A two-pronged approach to suppress psyllids

By Bryony C. Bonning and Lukasz L. Stelinski

Asian citrus psyllids (ACP) transfer the pathogen that causes citrus greening from plant to plant as they feed. After confirmation of citrus greening in Florida in 2005, growers intensified their use of insecticides against ACP to try to stop disease spread. However, this method alone has yielded variable success and increased costs. At times, it has resulted in unwanted consequences, such as populations of ACP with greater resistance to certain insecticides.

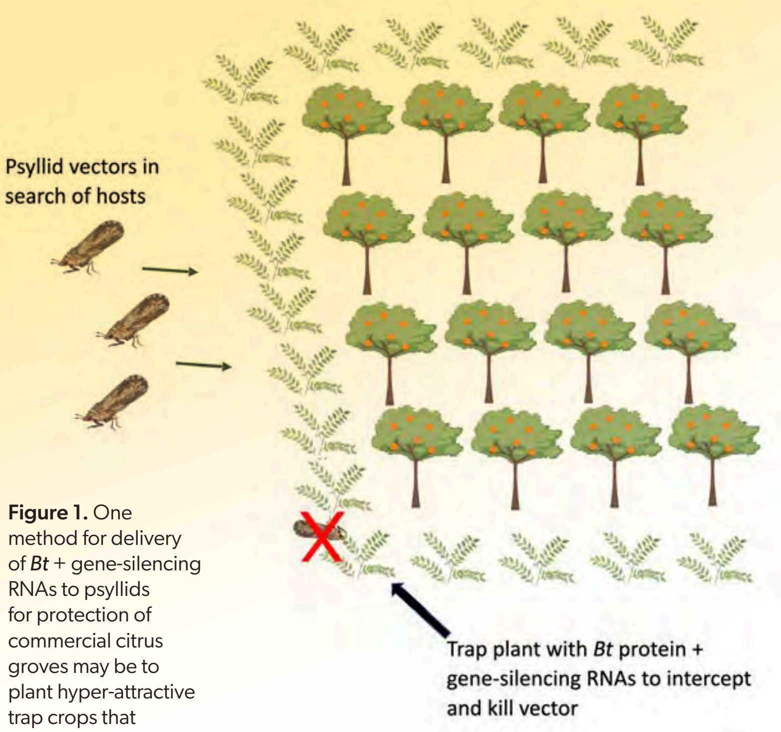

Figure 1. One method for delivery of *Bt* + gene-silencing RNAs to psyllids for protection of commercial citrus groves may be to plant hyper-attractive trap crops that express the active ingredients around groves. These trap crops would serve to intercept and kill the mobile psyllid vectors.

A new alternative approach for suppressing ACP populations is the use of pesticidal proteins produced by bacteria. The soil-dwelling bacterium, *Bacillus thuringiensis* (*Bt*), produces many proteins that are insecticidal against certain types of insects. These proteins allow *Bt* to enter the insect host where it divides, ultimately killing the insect. *Bt* is a valuable natural product for insect control and is widely used to control mosquito larvae residing in pools of water, and in organic agriculture.

In addition to using the whole bacterium, the pesticidal proteins that *Bt* produces also provide useful tools for pest control. Plants can be modified to produce these *Bt*-derived pesticidal proteins to target specific insect pests that feed on a particular crop, without putting non-target and beneficial organisms, such as pollinating insects, at risk.

These proteins are not only specific to insects in their activity, but each protein targets a limited spectrum of insects and is not harmful to humans. Therefore, the use of *Bt* pesticidal proteins for ACP control represents a more sustainable alternative for psyllid management.

Drawing on the use of *Bt* pesticidal proteins for managing other insects, such as the western corn rootworm in maize and the pink bollworm in cotton, researchers at the University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) have tested multiple *Bt* pesticidal proteins for toxicity against ACP. Several candidate proteins that were effective against ACP in the laboratory were selected for further investigation.

DELIVERING PROTEINS

The next challenge was to deliver the ACP-active pesticidal proteins to the plant phloem, where ACP feeds. Researchers explored two ways of accomplishing this.

One approach is to use a plant virus (citrus tristeza virus) that replicates in plant phloem as a delivery vehicle. These viruses naturally inhabit citrus phloem, and mild strains are known to inhabit and replicate within cultivated citrus without harming the trees. These viruses can be readily modified to produce *Bt* pesticidal proteins within trees, then used to infect cultivated citrus. After infecting citrus with the modified viruses, the ACP-killing *Bt* proteins are produced in the tree phloem, where the psyllids feed, until the viruses naturally die off. In at least one case, the *Bt* protein persists in the plant even after the virus has declined. Citrus could be periodically infected with these benign viruses that produce a highly specific ACP-killing protein instead of spraying trees frequently with broad-spectrum insecticides that negatively affect biological control agents.

