First Report of the Cereal Cyst Nematode *Heterodera filipjevi* on Winter Wheat in Montana

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Among the cereal cyst nematode complex, three species, *Heterodera avenae*, *H. filipjevi*, and *H. latipons*, are the most destructive to wheat (**Smiley and Yan 2015**). While *H. avenae* occurs in several U.S. states, *H. filipjevi* has only been reported from Washington in 2008 (**Smiley and Yan 2015**). *Heterodera latipons*has not been reported in the United States. In Chouteau County, Montana, a winter-wheat field reported to contain *H. avenae* was observed with areas of heavily stunted winter wheat 'Yellowstone'. Roots from affected plants displayed moderate numbers of nematode cysts. Given symptom severity, *H. filipjevi* was suspected (**Golinowski et al. 1997**). Living nematode cysts from wheat roots collected from the affected field were examined morphologically and by PCR for species identification. Observations of morphological characters critical for identification indicated that the specimens were *H. filipjevi*(Akinbade et al. 2010; **Subbotin et al. 2010**). Measurements of second-stage juveniles (J2, *n* = 17) included length of body (range = 490 to 615 µm, mean = 565.1 µm), stylet (24.0 to 25.0 µm, 24.3 µm) with anchor-shaped basal knobs, tail (50.0 to 70.0 µm, 61.4 µm), and hyaline tail terminus (35 to 43 µm, 38.5 µm). The lateral field had four lines, the inner two more distinct. Shapes of the tail, tail terminus, and stylet knobs were also consistent with *H. filipjevi*. The lemon-shaped cysts (*n* = 10) were light brown to yellow and had a zigzag pattern and bifenestrate vulval cone. Morphometrics included body length (L) excluding neck (475 to 718 µm, 643.0 µm); body width (W) (336 to 545 µm, 460.3 µm); L/W (1.3 to 1.6 µm, 1.4 µm); neck length (60 to 100 µm, 83.5 µm) and width (50 to 75 µm, 59.3 µm); fenestra length (40 to 55 µm, 48.0 µm) and µm width (20 to 30 µm, 24.8 µm); heavy underbridge (60 to 82.5 µm, 72.8 µm); vulval slit (7.5 to 12.5 µm, 9.4 µm); and many bullae. Molecular analysis of internal transcribed spacer (ITS1 and 2) and 28S rRNA confirmed identity as *H. filipjevi* (**Akinbade et al. 2014**). Amplicons generated from three J2 (two hatched from one cyst, one from another) were cloned and sequenced (**Akinbade et al. 2014**). Sequence from the 28S region (GenBank Accession No. KP878489) was >99.9% identical to those of *H. filipjevi* from China (GU083594, GU083592, and GU083597), and distinct from several populations of *H. avenae* (99.0 to 99.2%). The ITS rDNA from this population (KP878490) matched with 99.9% identity to several *H. filipjevi* sequences available from GenBank, including ones from Russia (AF274399), Tajikistan (AY148402), UK (AY148403), United States (GU079654), and China (GU083595). The next closest species match was *H. avenae*, with 96 to 97% identity to the Montana population. This is the first report of *H*. *filipjevi* from Montana. Eggs of *H. filipjevi* are noted for hatching in the fall during winter wheat planting, and therefore have the potential to affect winter

wheat more than *H. avenae*, whose eggs hatch only in the spring after a cold period. Moreover, resistance in wheat against these two nematode species is different and will require additional considerations.