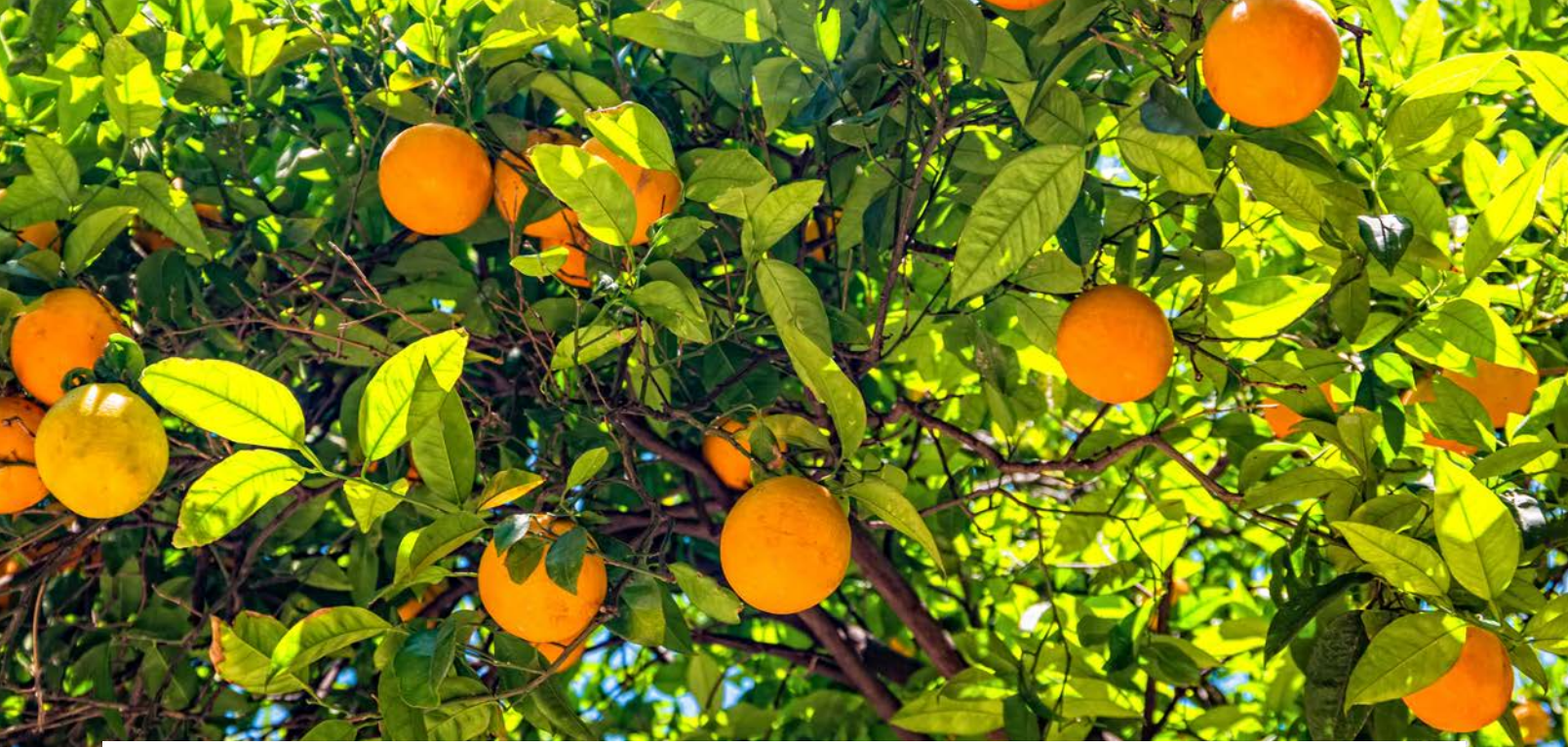

Citrus canopy health is highly important for HLB tolerance

By Amit Levy and Tripti Vashisth

In Florida, where almost all citrus trees are infected with huanglongbing (HLB), it is important to have an easy and reliable method to check the severity of the disease. Each tree responds differently to *Candidatus* liberibacter asiaticus (*C*Las), the bacterial pathogen that causes HLB. Compare it to COVID-19, where some people had the virus without any symptoms, while others became very sick. Similarly, it is important to know if your tree reacts mildly or severely to *C*Las. This helps growers make better decisions about treating trees by properly evaluating which treatments are working and which are not.

However, determining the severity of HLB is not as easy. Growers need to assess fruit yield. If the tree is sick, it will produce less fruit. But measuring the yield is laborious and time consuming. Disease Index Rating is the easiest technique to determine HLB's impact, but it can be problematic because it is subjective; each person will rate the symptoms a little differently. There are also microscopic, spectroscopic and imaging techniques, but they are more complicated and expensive.

Quantifying *C*Las with polymerase chain reaction (PCR) is the most used method for determining HLB severity levels. Regular PCR is used to detect *C*Las DNA in the plant sample, and quantitative PCR (qPCR) is used to measure the number of copies of *C*Las DNA corresponding to its population. With qPCR, the severity of HLB is usually determined by the Ct values. Basically, a higher Ct value means there are fewer bacteria in the sample. Trees with a higher Ct value are considered healthier than those with a low Ct value.

However, there is a big question here: Does the number of bacteria really determine the severity of the disease? Is a tree with less bacteria less sick? Going back to COVID-19 again, most cases with severe reactions were caused in large part by the person's immune response and not directly from the virus itself. If something similar is happening with HLB, would it be more important to kill the bacteria or strengthen the tree?

