RESEARCH NOTE/NOTA DE INVESTIGACIÓN

FIRST REPORT OF *MELOIDOGYNE INCOGNITA* INFECTING *CANNABIS SATIVA* IN FLORIDA, USA

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ABSTRACT

Coburn, J. D., D. Moreira, J. Freeman, M. Gu, H. X. Bui, and J. A. Desaeger. 2022. First report of *Meloidogyne incognita* infecting *Cannabis sativa* in Florida, USA. Nematropica 52:103-106.

Cannabis sativa (hemp) roots with small to medium galls were collected from a field in Gadsden County, Quincy, Florida in October 2020. Morphological characteristics of perineal patterns of mature females and second-stage juveniles were identified as *Meloidogyne incognita*. The species was also confirmed by species-specific primers Mi-F/Mi-R, and the mitochondrial COXII region with amplification of a PCR product using the primers C2F3/1108 which were sequenced to confirm the morphological identification. This is the first report of *M. incognita* on hemp in Florida.

Key words: Cannabis sativa, DNA sequence, *Meloidogyne incognita*, Mi-specific primers, southern root-knot nematode

RESUMEN

Coburn, J. D., D. Moreira, J. Freeman, M. Gu, H. X. Bui, y J. A. Desaeger. 2022. Primer reporte de *Meloidogyne incognita* afectando *Cannabis sativa* en Florida, USA. Nematropica 52:103-106.

Raíces de *Cannabis sativa* (cáñamo) con pequeñas a medianas agallas fueron recolectadas de un campo en el condado de Gadsden, Quincy, Florida en Octubre 2020. Características morfológicas de diseños perineales de hembras maduras y de juveniles en segundo estado fueron identificadas como *Meloidogyne incognita*. La especie también fue confirmada con los cebadores específicos de especie Mi-F/Mi-R, y se secuenció la región mitocondrial COXII con una amplificación de un producto de PCR con los cebadores C2F3/1108 para confirmar la identificación morfológica. Este es el primer reporte de *M. incognita* en cáñamo en Florida.

Palabras clave: Nematodo agallador del Sur, *Meloidogyne incognita*, *Cannabis sativa*, Secuencia de ADN, Mi-cebadores específicos.

Hemp (*Cannabis sativa*) is an annual dioecious crop of industrial importance due to the versatility of the plant, as it is cultivated for its fiber, seed oil, and cannabinoids (CBD) (Small and Marcus, 2002). With the removal of hemp from the controlled substance list, it is now an agricultural commodity in the United States, and because of this, it is a new crop that is now grown in Florida (Fike, 2016). In October 2020, CBD hemp 'Cherry Blossom' plants in a field in Gadsden County,

Quincy, FL, United States (30°32'44.6" N; 84°35'48.0" W), were observed to have numerous small to medium galls on the roots while no apparent aboveground symptoms were observed (Fig. 1). *Meloidogyne* females and egg masses were obtained from the symptomatic roots by dissecting the galls under a stereomicroscope. *Meloidogyne* second-stage juveniles (J2) were obtained by hatching egg masses in water for 48 to 72 hr at 25°C. The morphological characteristics of females and J2 were observed and measured with a Zeiss Axioscope 5 (Carl Zeiss Microscopy, Jena, Germany) at 400X and 1000X. Then, ImageJ software (Schindelin *et al*., 2012) was used to pairwise stitch multiple images and measure the morphological characteristics of J2 (Preibisch *et al.*, 2009). Perineal patterns of 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. The perineal patterns of females were oval with a high dorsal arch, weak lateral field, and wavy striae (Fig. 2).

