

CTV status in Florida

By S.J. Harper, S.J. Cowell, S.E. Halbert, R.H. Brlansky and W.O. Dawson

Citrus tristeza virus (CTV) is an almost ubiquitous pathogen of citrus. It is nearly impossible to prevent a tree from becoming infected during its productive lifespan in any major citrus-producing region where the brown citrus aphid (BCA) is present. Outbreaks of quick decline, the classic disease syndrome caused by CTV, had historically caused losses of more than 50 percent in some Florida groves, and led to a change in cultural practices, including the wide-scale abandonment of sour orange as a rootstock.

Over the past decade, this old enemy has received little attention due to the Huanglongbing (HLB) epidemic in Florida. CTV infection, unlike HLB, is not necessarily a death sentence to the tree. This is because the onset of tristeza diseases are dependent on three factors: the scion species or cultivar infected, the species of the rootstock on which the scion is grafted, and the infecting strain or isolate(s) of CTV. This is particularly relevant to Florida given that the bulk of new plantings are sweet orange cultivars and, with the advent of HLB, we see increasing use of sour orange rootstocks: up from 0.3 percent of new plantings in 2005 to 14.3 percent in 2014.

Will decline-inducing CTV strains, which are endemic throughout the state, cause another epidemic of decline on sour orange rootstock, aided and abetted by the continued presence of BCA? Here in this review we examine the status of CTV in Florida, highlighting recent findings and areas of future research.

CTV infection, unlike HLB, is not necessarily a death sentence to the tree.

STRAINS OF CTV

We began by examining a historical collection of samples, made by the U.S. Department of Agriculture over the past 40 years. When these samples were collected, the only means of differentiating one CTV isolate from another was through comparing symptoms on certain host species, a method which cannot reveal the complexity of the viral population present and often allows latent, mild infections to pass unnoticed. With the development of new molecular tools, we now have the opportunity to accurately determine the incidence of major CTV strains (Figure 1).

Using these tools, we found that three major strains were present in Florida over the past 40 years, each named after their type isolate: T36, some of whose isolates are known to induce decline; T30, the “mild” strain; and VT, a strain best known for causing stem pitting and decline in Asia and the Mediterranean. The first two strains have been present in Florida for a long time, and the incidence of these in infected trees has generally remained stable, while the incidence of VT-like strains has increased since the introduction of BCA in the mid-1990s. This is not to say that VT was a recent introduction; at least one variant of VT has been present since the 1960s in Meyer lemon, and there is anecdotal evidence suggesting that VT may have been introduced as early as sometime between 1900 and 1920.

But what about CTV today? Over the past year, we have conducted a series of surveys to determine the population composition and structure of CTV in Florida, with a particular emphasis on trees grown on sour orange rootstock — those most at risk from a resurgence in quick decline. We found while VT incidence spiked after arrival of BCA in 1995, it has since dropped to half by 2014 (Figure 1). It may very well be that many of the trees infected with VT in the decade after the arrival of the BCA are now dead from decline, greening or were removed during the canker eradication

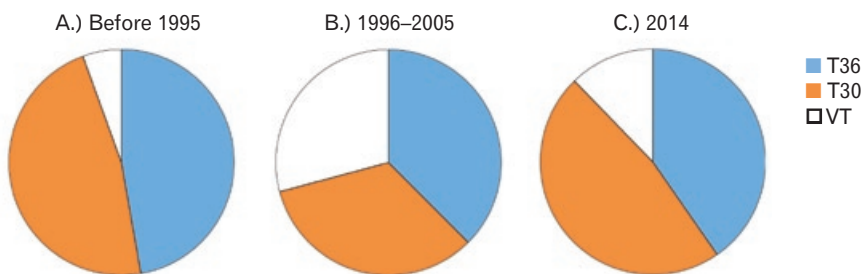


Figure 1. Percentage of incidence of the major *Citrus tristeza virus* strains T36 (blue), T30 (orange) and VT (white) present in samples collected before 1995 (A), and approximately one (B) and two (C) decades after the arrival of the brown citrus aphid, *Toxoptera citricida*.

