

# plant disease

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## Disease Notes

### First Report of *Xiphinema rivesi* (Nematoda, Longidoridae) in Washington State

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*e-Xtra*

Dagger nematode, *Xiphinema rivesi* Dalmaso, 1969 reportedly transmits several viruses in North America and Europe (2) leading to severe yield reduction in crops. Soil samples were collected in March 2013 during a survey of cherry orchards in Chelan County, WA; these historically suffer from cherry rasp leaf disease, caused by *Cherry rasp leaf virus* (CRLV) (genus *Cheravirus*). Soil samples were transported to the WSDA nematology laboratory in Prosser, WA, where 250-cc subsamples were processed using sucrose centrifugal flotation (1). Dagger nematodes were hand-picked and stored in 0.1% sodium chloride before being sent to the USDA-ARS Nematology Laboratory in Beltsville, MD, for morphological and molecular identification. The morphological and molecular analysis of adult females identified the dagger nematode species as *Xiphinema rivesi* Dalmaso, 1969 (4). Morphological characters used for identification included female body, and total stylet length (odontostyle and odontophore), location of guiding ring from oral aperture, head and tail shape, various tail measurements, and vulva percentage in relation to body length. Measurements of females ( $n = 10$ ) include a mean body length of  $1,902 \pm 162.4$  (1,832 to 2,203)  $\mu\text{m}$ , odontostyle  $83 \pm 3.5$  (80 to 90)  $\mu\text{m}$ , odontophore  $54.8 \pm 4.2$  (50 to 65)  $\mu\text{m}$ , total stylet  $137.8 \pm 4.2$  (130 to 145)  $\mu\text{m}$ , guiding ring from oral aperture  $70 \pm 5.1$  (60 to 75)  $\mu\text{m}$ , tail  $30.8 \pm 2.5$  (27.5 to 35.0)  $\mu\text{m}$ , body diameter at anus  $24.7 \pm 1.7$  (22 to 28)  $\mu\text{m}$ , J (hyaline portion of tail)  $6.0 \pm 0.9$  (5.0 to 7.5)  $\mu\text{m}$ , body diameter at beginning of J  $8.5 \pm 1.0$  (7.5 to 10.5)  $\mu\text{m}$ , body diameter at 5  $\mu\text{m}$  from tail terminus  $7.5 \pm 0.2$  (7.0 to 8.0)  $\mu\text{m}$ , and V%  $52.2 \pm 1.8$  (49.4 to 55.0)  $\mu\text{m}$ . Molecular diagnosis of *X. rivesi* was confirmed after DNA was extracted from two individual nematodes by mechanical disruption with a micro knife in 20  $\mu\text{l}$  worm lysis buffer containing 500 mM KCl, 100 mM Tris-Cl (pH8.3), 15 mM  $\text{MgCl}_2$ , 10 mM dithiothreitol (DTT), 4.5% Tween 20, and 0.1% gelatin. DNA extracts were stored at  $-80^\circ\text{C}$  until needed, then thawed, 1  $\mu\text{l}$  proteinase K (from 2 mg/ml stock) was added, and the tubes were incubated at  $60^\circ\text{C}$  for 60 min, followed by  $95^\circ\text{C}$  for 15 min. The 28S large ribosomal D2-D3 expansion segment was amplified with D2A (5'-ACAAGTACCGTGAGGGAAAGTT-3') and D3B (5'-TCGGAAGGAACCAGCTACTA-3'), and the internal transcribed spacer (ITS) region was amplified with primers TW81 (5'-GTTTCCGTAGGTGAACCTGC-3') and AB28 (5'-ATATGCTTAAGTTCAGCGGGT-3'), as previously described (3). To verify the identity of the sequences generated from PCR, sequenced products were subjected to a database search using BLAST. Sequences from the 28S region were >99% identical to several sequences of *X. rivesi* sampled from Spain (GenBank Accessions JQ990038, JQ990039, HM921357, and HM921358). Sequences from the ITS region were 97 to 98% identical to *X. rivesi* sequences (FR878063 to FR878066) obtained from the host *Vitis vinifera* from Italy. To the best of our knowledge, this is the first report of this nematode from the Washington. The quick and persistent spread of CRLV in most of the orchards visited calls for concern and there is need for urgent control measures against this vector nematode.

**References:** (1) W. R. Jenkins. Plant Dis. Rep. 48:692, 1964. (2) S. Sirca et al. Plant Dis. 91:770, 2007. (3) Skantar et al. J. Nematol. 44:58, 2012. (4) M. R. Wojtowicz. et al. J. Nematol. 14:511, 1982.

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## Supplemental Material



Photomicrographs of *Xiphinema rivesi* females. **A**, Entire female. **B and C**, Anterior region with arrows indicating tip of odontostylet, guiding ring, and basal flanges of stylet. **D**, Vulval region. **E and F**, Tail with arrows indicating anus.