Photo by Ozgur Batuman

Figure 1. Asian citrus psyllid adults, the insect vectors of HLB, feed on feather shoots of a young citrus tree.

Viruses in the gut of Asian citrus psyllid: Friends or foes?

By Ozgur Batuman and Amit Levy

Management options for hunaglongbing (HLB) are limited and rely heavily on insecticides for controlling Asian citrus psyllid (ACP) populations, even when integrated with other cultural control methods. Chemical strategies are expensive and, if not rotated, can contribute to the development of chemical resistance among ACP populations.

In some cases, long-term chemical applications may have negative effects on the environment and beneficial organisms. Protective screening, such as individual protective covers and citrus under protective screen, is one tactic researchers are exploring to protect new citrus plantings from HLB infection and to avoid the development of pesticide-resistant ACP populations. Other novel tactics that do not rely only on pesticides, including use of biological control agents for effective ACP management, are still much needed for long-term viability of the citrus industry.

VIRAL VECTORS

Viruses are the most abundant biological entity on Earth and can offer limitless biotechnological opportunities to explore and provide further knowledge of the *C*Las-vector interaction. Viral vectors have been documented as effective ways to deliver RNA interference (RNAi), a biological process in which RNA molecules inhibit gene expression by neutralizing targeted RNA molecules, into both plants and insects. They can be used for the biological control of the ACP and eventual disruption of HLB disease cycle.

Citrus tristeza virus (CTV) is currently used as a viral vector to deliver this RNAi technology into the citrus phloem and then into the ACPs that feed on citrus. However, these routes can be cumbersome, because they require an established infection in the citrus plant before delivering RNAi to the insect. Employing an insect virus that easily replicates and expresses proteins in the ACP can have the potential to deliver RNAi with higher efficiency and lower chances of deleterious, off-target effects.

The endemic establishment of both HLB and the ACP in Florida has greatly increased the need for alternative control options.

Development of knowledge-based and efficient biological control strategies to disrupt HLB spread by current technologies (i.e., RNAi) in the ACP vector itself is a strategy to control the disease without relying solely on chemical applications. The necessity of a precise and specific biological control for Florida ACP populations initiated an ongoing University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) study, with hopes to provide additional molecular tools.

In order to decrease or eliminate major dependence on broad-spectrum chemical controls, the study aims to investigate the Florida ACP body (i.e., gut) for an ACP-associated virus with the potential to be manipulated into a biocontrol agent against the HLB insect vector. The endemic establishment of both HLB and the ACP in Florida has greatly increased the need for alternative control options.

For this reason, the ongoing project was initiated in August 2017 in collaboration with many citrus growers to survey and characterize viruses infecting ACPs in Florida groves. The specific objectives of the project are:

Six Decades of Experience Speaks

By Rick Dantzler

This is the third entry in a series that shares what those in the citrus industry are doing to be successful in the HLB era. The first column was on Jim Snively and Southern Gardens, one of the largest citrus-growing companies in Florida. Phil Rucks' adaptation in growing nursery trees was the topic of the second article. This month, the focus is on a grower of fewer acres but one with a strong citrus background, Frank Thullbery of Lake Wales. At 89 years young, he isn't shy about sharing his thoughts.

"We have to get past the idea that greening kills trees because it doesn't. It makes them sick and other stuff kills them, and foot rot is the biggest problem of all," says Thullbery. "Growers aren't treating for foot rot nearly as much as they should. It's in all mature groves. It has to be dealt with or it will kill the trees. And some growers are watering too much. Why water during periods of heavy rainfall? Wet feet set the stage for foot rot. Your grove will let you know when it's thirsty."

There's no question that foot rot is a problem, says University of Florida plant pathologist Megan Dewdney. "Phytophthora foot rot is a perennial concern. Overwatering is a problem for rot management as *Phytophthora nicotianae* needs water to survive and infect," she says.

After graduating from the University of Florida with a degree in citrus horticulture in 1952, it was off to the war in Korea for Thullbery. When he returned, he went to work in the citrus business, ultimately starting Thullbery Caretaking, Inc. with his wife of 68 years, Catherine. They made a dynamic team, growing the business to a peak of 1,600 acres under their care.

They sold the business in 1981 and began overseeing approximately 360 acres for relatives and business partners. Today, Thullbery works only on the 10-acre block he and a nephew bought, but his 21 years as a production manager and 23 years as a caretaker have left him with a wealth of knowledge.

