Diseases Caused by Viruses

First Report of Watermelon crinkle leaf-associated virus 1 (WCLaV-1) and WCLaV-2 in Watermelon (*Citrullus lanatus*) Plants Coinfected with *Cucurbit chlorotic yellows virus* in Florida

Katherine E. M. Hendricks,¹ Regina N. Hernandez,² Pamela D. Roberts,^{1,†} Thomas Isakeit,² and Olufemi J. Alabi³

¹ Department of Plant Pathology, University of Florida, Southwest Florida Research and Education Center, Immokalee, FL 34142

² Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843

³ Department of Plant Pathology and Microbiology, Texas A&M AgriLife Research and Extension Center, Weslaco, TX 78596

Plant Dis. 106:339, 2022; published online as <https://doi.org/10.1094/PDIS-06-21-1141-PDN>. Accepted for publication 26 June 2021.

Watermelon (*Citrullus lanatus* L.) and other cucurbits are major crops in Florida. During the 2020 and 2021 seasons, watermelon plants with foliar virus-like symptoms of yellow mottling and chlorosis, mild leaf wrinkling, and thickened leaves were observed in commercial fields (40 to 150 ha) in five counties (Desoto, Glades, Osceola, Seminole, and Charlotte) at >50% field incidence. Initial screening of 13 field-collected samples (two to four per county) for potyviruses with the Agdia POTY ImmunoStrip (Agdia, Elkhart, IN) was negative. Total nucleic acid extracts from each sample (RNeasy Plant Mini Kit, Qiagen, Germantown, MD) were used in one-step RT-PCR (Qiagen OneStep RT-PCR kit) with species-specific primer targeting squash vein yellowing virus (SqVYV), papaya ringspot virus-W (PRSV-W) (Adkins et al. 2008), cucurbit yellow stunting disorder virus (CYSDV) (Polston et al. 2008), cucurbit chlorotic yellows virus (CCYV) (Hernandez et al. 2021a), watermelon crinkle leaf-associated virus 1 (WCLaV-1), and WCLaV-2 (Hernandez et al. 2021b). The samples were also tested for cucurbit leaf crumple virus (CuLCrV) as per Hagen et al. (2008). All 13 samples were negative for SqVYV, PRSV-W, CuLCrV, and CYSDV, but seven samples (53.8%) from four counties tested positive for CCYV, 12 (92.3%) from five counties were positive for WCLaV-1, and WCLaV-2 was detected in eight samples (61.5%) from five counties. Three samples were singly infected with WCLaV-1, whereas the remaining 10 were mixed infected with different combination of two or three viruses. Notably, symptoms on

all 13 plants were visually indistinguishable. To verify the results, two randomly chosen gene-specific fragments per virus, obtained with primers CCYV-v1330/c2369, CCYV-v4881/c5736, WCLaV-1vRP/1cRP, WCLaV-1vMP/1cMP, WCLaV-2vRP/2cRP, and WCLaV-2 vMP/2cMP, were excised from the gel, cloned, and Sanger sequenced as described (Hernandez et al. 2021a, 2021b). In pairwise comparisons, the ~1-kb partial ORF1a (GenBank accession nos. MZ325846 to MZ325847) and 753-bp complete coat protein cistron (MZ325848 to MZ325849) of CCYV from Florida shared 98.9 to 99.5%/98.4 to 99.6% nucleotide (nt)/amino acid (aa) and 99.4 to 99.8%/99.6 to 100% nt/aa identities, respectively, with the corresponding sequences of global CCYV isolates. The partial RNA1 (MZ325850 to MZ325851) and RNA2 (MZ325852 to MZ325853) sequences of WCLaV-1 from Florida shared 99.2 to 99.8%/100% nt/aa and 98.9 to 100%/99.3 to 100% nt/aa identities, respectively with the corresponding global sequences of WCLaV-1 isolates. Lastly, the partial RNA1 (MZ325854 to MZ325857) and RNA2 (MZ325858 to MZ325861) sequences of WCLaV-2 from Florida shared 96.4 to 99.8%/97 to 100% nt/aa and 96.5 to 100%/95.9 to 100% nt/aa identities, respectively, with the corresponding global sequences of WCLaV-2 isolates. This is the first report of WCLaV-1 and WCLaV-2 from Florida and the first documentation of the occurrence of CCYV in South Florida. CCYV has been reported previously from California (Wintermantel et al. 2019), Georgia (Kavalappara et al. 2021), and recently from North Florida (Jailani et al. 2021), but WCLaV-1 and WCLaV-2 have only been reported from Texas (Hernandez et al. 2021b), after their discovery in China (Xin et al. 2017). The results indicate further expansion of the geographical range of these cucurbit-infecting viruses, although their longer but undetected presence in Florida is plausible due to the resemblance of their associated symptoms with those attributed to known viruses.

References:

- Adkins, S., et al. 2008. Plant Dis. 92:1119.
- Hagen, C., et al. 2008. Plant Dis. 92:781.
- Hernandez, R. N., et al. 2021a. Plant Dis. 105:3313.
- Hernandez, R. N., et al. 2021b. Plant Dis. 105:2025.
- Jailani, A. A. K., et al. 2021. Plant Dis. <https://doi.org/10.1094/PDIS-03-21-0639-PDN>
- Kavalappara, S. R., et al. 2021. Plant Dis. 105:1862.
- Polston, J. E., et al. 2008. Plant Dis. 92:1251.
- Wintermantel, W. M., et al. 2019. Plant Dis. 103:778.
- Xin, M., et al. 2017. Front. Microbiol. 8:1514.

The author(s) declare no conflict of interest.

Keywords: viruses and viroids, pathogen detection, vegetables, cucurbits

[†]Indicates the corresponding author.
P. D. Roberts; pdr@ufl.edu