# Determining HLB infection levels using multiple survey methods

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*Methods used in a survey to determine HLB infection levels include, from top left clockwise: walking, tagging, all-terrain vehicles and platform trucks.*

Huanglongbing (HLB) or citrus greening is a very serious disease of citrus. It affects all citrus cultivars and causes lower fruit yields and subsequent tree decline. The disease is associated with the bacterium, *Candidatus* Liberibacter asiaticus (Ca. Las) and vectored by the Asian citrus psyllid (*Diaphorina citri* Kuwayama) in Florida.

Symptoms of HLB vary during the year between varieties and disease progression. They may resemble other disorders. Part of the tree may show symptoms while other sectors may remain healthy in appearance.

Visual leaf symptoms include asymmetrical chlorosis (blotchy mottle); yellow veins; vein corking; small upright leaves with chlorotic patterns; and/or leaves nearly devoid of green with only green spots (green islands).

Fruit symptoms include smaller than normal size; reduced fruit numbers; lopsided fruit; aborted seeds; irregular peel color; off-flavor juice; staining of the calyx; and increased fruit drop.

Overall tree decline will result in death after a number of years.

When HLB becomes established in an area, fruit yield and life span of citrus trees decrease over time. Citrus growers must implement multiple and aggressive production practices to detect the disease (scouting), minimize its spread and manage the vector in order to minimize economic losses. The costs associated with HLB management are significant not only in increased production costs, but also in lost revenue from greater tree and yield losses. Once the tree is infected, there is no known cure for the diseased tree.

## MANAGEMENT PRACTICES

Management practices for greening include:

1) use of disease-free budwood and the production of nursery trees in enclosed structures designed and built to exclude the citrus psyllid from entering the structure;

2) routine scouting and roguing of infected trees within groves;

3) use of diagnostic and visual methods to confirm the presence of the disease in suspected infected trees; and

4) management of the psyllid (vector).

In the field, visual detection of the disease is based primarily on leaf pattern or lopsided fruit, if present. However, visual diagnosis can be difficult and problematic because of uneven and varying symptoms associated with HLB within the tree. Without careful inspection of the entire tree, the disease can be missed, causing an underestimation of true disease incidence.

Laboratory diagnosis of the bacterium is done by quantitative real-time polymerase chain reaction (qPCR) testing of symptomatic tissue to determine if it is positive for the greening associated bacterium, Ca. Las.

The disease is known to be unevenly distributed within the tree, making de-tection by laboratory or field method less than 100 percent correct.

Other factors can also impact detection accuracy, including nutritional deficiencies, physical and environmental conditions, drought stress, and/or leaf diseases which visually cause symptoms that can be confused with HLB symptoms.

Florida citrus growers use various scouting methods to visually detect trees exhibiting greening symptoms. Scouting methods include walking, use of all-terrain vehicles (ATVs), platforms attached to vehicles (tractor or truck mounted), or combinations of several of the previous methods.

## FIELD DETECTION AND ACCURACY OF HLB SCOUTING CREWS

Given the various problems affecting visual detection of HLB in a citrus grove, this field study was conducted using multiple scouting methods to visually determine the presence of HLB within multiple 9-acre blocks planted to citrus. Five experienced scouting crews from different companies individually scouted the blocks

using their normal scouting procedures. Each survey was conducted in a manner to visually inspect each tree from both sides. After surveying and flagging each suspected tree, laboratory confirmation by qPCR testing was conducted to confirm the visual diagnosis for each survey crew.

Each surveyed block consisted of mature Valencia trees planted in north-south rows that were 25 feet apart and with an in-row spacing of 12.5 feet. The majority of the trees were 16 to 20 feet tall. The scouting was conducted over a two-week period prior to harvest in late January and early February 2009.

During the scouting period, several nights of cold weather (mid-20°F) occurred, producing some leaf damage that may have impacted surveying efficiency among the five crews. Scouting methods in this study included: 1) walking; 2) riding on all-terrain vehicles (ATVs); 3) riding on elevated platforms attached to a vehicle (tractor or truck); and/or 4) a combination of ATVs and elevated platform in the same survey trip.

Survey operations varied by block and consisted of multiple trips through the same block by different survey crews and survey methods. Not all crews surveyed each of the five blocks.

Each survey crew flagged the branch it suspected as being positive for greening symptoms as well as both the east and west side of the tree to aid in recording tree location within the block. After each survey was completed, the tree and row numbers were recorded as well as the position on the tree that was marked by the survey crew. After recording the suspected branch and tree, the flag was removed and discarded to allow the next crew to survey the same block without being aided by the previous survey flag being visible.

After all the crews had completed their surveys, samples were collected from each flagged tree and tested by qPCR to determine the presence or absence of HLB. As the samples were collected for PCR analysis, efforts were made to collect the most representative sample from each tree displaying HLB symptoms. In some cases, this sampling collection method may have affected the results as an exact sample of each crew's marked branch was not possible.

When the average visual detection for HLB was calculated for each scouting method, the percentage found by walking and platform/ATV averaged 47 percent, ATVs averaged 61 percent, and platforms without ATVs found 59 percent across all blocks and crews. Results varied significantly among survey crews.

Similar survey efficiency has also been found in Brazil when the same block was surveyed by four separate survey groups or teams. The Brazilian data indicated that of the 36 trees that were identified by multiple crews, individual crews found between 15 and 21 of the combined total trees in the block. Calculating the average efficiency from their data, approximately 50 percent of the trees were found with a range of 41.7 percent to 58.3 percent by each crew within the surveyed block.

## DETECTION EFFICIENCY

The efficiency for the identification of a specific HLB positive tree ranged from a low of 13 percent to a high of 100 percent. In a few cases, all survey crews marked a specific tree as suspect and subsequent laboratory testing indicated negative. This may indicate that neither the qPCR testing nor the survey method is always 100 percent accurate in determining positive HLB trees.

## CONCLUSIONS

A number of factors impact a survey crew's ability to find HLB positive trees including tree size, grove terrain (weeds and/or factors affecting the soil surface), weather conditions (temperature, wind and/or light intensity), overall disease incidence and distribution level in a given tree. In addition to physical and environmental conditions, the ability of the scout to detect symptoms varies between individuals as well as between methods.

This study indicated that no survey method or crew was 100 percent accurate in finding all the trees that exhibit various stages of the disease. Since not all visually positive trees were identified along with an unknown latency period of HLB within the tree, multiple scouting passes will be needed during the year.

To better understand the dynamics and multiple factors of scouting for HLB, additional studies need to be conducted over multiple locations and seasons to effectively determine optimum survey strategies. Extensive training, education in symptom recognition and evaluation of scouts are important considerations to maximize HLB detection within any grove. The success of any survey program is dependent on identifying a high percentage of positive trees rapidly and accurately.

For a complete report on this greening survey, please contact Steve Futch at shf@ufl.edu.

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