Diseases Caused by Viruses

First Report of Cucurbit Yellow Stunting Disorder Virus and Cucurbit Chlorotic Yellows Virus in Melon in the Central Valley of California

S. Mondal,¹ L. Jenkins Hladky,¹ P. L. Fashing,¹ J. D. McCreight,¹ T. A. Turini,² and W. M. Wintermantel^{1,†}

¹ USDA-ARS Crop Improvement and Protection Research Unit, Salinas, CA 93905

² Cooperative Extension Fresno County, Fresno, CA 93710

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In California, the whitefly-transmitted yellowing viruses, cucurbit yellow stunting disorder virus (CYSDV) and cucurbit chlorotic yellows virus (CCYV), both genus *Crinivirus*, family *Closteroviridae*, have been limited to the Sonoran Desert production regions of Imperial and Riverside counties since their emergence in 2006 and 2014, respectively (Kuo et al. 2007; Wintermantel et al. 2009, 2019), where losses to these viruses have nearly eliminated fall melon production. CYSDV and CCYV have never been identified in the Central Valley, but the aphid-transmitted cucurbit aphid-borne yellows virus (CABYV; genus *Polyomavirus*, family *Luteoviridae*), which produces symptoms nearly identical to those induced by CYSDV and CCYV (Lemaire et al. 1993), is common. As part of a larger study to monitor for whitefly-transmitted yellowing viruses in the southwestern United States, melon leaves exhibiting foliar mottling and interveinal chlorosis beginning near the crown and spreading outward along vines, typical of symptoms caused by yellowing viruses, were collected from 106 melon plants in four commercial fields and a research plot in Fresno County, California, during October 2020. Whiteflies (*Bemisia tabaci*) were present in all fields and confirmed as MEAM1 (biotype B) by PCR. Total RNA and DNA were extracted separately from the same leaf from each plant to determine the presence of RNA and DNA viruses. Total RNA was extracted as described in Tamang et al. (2021) and was used in RT-PCR with primer sets designed to amplify a 277-nt portion of the CABYV RNA dependent RNA polymerase (RdRp) gene (CABYV RdRp-F, 5'-AAGAGCGGCAGCTACAATAC-3'; CABYV RdRp-R, 5'-TGCCACA TTCCGGTTCATAG-3') and portions of the CCYV and CYSDV RdRp genes encoded on RNA1 of the latter two viruses (Kavalappara et al. 2021).

In addition, each CYSDV and CCYV infection was confirmed using a second set of primers that amplified 394- and 372-nt portions of the coat protein (CP) gene of each virus, respectively, encoded on RNA2 (Wintermantel et al. 2009, 2019). The 953-nt CCYV RdRp and 394-nt CYSDV CP amplicons were sequenced and found to share greater than 98% sequence identity to CCYV RNA1 (accession no. MH477611.1) and CYSDV RNA2 (accession no. LT992901.1), respectively. The CABYV infections were secondarily confirmed using a second set of primers designed to the CP gene (Kassem et al. 2007). Furthermore, four RNA samples from two separate fields that previously tested positive for CYSDV and CABYV and the only CCYV infection were confirmed using a recently developed multiplex RT-qPCR method (Mondal et al., *unpublished*). Total DNA was extracted using methods described in Mondal et al. (2016) and was used in PCR to test for the presence of the whitefly-transmitted begomovirus cucurbit leaf crumple virus (CuLCrV), which also occurs in the Sonoran Desert melon production region (Hagen et al. 2008) and is capable of inducing yellowing and leaf curl symptoms in melon. CABYV was by far the most prevalent virus, infecting 34/106 plants tested (32%) among the five fields. Four plants from three fields were infected singly with CYSDV (4%), and three more CYSDV-infected plants from two fields were coinfecting with CABYV (3%). Only one plant was found to be infected with CCYV as a single virus infection (1%). No triple infections nor any CuLCrV were detected in any of the plants sampled. This is the first report of CYSDV and CCYV in the Central Valley of California. In this survey, although CABYV was the predominant yellowing virus infecting melons in the Central Valley (32%), detection of CYSDV in fields distant from one another and the presence of CCYV even in a single field warrant more extensive monitoring of cucurbit crops and known alternate hosts of these viruses in the Central Valley.

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[†]Indicates the corresponding author.

W. M. Wintermantel; bill.wintermantel@usda.gov