First Report of the Fig Cyst Nematode, *Heterodera fici* Kirjanova, on Fig Tree, *Ficus carica*, in Ontario, Canada

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Abstract: Although fig trees are a popular ornamental fruit tree in subtropical regions, some hardy species, such as Ficus carica, have been grown in the west coast of British Columbia and southern Ontario in Canada. The fig cyst nematode, Heterodera fici Kirjanova, is a pest on fig plants, and the heavy infestation can cause retarded growth and yellowing of leaves (Maqbool et al., 1987). In the spring of 2016, a sample of rhizosphere from a potted fig (F. carica) seedling was submitted to the Nematology Laboratory, Canadian Food Inspection Agency. The sample was collected from a nursery in Niagara-on-the-Lake, Ontario, Canada, during an inspection to support export certification. The fig trees in the nursery had been grown in the outside fields during the growing seasons and potted and moved to indoor during the winters for last 3 years. The sample was subjected to a nematode extraction process, including decanting and sieving and misting, and lemon-shaped cysts and second-stage juveniles of Heterodera sp. were recovered from the sample examined. The morphological and molecular analyses of the cysts, vulval cone, and second-stage juveniles from both the roots and the crushed cysts identified the species as Heterodera fici Kirjanova. The cysts were characterized by their dark brown color and lemon shape, as well as distinct necks and vulval cones. The vulval cones were observed having an ambifenestrate fenestra (Fig. 1A), dome-shaped bullae scattered around the underbridge plane (Fig. 1B), well-developed underbridge (Fig. 1B), and coarse zig-zag ridges surrounding the fenestra on the surface. The cyst measurements (n = 3) were length $608.7 \pm 91.6 (506-682)$ µm, width = $395.3 \pm 40.9 \ (366-442) \ \mu m$, and length/width ratio = $1.3 \pm 0.2 \ (1.3-1.7) \ \mu m$. The morphometric characters of the vulval cone were fenestral length = $57.3 \pm 2.9 (54-59) \mu m$, fenestral width = $35.3 \pm 4 (33-40) \mu m$, and vulval slit = $50.7 \pm 1.2 (50-52) \mu m$. Second-stage juveniles tapering posteriorly (Fig. 1C). Stylet well developed, basal knobs rounded, directed slightly anteriorly (Fig. 1D). Tail tapering, with hyaline terminal about half of tail length (Fig. 1E). Second-stage juveniles (n = 16) showed the following morphometric characters: body length = $440.9 \pm 20 \; (408-470) \; \mu m$, $a = 20.3 \pm 2.3 \; (16.9-24)$, $b = 2.7 \pm 0.3 \; (2.3-3.3)$, $c = 8.1 \pm 0.6 \; (7.3-9.3)$, $c' = 3.8 \pm 1.00 \; (2.3-3.3)$ 0.3 (3.1-4.3), stylet length = $23.2 \pm 0.4 (23-24)$ µm, anterior end to median bulb = $71.8 \pm 4.5 (65-79)$ µm; maximum body width = $21.9 \pm 2 (17-25) \mu m$; body width at anus = $14.4 \pm 1.5 (12-17) \mu m$, tail length = $54.6 \pm 2.9 (50-60) \mu m$, and hyaline part of tail = $23.7 \pm 1.9 (12-17) \mu m$, tail length = $14.4 \pm 1.5 (12-17) \mu m$, t 2.9 (19–28) µm. These observations conform to the published descriptions of Heterodera fici Kirjanova (Kirjanova, 1954; Golden et al., 1988). The slides of the cyst vulval cone and juveniles were deposited in the Canadian National collection of Insects, Arachnids, and Nematodes (Accession no. 14851 to 14853 for the second stage juveniles and 14854–14855 for the cyst cones). For molecular analysis, DNA was extracted from individual juvenile (n = 4) from different cysts. A 1,151-bp fragment of ribosomal DNA containing ITS1-5.8S-ITS2 region was amplified and sequenced using primers 18S (5'-TTGATTACGTCCCTGCCCTTT-3') and 26S (5'-TTTCACTCGCCGT-TACTAAGG-3') (Vrain et al., 1992). The sequence was deposited into the GenBank database (Accession no. KY635986 and KY635987) and was compared with published sequences by means of BLAST search in the database (November 2016). The comparison revealed 99.0% to 100% similarity to the sequences of the same genomic region of H. fici from Iran (AF498385) and Georgia (AF274409). Subbotin et al. (2010) summarized the known occurrence of the fig cyst nematode from Belarus, Belgium, Estonia, France, Germany, Greece, Hungary, Italy, The Netherlands, Norway, Poland, Portugal, Russia, Spain, Yugoslavia, China, Georgia, Iran, Turkey, Uzbekistan, Australia, New Zealand, United States (California, Florida, Louisiana, Maryland, and Virginia), Brazil, Algeria, and South Africa. It is an exotic pest to Canada. To our knowledge, this is the first evidence of the occurrence of H. fici in

Key words: Canada, detection, fig cyst nematode, Heterodera fici Kirjanova.

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Fig. 1. Photomicrographs of *Heterodera fici* on fig tree from Ontario, Canada. A, B. Cyst vulval cones with the ambifenestrate fenestra in A) and well-developed underbridge and bullae in B). C–E. The second-stage juveniles from a crushed cyst with the whole body in C), the anterior region in D) and the posterior region in E).