OPEN ACCESS Freely available online

Journal of Citrus Pathology

Brief Report

A survey of Florida citrus viruses and viroids

SJ Cowell[1], SJ Harper[2], and WO Dawson[1]

[1]Department of Plant Pathology, Citrus Research and Education Center, University of Florida, Lake Alfred, Florida, USA; [2]Department of Plant Pathology, Washington State University, Prosser, Washington, USA.

*Correspondence to: scott.harper@wsu.edu

Citation: Cowell SJ, Harper SJ, Dawson WO. 2018. A survey of Florida citrus viruses and viroids. J Cit Pathol. iocv_journalcitruspathology_40701.

Abstract

Efficient disease management is critical in the production of citrus; a crop that is susceptible to several plant pathogens. The ongoing battle with citrus greening has led to a shift in cultural practices, which could lead to a resurgence of previously controlled diseases. Here we investigated the presence of several common citrus-infecting viruses and viroids (*Citrus leaf blotch virus*, *Apple stem grooving virus* (synonym: Citrus tatter leaf virus), *Citrus exocortis viroid, Hop stunt viroid* (synonym: Citrus viroid II), and *Citrus dwarfing viroid* (synonym: Citrus viroid III) in Florida citrus groves. All five viruses and viroids are still present, with varying incidence. It would be prudent to take them into consideration when developing citrus disease management strategies.

Keywords: *Apple stem grooving virus, Citrus dwarfing viroid, Citrus leaf blotch virus*, *Citrus exocortis viroid, Hop stunt viroid,* Florida, real-time RT-qPCR

Introduction

Nowhere in the US has citrus held more importance than in Florida, where 56% of the country's citrus is produced (Neupane 2016). Citrus production in Florida has often faced challenges from plant pathogens, but citrus greening is the foremost concern of the Florida citrus industry today. This has led to a shift in cultural practices, such as switching to different rootstocks, which may leave citrus crops susceptible to a resurgence of previously controlled or new diseases.

We previously showed that *Citrus tristeza virus* (CTV), which can pose a serious risk to citrus production, is still widespread throughout the state (Harper and Cowell 2016). But CTV is only one of the viruses and viroids that have been shown to affect tree health and productivity. Some variants of *Hop stunt viroid* (HSVd, synonym: Citrus viroid II) have been associated with cachexia disease, a discoloration and gumming of phloem tissues (Hadidi et al. 2017), while *Citrus leaf blotch virus* (CLBV) causes a bud union crease on trifoliate orange rootstocks (Vives et al. 2002). *Apple stem grooving virus* (ASGV, synonym: Citrus tatter leaf virus) and *Citrus dwarfing viroid* (CDVd, synonym: Citrus viroid III) have been found to stunt the growth of trees grown on trifoliate orange and hybrids (da Graça and Skaria 1996; Gillings et al. 1991). Finally, *Citrus exocortis viroid* (CEVd) causes bark scaling, dwarfing, and reduced yield on trifoliate orange and hybrid rootstocks (Broadbent and Garnsey 1987; Duran-Vila 2017). Most importantly, these citrus pathogens are rarely found as a single infection, which raises the potential for synergistic interaction and exacerbated symptom expression (Tessitori 2017).

Given the rapidly changing nature of the Florida citrus industry, a new framework for integrated disease management strategies must be developed. To do so, the presence and diversity of pathogens present must be assessed. While these pathogens have been reported as present in Florida previously (Garnsey et al. 2002), their current distribution and incidence is unknown. Therefore in this study we built upon the previous CTV survey, searching for common citrus-infecting viruses and viroids present in commercial citrus groves throughout Florida.

Materials and Methods

The incidence of CLBV, ASGV, CEVd, CDVd, and HSVd in Florida citrus was investigated. Sampling sites were selected on the basis of being older, well established, and on sour orange rootstock. Samples from these sites would be more likely to contain the viruses and viroids of interest, since trees on sour orange are more tolerant of these pathogens and therefore less likely to have been removed. A total of 133 samples were collected from commercial and research groves across ten counties, from the southern flatwoods to the northern limit of citrus production (Figure 1).

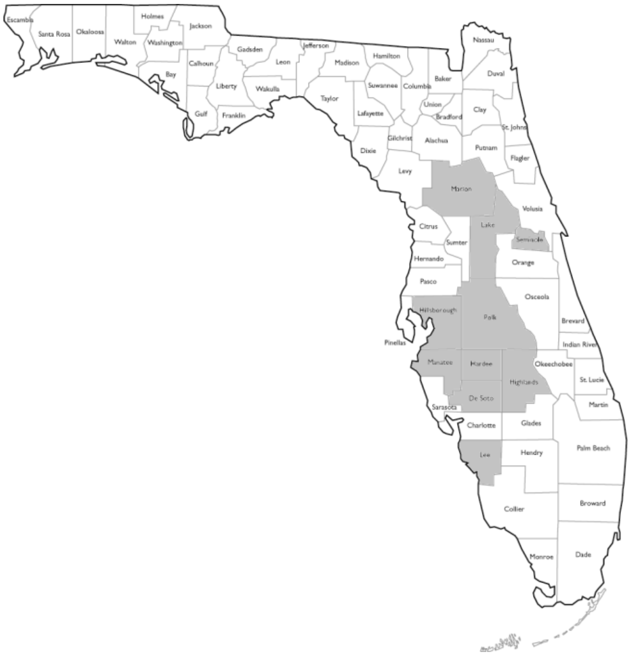

Fig. 1. Location of the counties sampled during this study.

