Diseases Caused by Viruses

First Report of Citrus Leaf Blotch Virus Infecting Bearss Lime Tree in California

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Between July 2017 and January 2018, the Citrus Clonal Protection Program (CCPP)-National Clean Plant Network, University of California, Riverside, received samples from 1,169 citrus trees surveyed in 20 different counties of California (CA) for virus and viroid testing under the Citrus Research Board project 5300-181 (CDFA permit 3096; USDA P526P-16-00352). Total RNA was extracted using a modified MagMax-96 Viral RNA Isolation Kit optimized for citrus tissues and tested with reverse transcription quantitative polymerase chain reaction (RT-qPCR) (Osman et al. 2015; Vidalakis and Wang 2013) and RT-PCR (Vives et al. 2013). Sample 0521612, originating from a nonsymptomatic Bearss lime (BL) (*Citrus latifolia* Tan.) at a residence in Los Angeles County, tested positive for citrus leaf blotch virus (CLBV), citrus vein enation virus (CVEV), and citrus exocortis viroid (CEVd). Citrus vein enation and exocortis diseases have been known to occur in CA (Wallace 1978). The only reference to CLBV (syn. Dweet mottle virus) in CA comes from the Citrus Variety Improvement Program, the forerunner of CCPP, from the interception of the virus during the reindexing on Dweet tangor (*C. reticulata* × *C. sinensis*) of a Florida citrus variety introduction in 1968 (Hajeri et al. 2010). The identity of CLBV-BL detected by RT-qPCR was verified by determining the preliminary genome sequence (5,925 nt, MK894852) generated by RT-PCR primer walking, cloning, and sequencing. The full CLBV-BL genome sequence was acquired with high-throughput sequencing using the TruSeq Stranded Total RNA Sample Library Prep with plant rRNA

depletion (Illumina, U.S.A.) from TRIzol (Thermo Fisher, U.S.A.) extracted RNA. The library was sequenced using the Illumina NextSeq500 platform (2 × 75 bp), which generated a total of 81,806,700 reads, after quality control, and low-quality reads were filtered with Fastp (Chen et al. 2018). CLBV reference genome (NC_003877) guided alignment using Bowtie2 version 2.3.4.1 (Langmead and Salzberg 2012) produced 135,574 reads that mapped to the virus genome. Reads were assembled using Trinity version 2.8.5 (Grabherr et al. 2011), which generated an assembled contig of 8,317 nt. 5' and 3' RACE was performed to produce the full-length virus sequence (8,477 nt, MT038390). BLASTn results showed that the CLBV-BL isolate had the highest (98.8%) identity to the NZ-G18 (EU857539) isolate in *Citrus limon* from New Zealand and covered 99% of the genome. De novo assembly performed with Trinity, on nonhost reads generated from Bowtie2 removing the reads mapped to citrus genomes as references, verified the presence of CEVd and CVEV in the sample 0521612. This note reports the first occurrence of CLBV in CA outside a citrus germplasm program (CDFA Pest Rating: <https://blogs.cdfa.ca.gov/Section3162/?p=5126>). The CLBV case in CA highlights the importance of the continuity of comprehensive citrus testing programs (germplasm production and field surveys) that include a combination of complementary biological indexing and molecular assays (Gergerich et al. 2015). In addition, this report emphasizes the need for the use of therapied and pathogen-tested citrus materials for tree propagation, regardless of residential or commercial use, to prevent citrus pathogens, especially CLBV and other related but uncharacterized graft or seed-transmissible agents capable of causing bud union crease on the commercial vital citrange rootstocks, from establishing in CA (Galipienso et al. 2000).

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