

Diseases Caused by Nematodes

First Report of Potato Cyst Nematode, *Globodera rostochiensis*, Infecting Potato (*Solanum tuberosum*) in Uganda

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Potato cyst nematodes (PCNs; *Globodera rostochiensis* [Wollenweber, 1923] and *G. pallida*) are quarantine pests (EPPO 2013) and have recently been reported from Kenya (Mburu et al. 2018) and Rwanda (Niragire et al. 2020). In East Africa, potato is an important staple food crop for millions of people, although current yields (10 t/ha) are far below potential (40 t/ha). A survey was conducted in Uganda to assess the incidence of PCNs in farmers' potato fields. Soil samples were collected from 124 fields in areas neighboring Kenya and Rwanda (November 2018 to April 2019). Within each field a bulk sample of 2 kg using 20 cores was collected following a zigzag transects. Soil was thoroughly mixed, air dried, and sieved (1-mm mesh). Nematode cysts were extracted by taking three subsamples of

200 cm³ per field, using a Fenwick can (EPPO 2013). Cysts were found in 17 fields (13.7%), with mean cyst counts of 2.6 cysts/200 cm³ of soil. One cyst each from two randomly selected fields (MBL 03 and MBL 07) were dissected under a stereo-microscope, and 20 eggs/cyst were inoculated separately onto three potato plants of cultivar 'Shangi' grown in 1-kg pots containing steam-sterilized loam soil. Plants were maintained in the greenhouse and harvested after 3 months. Using a Fenwick can, a mean of 12 cysts per pot (\bar{x} = 83 eggs/cyst) were extracted, of which 15 females and 31 second-stage juveniles (J2s) were used for morphometric analyses. Female length (L) ranged from 280.5 to 446.3 μ m (\bar{x} = 365.1 \pm 45.0 μ m), width (W) from 200.3 to 440.5 μ m (\bar{x} = 319.3 \pm 63.4 μ m), and the L/W ratio was 1.2 \pm 0.2; Granek's ratio (n = 7) varied from 1.57 to 3.52 μ m, (\bar{x} = 2.7 \pm 0.6 μ m); the distance from anus to vulval basin was 26.31 to 62.73 μ m (\bar{x} = 50.1 \pm 11.7 μ m). The J2 stylet length ranged from 14.93 to 22.59 μ m (\bar{x} = 19.37 \pm 1.86) with round-shape stylet knobs. Length of the hyaline tail was 12.64 to 27.63 μ m (\bar{x} = 23.05 \pm 3.80), and the true tail ranged from 30.06 to 54.48 μ m (\bar{x} = 43.33 \pm 4.87 μ m); body length of J2s varied from 332.02 to 427.29 μ m (\bar{x} = 394.00 \pm 19.30); all morphometric parameters matched those described for *G. rostochiensis* (Subbotin and Franco 2012). DNA was extracted (Qiagen DNeasy Blood and Tissue kit; Qiagen Group, U.S.A.) and amplified using candidate ITSFR primers targeting the ITS1-5.8S-ITS2 regions (modified from Mburu et al. 2018). One PCR reaction contained 0.5 μ M of each primer (forward and reverse), 5 μ l of 5 \times GoTaq Buffer (Promega), 2 mM MgCl₂, 200 μ M dNTPs, 0.125 μ l of GoTaq DNA polymerase (5 μ l/ μ l), and 1 μ l of DNA template (final volume = 25 μ l). PCR cycling included 2-min initial denaturation phase and 40 PCR cycles. The PCR amplicons (500 bp) were sequenced and edited using BioEdit Sequence Alignment Editor. DNA sequences were analyzed using the NCBI BLAST tool: sequences MN450308 and MN450309 showed similarity to the 5.8S (E = 4e⁻¹⁴⁰; 97.64% identity) and 18S (E = 5e⁻¹³⁹; 98.61% identity) ribosomal RNA gene of non-African *G. rostochiensis* isolates. A phylogenetic analysis showed that Ugandan populations cluster with Kenyan *G. rostochiensis* isolates but are less closely related to Rwandan populations or other *Globodera* species. Our findings highlight the need to conduct a comprehensive epidemiologic survey for developing a regional PCN-management strategy.

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