Diseases Caused by Viruses

First Report of Citrus Tristeza Virus Infecting Citrus Trees in Georgia, U.S.A.

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Citrus tristeza virus (CTV) (genus *Closterovirus*; family *Closteroviridae*) is one of the most important, economically devastating viruses of citrus worldwide. On citrus trees grafted onto sour orange rootstock, typical CTV symptoms include dieback and defoliation, stunting, curling and chlorotic leaves, stem pitting, and pinholes below the bud union on the inner face of the bark (Moreno et al. 2008). This single-stranded, positive-sense RNA virus is most efficiently transmitted by the brown citrus aphid (*Toxoptera citricida*), but it can also be transmitted by other aphid species and through grafting of infected plant material onto healthy plants (Herron et al. 2006; Moreno et al. 2008). In fall of 2020, leaf material for virus testing was collected from 13 navel orange trees (*Citrus × sinensis*) grafted onto *Poncirus trifoliata* rootstocks (including ‘Flying Dragon’) located in a citrus research orchard in Tifton, Georgia (GA). Trees ranged in age from 2 to 10 years, with the younger trees having been grafted from cuttings taken from the older trees. The oldest of these trees was derived from cuttings taken in 2009 from an orange tree growing locally in a residential yard in Tifton. This parent tree was more than 15 years old when these cuttings were obtained and was no longer available for sampling as of 2020. Symptoms of disease had not been noted on any of the tested trees, and trees were chosen for testing prior to the further dissemination of this plant material. The presence of CTV was verified via molecular and serological testing. CTV infection was initially confirmed in eight of 13 tested samples using the ImmunoStrip for CTV assay (Agdia, Elkhart, IN, cat no. ISK 78900/0025) according to the manufacturer’s instructions. RNA was extracted from leaf material collected from the 13 sampled

trees using the RNeasy Plant Mini Kit (Qiagen, Valencia, CA). Following cDNA synthesis, samples were tested for the presence of CTV by reverse transcription polymerase chain reaction (RT-PCR) using primer pair AR18F (5′-ATGTCAGGCAGCTTGGGAAATT-3′) and AR18R (5′-TTCTGTCTAAGTCRCGCTAAACA-3′), which produces a 511-bp amplicon (Roy et al. 2005). RT-PCR reactions confirmed the presence of CTV, with the same eight samples that had previously tested positive via ImmunoStrip producing amplicons of the expected size. Amplified products from two of these samples were then sequenced using Sanger sequencing (Retrogen, San Diego, CA) and subjected to BLAST analysis (Altschul et al. 1990) for further identification. The partial sequence of the p18 gene, generated from both samples, was deposited in GenBank with the accession number MW540805. The BLAST analysis showed 100% sequence identity with CTV complete genome accessions, MK779711.1 and EU937520.1, which group into the T30 genotype. Although previously reported in the nearby states of Florida, Alabama, and Louisiana, to the best of our knowledge, this is the first report of CTV infecting citrus in GA. The T30 genotype is considered to be a “mild” strain of CTV. However, its presence in GA suggests the possibility that more severe strains may also be present, which could pose a threat to the citrus industry if disseminated via infected plant material or vector transfer. Although the brown citrus aphid is not known to be widespread in GA, other CTV vectors are prevalent, including the cotton aphid (*Aphis gossypii*) and the black citrus aphid (*T. aurantii*). Georgia citrus growers and propagators should be aware of this virus and take appropriate precautions.

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