A second approach is to engineer the actual plant to produce the ACP-killing protein in its phloem. This approach could be used to create cultivated citrus that produces an ACP defense protein.

"Trap plants" also could be created that are more attractive to psyllids than cultivated citrus. In this case, trap plants would be planted around a cultivated grove and potentially serve as a barrier, deflecting psyllids from the citrus grove itself (Figure 1). This approach builds upon the idea of using artificial borders or windbreaks, which have proven to effectively reduce psyllid movement into groves. However, in this scenario, the *Bt* pesticidal protein produced by the trap plant would kill the psyllids, potentially further reducing the number of insects that reach the grove. A "hyper-attractive" trap plant, such as Indian curry leaf, would out-compete citrus in terms of its attractiveness to psyllids. UF/IFAS researchers have successfully created such Indian curry leaf plants that express *Bt* pesticidal proteins and kill psyllids.

UF/IFAS researchers also have produced citrus seedlings that express *Bt* pesticidal proteins and verified that these can kill feeding ACP. However, the selected *Bt* pesticidal proteins showed only moderate effects against ACP. The proteins by themselves may not represent a major advancement over control achieved with insecticides.

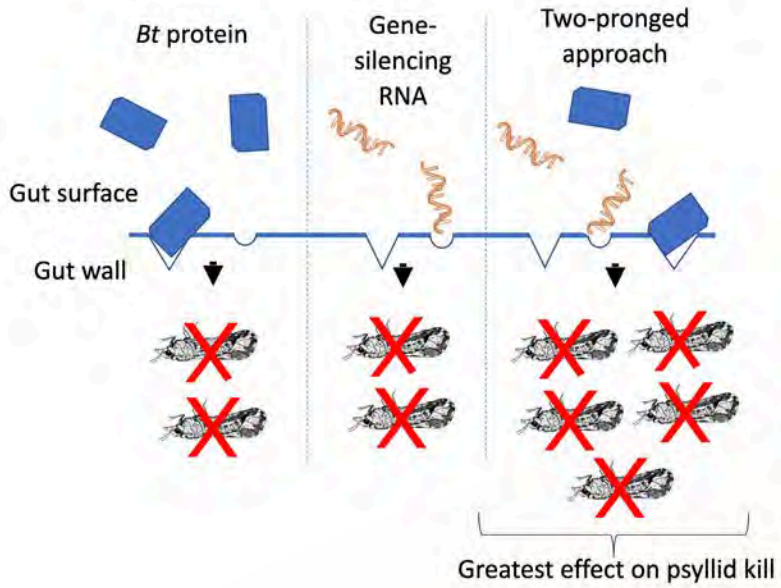

Figure 2. *Bt* proteins and gene-silencing RNAs are individually effective tools that kill Asian citrus psyllid. However, combining the two increases effectiveness above that seen for either approach alone.

A PSYLLID-KILLING COMBO

Researchers then tested the effects of the pesticidal protein in combination with gene-silencing RNAs. Gene-silencing RNAs are small molecules that reduce or block the expression of ACP genes that the psyllids need for survival. These RNAs have been investigated for some time as possible tools to manage ACP and can be delivered to the psyllid in the field by the same methods described above for *Bt* proteins.

The use of *Bt* pesticidal proteins in combination with gene-silencing RNAs has been shown to be significantly more effective than use of either technology individually against other insect pests, notably the western corn rootworm. First, when a *Bt* pesticidal protein is ingested by ACP, it creates a hole in the gut wall and causes the insect to stop feeding because of the damage. Then, the gene-silencing RNAs shut down the targeted genes in the ACP gut, effectively halting production of proteins from those "survival" genes. One possible explanation for the enhanced combined effect is that damage caused by the pesticidal *Bt* protein makes it easier for the gene-silencing RNAs to enter cells lining the psyllid gut where their targets are located (Figure 2).

Research on citrus greening management is comprised of a large portfolio of projects that focus on improving existing protocols, developing novel near-term tools, as well as projects that aim to have large impact, but that may require several years to implement. This project fits in the last category. For this project, researchers have identified *Bt* pesticidal proteins that kill ACP and combined this technology with a viable method to deliver the proteins to the psyllids infesting actual groves. This has been accomplished through cooperation among a large team of entomologists, molecular biologists, pathologists and plant geneticists, with oversight from growers and industry personnel.

Although the project has met several predetermined milestones with success in a laboratory setting, some remaining challenges must be overcome to deliver a field-ready technology to growers who need new tools now. Therefore, results are frequently shared with industry to elicit feedback on how to minimize potential regulatory challenges to acceptance of a field-ready product. A field-ready tool that combines *Bt* pesticidal proteins with gene-silencing RNAs will not by itself solve the problem of citrus greening. This technology is envisioned as working in concert with an integrated program that simultaneously focuses on plant health and potentially targets the pathogen itself. However, this novel two-pronged approach is expected to reduce reliance on insecticides and increase the capability to manage psyllids on a large scale.

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