"After getting rid of the bad trees and replanting, we applied a fungal treatment for phytophthora," explains Thullbery. "We apply two or three oil and nutritional sprays in the summer and inject probiotic materials. The oil sprays keep the psyllids down and allow beneficial insects to survive, which helps with the psyllids.

"We apply three applications of high-analysis fertilizer per year, hedge and irrigate with Microjets when necessary. But controlling phytophthora is the key."

I asked Thullbery if he'd be in the citrus business today if he were a young man. "I surely would. I'd be looking for a piece of land to buy that I could plant trees on," he answered. "The problem is too many people let their groves go and then want to sell them as real estate instead of grove."

And the secret to success? "Grow good quality, edible fruit that can be packed fresh, and turn what's left into great NFC (not from concentrate) juice."

It's a strategy that has worked well for him for a very long time.

Postscript: Frank Thullbery's great partner in life and business, his wife Catherine, died just prior to print.

Rick Dantzler is chief operating officer of the Citrus Research and Development Foundation.

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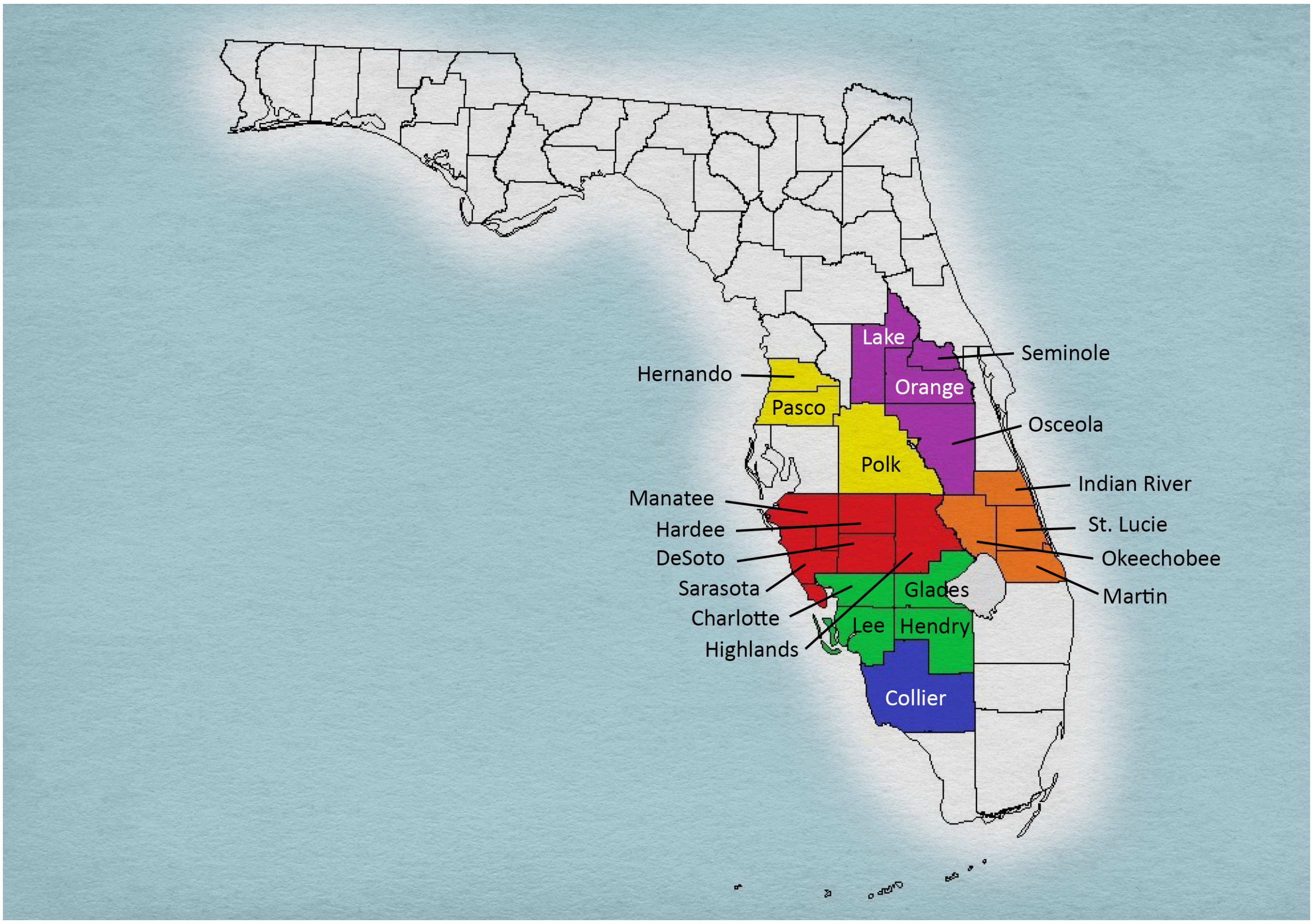

Figure 2. Surveys were conducted and ACP populations were collected monthly in 21 Florida counties in major citrus-production areas.

