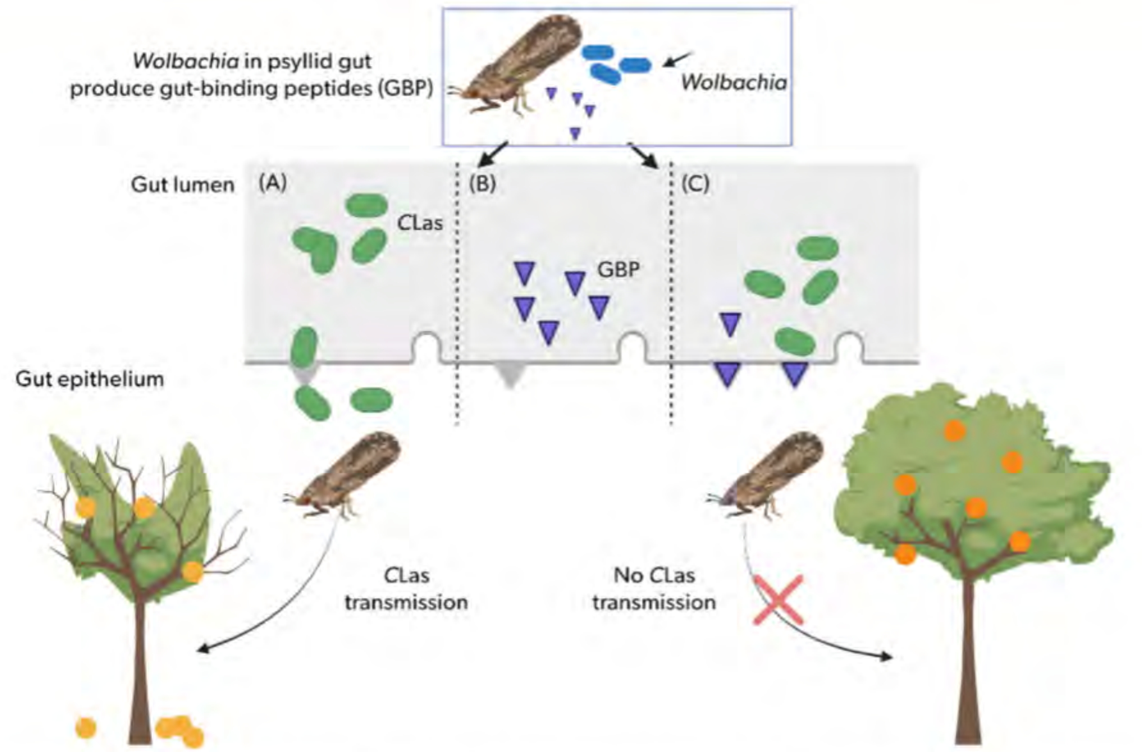

A novel strategy to block transmission of the *C*Las pathogen by 'gumming up' the psyllid gut includes the following steps: A) *C*Las binds to the gut surface and enters before it can enter into the psyllid and be transmitted. The psyllid becomes a vector. B) Gut-binding peptides (GBP) were selected for binding to the ACP gut to outcompete the pathogen. C) GBP 'gums up' the surface of the gut and prevents *C*Las binding, thereby blocking the bacterium from entering ACP. GBP produced by *Wolbachia* in the gut of the psyllid will be tested for *C*Las transmission blocking.

Graphic created with BioRender.com

Changing psyllids to make them incapable of spreading HLB

By Kirsten S. Pelz-Stelinski and Lukasz L. Stelinski

Managing Asian citrus psyllids (ACP) remains a consideration for growers because psyllids transmit the citrus greening disease pathogen, *Candidatus* Liberibacter asiaticus (*C*Las), to newly planted trees. There are several tools that can reduce psyllid populations or mitigate the symptoms of HLB and improve the health of infected trees. However, additional methods that could help prevent transmission of *C*Las to newly planted trees could serve as an important complement. Replanted citrus groves could get into production faster if pathogen transmission could be prevented. Although ACP can be suppressed with insecticides, these tools are expensive and have undesirable consequences such as resistance and collateral death of natural enemies.

A potential new method for managing greening is development of non-transmitting ACP. Several bacteria, called endosymbionts, naturally live inside ACP. These bacteria cannot be transmitted to citrus and do not act as pathogens. They also cannot survive outside of the insect. The insect and bacteria mutually benefit one another.

WORK WITH WOLBACHIA

One novel approach for developing non-transmitting ACP is modifying one of the endosymbionts living in psyllids, called *Wolbachia*, to produce proteins that target the CLas pathogen after the ACP ingests it. The process of modifying naturally occurring *Wolbachia* to produce new proteins is called paratransgenesis.

> *A potential new method for managing greening is development of non-transmitting ACP.*

Scientists have specifically investigated *Wolbachia* for many years as a potential tool to control spread of human-borne pathogens by mosquitos. Modifying this bacterium in mosquitos has been shown to reduce the transmission of several viruses among people with no adverse effects on humans or the environment.

The first step in this approach with ACP was isolating their specific *Wolbachia*. Based on the methods used with mosquitos, researchers established cultures of *Wolbachia* taken from ACP and grew them in large quantities. The second step was introducing genes into *Wolbachia* that would modify ACP in the desired way. Modified *Wolbachia* are then reintroduced into the psyllids by injecting young ACP. These modified *Wolbachia* produce new proteins inside the ACP that inhibit transmission of the CLas pathogen to citrus.

THE ROAD TO COMMERCIALIZATION

This project has met several technological milestones in the laboratory, including establishing a *Wolbachia* system. Certain challenges must be overcome before delivering a field-ready tool to commercial groves. First, researchers are still testing possible genes that would most effectively disrupt the pathogen. A goal of the current project is to identify peptides that compete with the citrus greening pathogen for a limited number of binding sites located in the ACP gut. These peptides are intended to outcompete the pathogen and 'gum up' the psyllid gut, preventing the pathogen from colonizing the insect. Normally, such colonization must occur before the pathogen can be transmitted. Ultimately, this strategy would break the cycle of pathogen transmission at the source by neutralizing the psyllid.

A psyllid population that cannot transmit CLas would be mass-reared for release in commercial citrus groves to replace local ACP populations. The existing ACP population could be reduced locally with insecticides before releasing non-transmitting ACP. Any remaining resident ACP would mate with non-transmitting ACP, and the *Wolbachia* modified with the anti-pathogen protein would be passed to the offspring. *Wolbachia* stay in the ACP throughout its lifetime and are naturally passed to offspring.

In this way, the local population of ACP would be changed and no longer transmit the greening pathogen. Periodic releases of non-transmitting psyllids would be necessary to maintain this in the local population. Although this technology would not likely solve the greening problem, we expect it could serve as an important complement to current tools by reducing the need for broad-spectrum insecticides and facilitating biological control agents.

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