Diseases Caused by Viruses

First Report of Orchid Fleck Virus Associated with Citrus Leprosis Symptoms in Rough Lemon (*Citrus jambhiri*) and Mandarin (*C. reticulata*) the United States

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Citrus leprosis is an economically important disease of citrus in South and Central America. The disease can be caused by several nonsystemic viruses belonging to the genera *Cilevirus* (family *Kitaviridae*) and *Dichorhavirus* (family *Rhabdoviridae*) (Freitas-Astúa et al. 2018; Roy et al. 2015). In February 2020, lesions consistent with citrus leprosis were observed on the leaves and stems of rough lemon (*Citrus jambhiri*) and mandarin (*C. reticulata*) trees in Hilo, Hawaii. Brevipalpus mites, vector of orchid fleck virus (OFV), were also present on these trees (Freitas-Astúa et al. 2018). To identify the virus associated with the symptoms, total RNA was isolated using a NucleoSpin RNA Plus kit (Macherey-Nagel) and underwent reverse transcription (RT)-PCR with two newly designed universal primers specific for dichorhavirus (Dichora-R1-F1, 5'-CAYCACTGYGCBRTNGCWGATGA / Dichora-R1-R1, 5'-AGKATRTSWGCCATCCKGGCTATBAG). The expected ~350-bp amplicon was obtained and directly sequenced in both directions. BLASTn and BLASTx searches revealed that the primer-trimmed consensus sequence (MT232917) shared 99.3% nucleotide (nt) and 100% amino acid (aa) identity with an OFV isolate from Germany (AF321775). OFV has two orchid- (OFV-Orc1 and OFV-Orc2) and two citrus- (OFV-Cit1 and OFV-Cit2) infecting strains (Roy et al. 2020). However, an isolate of OFV-Orc1 has recently been associated with citrus leprosis in South Africa (Cook et al. 2019). To confirm the presence of OFV in Hawaiian citrus and identify the strain, symptomatic

tissue was submitted to USDA-APHIS-PPQ-S&T, where total RNA was extracted from the symptomatic tissue using an RNeasy Plant Mini kit (Qiagen). The RNA samples were tested with OFV-Orc and OFV-Cit generic and specific primers in a conventional RT-PCR assay following optimized RT-PCR protocols (Roy et al. 2020). Two additional sets of generic primers (OFV-Orc-GPF, 5'-AGCGATAACGACCTTGATATGACACC / OFV-Orc-GPR, 5'-TGAGTGGTAGTCAATGCTCCATCAT; and OFV-R2-GF1, 5'-CARTGT CAGGAGGATGCATGGAA / OFV-R2-GR, 5'-GACCTGCTTGATGTAAT TGCTTCCTTC') were designed based on available OFV phospho (P) and large (L) polyprotein gene sequences in GenBank. These assays detected OFV-Orc2 in the symptomatic citrus samples, with the nucleocapsid (1,353-bp), P (626-bp), and L (831-bp) gene sequences sharing 97 to 98% identity with published OFV-Orc2 sequences (AB244417 and AB516441). Ribo-depleted RNA (Ribo-Zero, Illumina) was prepared using a TruSeq Stranded Total RNA Library Prep kit (Illumina) and underwent high-throughput sequencing on a MiSeq platform (Illumina). The resulting 19.6 million 2 × 75-bp reads were de novo assembled using SPAdes version 3.10.0 (Bankevitch et al. 2012). In addition to sequences corresponding to citrus tristeza virus and citrus vein enation virus, two contigs of 6,412 nt (average depth, 18,821; MW021482) and 5,986 nt (average depth, 19,278; MW021483) were found to have ≥98% identity to RNA1 (AB244417) and RNA2 (AB244418) of OFV isolate So (Japan), respectively. This is the first report of OFV in Hawaii and the first time leprosis has been observed in the United States since it was eradicated from Florida in the 1960s, although that outbreak was attributed to infection by citrus leprosis virus-N0, a distant relative of OFV (Hartung et al. 2015). The recent detection of citrus leprosis associated with OFV infection in South Africa (Cook et al. 2019) and now Hawaii underscores the threat this pathogen poses to the global citrus industry.

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