RESEARCH NOTE/ NOTA DE INVESTIGACIÓN

FIRST REPORT OF *MELOIDOGYNE JAVANICA* INFECTING INDUSTRIAL HEMP (*CANNABIS SATIVA*) IN FLORIDA, USA

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ABSTRACT

Brito, J. A., M. R. Moore, S. J. Vau, R. Xue, L. A. Combee, R. Leahy, C. G. Roberts, and J. Stanley. 2023. Nematropica 53:1-5.

Industrial hemp (*Cannabis sativa*) is an emerging crop in the USA, including Florida. A root-knot nematode identified as *Meloidogyne javanica* was found infecting industrial hemp in a field in Marion County, FL. Symptoms included leaf yellowing and stunted plant growth in a patchy distribution. Infected roots were severely galled and egg masses were clearly visible outside the roots. Species identification was performed using biochemical (EST and MDH), DNA (*NADH5* and *COX2*) and morphological analyses. To our knowledge, this is the first report of *M. javanica* infecting industrial hemp under field conditions in Florida, USA.

Key words: Identification, Javanese root-knot nematode, *Meloidogyne floridensis*, plant-parasitic nematodes

RESUMEN

Brito, J. A., M. R. Moore, S. J. Vau, R. Xue, L. A. Combee, R. Leahy, C. G. Roberts, y J. Stanley. Primer reporte de *Meloidogyne javanica* infectando cánamo industrial (*Cannabis sativa*) en Florida, EUA. Nematropica 53:1-5.

El cáñamo indrustrial (*Cannabis sativa*) es un cultivo emergente en los EUA, incluyendo Florida. Un nematodo formador de agallas identificado como *Meloidogyne javanica* fue encontrado infectando cáñamo industrial en un campo en el condado de Marion County, FL. Los síntomas incluyeron amarillamientos de las hojas y retraso en el crecimiento de la planta en una distribución en parches. Raíces infectadas estaban severamente agalladas y las masas de huevos estaban claramente visibles fuera de las raíces. La identificación de la especie se realizó usando análisis bioquímicos (EST and MDH), ADN (*NADH5* and *COX2*) y morfológicos. A nuestro conocimiento este es el primer reporte de *M. javanica* infectando cáñana industrial bajo condiciones de campo en Florida, EUA.

Palabras chave: Identificación, el nematodo Javanés formador de agallas, *Meloidogyne floridensis*, nematodos parásitos de plantas

In 2018, the United States Government (2018 Farm Bill, PL 115-334) removed hemp (*Cannabis sativa*) and cannabis-derived products with very low concentrations of the psychoactive compound delta-9-tetrahydrocannabinol (≤ 0.3% THC dry weight) from the definition of marijuana in the Controlled Substances Act. These cannabis products are currently treated as any other agricultural commodity and must meet applicable Food and Drug Administration requirements and standards (Anonymous, 2018). Additionally, other regulations might be imposed on hemp by certain states in the USA. In Florida, industrial hemp is an emerging crop and can be used for plant fiber, essential oil, and seed production. As with many other crops, hemp is prone to damage by insects and pathogens, including plant-parasitic nematodes (PPN). For instance, cannabis was reported to be susceptible to *Meloidogyne* spp., including *M. incognita* (McPartland, 1996; Van Biljon, 2017), *M. javanica* (Van Biljon, 2017; Esfahani and Ahmadi, 2010; Pofu *et al*., 2010; Song *et al*., 2017), and *M. enterolobii* (Ren *et al*., 2020). A comprehensive literature review focusing on PPN interactions with hemp was recently published (Bernard *et al*., 2022). Due to the recent legalization of hemp in the USA, very little is known about the best cultivars of industrial hemp suitable for production, crop management, and susceptibility to diseases, including PPN. Recently, *M. incognita* was reported from an industrial hemp field in Alabama (Lawaju *et al*., 2021). In a Florida greenhouse study, *M javanica* was inoculated onto six hemp varieties with results indicating 6 to 60 juveniles per root system with some infection variability among varieties (Coburn and Desaeger, 2019).

