RESEARCH NOTE/NOTA DE INVESTIGACIÓN

INTERCEPTION OF *MELOIDOGYNE ENTEROLOBII* ON SWEETPOTATO IN LOUISIANA

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ABSTRACT

Santos Rezende, J., C. A. Clark, M. W. Sistrunk, and T. Watson. 2022. Interception of *Meloidogyne enterolobii* on sweetpotato in Louisiana. Nematropica 52:1-5.

In 2018, a Louisiana sweetpotato grower submitted severely deformed sweetpotato storage roots imported from North Carolina to the LSU AgCenter Nematode Advisory Service for further examination. Developing root-knot nematode females and egg masses were observed after peeling away the periderm and outer cortical cells. DNA was extracted from the mature females and used for species-level identification using polymerase chain reaction (PCR) and Sanger sequencing. PCR amplicon size and sequence data confirmed the identity as *Meloidogyne enterolobii*. This is the first report of *M. enterolobii* being intercepted on sweetpotato in Louisiana.

Key words: Guava root-knot nematode, *Ipomoea batatas*, Louisiana, *Meloidogyne enterolobii*, nematode interception, sweetpotato

RESUMEN

Santos Rezende, J., C. A. Clark, M. W. Sistrunk, y T. Watson. 2022. Intercepción de *Meloidogyne enterolobii* en camote en Louisiana. Nematropica 52:1-5.

En el 2018, un productor de camote en Louisiana envió unas raíces de almacenamiento de camote deformadas severamente importadas de North Carolina al LSU AgCenter Nematode Advisory Service. Hembras en desarrollo y masas de huevos del nematodo agallador se observaron luego de pelar el peridermis y las células corticales externas. Se extrajo ADN de las hembras maduras y se utilizaron para la identificación con la reacción en cadena de la polimerasa (PCR) y secuenciación Sanger. El tamaño del producto de PCR y la información de las secuencias confirmaron la identidad como *Meloidogyne enterolobii*. Este es el primer informe de *M. enterolobii* siendo interceptado en camote en Louisiana.

Palabras clave: El nematodo agallador de la guayaba, *Ipomoea batatas*, Louisiana, *Meloidogyne enterolobii*, intercepción de nematodos, camote

The introduction and dissemination of plant-parasitic nematodes pose an increasing threat to agricultural production systems as the world becomes more interconnected. Global and regional trade of seeds and plants creates a risk of introducing invasive nematodes. The introduction of a plant-parasitic nematode to a new area can disturb and sometimes completely render

unprofitable the production of susceptible agricultural crops. Root-knot nematodes (*Meloidogyne* spp.) are one of the most widespread and damaging genera of plant-parasitic nematodes, causing substantial economic losses globally. Among the root-knot nematodes, *Meloidogyne enterolobii* (Yang and Eisenback, 1983) is receiving increasing attention worldwide due to the yield loss that this nematode can cause and a rapidly expanding distribution (Castillo and Castagnone-Sereno, 2014; Philbrick *et al.*, 2020).

Meloidogyne enterolobii is considered one of the most damaging root-knot nematodes in the world due to its high reproductive rate and ability to overcome root-knot nematode resistance in many important crops, including cotton, pepper, soybean, sweetpotato, and tomato (Berthou *et al.*, 2003; Brito *et al.*, 2007; Cetintas *et al.*, 2008; Castagnone-Sereno, 2012; Brito *et al.*, 2020). This nematode has a broad host range, parasitizing various fruit trees, ornamentals, vegetable crops, and is known to cause significant damage and economic losses on most agronomic crops (Castillo and Castagnone-Sereno, 2014). *Meloidogyne enterolobii* is found in tropical and sub-tropical growing regions throughout the world, including Central and South America, Africa, Asia, and Europe (Castillo and Castagnone-Sereno, 2014). *Meloidogyne enterolobii* has also been found in North America parasitizing ornamental plants grown in Florida (Brito *et al.*, 2004), and sweetpotato, cotton, and soybean crops grown in North Carolina and South Carolina (Ye *et al.*, 2013; Rutter *et al.*, 2018).

