RESEARCH NOTE/NOTA DE INVESTIGACIÓN

FIRST REPORT OF *MELOIDOGYNE ENTEROLOBII* ON SWEET POTATO IN FLORIDA, USA

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ABSTRACT

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Sweet potatoes with galled roots were collected from a commercial Asian vegetable farm located in Wimauma, FL in July 2020. Morphological characteristics of second-stage juveniles and perineal patterns of mature females were consistent with those of *Meloidogyne enterolobii*. Intergenic space region 2 (IGS2) and D2-D3 regions on the rDNA, and the COXII region on mtDNA were sequenced to confirm this root-knot nematode species as *M. enterolobii*. This is the first report of this species found on sweet potato in Florida.

Key words: DNA sequencing, Guava root-knot nematode, *Meloidogyne enterolobii*, polymerase chain reaction, species-specific primers, sweet potato

RESUMEN

Gu, M., H.X. Bui, W. Ye, y J. A. Desaeger. 2021. Primer reporte de *Meloidogyne enterolobii* en camote en Florida, EE.UU. Nematropica 51:36-40

Raíces de camote con agallas fueron recolectadas en una finca comercial de vegetales asiáticos, ubicada en Wimauma, Florida en Julio 2020. Características morfológicas de juveniles en segundo estado y diseños perineales de hembras maduras fueron consistentes con las de *Meloidogyne enterolobii*. La región espaciadora intergénica 2 (IGS2) y la región D2-D3 del ADNr, y la región COXII del ADNmt fueron secuenciadas para confirmar esta especie como *M. enterolobii*. Este es el primer reporte de esta especie en camote en Florida.

Palabras clave: secuenciación de ADN, El nematodo agallador de la guayaba, *Meloidogyne enterolobii*, reacción en cadena de la polimerasa, imprimadores especie-específicos, camote

Sweet potato (*Ipomoea batatas* L.) is native to the tropical regions of America and is grown worldwide (Hahn, 1977). Tuberous roots are mainly the edible part, but young shoots and leaves can be also used in many Asian cuisines. Florida was ranked fourth in the United States for sweet potato production in 2018 with nearly 2,428 ha of

sweet potato grown in Miami-Dade and Suwannee counties (National Agricultural Statistics Service, 2019). In July 2020, wilting and stunted sweet potato plants with galled root symptoms (Fig. 1A) were observed in a commercial Asian vegetable farm in Wimauma, FL (27°44.834' N, 82°15.723' E). No visible damage was observed on sweet potato storage roots.

Root-knot nematode (RKN) infected roots and soil were collected for nematode extraction. Second-stage juveniles (J2) of RKN were extracted from a 200 cm^3 soil sample collected from the rhizosphere of symptomatic sweet potato roots by the modified Baermann funnel technique with a two-day incubation period; the population density was 204 RKN J2/200 cm^3 soil. Images of RKN J2 were taken using a ZEISS Axioscope 5 microscope at a magnification of 400X. Then, ImageJ software was used to pairwise stitch multiple images and measure the morphological characteristics of the RKN J2 (Fig. 1B) (Preibisch *et al*., 2009). Morphological measurements of J2 (n = 17, mean ± standard deviation, range) were: body length = 434.6 ± 27.4 (387.6 - 482.9) µm, body width = 15.0 ± 1.4 (11.7 - 18.3) µm, and stylet length = 14.3 ± 0.7 (13.1 - 15.8) µm. Perineal patterns of RKN mature females (n = 5) were cut and cleaned in 45% lactic acid, then mounted in glycerin on glass slides (Abrantes and Santos, 1989). The perineal patterns were photographed using a ZEISS Axioscope 5 at a magnification of 400X (Fig. 1C). Morphological characteristics and measurements of the J2 and perineal patterns of females were consistent with those described for *Meloidogyne enterolobii* Yang and Eisenback, 1983 (Yang and Eisenback, 1983; Philbrick *et al*., 2020).