For this first report, a root sample collected from two symptomatic plants in an industrial hemp field, Marion County, Florida, USA was submitted to the Nematode Diagnostic Laboratory at the Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, Florida, USA (FDACS-DPI). The infected plants showed yellowing leaves, stunted growth, and incipient wilting; the secondary and tertiary roots were clearly galled, a typical symptom induced by root-knot nematodes. The bases of the taproots were swollen and many females were observed upon dissection. Nematode species identification was performed using biochemical, morphological, and DNA-based methods. Isozyme analyses, esterase (EST) and malate dehydrogenase (MDH), were carried out on egg-laying females (N = 50) (Brito *et al*., 2008; 2021). The morphology of the female perineal patterns (N = 9) and morphometrics of selected characters of second-stage juveniles (J2) (N = 20) (isolate 08222022-7643-1-1R) were examined. The species-specific phenotype EST = J3 for *M. javanica* and MDH = N1 were detected in all the females analyzed. Similarly, both the morphology of the perineal patterns and the morphometrics obtained from the J2, including body length (431.3 ± 16.4 µm; range 406.1 – 453.3 µm), body width (16.1 ± 1 µm; 14.2 – 17.3 µm), tail length (53 ± 2.2 µm; 48.2 – 56.9 µm), hyaline tail length (15.3 ± 0.7 µm; 14.1 – 16.4 µm), length of excretory pore to head end (87.1 ± 1.4 µm; 84.5 – 89.3 µm), stylet length (9.9 ± 0.5 µm; 9.1 – 10.9 µm), stylet knob height (1.1 ± 0.2 µm; 0.8 – 1.4 µm), stylet knob width (2.5 ± 0.3 µm; 2 – 3.1 µm), dorsal esophageal gland opening (3.2 ± 0.4 µm; 2.5 – 3.7 µm), and the De Man ratios a (27 ± 2 µm; 24.6 – 31.6 µm), c (8.2 ± 0.3 µm; 7.7 – 8.9 µm) were in agreement with previous reports for *M. javanica* (Chitwood, 1949; Whitehead, 1968; Jepson, 1987). Nematode species identification determined with DNA sequencing was performed using J2 of the isolate 08222022-7643-1-1R collected from hemp and mentioned above. In addition, *M. floridensis* (MFGNV14) (Maquilan *et al*., 2018) was included in this DNA analysis because it is an emerging nematode isolate in Florida and lacks cytochrome c oxidase II (*COX2*) and NADH dehydrogenase subunit 5 (*NADH5*) sequence data useful for species identification (Janssen *et al*., 2016). Second-stage juveniles were picked into 260 µl of 1× phosphate-buffered saline and DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). Kapa Hifi HotStart ReadyMix PCR Kit (Roche Diagnostics, Risch-Rotkreuz, Switzerland) was utilized for PCRs and targeted fragments of the mitochondrial genes *COX2* and *NADH5*. PCR primer sets (*COX2*F/*COX2*R and *NAD5F2*/*NADH5R1*) and thermocycle conditions followed Janssen *et al*. (2016). Positive PCRs were verified by gel electrophoresis and PCR products were purified for bidirectional sequencing on the Applied Biosystems SeqStudio platform with BigDye Terminator v3.1 cycle sequencing chemistry (Applied Biosystems, Foster City, CA).

Chromatograms were trimmed and assembled in Geneious Prime 2022.2.2. Newly generated consensus sequences (*M. floridensis NADH5*: OP612782; *M. floridensis COX2*: OP612784; *M. javanica NADH5*: OP612783; *M. javanica COX2*: OP612785) were aligned in MEGA7 (Kumar *et al*., 2016) using the default settings of MUSCLE (Edgar, 2004). New sequences were compared to the GenBank *COX2* and *NADH5* (PopSets: 1005136704 and 1005137048) datasets produced by Janssen *et al*. (2016) and Moore *et al*. (2020). Only *Meloidogyne* isolates with both *COX2* and *NADH5* sequences were further analyzed. The resulting concatenated data matrix (771 bp wide; trimmed to 100% data coverage per terminal taxon) included exemplars of 11 *Meloidogyne* species. Datafiles (concatenated alignment, partition file, Newick tree file, and list of GenBank accessions) were deposited at Zenodo (10.5281/zenodo.7331034).

Maximum-likelihood analysis of the concatenated data matrix was performed in the online W-IQtree portal (Trifinopoulos *et al*. 2016). The best fit model of sequence evolution for each