Meloidogyne enterolobii is particularly damaging to sweetpotato production. Total loss of a sweetpotato field has been reported due to *M. enterolobii* infestation in North Carolina (NCDA&CS, 2017). Symptoms caused by this nematode can result in severely deformed and often unmarketable storage roots showing large knots, cracks, and unsightly dark spots in the flesh of the root (Fig. 1). The use of contaminated sweetpotato storage roots is a primary means of dissemination of *M. enterolobii* into new production fields, endangering not only sweetpotato but other nearby and rotational crops.

In 2018, a Louisiana sweetpotato grower noticed unusual symptoms on sweetpotato storage roots that were imported from North Carolina and had recently been bedded in Morehouse Parish, LA for slip production. The storage roots showed large bumps and cracks on the surface and dark spots were observed within the root cortical tissue. The grower submitted the symptomatic sweetpotato storage roots to the LSU AgCenter Nematode Advisory Service (NAS; Baton Rouge, LA) for further examination. Developing root-knot nematodes and unusually large numbers of females and egg masses were observed after peeling away the periderm and outer cortical cells.

Single egg masses removed from the contaminated sweetpotatoes were used to inoculate 'Rutgers' tomato planted in sterilized field soil and grown in a greenhouse to observe galling symptoms and culture nematodes for further investigation. Thirty days after inoculation, severe galling was observed on the root system. Three individual females were excised from the infected roots and DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit (Hilden, Germany). Extracted DNA was used for species identification through analysis of PCR band size and Sanger sequencing. PCR amplicons were obtained using

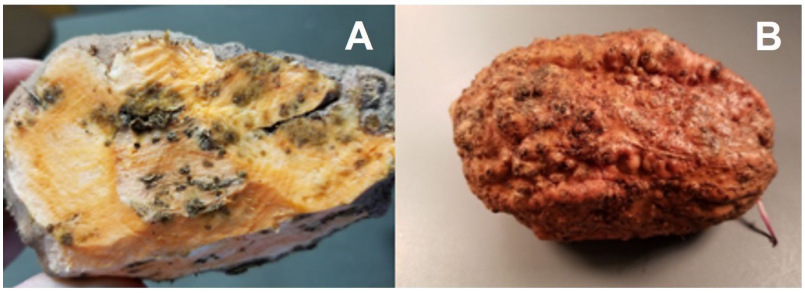

Figure 1. Sweetpotato storage roots intercepted in Morehouse Parish, LA. A) Damage caused by *Meloidogyne enterolobii* inside storage roots of sweetpotato. B) Sweetpotato showing pronounced bumps and cracking associated with *M. enterolobii* infestation.

rDNA-IGS2 internal primers (Me-F 5′-AACTTTTGTGAAAGTGCCGCTG-3′ and Me-R 5′-TCAGTTCAGGCAGGATCAACC-3′) described by Long *et al.* (2006) and thermocycle conditions described by Hu *et al.* (2011). PCR band size results were consistent with the literature for *M. enterolobii*, producing a 236 bp DNA fragment (Long *et al.*, 2006) (Fig. 2). *Meloidogyne incognita* race 3 genomic DNA and *M. incognita* specific primers MiF/MiR described by Meng *et al.* (2004) were used to show specificity of PCR amplification. Following PCR amplification and visualization, one *M. enterolobii* DNA fragment was purified, sequenced, and nematode identity was confirmed through BLAST analysis.

Following nematode species confirmation, a containment protocol was implemented in the contaminated field in an attempt to prevent the establishment of *M. enterolobii* where symptomatic sweetpotato storage roots were bedded. The contaminated sweetpotato bed was sprayed with an herbicide mixture (Roundup® (Monsanto, St. Louis, MO, USA) and Liberty® (Bayer CropScience, Research Triangle Park, NC, USA)) and immediately covered with a second layer of clear plastic, which remained in place for approximately 4 months to rot the contaminated storage roots directly in the bedded field. After the clear plastic was removed, a soil fumigant (Telone® II [TriCal Inc., Hollister, CA, USA] at 10 gal/A) was broadcast applied to a depth of 50 cm twice over a 14-day application interval. The field was then kept free of weeds through periodic application of herbicides from 2018 to 2020. In spring 2021, a non-host corn crop was planted in the field.