1) To survey and determine the prevalence of viruses in ACPs in Florida citrus groves
2) To identify novel ACP-associated viruses
3) To explore the newly identified virus(es) as a potential biological agent and RNAi vector for ACP control.

VIRUSES IDENTIFIED

Beginning in August 2017, ACP adults and nymphs were collected monthly from more than 20 different commercial citrus groves, each representing a major citrus-producing county throughout south-central Florida (Figure 2). Five ACP viruses that were previously identified in ACPs from different parts of the world by Bryce Falk's group at University of California, Davis were successfully detected in adult and nymph ACP samples from surveyed Florida groves. These viruses include *Diaphorina citri*-associated C virus (DcACV), *Diaphorina citri* picorna-like virus (DcPLV), *Diaphorina citri* flavi-like virus (DcFLV), *Diaphorina citri* reovirus (DcRV) and *Diaphorina citri* densovirus (DcDNV). Genome analysis of these viruses detected in Florida citrus groves revealed that these viruses were very similar to those viruses reported around the world.

Among these five viruses detected in Florida, the DcACV has consistently remained the most prevalent virus in ACP populations screened during the entire study. DcACV, an RNA virus, was continuously detected in both adult and nymph ACP samples coming from each of the surveyed sites throughout the year, whereas the other viruses were not found as consistently or were primarily found in nymphs.

The second most prevalent virus in Florida ACP was the DcPLV, another RNA virus. The virus was detected in ACP adults and nymphs from all surveyed sites, but less frequently than DcACV.

DcFLV was detected at similar levels to DcPLV in adults and nymphs from all surveyed sites. DcDNV, the only DNA virus found in this study, was detected in ACP from all surveyed sites and appears to infect mainly nymphs. This virus has not previously been observed in Florida. The reovirus, DcRV, was found only in ACPs from two sites during the study. It is possible that DcRV is lethal to ACP, which would explain the scarcity of ACP with this virus in our surveys.

Further studies conducted on grove-collected ACPs identified two new ACP-infecting viruses: another reo-like virus and a picorna-like virus. The new reo-like virus was later surveyed and detected in both adult and nymph ACP populations in Florida. One of the unexpected outcomes of the results, however, was the discovery that CTV, another devastating citrus disease (see the May 2019 Citrus Industry issue), is extremely abundant in the ACP guts. These results cautiously suggest that, although CTV does not cause noticeable decline or induce symptoms in citrus trees, the virus persists throughout Florida. The abundance of CTV and other unknown viral sequences detected in ACP guts warrants further research to determine the function of these viruses.

WHAT NEXT?

Identifying novel and other previously known ACP-associated viruses, as well as conducting a comprehensive survey to better understand the prevalence of these viruses in ACP populations for the first time since introduction of the ACP into Florida in 1998, is a major step forward. However, it is not known yet whether these viruses are friends or foes and whether they are contributing to or altering the spread of HLB. Finding an ACP-virus that behaves as a lethal pathogen (e.g., DcRV) or may be present in all psyllids in Florida (e.g., DcACV) will provide foundational support for the ability to identify a widespread ACP-infecting virus and use it as a viral vector for the modification and biological interruption of the HLB pathosystem.

The hope is to determine the potential effectiveness of viruses to deliver therapies into the insects (i.e., as vectors of RNAi) for ACP and HLB management.

Ultimately, the goal is to find a virus, like the densovirus found in cricket (paralysis virus), that can immobilize ACP so that it could be used as a biological control agent. Unknown additional viral sequences detected in the study will be characterized in the future, as well as pursued for the potential biological control of ACP. The hope is to determine the potential effectiveness of viruses to deliver therapies into the insects (i.e., as vectors of RNAi) for ACP and HLB management.

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Ozgur Batuman and Amit Levy are UF/IFAS assistant professors at the Southwest Florida Research and Education Center in Immokalee and the Citrus Research and Education Center in Lake Alfred, respectively.