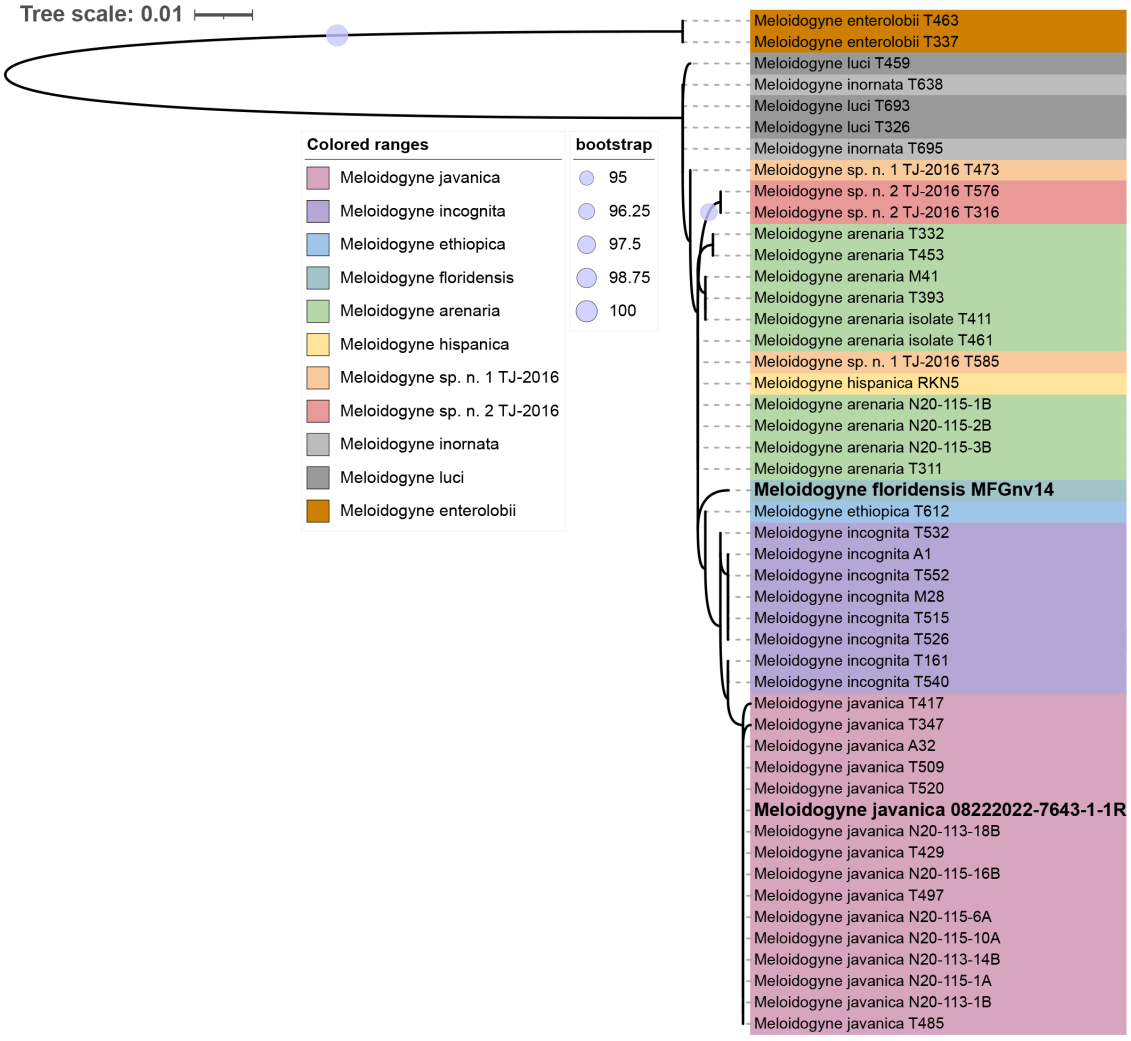

Figure. 1. Maximum-likelihood bootstrap consensus tree of the concatenated *NADH5* and *COX2* dataset. Terminal taxa are highlighted by species. Ultrafast bootstrap support values ≥ 95 are labeled on their corresponding node. *Meloidogyne javanica* isolate 08222022-7643-1-1R from the roots of *Cannabis sativa* and *M. floridensis* isolate MFGnv14 from peach are presented in bold text.

partition (TN+F+I for *COX2*; TIM+F+G4 for *NADH5*) was selected by ModelFinder (Kalyaanamoorthy *et al*., 2017) using Bayesian information criteria. Node support values were calculated using 5,000 ultrafast ML bootstrap replicates (Hoang *et al*., 2017). The resulting bootstrap consensus tree (log-likelihood of -371.619558) was imported into the Interactive Tree of Life (Letunic and Bork, 2021) online portal where the topology was aesthetically arranged, node support values were added, and terminal taxa were highlighted (Fig. 1). Though bootstrap support for recovered clusters was generally low, the *Meloidogyne* isolate from hemp was a 100% match to previously reported *M. javanica* isolates (Janssen *et al*., 2016; Moore *et al*., 2020) (Fig. 1). Considering that *M. javanica* is commonly found in Florida, breeders and growers of industrial hemp should ponder this finding in their decisions. To our knowledge, this is a first report of *M. javanica* infecting industrial hemp under field conditions in Florida, USA.

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Received: *Accepted for publication:*

28/XI/2022 *26/I/2023*

Recibido: *Aceptado para publicación:*