Using tristeza virus to provide citrus with anti-microbial or insecticidal protection

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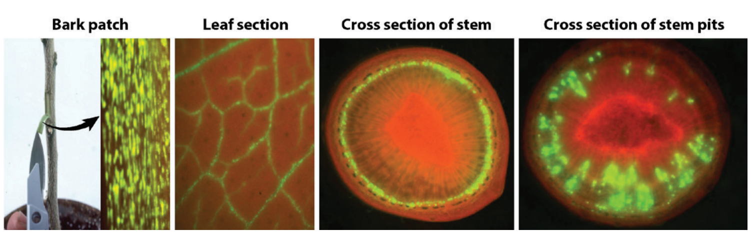
What is the technique?

A novel way to kill Asian citrus psyllids is to insert into the citrus plant insect-derived or plant-derived peptides. These are short portions of protein that have insecticidal (kill the insect) or antimicrobial (kill *C*Las) activity. An example of a plant-based insecticidal peptide is lectins, that act against phloem-feeding insects. Lectins affect receptors in the insect gut, causing lesions that prevent the insect from feeding, and so it dies. Antimicrobial peptides affect the internal communities of endosymbiotic (helpful) bacteria in psyllids, reducing psyllid fitness. Antimicrobial peptides also have the potential to kill *C*Las bacteria directly, if selective antimicrobials can be found, engineered into plants, and delivered to the psyllid.

Using viruses as delivery tools

Antimicrobial peptides could be inserted into citrus via genetic engineering, but this approach requires replanting of existing citrus trees and the process would need to be repeated for each variety of citrus. An alternative method to deliver peptides, is to engineer a mild strain of the citrus tristeza virus (CTV) that, in itself is not damaging to citrus, but it can express insecticidal or antimicrobial peptides. This altered CTV would act as a carrier to inoculate trees and so would not require the trees to be replanted – creating an approach that is much faster than engineering the plant.

In order to show visually where the virus ends up in the tree, Dawson created a virus that makes a visual green fluorescent protein (GFP). CTV was shown to produce GFP (green color) to very high levels (below) throughout the tree. Recently,



Expression of green fluorescent protein by the CTV vector in citrus trees

Dawson and his collaborators have used the vector to produce a wide range of antimicrobial peptides to control HLB.

Who is working on the Project?

Bill Dawson at the University of Florida, has pioneered research on development of CTV vectors to transport foreign genes within plants. Through his laboratories' research, CTV vectors have been developed to express foreign genes throughout citrus trees, acting like 'vehicles' to deliver antimicrobials into the tree. Dr. Kirsten Pelz-Stelinski, also of the University of Florida is collaborating in learning how the insecticidal peptides affect psyllids.

What are the challenges and opportunities?

Citrus budwood, containing a mild strain of CTV that makes insecticidal or antibacterial peptides specific for psyllids, could be grafted onto nursery trees. Or, the CTV budwood could be graft-inoculated onto existing trees, negating the need for tree removal and replanting, and would not require developing new technologies for different varieties. Because making the peptide is not a permanent ability of the virus, it eliminates the possibility of the trait escaping in pollen. The major limiting factors in developing this technology are finding effective antimicrobial and insecticidal peptides to incorporate into the CTV and obtaining approval for their use from the FDA and EPA. To this end, on May 10, 2018 USDA APHIS released a draft Environmental Impact Statement for a 45 d period for public comment (https://www.regulations.gov/docket?D=APHIS-2017-0018). This document describes the environmental impacts that might result from the environmental release throughout Florida of genetically engineered Citrus tristeza virus. They also included an analysis of the plant pest risks associated with using this engineered virus as a biological control agent to help manage HLB.

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