Flush bark, petiole, midrib, and leaf blade tissue was taken from 3-4 sites on each tree, pooled and extracted using TRIzol (Life Technologies, Carlsbad, CA) as per the manufacturer's instructions. Extracts were then diluted 1:10 to reduce the effect of inhibitory substances present prior to amplification. Detection of targeted viruses and viroids were performed using published real-time RT-qPCR assays for ASGV (Cowell et al. 2017), CEVd (Monger et al. 2010), CDVd (Vidalakis et al. 2011), and HSVd (Papayiannis 2014). A new assay for the detection of CLBV was developed for this survey, which was performed using the Superscript III Platinum One-Step qRT-PCR kit (Life Technologies) with a final optimized concentration of 3 mM $MgSO_4$, 400 nM of sense (5'-GGGAACGAAGTTTGCAGCTTT-3') and antisense (5'- CGCTCCCATCAGCTTCAGT-3') primers, 100 nM of Taqman (5'-6-FAM-TATGCAGTAGGTAGCAATGCAGCA-BHQ1-3') probe, and 2 µl of diluted RNA template in a reaction volume of 10 µl. Cycling conditions were: 50 °C for 5 minutes, 94 °C for 2 minutes, then 40 cycles of 94 °C for 10 seconds and 60 °C for 40 seconds. All survey samples were tested in technical replicates of 3, and all assays included negative (no template) controls. Samples with a Ct <36.00 were considered positive.

Results and Discussion

From the survey of commercial and research groves throughout the major citrus producing counties in Florida, we found that unlike CTV, which is prevalent throughout the state (Harper and Cowell 2016), other citrus-infecting viruses and viroids are more scattered in their distribution. For example, none of the surveyed viruses and viroids were detected in the samples from Marion and Hardee counties, whereas they were much more prevalent in Hillsborough, Seminiole, and DeSoto counties; sample sizes from some counties were too low to gain a clear picture of overall incidence (Table 1).

In terms of the pathogens assayed for, ASGV was the rarest, being detected in only 9 of the 133 trees sampled, and these positives were largely from Polk County. CLBV was similarly rare, in 12 of the 133 trees, and again, located primarily in Polk County. Given that both of these viruses do not have known insect vectors, it is possible that this localized spread is due to graft transmission through propagation.

In contrast, the three viroids assayed for were, in general, more widely distributed across the state, and in the cases of CEVd and HSVd, at higher incidence than the other pathogens tested. CEVd was found in 31 of 133 trees surveyed, a total of 23% incidence, while HSVd was found in 40 of 133 trees, an incidence of 30%. CDVd was less frequently found, in only 12 of 133 trees tested. Overall incidence of these viroids was higher in individual groves (data not shown), possibly due to the ease by which these can be transmitted through mechanical means such as hedging or topping of rows (Barbosa et al. 2005).

As with CTV, it is difficult to pinpoint where these viruses and viroids came from, for many of the groves sampled were older, in one case up to eighty years old. This would indicate however, that these viruses and viroids have been present in Florida for a long time. In most cases, the trees sampled did not show obvious signs of disease. It is fortunate that cultural practices, particularly in older groves on sour orange rootstock, have limited the potential risks of disease from the pathogens surveyed, for all five can cause detrimental effects on trifoliate orange, or trifoliate orange hybrid rootstocks (da Graça and Skaria 1996; Broadbent and Garnsey 1987; Gillings et al. 1991; Guardo et al. 2015; Vernière et al. 2004). However, the potential for disease exists, for example CEVd induced stunting, bark cracking and scaling was observed on a new planting on X639 (*Poncirus trifoliata* x *Citrus reshni*) rootstock in central Florida (Harper and Brlansky, unpublished).

While eradication efforts for citrus canker and, more recently, for citrus greening may reduce the incidence, and hence potential for spread of many of these pathogens, growers should be aware that they are still present, and can rapidly spread through infected planting stock, or by cultural practices such as hedging. Furthermore, their continued presence should be considered as part of an integrated disease management strategy, especially in trifoliate orange hybrid rootstocks, some of which have been promoted due to citrus greening tolerance (Bowman and McCollum 2015).

Table 1
Incidence of *Apple stem grooving virus*, *Citrus leaf blotch virus*, *Citrus exocortis viroid*, *Citrus dwarfing viroid*, and *Hop stunt viroid* in plants sampled from commercial citrus groves throughout Florida.

