

First Report of the Spiral Nematode *Helicotylenchus microlobus* Infecting Soybean in North Dakota

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Abstract: Spiral nematodes (*Helicotylenchus* spp.) are common plant-parasitic nematodes in fields of many crops. In June 2015, two soil samples were collected from a soybean field in Richland County, ND. Nematodes were extracted from soil using the sugar centrifugal flotation method (Jenkins, 1964). Plant-parasitic nematodes were identified to genus based on morphological features and counted. Both samples contained spiral nematodes from 1,500 to 3,300 per kilogram of soil. In June and August 2016, 10 soil samples were collected from the same field. Nematodes were extracted, and nine of the samples had spiral nematodes ranging from 125 to 3,065 per kilogram of soil. One soil sample with 1,500 spiral nematodes per kilogram was used to inoculate two soybean cultivars Sheyenne and Barnes each in four replicates. After 15 wk of growth at 22°C in a greenhouse room, the population of spiral nematodes was found to have increased greatly. The final density was 9,300 ± 1,701 spiral nematodes per kilogram of soil for Sheyenne and 9,451 ± 2,751 for Barnes. The reproductive factor in Sheyenne and Barnes was 6.2 and 6.3, respectively, indicating that this spiral nematode infects and reproduces well on these two soybean cultivars. Infected soybean roots had small brown lesions on the surface. Individual spiral nematodes were handpicked and examined morphologically and molecularly for species identification. Morphological measurements of adult females ($n = 15$) included body length (mean = 708.5 µm, range = 600.0–812.0 µm), stylet (27.6, 26.0–29.0), body width (28.3, 25.0–33.0), lip region end to posterior end of pharyngeal glands (142.5, 130.0–152.0), anal body width (15.8, 14.0–17.5), tail length (20.3, 15.0–25.0), tail annules (11.6, 10.0–14.0), a (25.0, 21.4–27.1), b (5.0, 4.4–5.7), c (35.4, 30.2–41.7), c' (1.3, 1.0–1.6), and V (61.8%, 60.0–63.7). The spiral nematode was identified as *Helicotylenchus microlobus* according to morphological and morphometric characteristics (Subbotin et al., 2015). DNA was extracted from single nematodes ($n = 8$) using the Proteinase K method (Kumari and Subbotin, 2012). The internal transcribed spacer (ITS) region of rDNA was amplified with the primers rDNA2/rDNA1.58S (Cherry et al., 1997). The PCR products were then purified and sequenced. The consensus ITS rDNA sequence (accession no. KY271078, 822 bp) that was deposited into the GenBank shared 99% identity with two isolates of *H. microlobus* from California (KM506860.1 and KM506859.1) and one isolate of *H. microlobus* from Spain (KM506862.1) (Subbotin et al., 2015). It had only 91% sequence identity with seven isolates of *H. pseudorobustus* (KM506875.1, KM506880.1, KM506876.1, KM506874.1, KM506872.1, KM506879.1, and KM506878.1) from California, Switzerland, and New Zealand, a spiral nematode species very closely related to *H. microlobus* in morphology. The molecular tests confirmed the identity of this spiral nematode as *H. microlobus*. The *H. microlobus* nematode was reported as one of the most commonly observed spiral nematodes in soil samples in the state of Minnesota, and all 13 soybean cultivars tested except Hawkeye were rated as hosts (Taylor, 1960). To our knowledge, this is the first report of *H. microlobus* in North Dakota.

Key words: first detection, *Helicotylenchus microlobus*, identification, North Dakota, soybean.

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