Diseases Caused by Viruses

Smilax auriculata: A New Host for Orchid Fleck Dichorhavirus Identified in Florida, USA

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In October 2020, two dune/earleaf greenbrier vines, *Smilax auriculata* (Smilacaceae: Liliales), showing chlorotic mottling and bleached ring pattern symptoms on leaves were collected from a residential landscape (29.6075° N, 82.4393° W) and an additional two from a nearby upland pine wooded area (29.6071° N, 82.4403° W) in Alachua County, Florida. All four symptomatic and one asymptomatic *S. auriculata* leaf samples were collected by the Florida Department of Agriculture and Consumer Services, Gainesville, FL. Total RNA was extracted using an RNeasy Plant Mini Kit (Qiagen, Valencia, CA) and used in downstream applications. Two pairs of orchid fleck virus (OFV) specific primers successfully amplified 350-bp N (Kubo et al. 2009) and 500-bp L gene fragments (Ramos-González et al. 2016) only in the four symptomatic *S. auriculata* samples by conventional RT-PCR assay. The obtained amplicon sequences (accession nos. MZ645935 and MZ645938) shared 99% nucleotide (nt) identity with OFV infecting orchid (LC222629) and citrus (MK522804). The OFV subgroup I (OFV-Orc1) and II (OFV-Orc2) specific primers (Kondo et al. 2017) were utilized to confirm the presence of OFV type strains infecting *S. auriculata*. The subgroup I specific amplicon sequence (MZ645934) shared 99% nt identity with OFV-Orc1 (LC222629), and the subgroup II specific amplicon (MZ645930) shared 98 to 99% nt identity with OFV-Orc2 (AB244417). To further confirm the presence of an OFV strain in *S. auriculata*, symptomatic tissue and RT-PCR positive RNA were sent to the USDA-APHIS-PPQ-Plant Pathogen Confirmatory Diagnostics Laboratory. Total RNA was extracted from both *S. auriculata* samples and

tested using optimized conventional RT-PCR protocols (Roy et al. 2020) with additional sets of OFV generic primers (Olmedo-Velarde et al. 2021). RT-PCR assays amplified 626- and 831-bp amplicons of the P and L genes, respectively, in the symptomatic *S. auriculata* samples. The L gene sequence shared 99% nt identity with both OFV-Orc strains, whereas the P gene sequence shared 98% nt identity with OFV-Orc2 infecting orchids (AB244417) and citrus (MW021482). A TruSeq Stranded Total RNA Library Prep kit (Illumina) was used to prepare a Ribo-depleted RNA library from one of the residential samples for sequencing on a NextSeq550 Illumina platform (2 × 75 cycles). The resulting 28.2 million reads were de novo assembled using SPAdes v.3.10.0 (Bankevich et al. 2012). Assembled reads identified seven nonoverlapping viral contigs. Five RNA1 and two RNA2 contigs covered more than 97% of the bipartite OFV genome with an average coverage depth of 5,297.61 and 5,186.04, respectively. The contigs of RNA1 (OL589310) and RNA2 (OL606757) shared 98 to 99% nt identity to OFV-Orc2-RNA1 (AB244417) and OFV-Orc-RNA2 (AB244418 and LC222630), respectively. No other virus sequences were identified. This is the first time a species in the genus *Smilax* has been identified as a natural host of OFV even though known flat mite (*Brevipalpus* sp.) vectors of OFV were not observed. Both OFV-Orc strains are known to infect citrus and cause leprosis disease in South Africa (Cook et al. 2019) and Hawaii (Olmedo-Velarde et al. 2021). Citrus leprosis (CiL) was first reported from Florida in the 1860s but has not been reported since the mid-1960s. CiL in Florida was attributed to infection by an ancestor of OFV, citrus leprosis virus-N0 (Hartung et al. 2015). Very recent findings of OFV-Orc in Florida in *Liriope*, *Aspidistra*, and *Ophiopogon* among the Asparagaceae family members (Fife et al. 2021) and now in the Smilacaceae family suggest a broader host range of the virus than previously known. Further research is needed to better characterize the potential risk of OFV to citrus production in Florida and other citrus-producing countries.

References

- Bankevich, A., et al. 2012. J. Comput. Biol. 19:455.
- Cook, G., et al. 2019. Eur. J. Plant Pathol. 155:1373.
- Fife, A., et al. 2021. J. Integr. Pest Manag. 12:1.
- Hartung, J. S., et al. 2015. Phytopathology 105:1277.
- Kondo, H., et al. 2017. Virology 508:18.
- Kubo, K. S., et al. 2009. J. Gen. Plant Pathol. 75:250.
- Olmedo-Velarde, A., et al. 2021. Plant Dis. 105:2258.
- Ramos-González, P. L., et al. 2016. J. Phytopathol. 164:342.
- Roy, A., et al. 2020. Phytopathology 110:106.

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