Diseases Caused by Nematodes

First Report of *Heterodera trifolii* on White Clover and *Rumex obtusifolius* in Costa Rica

L. A. Núñez-Rodríguez, L. Flores-Chaves, and D. A. Humphreys-Pereira[†]

Laboratorio de Nematología, Centro de Investigación en Protección de Cultivos, Escuela de Agronomía, Universidad de Costa Rica, San José, 2060, Costa Rica

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In 2018, during a survey sampling for cyst-forming nematodes, two populations of *Heterodera* sp. were found in the north region of Cartago (Llano Grande and Oreamuno), Costa Rica. White females and cysts were attached to the plant roots of two weeds, white clover (*Trifolium repens* L.) and *Rumex obtusifolius* L. Plants were asymptomatic aboveground. Cysts were extracted from soil samples using the Fenwick method (Fenwick 1940) and cut to release the second-stage juveniles (J2s). Cysts were lemon-shaped with a prominent vulval cone, light to dark brown coloring, and ambifenestrate with developed underbridge and bifurcated at the end. Measurements combining both populations are given in micrometers and provided in the following format: mean $\pm$ SD (range). Cysts ($n = 20$): length excluding neck = 744.3 ± 61.6 (648.5 to 837.4); width = 495.9 ± 60.5 (421.9 to 621.1); fenestral length = 50.7 ± 6.4 (40.3 to 61.3); and semifenestral width = 36.2 ± 5.6 (24.1 to 45.7). J2s ($n = 60$): length = 541.1 ± 25.6 (492.5 to 604.9); width = 22.9 ± 1.7 (20.5 to 27.3); stylet = 27.9 ± 0.9 (26.2 to 29.6); labial region height = 4.7 ± 0.3 (3.8 to 5.5); labial region diameter = 9.4 ± 0.5 (8.5 to 10.9); DGO = 5.9 ± 0.5 (4.6 to 7.3); anterior end to excretory pore = 122.2 ± 8.3 (106.4 to 148.7) and to median bulb valve = 81.2 ± 5.3 (67.3 to 92.6); tail length = 66.6 ± 4.9 (55.9 to 77.3); and hyaline region of tail length = 37.1 ± 3.7 (27.7 to 44.5). No males were found. The morphological and morphometrical features of the two *Heterodera*

populations overlapped with each other and within other *H. trifolii* populations reported worldwide (Sekimoto et al. 2017; Subbotin et al. 2010). The primer sets TW81/AB28 (Subbotin et al. 2001), D2A/D3B (De Ley et al. 1999), and HTcox1F2 (5'-GCTTCTGATCTTTCCCTTTCCACGTA-3', this study)/JB4 (Derycke et al. 2005) were used for amplification of the internal transcribed spacer (ITS) rRNA, the D2-D3 expansion segments of the 28S rRNA (28S), and the partial mitochondrial *cox1* gene (*cox1*), respectively. The resulting sequences were submitted to GenBank (ITS, MT020783; 28S, MT010295; and *cox1*, MT007852). A BLASTn search of the ITS, 28S, and *cox1* gene sequences of *H. trifolii* from Costa Rica revealed a 100% identity with sequences of *H. trifolii* from Japan (LC208684), South Korea (MN720070), and the United States (MK093174), respectively. A greenhouse assay was established to confirm the reproduction of *H. trifolii* on each host. Using 10 replicates (individual pots) per treatment (hosts), pots were inoculated with 1,000 individuals (eggs + infective J2s). The experiment was conducted twice. The average greenhouse temperature was $22.1 \pm 3.8^\circ\text{C}$, and plants were harvested 75 days after inoculation. Results showed that *H. trifolii* was able to reproduce successfully on both hosts. Final average population (roots + soil) in *R. obtusifolius* and *T. repens* was 21,516 and 2,626 nematodes (all stages and cysts), respectively. These two weed species can be a source of inoculum for economically important crops. Therefore, studies on life cycle and pathogenicity assays are being performed on agricultural crops. To our knowledge, this is the first report of *H. trifolii* on white clover and *R. obtusifolius* in Costa Rica.

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[†]Indicates the corresponding author.

E-mail: danny.humphreys@ucr.ac.cr (D.A.H.-P.)