Diseases Caused by Nematodes

First Report of *Meloidogyne enterolobii* Infecting *Ixora chinensis* in China

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Ixora chinensis is a common flowering ornamental shrub native to China and southeast Asia. It has been introduced to tropical and subtropical regions of the world. In August 2021, the Plant Nematology Laboratory at Fujian Agriculture and Forestry University received heavily galled roots of cutting plants from Lishui Town (23°17'1.8564" N, 113°5'34.89" E, Guangdong Province, China, for disease diagnosis. The roots presented galls similar to those associated with root-knot nematodes (RKN), *Meloidogyne* spp. Upon inspection, there were one to several female RKN in each gall, and egg masses were often completely embedded within the gall. Males were found in the galls. Symptoms included severely reduced plant growth and leaf yellowing. Morphological and molecular analyses were conducted to identify the species. Females and egg masses were collected from the roots, and egg masses were incubated in water at 25°C to obtain second-stage juveniles (J2s). Morphological measurements from 20 J2s and perineal patterns from 20 adult females matched the original description of *Meloidogyne enterolobii* (Yang and Eisenback 1983). The perineal patterns were round to oval with fine, dense stripes; dorsal arches were moderately high to high and mostly lacking lateral lines, but when present, the lines were not conspicuous. Measurements of J2s included: stylet = $13.2 \pm 0.5 \mu\text{m}$ (12.4 to 14.0 μm), dorsal pharyngeal gland orifice to stylet base = $51.8 \pm 3.0 \mu\text{m}$ (47.0 to 56.8 μm), tail = $51.8 \pm 3.0 \mu\text{m}$ (47.0 to 56.8 μm), and hyaline tail terminus = $16.0 \pm 2.0 \mu\text{m}$ (11.3 to 17.7 μm). Amplification and sequencing of the rDNA internal transcribed spacer (ITS; ITS1_5.8S ITS2) region was done with primers V5367/26S (TTGATTACGTCCCTGCCCTTT/TTTCACTCGCGTTACTAAGG) (Vrain et al. 1992) and the D2-D3 region of the 28S rDNA with primers D2A/D3B (ACAAGTACCGTGAGGGAAAGT/TCGGAAGGAACAGCT

ACTA) (Subbotin et al. 2006). A 765-bp DNA fragment was obtained for the ITS region, and the sequences (GenBank accession nos. OL444880 to OL444882) were compared with those in GenBank using BLAST and showed 99.16 to 99.87% identity with known sequences of *M. enterolobii* from China (MK850138, MK850135, and MT406251). The D2-D3 region sequences of the 28S rDNA (GenBank accession nos. OL455021 to OL455023) were 99.21 to 100% identical to *M. enterolobii* sequences from China (MN017115, MN017118, and MT193450). Identification was further confirmed using PCR with a species-specific primer set Me-F/Me-R (AACTTTTGTGAAAGTGCCGCTG/TCAGTTCAGGCAGGATCAACC) (Long et al. 2006). For pathogenicity tests, single seedlings (12 to 15 cm) from stem cuttings of *I. chinensis* were maintained in 16-cm diameter pots with sterilized sandy loam. Six replicates were inoculated with 5,000 freshly hatched J2s of the original population, and another six replicates without inoculation were control plants. The plants were maintained in a greenhouse at 25 to 30°C and 65 to 80% relative humidity. After 60 days, all inoculated plants were stunted and yellowing; galling symptoms on root tips and secondary feeder roots were similar to those observed in the field, and females and egg masses were obtained by dissecting galls. No gall symptoms were observed on control plants. These results confirmed the nematodes' pathogenicity. *M. enterolobii* is one of the most damaging species of RKN due to its wide host range, pathogenicity, and ability to develop and reproduce on several crops carrying resistance genes (Castagnone-Sereno 2012). To our knowledge, this is the first report of infection of *M. enterolobii* on *I. chinensis*. As cuttings are generally used for the propagation of *I. chinensis*, the spread of this nematode through infested soil and infected cutting seedlings of *I. chinensis* is likely. Considering the increasing impact of this nematode in China, this report is essential for control measures to be taken to avoid its spread to other regions or host crops.

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