Using antibodies for early detection of HLB infection

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What is the technique?

Huanglongbing (HLB) is a disease caused by a bacterium called *Candidatus* Liberibacter asiaticus (*C*Las) that colonizes the phloem (nutrient conducting pathway) of citrus trees. To limit the spread of the disease, it is crucial to identify and remove infected trees as early in the infection process as possible. However, determining whether a tree is infected or not is very challenging. Visual inspection is not a suitable option because by the time symptoms appear (9 months or more), the Asian citrus psyllid insect vector has already moved the disease on to the next tree. The official biochemical method to detect the disease uses polymerase chain reaction (PCR). However, the PCR method requires that the leaf sample taken from the tree contains the bacterium and the bacterium might not always be in the leaf chosen, as *C*Las is often unevenly distributed in infected trees. Another approach could be to identify and detect molecules that the bacterium releases into the phloem early in the infection process. These molecules, which are smaller than the bacterium, would spread throughout the tree with the phloem flow, where they could be detected using an immunological method. Wenbo Ma and her collaborators have exploited this approach using released bacterial molecules called 'effectors' and a detection method called enzyme-linked immunosorbent assay (ELISA).

What are bacterial effectors?

Effectors are protein molecules secreted by pathogens such as bacteria to help promote disease in the plants that they infect. Effectors bind to other proteins and manipulate plant cell activity. Early in the infection process, as *C*Las colonizes the phloem of citrus plants, it secretes effectors into the phloem sap which then move throughout the plant in the vascular conducting tissue. Therefore, the effector molecules could be detected in areas of the plant where the bacterium is not present yet, before the plant shows any symptoms.

How can they be detected?

Bacterial effectors can be detected using antibodies, which are a type of protein from the animal immune system that can identify specific protein structures, like those in the effectors. By artificially exposing antibodies to the bacterial effectors, they can be 'trained' to detect them. ELISA (enzyme-linked immunosorbent assay) is a technique that combines these 'trained' antibodies with a color reaction, so that a visual signal is released if the antibodies detect the bacterial effector. A sample would be taken from the phloem tissue of a possibly infected tree and incubated with the antibodies in a small well of a plastic plate, in which the liquid would change color if the tree was infected with *C*Las. ELISA has been widely used for disease diagnosis because it is highly sensitive, rapid and inexpensive.

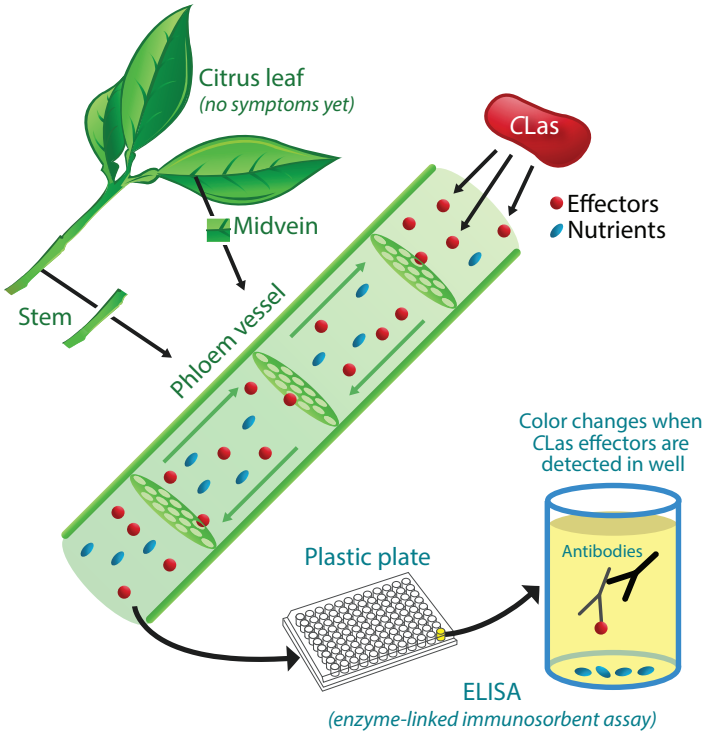

Figure drawn by Barbara Alonso

Who is working on the project?

Wenbo Ma from the University of California, Riverside, has been leading this project with funding from the Citrus Research Board, USDA-NIFA and USDA-APHIS and the Office of Research, UC Riverside.

What are the challenges and opportunities?

Since effectors are essential for pathogenic bacteria to cause disease in plants, they should be present and detectable early in the infection process, before the plant shows any symptoms. And because they move systemically through the phloem, they could be detected in areas of the plant that the bacterium has not yet colonized, overcoming the problem of uneven distribution inherent to the PCR-based detection method. The team led by Wenbo Ma is working to determine how early in the infection process this technique would detect the bacterial effectors, validating it with different citrus

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varieties, tree ages and sampling protocols. Another challenge is the expense and time requirements of ELISA, which limits how fast samples could be processed. It would be difficult to use it as a rapid screen for entire citrus orchards. Further experiments will need to be conducted before this early detection technique is deployed for field use, but it could be a valuable complement to other HLB diagnostic tools.

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