Soil samples were collected from the contaminated field in August 2020 and September 2021. The field was divided into four 0.4-ha sections, from which approximately 20 soil cores

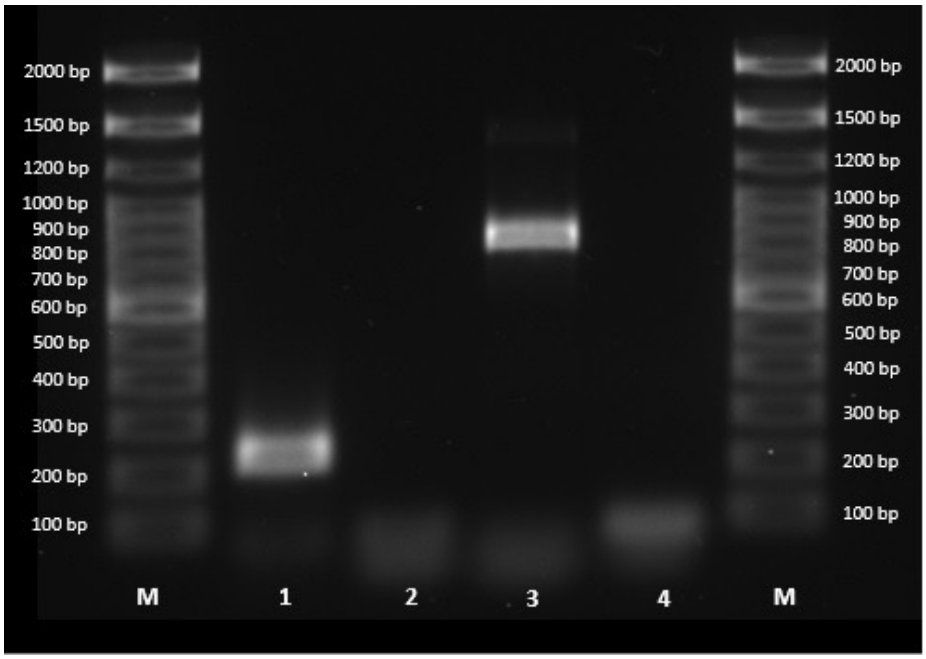

Figure 2. PCR amplification products using *Meloidogyne enterolobii* (Me) and *Meloidogyne incognita* (Mi) specific primer sets. 1) PCR product (~ 250 bp) of *M. enterolobii* using genomic DNA extracted from a root-knot nematode female excised from tomato roots inoculated with a single egg mass from an infected Morehouse Parish storage root and amplified using MeF/MeR primers. 2) No PCR product of *M. enterolobii* using genomic DNA extracted from a root-knot nematode female excised from tomato roots inoculated with a single egg mass from an infected Morehouse Parish storage root and MiF/MiR primers. 3) PCR product (~ 850 bp) of *M. incognita* genomic DNA extracted from a root-knot nematode female amplified using MiF/MiR primers. 4) No PCR product of *M. incognita* genomic DNA extracted from a root-knot nematode female of a race 3 population using MeF/MeR primers. M) 100bp DNA ladder.

(2.5-cm-diam. by 25 cm deep) were collected per section and subsequently processed at the LSU AgCenter NAS. No plant-parasitic nematodes were detected at either sampling date. Extensive surveying conducted from 2019-2021 in sweetpotato, soybean, cotton, and sugarcane fields (n = 291) has failed to detect *M. enterolobii* in any Louisiana production field (Watson, unpublished data), suggesting that this pest has not successfully established in Louisiana. Additionally, the Louisiana Department of Agriculture and Forestry (LDAF) enacted a quarantine to restrict entry of plant material and farming equipment from Florida, North Carolina, and South Carolina. Since the 2018 interception, LDAF has implemented a *M. enterolobii* quarantine that prohibits the importation of nursery stock and sweetpotatoes from Florida, North Carolina, and South Carolina, with the only exception being nursery stock that is certified free of *M. enterolobii* by the state of origin's regulatory agency. In addition, LDAF receives notifications for nursery stock shipped from the three quarantined states and does periodic nematode sampling of the incoming material.

In Louisiana, *M. enterolobii* is considered an important and threatening new nematode pest. The rapid implementation of quarantine restrictions on states where this nematode has established, and use of certified clean planting material will undoubtably reduce the risk of introducing *M. enterolobii* into Louisiana. Although *M. enterolobii* has not established in Louisiana cropping systems, it is important that growers perform routine inspections of their fields and submit soil samples to the LSU AgCenter NAS for examination. This will increase the chances of early detection of *M. enterolobii* and implementation of successful management strategies.

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