterolobii* has existed in these fields. *Meloidogyne enterolobii* may have been spread across these Asian vegetable farms with infested plant material, which is a common practice among Vietnamese farmers. Also, these farmers are very isolated due to

language barriers and have no or very limited access to extension and pest management information. None of the farmers that we visited had ever heard about plant-parasitic nematodes and the risk they can pose to their crops. As a result, there is no nematode management being practiced, and there is no information on losses that nematodes cause on their farms. The diversity and continuous production of crops, sometimes more than 10 crops are grown on the same farm, further complicates management recommendations. Rotation and cover crop options are limited, especially considering that the majority of the crops are good hosts to *Meloidogyne* spp. and that fields are being planted almost year-round.

Figure 1. Galled roots of different Asian vegetables collected from the surveyed farms ranged from small to big size (A-E). A: Thai basil; B: Ginger, C: Sweetpotato, D: Luffa and E: Water spinach.

Table 4. Occurrence of *Meloidogyne* spp. infecting Asian vegetables in Wimauma, Hillsborough County, Florida.

Meloidogyne spp.	Accession number	Number of samples detected	Number of farms detected
M. enterolobii	MW802190, MW804235, MW507374, MW488150, MW507374	46	7
M. arenaria	MZ892613	6	3
M. incognita		6	4
M. haplanaria		4	2
Total samples		62	10

Table 5. *Meloidogyne* spp. associations with Asian vegetables in Wimauma, Hillsborough County, Florida.

Meloidogyne spp.	Host plant	Scientific name of host plant	Botanical family name
M. arenaria	Ginger	*Zingiber officinale*	Zingiberaceae
	Vietnamese muskmelon (Indian cream cobra melon)	*Cucumis melo*	Cucurbitaceae
	Jute	*Corchorus olitorius*	Malvaceae
	Okra	*Abelmoschus esculentus*	Malvaceae
M. enterolobii	Thai Basil	*Ocimum basilicum*	Lamiaceae
	Vietnamese eggplants	*Solanum macrocarpon*	Solanaceae
	Jute	*Corchorus olitorius*	Malvaceae
	Luffa	*Luffa cylindrica*	Cucurbitaceae
	Malabar spinach	*Basella alba*	Basellaceae
	Perilla	*Perrila frutescens var. crispa*	Lamiaceae
	Pumpkin	*Cucurbita pepo*	Cucurbitaceae
	Sweet potato	*Ipomoea batatas*	Convolvulaceae
M. haplanaria	Vietnamese muskmelon (Indian cream cobra melon)	*Cucumis melo*	Cucurbitaceae
	Okra	*Abelmoschus esculentus*	Malvaceae
M. incognita	Amaranth	*Amaranthus sp.*	Amaranthaceae
	Luffa	*Luffa cylindrica*	Cucurbitaceae
	Guava	*Psidium guajava*	Myrtaceae
	Thai Basil	*Ocimum basilicum*	Lamiaceae
	Water spinach	*Ipomoea aquatica*	Convolvulaceae

CONCLUSIONS

This is the first report on *Meloidogyne* spp. infecting Asian vegetables in Florida and in the U.S. Four different *Meloidogyne* spp. were identified, with *M. enterolobii* being the most common, found on eight different crops. More survey work and additional studies are needed to create awareness among Asian vegetable growers and to help them with nematode management, including limiting the spread of nematodes like *M. enterolobii*.

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