

First Report of Cucurbit Chlorotic Yellows Virus in Association with Other Whitefly-Transmitted Viruses in Yellow Squash (*Cucurbita pepo*) in Georgia, U.S.A.

Saritha Raman Kavalappara,¹ Hayley Milner,¹ Alton Sparks,² Cecilia Mcgregor,³ William M. Wintermantel,⁴ and Sudeep Bag^{1,†}

¹ Department of Plant Pathology, University of Georgia, Tifton, GA

² Department of Entomology, University of Georgia, Tifton, GA

³ Department of Horticulture, University of Georgia, Athens, GA

⁴ United States Department of Agriculture-Agricultural Research Service, Salinas, CA

Funding: Funding was provided by USDA-NIFA-Hatch project (1020319) and U.S. Department of Agriculture (AWD00011012). Plant Dis. 0:1, 2021; published online as <https://doi.org/10.1094/PDIS-11-20-2429-PDN>. Accepted for publication 6 January 2021.

Viruses transmitted by whiteflies (*Bemisia tabaci*) cause severe damage to cucurbits in the southern United States. In the fall of 2020, samples of yellow squash plants (*Cucurbita pepo*) exhibiting symptoms of yellow mottle, interveinal yellowing, and leaf crumple were collected from an insecticide trial in Tifton, Georgia. Total nucleic acid was isolated using the MagMax 96 Viral RNA Isolation Kit (ThermoFisher Scientific) following the manufacturer's instructions but without DNase treatment. Polymerase chain reaction (PCR) and reverse transcription (RT)-PCR were carried out to determine the presence of whitefly-transmitted viruses. We identified infection by cucurbit chlorotic yellows virus (CCYV) using primers targeting a 953-nt segment of CCYV RNA1 encoding the RNA dependent RNA polymerase gene (RdRp) (CCYV-RDRP-1515F, 5'-CTCCGAGTA GATCATCCCAAATC-3'; CCYV-RDRP-1515R, 5'-TCACCAGAACTC CACAATCTC-3') along with other whitefly-transmitted viruses previously reported in Georgia. CCYV was detected from 27 of the 28 samples tested, and cucurbit yellow stunting disorder virus (CYSDV; Polston et al. 2008) and cucurbit leaf crumple virus (CuLCrV; Gadhave et al. 2020) were detected

from 23 and 28 squash samples, respectively, with all three viruses regularly occurring as mixed infections. The presence of CCYV was further confirmed by amplification of portions of two different genomic segments from RNA2, including a section of the heat-shock protein (HSP) homolog gene (Bananej et al. 2013) as well as a portion of the coat protein (CP) gene, which was amplified using primers CCYV_CPF (5'-TCCCGGTGCCAACT GAGACA-3') and CCYV_CPR, 5'-TACGCGCGCAGAGGAATTT-3'). The respective 462-bp HSP and 375-bp CP amplicons were cloned and sequenced. The partial CP gene sequence (MW251342) was 97.86% identical to a CCYV isolate from Shanghai (KY400633). The partial HSP sequence (MW251341) shared 99.73% identity with the recently identified CCYV isolate from California (MH806868). Criniviruses are an emerging group of whitefly-transmitted viruses responsible for worldwide losses of billions of dollars annually (Tzanetakis et al. 2013). CCYV, a member of the genus *Crinivirus*, was believed to be restricted to Asia, Africa, and the Mediterranean regions of Europe (Bananej et al. 2013; Orfanidou et al. 2014) until it was recently identified in the Imperial Valley of California (Wintermantel et al. 2019). Southern Georgia has been experiencing high whitefly populations, resulting in the emergence of CuLCrV and CYSDV on vegetables in recent years. Because CCYV can produce symptoms virtually identical to those of CYSDV and occurs in mixed infections in cucurbits with other whitefly-transmitted viruses, its epidemiology, role in disease incidence, severity, and impact on economically important crops in the southeastern United States will require further investigation.

References:

- Bananej, K., et al. 2013. Plant Dis. 97:1005.
Gadhav, K. R., et al. 2020. Phytopathology 110:1235.
Orfanidou, C., et al. 2014. Plant Dis. 98:1446.
Polston, J. E., et al. 2008. Plant Dis. 92:1251.
Tzanetakis, I. E., et al. 2013. Front. Microbiol. 4:119.
Wintermantel, W. M., et al. 2019. Plant Dis. 103:778.

The author(s) declare no conflict of interest.

Keywords: cucurbit chlorotic yellows virus, crinivirus, Georgia, squash (*Cucurbita pepo*)

[†]indicates the corresponding author.
S. Bag: Sudeepbag@uga.edu