THE ORIGINAL
Tropicana
PURE PREMIUM

Tropicana Sr. Procurement Manager
 Eddie Wertz
 Office: (941) 742-2646 or (863) 494-5658
 Mobile: (863) 990-1852

Buyer
 Steve Pfleger
 Office: (941) 742-2343
 Mobile: (863) 899-5345
 Northern Area

Buyer
 Steve Riffle
 Office: (863) 453-4359
 Mobile: (863) 214-3222
 Southern Area

Table 1. The incidence of three different *Citrus tristeza* virus strains (T36, T30 and VT) as identified by real-time PCR in Florida in 2014, sorted by county. Values represent the number of trees positive for each strain, over the total number sampled.

County	No. trees CTV positive / No. trees tested	No. trees positive for strains		
		T36	T30	VT
Marion	19 / 19	19 / 19	19 / 19	11 / 19
Lake	14 / 14	11 / 14	14 / 14	0 / 14
Polk	9 / 9	9 / 9	6 / 9	9 / 9
Collier	16 / 18	10 / 16	15 / 16	2 / 16
Hardee	17 / 17	17 / 17	17 / 17	1 / 17
Desoto	33 / 33	13 / 33	30 / 33	0 / 33
St. Lucie	30 / 30	16 / 30	30 / 30	0 / 30

program. However, when we divide the results by county (Table 1), we have a clearer picture of what is occurring. The T36 and T30 strains are found in almost all infected trees throughout the state, whilst VT distribution is more sporadic. We also found that VT was either present in all infected trees within a grove, or not present at all, suggesting spread within, but not between, groves in an area. It is likely that the pesticide spray regimes in place to control the spread of HLB are also effective at reducing CTV spread.

The continued presence of the T36 and VT strains in Florida is a cause for concern, particularly for groves replanted on sour orange rootstock. Many isolates containing these two strains will stunt sour orange seedlings in greenhouse studies, a potential indicator for decline. The persistence of T36 in particular is unfortunately helped by use of decline-tolerant rootstocks such as Swingle citrumelo, which, while not susceptible to decline themselves, readily support T36 infection and so form an excellent reservoir for further viral spread to neighboring trees.

It should also be noted here that T30, the so-called “mild” strain, is not as mild as it seems. New methods of discovering all CTV strains present in an infected plant showed that most plants thought to be infected with T30 alone actually contain T30 with small amounts of T36 that are largely undetectable by the MCA13 antibody screen. Furthermore, several of these

“mild” isolates have been found to cause severe stunting and stem pitting of grapefruit in greenhouse studies.

MIXTURES AND POPULATIONS

Our surveys also illustrated an important point: Nearly all trees infected by CTV in Florida contain a population of two or three strains. The strains present in these populations have the potential to interact, and it is these interactions that will dictate the outcome of the infection, determining the type of symptoms produced and their severity. For example, simply having T36 in a population does not guarantee the onset of quick decline of sweet orange on sour orange rootstock, nor does having T30 guarantee a symptomless infection in sweet orange or grapefruit. On the other hand, we have observed that T36 establishes a more extensive infection in the presence of VT, potentially exacerbating symptom expression.

The interaction between strains of a population is also the basis of cross-protection, the deliberate inoculation of trees with a “mild” isolate in the hope of preventing damage from more “severe” isolates. If we look at the most successful cross-protection schemes used around the world — those in Pera sweet orange in Brazil and grapefruit in South Africa — we see that the cross-protecting isolates are populations comprised of what are individually very severe and damaging strains. The strains of each population

VIGIRON
Simple = COMPLEX
 Simple natural affinity
 is the COMPLEX...
Iron Humate
Carbon and Iron
 That's it, except for... the simple
 natural recommendation:
 “Serve some to your soil today, and it
 will stand to serve your crop tomorrow.”

Available bulk, F.O.B., Ruskin, FL
 813.645.6911 www.Vigiron.com
 3033 Cockroach Bay Rd., Ruskin, FL 33570

have reached an equilibrium with each other and with their host, reducing the effects of CTV infection. Yet, this balance is easily upset. An introduction of new strains, a change in host species or cultivar, or even a change in environmental conditions has been shown to break cross-protection and lead to disease.