Molecular methods were employed to further identify the RKN population to the species level. DNA was extracted from a single female picked from infected sweet potato roots using the NaOH digestion method (Hübschen *et al*., 2004). DNA specimens were replicated three times and labeled as 113, 196, and 197. PCR was performed using a Eppendorf Mastercycler® Pro Thermal Cycler (Enfield, CT) in a 25 µl reaction volume consisting of 12.5 µl of 2× Apex™ Taq Red DNA Polymerase

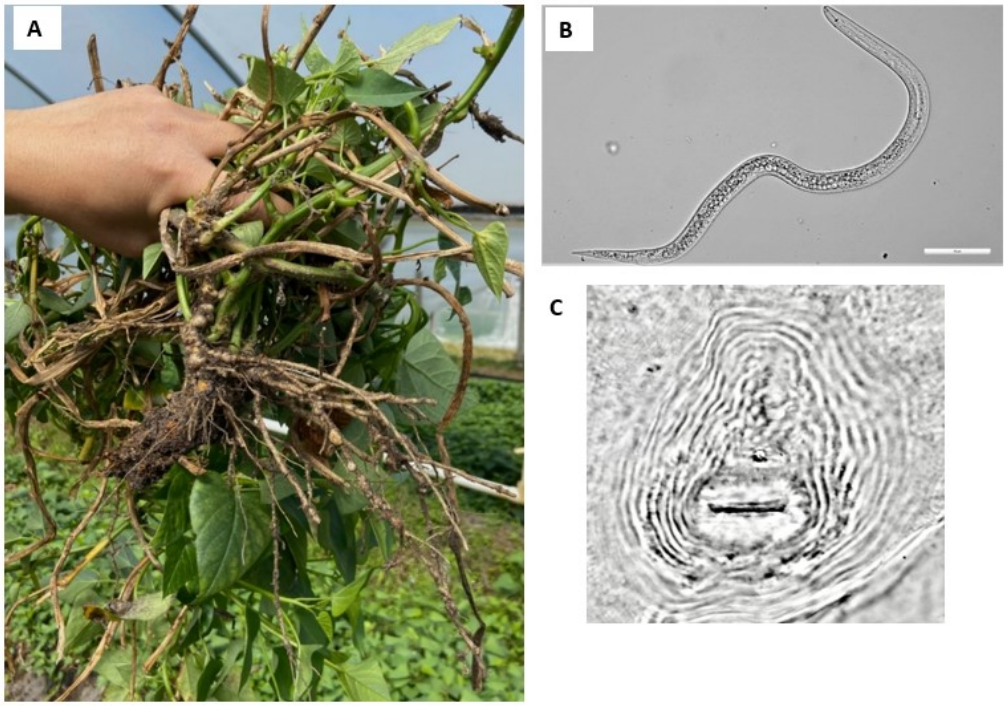

Figure 1. (A) Galled roots of sweet potato, *Ipomoea batatas* L., infested by *Meloidogyne enterolobii* Yang and Eisenback, 1983 collected from a commercial Asian vegetable farm located in Hillsborough County, Florida, USA. (B) The second-stage juvenile of *M. enterolobii* extracted from soil. (C) The perineal pattern of a *M. enterolobii* female isolated from infested sweet potato root.

Master Mix (Genesee Scientific, San Diego, CA), 1 µl of DNA extract, 0.5 µl of each 10 µM primer stock, and 10.5 µl of sterile water. The amplification conditions included an initial denaturation at 95°C for 4 min followed by 35 cycles of 94°C for 30 sec, 68°C for 30 sec, and 72°C for 30 sec, and a final extension at 72°C for 10 min; annealing temperature was set according to the primer set. The amplicons were separated by gel electrophoresis using a 1.2% Apex general purpose agarose (Genesee Scientific, San Diego, CA) gel stained with 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). Species identification was confirmed with the species-specific primer set Me-F/Me-R (Long *et al*., 2006), and resulted in a 236 bp DNA fragment (Fig. 2). The results of the molecular identification were consistent with the literature for *M. enterolobii* (Long *et al*, 2006; Villar-Luna *et al*., 2016). The D2-D3 expansion segment of the 28S rDNA and the mtDNA CoxII region DNA were amplified by PCR using the primer sets 28S391a/28S501 and C2F3/1108 (Ye *et al*., 2021). The sequencing results were submitted to the National Center for Biotechnology Information (NCBI) with GeneBank Accession Nos. MW804235 and MW802190. The molecular sequences had 100% identity with *M. enterolobii* for D2/D3 (MW488150, KP901079, KP411230) and CoxII (MW507374, MN809527, KX214350).

