# The value of early detection technologies (EDTs) for HLB management

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http://ucanr.edu/sites/scienceforcitrushealth/

## What is the technique?

Early detection technologies (EDTs) are tests that indicate the presence of disease before signs or symptoms of the disease can be seen. In the same way that a doctor measures a patient's blood pressure to look for heart problems, a grower might use a trained "sniffer" dog to detect changes in a tree that looks healthy but has huanglongbing (HLB) disease. By using the EDT, the grower is able to uncover HLB earlier, and can decide on an early, cost-saving course of action.

In the case of HLB, there are many EDTs under development. Some of them look for patterns in the microorganisms that live on the citrus leaves (Leveau snapshot); some look for patterns in the chemicals that are produced by the tree in response to HLB (Pourreza, Davis and Slupsky); and others look for the molecules that the bacterium injects in the tree to cause disease (Ma). A description of some of the EDTs can be found on the Science for Citrus Health website.

## Why do we need EDTs for HLB?

To understand why EDTs are needed and what their potential value is, it is necessary to understand the difference between the *incubation period* for a disease and the *latent period*. The incubation period is the time between exposure to the pathogen and the appearance of symptoms. The latent period is the time between exposure and the newly-infected host becoming infectious. Huanglongbing (HLB) has a long incubation period and a very short latent period, which means that a tree can be diseased for a long time without showing any visible symptoms, while being infectious for a large fraction of that time. Even if a tree does not seem diseased, it can serve as a home for the bacterium (*Candidatus* Liberibacter asiaticus, *C*Las) that causes HLB. If a psyllid feeds on the infected tissue of a tree (with or without symptoms), *C*Las that is present in the leaf tissue can be picked up by the insect and transmitted to other trees when the psyllid moves on to feed. Information from an EDT can help a grower detect the disease in a tree a long time before it would be detected by eye. This cuts down the time psyllids are able to feed on it and transmit the disease, slowing the spread of HLB to neighboring trees.

## Why is it important to remove infected trees as early as possible?

If a tree that tests positive for *C*Las is not treated or removed, the bacterium will spread throughout the tree. Over time, an increasing proportion of the tree's tissues will become infected, increasing the chances that a psyllid will become infected upon feeding, and subsequently spread the infection to healthy neighboring trees. If the infected tree is removed, there is no opportunity for psyllids to feed on the infected tissue and spread the disease. Once *C*Las is detected, tree removal is the only surefire way to prevent the spread of the infection, and it is extremely time-sensitive. The sooner an infected tree is removed, the lower the chances that psyllids will get infected. The savings associated with early infected tree removal will be proportional to the amount of surrounding trees that would have been infected with *C*Las due to that tree, and the number of months that it would be left on the ground.

## Who is working on this project?

Several research teams in different universities and research stations, supported by a variety of funding organizations, have been working on the development of a variety of EDTs. These EDTs, designed under laboratory and greenhouse conditions, are being validated under field conditions in Texas and Florida. In California, where HLB has not been detected in citrus orchards, samples of different citrus varieties have been collected from healthy trees and trees affected by other diseases from all over the state. These samples are being used to calibrate the EDTs, and to test if they can distinguish between healthy and HLB-diseased trees, and between HLB-diseased trees and trees affected by other common citrus diseases. Dr. Neil McRoberts and his team at UC Davis are evaluating the data from these experiments and providing support to the EDT researchers.

## What are the challenges and opportunities?

Currently, regulations require HLB infected trees to be removed if a certain amount of *C*Las DNA is detected in leaf samples through polymerase chain reaction (PCR). However, *C*Las is unevenly distributed in the sap of citrus trees, and the leaf samples collected might not be PCR-positive even though the bacterium is already present elsewhere in the tree. EDTs offer the possibility to detect infected trees before they are PCR-positive, so they could be removed earlier in the HLB epidemic. Therefore, the value of EDTs relies on the voluntary removal of EDT-positive trees before the law requires them to be removed.

No EDT gives perfect diagnostic results. Sometimes healthy trees will produce EDT scores that look like diseased trees (so-called "false positives" - see Table 1). Removing such trees will result in an immediate financial loss. However, because

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the economic damage caused by leaving an infected tree in place is much bigger than the value of a healthy tree, using an EDT to guide decisions has the potential to result in a long-term economic benefit to individual growers and communities, by reducing the spread of HLB. Losing a few healthy trees along the way is the unavoidable cost of stopping the disease from spreading. Likewise, some trees will seem healthy based on EDT scores but might end up showing symptoms ("false negatives"- see Table 1). The proportion of true positives, false positives, true negatives and false negatives represents the accuracy of a diagnostic test. Dr. McRoberts' team is analyzing the accuracy of the EDTs, and preliminary results suggest that the best performing EDTs could be correctly determining the status of the trees 95% of the time.

The results of this analysis could be used to foster the adoption of EDTs among the citrus grower community, promoting the idea that the sooner infected trees are detected and removed, the smaller impact HLB will have on California's citrus production. Unless there is sufficient cooperation in integrated management of HLB by removing infected trees as early as possible, controlling the ACP on an area-wide scale, and using certified plant material, the California citrus industry is likely to suffer unsustainable economic losses to HLB.

## Comparison of the EDT score with disease status

| | True status of the tree | | |
|---|---|---|---|
| **EDT score** | **Has HLB** | **Does not have HLB** | |
| **Positive** | **True Positives (TP)**<br>*EDT positive and develops symptoms* | **False Positives (FP)**<br>*EDT positive but does not develop symptoms* | **Positive predictive value: TP/(TP+FP)**<br>*Probability of HLB given a positive EDT score* |
| **Negative** | **False Negatives (FN)**<br>*EDT negative but develops symptoms* | **True Negatives (TN)**<br>*EDT negative and does not develop symptoms* | **Negative predictive value: TN/(TN+FN)**<br>*Probability of no HLB given a negative EDT score* |
| | **Sensitivity: TP/(TP+FN)**<br>*Probability a tree with HLB will have a positive EDT score* | **Specificity: TN/(TN+FP)**<br>*Probability a tree without HLB will have a negative EDT score* | |

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