In Florida, there has been some use of cross-protective isolates, most of which we have found to be a mixture consisting of a majority T30

strain with a latent T36 strain (Figure 2). This population appears effective at preventing decline amongst the groves we have visited, although we did notice slow decline symptoms breaking through in some of the protected plants. When we examined this further, we found that healthy or asymptomatic plants (Figure 2) maintained the expected high T30-low T36 population structure while those expressing symptoms had shifted, with T36 becoming the

dominant member of the population while T30 decreased (Figure 2). What causes such a shift is unknown and is a major focus of our research into the behavior of virus populations.

APHID TRANSMISSION

There are a number of aphid species found on citrus in Florida, but only four of these — BCA (*Toxoptera citricida*), the black citrus aphid (*Toxoptera aurantii*), the spirea aphid (*Aphis spiraecola*) and the melon aphid (*Aphis gossypii*) — can transmit CTV. Of these, BCA is the most effective vector; after its arrival in Florida, CTV infection rates increased from 10 percent to 20 percent to more than 80 percent in some areas. During the decline epidemics of the late 1990s, BCA incidence was extremely high and had largely displaced the previously abundant *T. aurantii* (Figure 3). Over the past few years, the BCA population has declined (Figure 4) and stabilized at approximately 25 percent of the aphid population found on citrus in Florida, due in part to this species' sensitivity to pesticides. Should conditions permit, however, BCA populations can increase exponentially, as they reach maturity within a week, and each adult can give birth to approximately 30 to 40 nymphs.

The transmission rate of endemic Florida CTV isolates varies considerably due to host species and population composition. We have found groups of as few as 10 BCA can transmit the two major CTV populations found in Florida groves, T36-T30 and T36-T30-VT, at rates of nearly 20 percent. This means that if conditions are suitable and no control measures are applied, CTV can spread very quickly into new plantings. Even with spray regimes in place, growers should be aware that the virus can still spread, albeit at a reduced rate. During our 2014 surveys, we observed that most replants and new plantings in the vicinity of older, infected trees were infected with CTV.

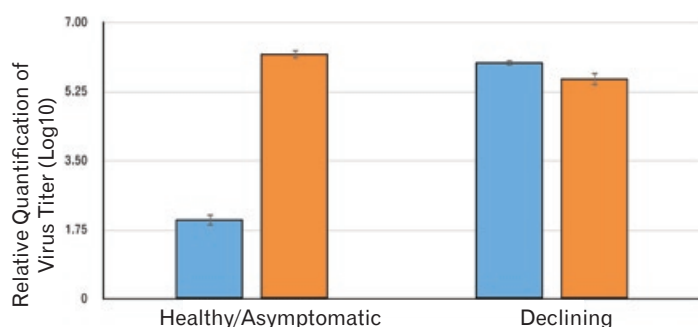


Figure 2. A comparison of the proportions of the typical Florida population of strains T36 (blue) and T30 (orange) in Valencia sweet orange on sour orange rootstock in asymptomatic trees and declining trees.

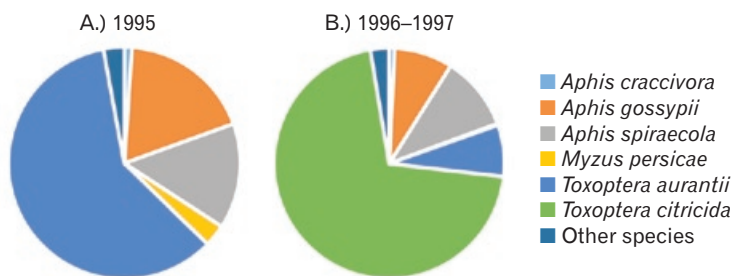


Figure 3. The incidence of aphid species on citrus from collection traps before (A: 1995) and after (B: 1996-1997) the arrival of the brown citrus aphid, *Toxoptera citricida*, in Florida. Note that all named species, with the exception of *Aphis craccivora*, can transmit CTV.

