Diseases Caused by Nematodes

First Report of Northern Root-Knot Nematode *Meloidogyne hapla* on *Bupleurum chinense* in Gansu Province, China

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Bupleurum chinense is an important traditional medicine in China with anti-inflammatory and immunomodulatory effects (Navarro et al. 2001). Diseases of *B. chinense* have been caused by fungi (rust and root rot) and viruses (cucumber mosaic virus and broad bean wilt virus 2) (Zhang et al. 2009) but not by nematodes. Root-knot nematodes (RKNs, *Meloidogyne* spp.) are destructive plant-parasitic nematodes with high adaptability and diversity, infecting >5,500 species (Azevedo de Oliveira et al. 2018). In October 2020, symptoms of dwarfing, leaf yellowing, and roots with many knots on *B. chinense* were seen in fields in Dingxi, Gansu, northwest China (N 35° 19' 42"; E 104° 2' 24"). Hundreds of eggs, mature males, and females were exuded from dissection of washed root knots. Morphology was examined with an optical microscope. Female ($n = 15$) perineal patterns were oval with slightly dorsal arches, and lateral lines and punctations on the anus in some specimens. Measurements (mean $\pm$ SD [range]) of females ($n = 20$): body length (L) = 525.23 ± 59.88 μ m (439.72 to 659.93 μ m), maximum body width (W) = 403.92 ± 57.17 μ m (311.01 to 513.34 μ m), stylet length (St) = 11.28 ± 1.05 μ m (9.82 to 12.91 μ m), median bulb width (MBW) = 31.13 ± 3.32 μ m (23.66 to 35.55 μ m), distance from anterior end to center of median esophageal bulb valve (MB) = 64.45 ± 3.44 μ m (58.62 to 71.92 μ m), and dorsal gland orifice to stylet (DGO) = 3.79 ± 0.60 μ m (2.72 to 5.00 μ m). Males ($n = 20$): L = $1,038.25 \pm 90.34$ μ m (877.28 to 1,206.12 μ m), St = 18.13 ± 1.48 μ m (15.10 to 20.12 μ m), body length divided by greatest body width (a) = 31.77 ± 4.03 (23.29 to 41.16), MBW = 10.97 ± 0.78 μ m (9.05 to 12.31 μ m), MB = 64.81 ± 3.45 μ m (59.59 to 71.38 μ m), DGO = 4.05 ± 0.47 μ m

(3.11 to 5.08 μ m), and spicule length (Spic) = 22.57 ± 1.91 μ m (19.26 to 26.43 μ m). J2s ($n = 25$): L = 381.73 ± 25.85 μ m (336.96 to 419.98 μ m), St = 10.52 ± 1.03 μ m (9.15 to 12.14 μ m), a = 24.35 ± 2.10 (20.45 to 28.29), DGO = 3.02 ± 0.42 μ m (2.42 to 3.79 μ m), body length divided by tail length (c) = 8.90 ± 0.86 (7.71 to 10.48), and tail length divided by body width at anus (c') = 4.18 ± 0.50 (3.47 to 5.04). According to morphological characteristics, it was preliminarily identified as *Meloidogyne hapla* Chitwood, 1949 (Whitehead 1968). DNA was extracted from 10 females, and the ITS region and the D2-D3 region of 28S rDNA were amplified using primers TW81/AB28 (GTTTCCGTAGGTGAACCTGC/ATATGCTTAAGTTCAGCGGGT) (Subbotin et al. 2000) and D2A/D3B (ACAAGTACCGTGAGGGAAAGTTG/TCGGAAGGAACCAGCTACTA) (De Ley et al. 1999), respectively. PCR products were purified and sequenced. The ITS and D2-D3 regions were 557 and 762 bp, respectively. The ITS region sequence (GenBank OK030559) was 99.46 to 99.82% identical to *M. hapla* from China (MT490918), New Zealand (JX465560), Australia (AF516722), and Japan (LC030357). The D2-D3 sequence (OK030558) was 99.58 to 100.00% identical to *M. hapla* from Canada (MW182329), Ethiopia (KJ645432), the United States (KP901086), and China (MN446015). Fragments obtained using the specific primers of *M. hapla* (Mh-F/Mh-R) were 462 bp, which was consistent with *M. hapla* (Feng et al. 2008). Thus, the RKNs on *B. chinense* in China were identified as *M. hapla*. Six *B. chinense* seedlings were planted in sterilized soil in 16-cm-diameter, 20-cm-deep plastic pots in the greenhouse at 20 to 25°C. After 21 days, 2,000 J2s/pot were inoculated; six uninoculated seedlings were a control. After 90 days, all inoculated plants showed similar symptoms to those in the field, and the reproduction factor (final population density/initial population density) was 1.47. No symptoms were seen on controls. These results proved that the nematode infecting *B. chinense* is *M. hapla*. This is the first report of *B. chinense* as a host of *M. hapla* in China. *B. chinense* is cultivated on ~19.1 million ha in Gansu, yielding 40% of China's output (Wang et al. 2017). The short, stunted roots of *B. chinense*-infected *M. hapla* harm its medical quality and restrict development of the local Chinese herbal medicine industry.

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