Meloidogyne enterolobii has been reported in North and South Carolina, Florida, and Louisiana (Brito *et al*., 2008; Ye *et al*., 2013; Overstreet *et al*., 2019; Rutter *et al*., 2019; Moore *et al*., 2020a, 2020b). Due to its emerging threat to many crops in tropical and subtropical areas, quarantine

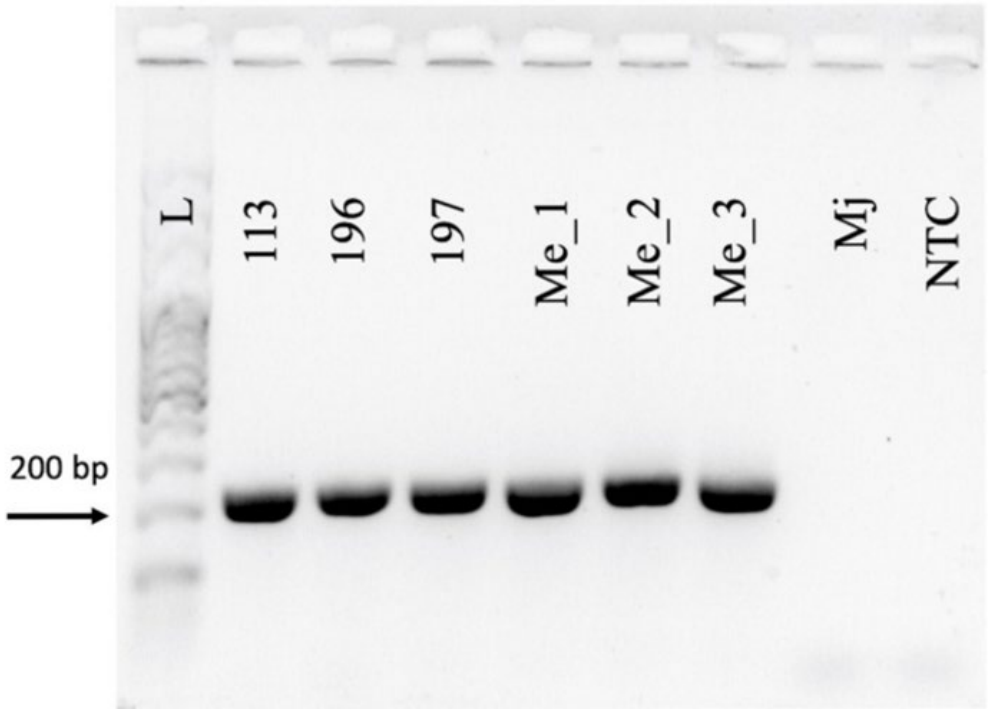

Figure 2. PCR amplification products of DNA extracted from matured root-knot nematode (RKN) females using *Meloidoogyne. enterolobii* Yang and Eisenback, 1983 specific primers Me-F/Me-R. Samples 113, 196, and 197 were RKNs collected from the infested field; samples Me_1, Me_2 and Me_3 were *M. enterolobii* collected from greenhouse cultures from Nematology Lab in the University of Florida Gulf Coast Research and Education Center (GCREC); sample Mj was *M. javanica* (Treub 1885) Chitwood, 1949 culture kept in the GCREC; NTC = negative control; L = Quick-Load® Purple 100 bp DNA ladder (New England BioLab, Inc., Ipswich, MA).

regulations for *M. enterolobii* have been strictly applied in Arkansas, Louisiana, and Mississippi (Moore *et al.*, 2020a). *M. enterolobii* has been detected on sweet potato in Louisiana, North Carolina and South Carolina (Overstreet *et al*., 2019; Rutter *et al*., 2019; Ye *et al*., 2021), however, this is the first detection of this RKN species on sweet potato in Florida and it could have a large impact to sweet potato production in this state.

It is unclear whether *M. enterolobii* is native or introduced in Florida, but the first report of this species (then called *M. mayaguensis*) in the continental United States was from Florida (Brito *et al*., 2004). No prior nematode surveys have been done on Asian vegetable farms in Florida, therefore, we do not know if the presence of *M. enterolobii* on these farms is due to recent introduction(s) or not. Asian vegetable farms in Florida are typically small (< 20 ha) and isolated due to the language barrier. Most farmers are unaware of even the existence of nematode pests. More extensive nematode surveys and dissemination efforts will be conducted on vegetable farms (Asian and other) in Florida to further study the distribution and importance of *M. enterolobii* in Florida. The implications of this finding are significant, not only for Asian vegetable growers in Florida, but for sweet potato and vegetable growers in the southeastern United States as a whole.

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