First Report of Little Cherry Virus 1 Infecting Sweet Cherry in Ontario, Canada

A. Simkovich,^{1,2} S. E. Kohalmi,² and A. Wang^{1,†}

¹London Research and Development Centre, Agriculture and Agri-Food Canada, London, Ontario, Canada

²Department of Biology, University of Western Ontario, London, Ontario, Canada

Funding: Funding was provided by Agriculture and Agri-Food Canada. Plant Dis. 105:4173, 2021; published online as <https://doi.org/10.1094/PDIS-04-21-0798-PDN>. Accepted for publication 12 July 2021.

The Niagara fruit belt is one of the richest fruit-producing areas in Canada, contributing to 90% of Ontario's tender fruits such as peach, plum, and sweet cherry. Little cherry virus 1 (LCV1) of the genus *Velarivirus* is a causal agent of little cherry disease, which has devastated cherry crops in many regions (Eastwell and Bernardy 1998; Jelkmann and Eastwell 2011). From 2013 to 2018, foliar symptoms indicative of viral infection such as leaf deformation, ringspot, mottling, vein clearing, and reddening were found on sweet cherry trees grown in the Niagara region. To determine if these trees were infected by a virus, small RNAs (sRNAs) were isolated from separately pooled asymptomatic and symptomatic leaves using the mirPremier microRNA isolation kit (Sigma Aldrich Canada, Oakville, ON). The sRNAs were used to create two libraries (four leaves per library) with the TruSeq Small RNA Sample Prep Kit (Illumina, San Diego, CA). The sRNA libraries were separately sequenced with the MiSeq Desktop Sequencer (Illumina). In total, 5,380,196 reads were obtained, and Trimmomatic (Bolger et al. 2014) was used to remove adaptors. The remaining 4,733,804 clean reads were assembled into contigs using Velvet 0.7.31 (Zerbino and Birney 2008) and Oases 0.2.09 (Schulz et al. 2012) with minimum length of 75 nt. A BLASTn search (Altschul et al. 1997) of the contigs identified the presence of cherry virus A (CVA; genus: *Capillovirus*) and two members of the *Ilarivirus* genus (*Prunus necrotic ringspot virus* [PNRSV] and *prune dwarf virus* [PDV]) in both

libraries. LCV1 was only found in contigs derived from the symptomatic library. Of the clean reads, 22,016 were assembled into six contigs (with lengths ranging from 86 to 116 nt) mapping to LCV1, covering 7.07% of the viral genome. To confirm LCV1 infection, primers were designed from the assembled contigs and used for reverse transcription polymerase chain reaction (RT-PCR). Amplicons were sequenced, and the terminal sequences were determined using 5' and 3' RACE Systems (Invitrogen, Burlington, ON). Degenerate primers were designed from multiple sequence alignments of published LCV1 genomes for amplification and primer walking to obtain the sequence of LCV1. The complete genome sequence of LCV1 has a length of 16,934 nt and was deposited in GenBank (accession no. MN508820). A BLASTn search showed that this isolate is nearly identical (99.6% sequence identity) to an isolate from California (accession no. MN131067). To determine the incidence of infection, a field survey was performed at the same location during the spring months of 2014 to 2018 using RT-PCR with primers specific to the viral coat protein gene. Among 46 cherry trees sampled, two (4.3%) trees were infected with LCV1 and showed negative results with CVA, PNRSV, and PDV. Both trees displayed mild suturing of primary and secondary veins. LCV1 has been identified in Western stone fruit producing regions (British Columbia in Canada, and Washington, California, and Oregon in the United States of America). To the best of our knowledge, this is the first report of LCV1 in any eastern region of Canada. The low incidence of LCV1 suggests that this virus is not widespread in this region. Routine monitoring and detection of LCV1 is required to prevent this devastating cherry disease from spreading in this region.

References:

- Altschul, S. F., et al. 1997. Nucleic Acids Res. 25:3389.
- Bolger, A. M., et al. 2014. Bioinformatics 30:2114.
- Eastwell, K., and Bernardy, M. 1998. Acta Hort. 472:305.
- Jelkmann, W., and Eastwell, K. C. 2011. Page 153 in: Virus and Virus-Like Diseases of Pome and Stone Fruits. APS, St. Paul, MN.
- Schulz, M. H., et al. 2012. Bioinformatics 28:1086.
- Zerbino, D. R., and Birney, E. 2008. Genome Res. 18:821.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: fruit tree virus, pathogen detection, Prunus, viruses and viroids

[†]Indicates the corresponding author.
A. Wang; Aiming.Wang@AGR.GC.CA