# Disease Note

## Diseases Caused by Viruses

### First Report of Tobacco Ringspot Virus in Highbush Blueberry in Washington State

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Since 2015, several highbush blueberry plants (*Vaccinium corymbosum*) of cultivars Draper and Top Shelf in an organic farm in eastern Washington State showed reduced growth with deformed leaves displaying chlorotic spots, rings, and red blotches and producing small and poorly ripened berries. The symptomatic plants showed gradual decline within 2 to 3 years postplanting. In ELISA using antibodies (Agdia, U.S.A.) to blueberry leaf mottle virus, cherry leaf roll virus, peach rosette mosaic virus, strawberry latent ringspot virus, tomato black ring virus, tomato ringspot virus, and tobacco ringspot virus (TRSV), leaf samples from six symptomatic plants tested positive only to TRSV (*Secoviridae*: *Nepovirus*). Subsequently, total RNA was isolated from leaves of a symptomatic plant using the Spectrum Plant Total RNA Kit (Sigma-Aldrich, U.S.A.). High-quality RNA was subjected to high-throughput sequencing (HTS) on the Illumina NovaSeq platform (Huntsman Cancer Institute, U.S.A.). An average of ~28 million 150-bp paired-end reads obtained were subjected to quality filtering followed by de novo assembly using CLC Genomics Workbench (version 12.0) and BLASTn analysis (https://www.ncbi.nlm.nih.gov/blast). Two contigs of 2,778 bp (average coverage: 11,031.7) and 3,589 bp (average coverage: 11,882) showed, respectively, a maximum of 97.3 and 97.6% nt identity with TRSV RNA1 of a South Korean isolate (KJ556849). Another contig of 3,615 bp (average coverage: 7,072.1) showed a maximum of 92.8% nt identity with TRSV RNA2 of an isolate from Iowa (MT563079). The HTS data revealed no other viral sequences reported from blueberry plants (Martin and Tzanetakis 2018). To further confirm the presence of TRSV, extracts of leaf samples from seven symptomatic and 10 asymptomatic plants collected randomly from cultivars Draper and Top Shelf were tested by RT-PCR using primers specific to a region of the helicase gene of TRSV RNA1 (forward, GACTACTGAGCAACATTGCAACTTCC; reverse, GTCCCCTAACAGCA TTGACTACC) and the coat protein gene of TRSV RNA2 (forward, GCT GATTGGCAGTGTATTGTTAC; reverse, GTGTTCGCATCTGGTTTCAAA TTGG). An approximately 360-bp fragment specific to RNA1 and ~640-bp fragment specific to RNA2 were amplified only from symptomatic samples. Sanger sequence analysis of amplicons specific to RNA1 and RNA2 showed 98.1 and 96.8% nt identity with corresponding sequences of TRSV isolates from South Korea (KJ556849) and Iowa (MT563079), respectively. These results confirmed the presence of TRSV in symptomatic blueberry plants. The complete sequence of RNA1 (7,512 nt, MW495243) and RNA2 (3,925 nt, MW495244) genome segments of the blueberry isolate determined in this study showed 95.9 and 93.2% nt sequence identity, respectively, with corresponding TRSV sequences from South Korea (KJ556849) and Iowa (MT563079). Based on previous reports (Converse and Ramsdell 1982; Martin and Tzanetakis 2018; Martin etal. 2012), this study represents the first report of TRSV infecting highbush blueberry in Washington State. Because the state has emerged as the national leader in blueberry production, the results will strengthen plant health certification standards to provide virus-tested propagative materials for domestic growers and export to the European Union.

*References*:

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