Disease Notes

Diseases Caused by Nematodes

First Report of the Hop Cyst Nematode, *Heterodera humuli*, in Two Counties of the Yakima Valley Region, WA, U.S.A.

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Cyst nematodes are ranked as the second most damaging plant-parasitic nematode genus of crops worldwide (Jones et al. 2013). The hop cyst nematode, *Heterodera humuli*, has been reported to cause up to 38% reduction in dry hops per bine (Hay and Pethybridge 2003). America is the top hop producing country worldwide, with 75% of production occurring in Washington State, with the majority of this production occurring in the Yakima Valley region (USDA-NASS 2019). In late 2019, 30 soil samples from 15 different fields were collected from the hop cultivars HBC 394, HBC 369, and YCR 14. Nematodes were extracted using an adapted centrifugal floatation method (Jenkins 1964) from 100-cm^3 subsamples of soil. Twenty of these samples contained at least one cyst, and 23 contained at least one juvenile. Body length of juveniles (n = 5; average ± standard deviation) was 377.62 ± 4.76 µm, which is consistent with *H. humuli* juvenile body measurements (Sen 1968). Three samples from Yakima County and two from Benton County were identified to the species level using sequences from the internal transcribed spacer (ITS) region of the 5.8S gene. The sequences (GenBank accession nos. MT840678 to MT840682) were amplified using forward primer 5.8S-F (5′-GTGATTC-CATTCACCAHCTACCTG-3′), and reverse primer 5.8S-R (5′-TTCGCAC-TAATTATCGCAGTTGG-3′). Sequence comparison with available ITS (5.8S) sequences in GenBank using BLAST showed 99.85% identity to *H. humuli* for all five samples. Because COI sequences of *H. humuli* are not available, to provide an additional marker for species identification, we amplified the COI sequences by using forward primer Hete-COI-F (5′-TTTGGDCAYCCHGARGTTTATGTT-3′), and reverse primer Hete-COI-R (5′-AYWGTAAAAAGGRRAATAAAACC-3′) for these samples. Four COI sequences (GenBank accession nos. MT840683 to MT840686) were obtained. These COI sequences will be used to identify future *H. humuli* samples. To confirm pathogenicity, eight 1-gal pots were filled with a 90:10 play sand to potting soil mixture, and one hop rhizome (cv. 'Centennial') was planted in pots and maintained in a greenhouse. After above-ground plant growth was observed, half the pots were inoculated with hand-picked *H. humuli* cysts from Yakima soil samples at a density of 10 cysts/100 cm^3 of soil. The life cycle of *H. humuli* in potted experiments is 40 days (Mende and McNamara 1995). Forty-five days after inoculation, plant measurements were recorded and nematodes extracted from five 100-cm^3 soil samples per pot as described above. Soil samples revealed that *H. humuli* populations had an average reproductive factor (RF = final nematode population/initial nematode population) of 2.08. Five cysts were crushed to determine eggs/cyst, which yielded an average of 101 eggs/cyst. Young infected hops lacked vigor, with all replicates stunted both in bine height and leaf length compared with healthy controls. Bine heights were reduced by an average of 40.4% in pots inoculated with *H. humuli* compared with control plants (P = 0.0016). Distribution of hop cyst nematodes within the United States is limited to the top four states for hop production: Washington, Oregon, Idaho, and Michigan (Cobb 1962; Hafez et al. 2010; Sen and Jensen 1967; Warner and Bird 2015). In 1962, Cobb reported *H. humuli* in Pierce County, Washington, but it had not been reported in Benton County and Yakima County until now. This is a significant finding that has the potential to impact the Washington State hop industry, valued at $475.7 million in 2019 (USDA-NASS 2019). Due to the lack of known effective nematode control measures, the discovery of *H. humuli* in the major hop-growing region of Washington warrants concern.

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