Disease Note

Diseases Caused by Nematodes

First Report of Meloidogyne enterolobii Galling Amaranthus hybridus Roots in Sinaloa, Mexico

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In May 2024, after the cucumber winter crop cycle, the mulched raisedbeds were infested with Amaranthus hybridus L. weed. Roots from 20% of the plot area showed galling symptoms (≈50% of each root system was galled). Root systems from five plants were collected and washed. Female root-knot nematodes (RKNs) were found and dissected for individual DNA extractions (n = 20) with NaOH. PCR identification was carried out with universal Meloidogyne primers (MF/MR) and species-specific primers for M. incognita (Mi-F/Mi-R), Meloidogyne enterolobii (Me-F/Me-R), and M. javanica (Fjav/Rjav) (Hu et al. 2011; Long et al. 2006; Meng et al. 2004; Zijlstra et al. 2000). Only the universal and M. enterolobii-specific primers amplified the expected size fragments (≈230 bp). To confirm the RKN identity, root samples of 3 g (n = 10) were taken for DNA extraction with CTAB. The 28S rRNA D2-D3 expansion domains, 5S ribosomal RNA gene, and intergenic spacer were amplified using MF/MR and Me-F/Me-R primers, respectively. To obtain RKN inoculum, egg masses (n = 40) were dissected from A. hybridus roots and individually placed into a 2-week-old cucumber seedling substrate. Seedlings were transplanted into 1.0-liter pots filled with sterilized sand and substrate (1:1 v/v) and grown for 30 days in a nursery. Eggs were extracted from the cucumber roots by stirring in NaOCl (Gómez-González et al. 2021). For pathogenicity tests, A. hybridus seeds

were spread on a germination tray, watered, and placed in a nursery. After germination, 20 individual plants were transplanted in 1.0-liter pots as described above. A 1-ml aliquot containing 1,000 eggs of RKNs was pipetted into each pot. Control plants received 1.0 ml of sterilized tap water. Pots were set in a completely randomized design in a greenhouse ($28 \pm 7^{\circ}$ C, $60 \pm$ 4% relative humidity, and 13.5-h photoperiod) for 35 days. Subsequently, the roots were extracted and washed. The galling levels were determined as percentages. Root samples of 10 g were stirred in NaOCl to determine the final egg numbers (Pf). The reproduction factor was determined by dividing the inoculum number (Pi) (Garabedian and Van Gundy 1983). Pathogenicity assays were conducted two times. The identity of M. enterolobii was confirmed by PCR from a female from each pot as described before (n = 20). The A. hybridus root systems had 32% of galled roots and 387 ± 10 eggs per gram of root. Galling was absent on control plants. The RKN reproduction factor was 1.3. The reidentification by PCR was positive for M. enterolobii in all samples. A representative amplified fragment was sequenced using the MeF/MeR primers. The 28S RNA sequence (accession no. PQ834249) had 99.87% identity with 100% query coverage to 30 sequences of M. enterolobii. The 5S rRNA sequence (PQ300130) had 99.53% identity with 100% query coverage to 23 sequences of M. enterolobii. The weed A. hybridus is widely distributed in Mexico (Villaseñor Rios and Espinosa García 1998). Key horticultural crops such as tomato, pepper, cucumber, and eggplant have been reported as hosts of M. enterolobii in Sinaloa (Castro-López et al. 2024). During fallow periods between horticultural crop cycles, weeds such as A. hybridus invade mulched beds and take advantage of moisture and fertilizer remnants, serving as alternative hosts for pathogens. To our knowledge, this is the first report of M. enterolobii parasitizing A. hybridus in Sinaloa, Mexico. These results will help farmers to take precautions in fields with a record of this RKN.

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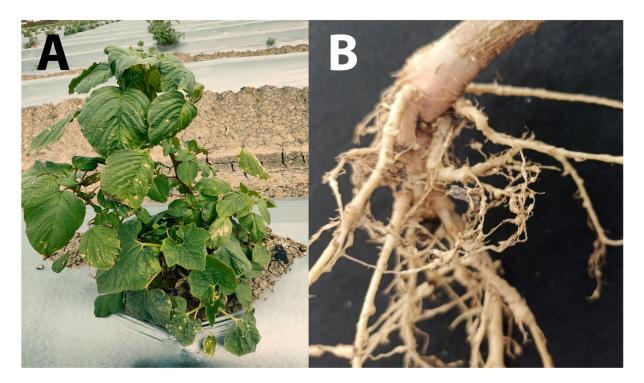


Fig. S1. A) Mulched beds invaded by *Amaranthus hybridus*. B) Galled roots of *A. hybridus* by *Meloidogyne enterolobii*.