Diseases Caused by Nematodes

First Report of *Meloidogyne enterolobii* on Industrial Hemp (*Cannabis sativa*) in China

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Industrial hemp (*Cannabis sativa* L.) is an important annual herbaceous plant that has great economic value. In March 2020, many small to large galls were observed on the roots of industrial hemp plants growing in a field in Tianya District, Sanya City, Hainan Province, China. The diseased plants did not show obvious aboveground symptoms. Females were obtained by dissecting the galls under a stereomicroscope. Second-stage juveniles (J2s) were collected for 24 to 48 h from egg masses hatching at 25°C. The morphological characteristics of females and J2s were observed and measured with a Nikon E200 microscope at 100× and 400× magnification. The perineal patterns of females were oval, with coarse and smooth striae, moderately high to high dorsal arches, and lacking distinct lateral lines. Measurements of females ($n = 20$) included vulval slit length = 26.4 ± 2.7 (23.6 to 31.2) μm and vulval slit to anus distance = 22.1 ± 2.4 (18.9 to 24.7) μm . The J2s had long and narrow tails with bluntly rounded tail tips and distinct hyaline tail termini. Measurements of J2s ($n = 20$) were body length = 432.4 ± 23.1 (386.8 to 465.3) μm , body width = 16.2 ± 1.8 (14.2 to 18.9) μm , stylet = 12.8 ± 0.5 (11.6 to 13.7) μm , dorsal esophageal gland orifice to stylet base = 3.6 ± 0.4 (3.1 to 4.8) μm , tail = 52.9 ± 3.8 (46.3 to 61.4) μm , and hyaline tail length = 15.7 ± 2.6 (12.5 to 19.2) μm . These morphological characteristics were consistent with the original description of *Meloidogyne enterolobii* (Yang and Eisenback 1983). Molecular biology analyses were also conducted to confirm species identification. Genomic DNA was extracted from a single J2 (Song et al. 2017).

The ITS rRNA gene and D2-D3 region of the 28S rRNA gene were amplified using the primers 18s/26s (TTGATTACGTCCCTGCCCTTT/TTTCACTCGCCGTACTAAGG) and D2A/D3B (ACAAGTACCGTGAGGGAAAGT/TCGGAAGGAACCAGCTACTA), respectively (Subbotin et al. 2006; Vrain et al. 1992). The ITS rRNA gene sequence (765 bp, GenBank accession no. MT654126) was 100% identical to *M. enterolobii* sequences previously obtained from Fujian, China (MT406251) and Vietnam (MG773551), and the D2-D3 region sequence of 28S rRNA gene (759 bp, MT654127) revealed 99% identity with *M. enterolobii* sequences from Fujian (MT193450) and Taiwan (KP411230), China, and South Carolina, U.S.A. (MH800969). In addition, species identification was further confirmed using the *M. enterolobii*-specific primers Me-F/Me-R (AACTTTTGTGAAAGTGCCGCTG/TCAGTTCAAGGCAGGATCAACC) and MK7-F/MK7-R (GATCAGAGGCGGCGCATTGCGA/CGAACTCGCTCGAACTCGAC), respectively (Long et al. 2006; Tigano et al. 2010). The PCR products had the expected fragment lengths of 236 and 520 bp, which were consistent with those previously reported for *M. enterolobii* (Long et al. 2006; Tigano et al. 2010). The pathogenicity test of this nematode was performed in a greenhouse at 25°C. Ten industrial hemp seedlings (cv. Longma No. 5) maintained in 12-cm diameter and 12-cm high pots containing autoclaved soil, were inoculated with 800 freshly hatched J2s of the original population of *M. enterolobii* per plant, and eight noninoculated seedlings were used as controls. At 60 days after inoculation, all inoculated plants exhibited gall symptoms on the roots similar to those in the field, and the nematode reproduction factor (final population density/initial population density) was 18.2. No symptoms were observed on control plants. These results confirmed the pathogenicity of *M. enterolobii* on industrial hemp. To our knowledge, this is the first report of industrial hemp as a new host of *M. enterolobii* in China. Because *M. enterolobii* has a broad host range, a strong pathogenicity, and a high reproduction rate, it could become a major threat to industrial hemp production. Further monitoring and research on effective control strategies are needed.

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