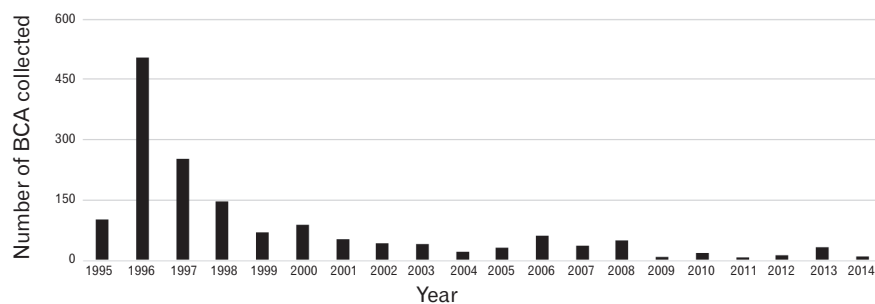


Figure 4. Numbers of samples of the brown citrus aphid, *Toxoptera citricida*, collected by the Division of Plant Industry on citrus since 1995.

SUMMARY AND FUTURE DIRECTIONS

While CTV is not currently as significant a problem as HLB, virus populations remain as diverse and widespread as they did before the decline epidemics of the late 1990s. With changes in scions grown, rootstock selections and cultural practices, the potential for a resurgence

present in the state have been found to induce severe stem pitting under experimental conditions, stem pitting has not been observed in productive groves. Nevertheless, the potential for an outbreak exists. Illegal importation of budwood could introduce a new CTV strain, or changes in cultural practices may change already existing CTV populations in Florida groves.

There is hope that future research will lead to long-term management strategies for citrus tristeza diseases.

in tristeza disease is very real. This is particularly true of stem pitting in sweet orange and grapefruit cultivars, which is the primary CTV disease syndrome in citrus in Australia, Japan, South America and South Africa. The Florida industry has been fortunate thus far in that while many isolates

Controlling future outbreaks of stem pitting through cross protection is one solution that has been successfully applied by major citrus producers in Australia and South Africa in grapefruit, and in sweet orange in Brazil. It should be noted, however, that these solutions are specific to local

host and environmental conditions. Despite having successful cross-protection schemes in grapefruit, both Australia and South Africa are beset by stem pitting in orange cultivars, while in Brazil the cross protective isolate only works in Pera, and not at all in Valencia or Hamlin sweet orange cultivars. In time, it may be possible to develop viable cross-protection isolates for Florida citrus against stem pitting, but as overseas experience shows, these will be cultivar-specific and not a one-size-fits-all solution.

With the increased use of sour orange as a rootstock for sweet orange cultivars to promote rapid tree growth in the face of greening, a resurgence of decline is a possibility. However, unlike stem pitting, our research has shown that decline has the possibility of being managed in Florida, for there is a correlation between the relative titers of T36 and T30 and disease onset. When T36 is at low titer and T30 is abundant in a tree, trees remain asymptomatic, whereas when T36 titer increases, trees decline. There is hope that with our growing understanding of the biology of CTV and its interaction with its hosts, we can determine the threshold level beyond which T36 causes damage, and identify the host and environmental factors that will allow us to manipulate the population to keep its titer low.

To summarize, CTV remains a real threat to the continued viability of the Florida citrus industry, and growers should be aware of cultural practices that could cause a resurgence of decline disease or an outbreak of stem pitting on sweet orange or grapefruit. There is, however, hope that future research will lead to long-term management strategies for citrus tristeza diseases. 🍊

S.J. Harper and S.J. Cowell are post-doctoral associates, R.H. Brlansky is a professor, and W.O. Dawson is an eminent scholar – all at the Citrus Research and Education Center in Lake Alfred. S.E. Halbert is courtesy assistant professor with the Division of Plant Industry in Gainesville.

We keep you growing...

- Over thirty years of industry experience
- Florida's largest citrus crop insurance provider
- Knowledgeable & professional service
- Products for all your risk management needs

Carden & Associates, Inc.
888.296.7533 toll free
info@cardeninsurance.com
www.cardeninsurance.com