Morphological measurements of J2 (n = 20; mean ± standard deviation, range) included body length = 420.8 ± 23.4 (382.3 to 469.4) µm, body width = 16.4 ± 0.7 (15.0 to 17.4) µm, and stylet length = 14.5 ± 0.7 (13.1 to 15.4) µm. The perineal patterns of females and J2 were consistent with those described for *Meloidogyne incognita* (Kokoid and White, 1919; Chitwood, 1949). To confirm the identification of *M. incognita*, DNA was extracted from females (n = 5) that were dissected from symptomatic roots using NaOH digestion method (Hübschen *et al*., 2004). The mitochondrial DNA COXII region was amplified by PCR using the primers C2F3/1108 (Powers and Harris, 1993); the species was also confirmed with species-specific primers Mi-F/Mi-R (Meng *et al*., 2004). PCR products of COXII region were sequenced by the GENEWIZ (South Plainfield, NJ, USA), and the results were recorded in the NCBI with GeneBank Accession No. MZ066384. The sequences had 100% identity with *M. incognita* in LC547506, MK102799, and MK102798.

Figure 1. Galled root system of hemp caused by *Meloidogyne incognita*.

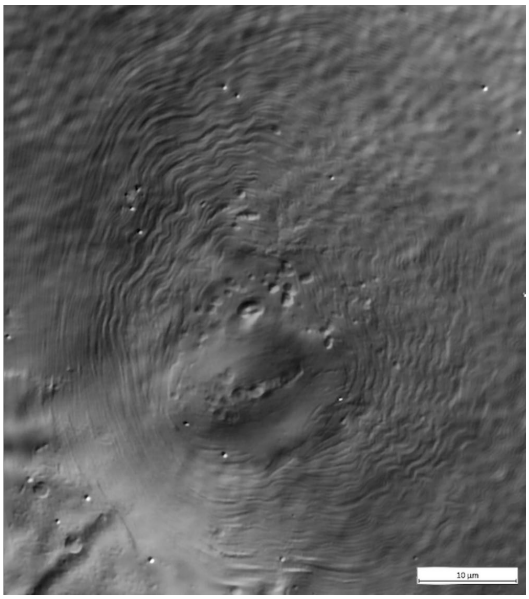

Figure 2. The perineal pattern of *Meloidogyne incognita* female isolated from infected hemp root.

The pathogenicity of this nematode was tested in a greenhouse at 24°C in March 2021. Five one-month-old hemp seedlings ('Cherry Blossom' x 'T1') were inoculated with 1,500 *M. incognita* J2 hatched from egg masses. Another five uninoculated hemp seedlings were used as controls. At 60 days after inoculation, all five inoculated plants displayed similar galling characteristics on the roots to those observed in the field. The average gall index (scale 0-10; Bridge and Page, 1980) was 2.0 ± 0.7 on inoculated plants. Using the NaOCl method (Hussey and Barker

1973) 533 ± 350 *M. incognita* eggs were extracted from roots. Using a modified Baermann funnel technique (Hooper and Evans, 1993; Forge and Kimpinski, 2007; Saikai *et al*., 2021) 55.6 ± 15.4 *M. incognita* J2/200 cm^3 of soil were recovered. The nematode reproduction factor (the final population density divided by the initial population density) was 1.19 ± 0.36 (Nicol *et al*., 2010). No symptoms were observed on the control plants. These results confirmed the pathogenicity of *M. incognita* on hemp.

Several recent studies have demonstrated that *Meloidogyne* spp. can reproduce on hemp (Kotcon *et al*., 2018; Coburn and Desaeger, 2019; Bernard and Chaffin, 2020; Hansen *et al*., 2020; Bernard *et al*., 2022), but other than anecdotal, few reports exist on *Meloidogyne* species infecting hemp in the field (Song *et al*., 2017; Ren *et al*., 2021; Lawaju *et al*., 2021; Bernard *et al*., 2022). This is the first detection of *M. incognita* on hemp in Florida. In Florida, *Meloidogyne* spp. are widespread and have the potential to be an important biotic threat to the commercial production of hemp. Additional studies to evaluate the impact of *M. incognita* and other nematodes on hemp in Florida are ongoing.

ACKNOWLEDGMENTS

The authors thank the staff and students at the nematology laboratory at the University of Florida's Gulf Coast Research and Education Center, Wimauma, Florida for technical support.

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Received: *Accepted for publication:*

30/V/2022 *4/X//2022*

Recibido: *Aceptado para publicación:*