County	ASGV	CLBV	CEVd	CDVd	HSVd
DeSoto	0/33	0/33	0/33	0/33	13/33
Seminole	0/32	0/32	18/32	4/32	13/32
Lake	0/8	0/8	0/8	0/8	0/8
Hillsborough	1/13	2/13	7/13	8/13	11/13
Marion	0/19	0/19	0/19	0/19	0/19
Hardee	0/17	0/17	0/17	0/17	0/17
Polk	5/8	8/8	4/8	0/8	2/8
Highlands	1/1	0/1	0/1	0/1	0/1
Lee	1/1	1/1	1/1	0/1	1/1
Manatee	1/1	1/1	1/1	0/1	0/1
Total	**9/133**	**12/133**	**31/133**	**12/133**	**40/133**

Acknowledgments

This research was supported in part by an endowment from the JR and Addie S Graves family and the Florida Agricultural Experiment Station.

References

Barbosa CJ, Pina JA, Pérez-Panadés J, Bernad L, Serra P, Navarro L, Duran-Vila N. 2005. Mechanical transmission of citrus viroids. Plant Dis. 89:749-754.

Bowman K, McCollum G. 2015. Five new citrus rootstocks with improved tolerance to huanglongbing. Hort Sci. 50(11):1731-1734.

Broadbent P, Garnsey SM. 1987. Citrus Exocortis. In: Diener TO, editor. The Viroids. The Viruses. Boston (MA): Springer. p. 235-245.

Cowell SJ, Harper SJ, Dawson WO. 2017. A real-time RT-qPCR assay for the detection of *Citrus tatter leaf virus*. J Virol Meth. 244:29-31.

da Graça JV, Skaria M. 1996. *Citrus Tatter Leaf virus* in the Rio Grande Valley of South Texas. In: da Graça JV, Moreno P, Yokomi R, editors. Proceedings of the 13th International Organization of Citrus Virologists; China. Riverside (CA): IOCV. p. 198-200.

Duran-Vila N. 2017. Citrus exocortis viroid. In: Hadidi A, Flores R, Randles JW, Palukaitis P, editors. Viroids and Satellites. San Diego (CA): Elsevier. p. 169-179.

Garnsey SM, Zies DL, Irey M, Sieburth PJ, Semancik JS, Levy L, Hilf ME. 2002. Practical field detection of citrus viroids in Florida by RT-PCR. In: Duran-Vila N, Milne RG, da Graça JV, editors. Proceedings of the 15th International Organization of Citrus Virologists; Cyprus. Riverside (CA): IOCV. p. 219-229.

Gillings M, Broadbent P, Gollnow BI. 1991. Viroids in Australian Citrus: Relationship to Exocortis, Cachexia and Citrus Dwarfing. Funct Plant Biol. 18:559-570.

Guardo M, Sorrentino G, Caruso A. 2015. Characterization and incidence of *Citrus leaf blotch virus* (CLBV) in southern Italy. Acta Hortic. 1065:825-830

Hadidi A, Vidalakis G, Sano T. 2017. Economic significance of fruit tree and grapevine viroids. In: Hadidi A, Flores R, Randles JW, Palukaitis P, editors. Viroids and Satellites. San Diego (CA): Elsevier. p. 15-25.

Harper SJ, Cowell SJ. 2016. The past and present status of *Citrus tristeza virus* in Florida. J Cit Pathol. iocv_journalcitruspathology_32387.

Monger W, Tomlinson J, Booonham N, Marn MV, Plesko IM, Molinero-Demilly V, Tassus X, Meekes E, Toonen M, Papayiannis L, Perez-Egusquiza Z. 2010. Development and inter-laboratory evaluation of real-time PCR assays for the detection of pospiviroids. J Virol Meth. 169:207-210.

Neupane D, Moss CB, van Bruggen AH. 2016. Estimating citrus production loss due to citrus huanglongbing in Florida. In: Annual Meeting, Southern Agricultural Economics Association. p. 6-9.

Papayiannis LC. 2014. Diagnostic real-time RT-PCR for the simultaneous detection of Citrus exocortis viroid and Hop stunt viroid. J Virol Meth. 196:93-99.

Tessitori M. 2017. Apscaviroids infecting citrus trees. In: Hadidi, A, Flores, R, Randles JW, Palukaitis P, editors. Viroids and Satellites. San Diego (CA): Elsevier. p. 243-249.

Vernière C, Xavier P, Dubois C, Dubois A, Botella L, Chabrier C, Bové J, Duran-Vila N. 2004. Citrus viroids: symptom expression and effect on vegetative growth and yield of clementine trees grafted on trifoliate orange. Plant Dis. 88:1189-1197.

Vidalakis G, Pagliaccia D, Bash JA, Afunian M, Semancik JS. 2011. Citrus dwarfing viroid: effects on tree size and scion performance specific to Poncirus trifoliata rootstock for high-density planting. Ann Appl Biol. 158(2):204-217.

Vives MC, Galipienso L, Navarro L, Moreno P, Guerri J. 2002. *Citrus leaf blotch virus*: a new citrus virus associated with bud union crease on trifoliate rootstocks. In: Duran-Vila N, Milne RG, da Graça JV, editors. Proceedings of the 15th International Organization of Citrus Virologists; Cyprus. Riverside (CA): IOCV. p. 205-212.