Diseases Caused by Viruses

First Report of Hop Stunt Viroid Infecting Citrus Trees in Georgia, USA

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Plant Dis. 105:515, 2021; published online as <https://doi.org/10.1094/PDIS-07-20-1537-PDN>. Accepted for publication 10 September 2020.

Citrus production has rapidly increased in the state of Georgia (USA); there are now citrus plantings in ≥32 counties in residential, production, and nursery settings. Viroids (circular, single-stranded RNA, 246 to 463 nt [Ito et al. 2002]) can infect citrus. Hop stunt viroid (HSVd) is one of several viroids that infect citrus. It has been previously reported in Arizona, California, Florida, Texas, and Washington (USA) and in other countries (EPPO 2020; Hataya et al. 2017). HSVd is often spread on contaminated tools or through grafting. With a wide host range that includes Moraceae, Rosaceae, and Rutaceae (citrus), this viroid can spread through a nursery (Hataya et al. 2017). In the USA, HSVd has been found on many varieties of grapefruit, oranges, and tangelos (Kunta et al. 2007). Two variants of HSVd infect citrus: cachexia-inducing (CI) and non-CI. These can be differentiated by five nucleotide differences (Palacio-Bielsa et al. 2004). The CI variant commonly causes damage on susceptible varieties; the non-CI variant typically does not. Symptoms of HSVd include discoloration and gumming of phloem tissues, stem pitting, bark splitting, and chlorotic and stunted growth in susceptible citrus varieties including tangerines and their hybrids (Hataya et al. 2017). There are not typically symptoms on leaves or fruits; however, lime plants have shown some yellowing on leaves (Hataya et al. 2017). In May and June 2020, leaf samples were collected from 12 different citrus plants from two nurseries located in Berrien and Mitchell counties in Georgia. Three to five leaves were collected from the canopy of each plant, and a 0.5-mm-diameter square was taken from each leaf cut into 1-mm squares. These pieces were combined into a single sample per plant, with a 200-mg portion used for extraction of nucleic acids. The cultivars sampled included *Citrus reticulata* ‘Dekopon’ as well as *Citrus unshiu* ‘Miho Wase’, ‘Owari’, and ‘Brown Select’. The sampled trees looked

relatively healthy but were tested to ensure that they were negative for target viroids. RT-PCR was initially used to verify infection with HSVd. Genomic RNA was extracted from the leaf tissue of 12 plants using TRIzol reagent (Thermo Fisher). Following cDNA synthesis, samples were tested for HSVd using the primer pair HSVd-F (5'-GGCAACTCTTCTCA GAATCCAGC-3') and HSVd-R (5'-CCGGGGCTCCTTTCTCAGG TAAGT-3'), which produces a 302-bp amplicon (Sano et al. 1988). PCR reactions for nine of the samples did not result in the production of bands; however, the other three samples, all *C. reticulata* ‘Dekopon’, produced the amplicon for HSVd. The amplified products were sequenced using Sanger sequencing (Retrogen), and the identity of the sequences was confirmed with BLAST. Full genome sequences from these amplicons (deposited as MT632007) were identical to one another and shared 99% identity to corresponding HSVd sequences in GenBank (KY654683.1). The resulting sequences were checked for the five nucleotide differences between the CI and non-CI variants, and all three samples were non-CI variants. In addition to RT-PCR and sequencing, the recombinase-polymerase-amplification technology-based AmplifyRP Acceler8 end-point detection assay (Agdia; cat no. XCS64200) was performed on the 12 samples. This assay confirmed HSVd in the same three samples previously confirmed via RT-PCR. This is the first report of HSVd infecting *C. reticulata* ‘Dekopon’ in Georgia. The presence and dissemination of viroids or viruses within citrus nursery stock represent a significant threat to the Georgia citrus industry. Nursery stock infected with this viroid should be destroyed, and Georgia nursery producers and citrus growers must take appropriate precautions, including properly sanitizing tools used for citrus grafting and pruning, to prevent the spread of this and other viroid diseases. Further research is needed to determine the distribution of HSVd and its potential to impact commercial citrus production in Georgia.

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The author(s) declare no conflict of interest.

Keywords: hop stunt viroid, citrus, Georgia

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