Diseases Caused by Nematodes

First Report of the Root-Knot Nematode *Meloidogyne incognita* Infecting *Ixeris chinensis* in Hebei Province, China

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Ixeris chinensis (Thunb.) Nakai is an important medicinal plant, and young seedlings are also used as a wild vegetable in China. In recent years, *I. chinensis* has been cultivated in Shandong and Hebei Provinces, China. In August 2018, *I. chinensis* plants growing in greenhouses of Langfang City (116°36'28.62" N; 39°36'36.44" E), Hebei Province, exhibited typical symptoms caused by root-knot nematodes (RKNs; *Meloidogyne* spp.). The plants grew slowly, leaves were small and few, and color became lighter. Galls and egg masses were visible on roots and white, pear-shaped adult females observed inside the roots. The number of galls per plant ($n = 15$) was 62.87 ± 11.88 (42 to 95) and egg masses 16.53 ± 4.70 (9 to 26). Nematodes were extracted from soil and root samples ($n = 15$) using standard procedures (Barker 1985) and RKNs found in 100% of samples. Morphological and molecular studies were conducted on second-stage juveniles (J2s), eggs, and females with eggs. Females ($n = 30$) were pear-shaped, 505.81 to 799.81 (665.03 ± 76.48) μm in length, and 276.81 to 584.50 (397.98 ± 70.42) μm in width, with a round perineal pattern, and the dorsal arch is relatively high, squarish and with wavy striae. Body length of J2s ($n = 20$) varied from 349.39 to 416.51 (382.15 ± 16.99) μm , body width was 14.13 to 17.97 (15.68 ± 1.19) μm , stylet length 11.52 to 14.89 (13.48 ± 0.92) μm , tail length 36.12 to 52.15 (42.69 ± 4.46) μm , hyaline tail terminus length 8.91 to 15.30 (12.49 ± 2.03) μm , and presented well-developed esophageal glands. Eggs ($n = 20$) were oval with length/width $84.05 \pm 4.14/38.21 \pm 1.84$ μm . These morphological characteristics were similar to the description of *M. incognita* according to Zeng et al. (2014) and Whitehead (1968). The sizes of observed females in *I. chinensis* plant were smaller compared with *M. incognita* isolate characterized by Zeng et al. (2014), but they were similar to Whitehead (1968). Species

identification was further confirmed by PCR with *M. incognita*-specific primers Mi-F/Mi-R (5'-GTGAGGATTTCAGCTCCCCAG-3', 5'-ACGAGGAACATACTTCTCCGTCC-3') (Meng et al. 2004) and 28S rDNA D2/D3 region primers MF/MR (5'-GGGGATGTTTGAGGCAGATTG-3', 5'-AACCGCTTCGGACTTCCACCAG-3') (Hu et al. 2011). A 995-bp and a 478-bp DNA fragment were obtained with primers Mi-F/Mi-R and MF/MR, respectively. Fragments were sequenced (MK410954 and MK410953, respectively) and compared with available sequences in NCBI. Sequences were 100% identical to the JN005841, JN005843, JN005844, and JN005845 and the AF435794, MH332653, and MH332652 *M. incognita* sequences, respectively. Morphological and molecular characterization supports the identification of the isolate found on *I. chinensis* as *M. incognita*. Fifteen *I. chinensis* seedlings (five- to six-leaf stage) grown in sterilized soil individually were also inoculated with 1,200 *M. incognita* J2s (Pi) per seedling, using a sterilized micropipette and maintained at 25 to 28°C in a greenhouse. The test was repeated three times independently. After 7 weeks, plants were uprooted; the number of galls (196.3 ± 2.5), egg masses (43.6 ± 0.5), and final population density were evaluated from the eggs and J2s collected per plant from roots (Pf; $6,905.6 \pm 81.0$); and the reproduction factor ($R_f = \text{Pf}/\text{Pi}$; 5.76 ± 0.1) was calculated. *I. chinensis* was considered a good host for *M. incognita* in Langfang. Previous reports showed that *I. chinensis* was moderately resistant to *M. incognita* (Tsay et al. 2004). Our results are somewhat in contradiction, probably due to differences in environmental conditions or genetic diversity of RKNs or *I. chinensis*. *M. incognita* is the most frequent RKN species in China, with a wide distribution and range of host plants, and it causes severe yield losses. To our knowledge, this is the first report of *I. chinensis* as a host of *M. incognita* in Hebei Province, China.

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