BACTERIA LEVELS AND PHLOEM PLUGGING

To address this question, a transmission electron microscope was used to quantify the amount of *C*Las in young leaves, feeder roots and seeds of sweet orange and grapefruit. The electron microscope provides an accurate visualization of the bacterial cells inside the phloem tissue. Surprisingly, no bacterial cells were seen in the leaves of infected trees. There were some bacteria present in the roots, but most were concentrated in the seeds (Figure 1, page 12).

The mirror image was seen in the plant response, which was determined by phloem plugging. There was a lot of phloem plugging in the leaves (where there were almost no bacteria), some plugging in the roots (that had some bacteria) and no plugging in the seeds (that had a lot of bacteria). This finding tells us that plant response is not directly related to the number of bacteria in the tissue. Bacteria levels are actually very low or not present at all in the leaves where symptoms are seen. So maybe the plant response, such as the

Leaf – No *C*Las, high plugging **Root** – Some *C*Las, some plugging **Seed** – High *C*Las, no plugging

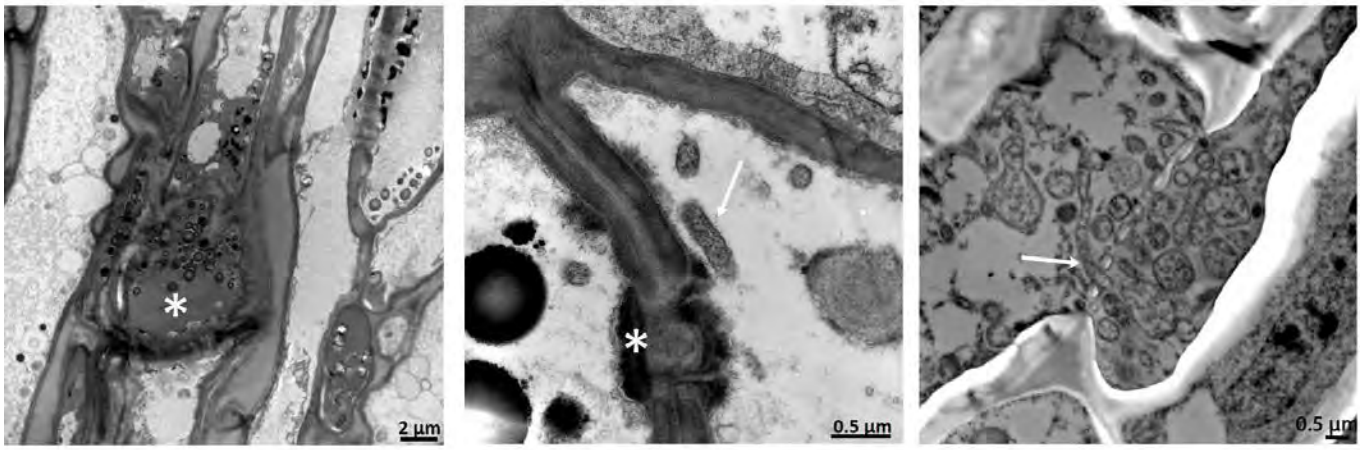

Figure 1. Transmission electron microscopy of phloem from leaves, roots and seeds. In leaves, phloem is completely plugged, with no bacteria present. In seed coats, phloem is free of plugging, with many bacterium cells. Roots display an intermediate status. Arrows point to *Candidatus* liberibacter asiaticus (*C*Las). Asterisks show phloem plugs.

phloem plugging, is more important than the bacteria levels.

WHAT AFFECTS YIELD

To test this, we looked at which measurements are correlated to the disease in the field. As mentioned, the most important way to determine the disease level for the grower is the tree's fruit yield. We wanted to see whether any other measurements correlate with this yield. We were able to identify a few correlated characteristics, but the Ct value was surprisingly not one of them, meaning that a higher or lower Ct value did not mean a higher or lower yield.

One of the values that positively correlated with fruit yield was the percentage of photosynthetically active radiation (PAR) interception in the canopy (%INT). Intercepted PAR indicates the amount of light caught by various canopy layers as the light travels through the canopy. A higher %INT indicates higher canopy density. In a sick tree, lower canopy density is expected, and more light will go

		%INT	Yield (pounds/tree)	Clas cells/gram plant tissue
Valencia on Swingle, 10-year-old	Severe	85.28	**52.18**	4.87E+09
	Mild	94.18	**90.39**	7.82E+09
Hamlin on Swingle, 20-year-old	Severe	79.01	**83.78**	8.34E+09
	Mild	92.23	**133.93**	4.15E+09
Valencia on Swingle, 21-year-old	Severe	80.83	**80.47**	2.50E+09
	Mild	91.14	**138.34**	2.37E+09

Table 1. HLB disease severity according to percentage of photosynthetically active radiation interception in the canopy (%INT). Bold values of yield indicate a statistically significant difference between severe and mild ($p<0.05$; paired t-test). There is no difference in *Candidatus* liberibacter asiaticus (CLas) numbers between the severe and mild trees.

through the canopy, meaning less light will be absorbed by the leaves and %INT will be lower.

Table 1 shows results from three different field trials. In each trial, we divided the trees into two groups according to the %INT. Above 90 means trees had mild HLB symptoms and below 90 means the trees were severely sick. Yield measurements showed that dividing trees this way was very accurate. Trees with low %INT, which were included in the severe HLB group, produced about half the yield compared to those with high %INT (mild HLB). The number of bacteria in these trees was calculated according to the Ct value. Surprisingly, there was no statistical difference between the high-producing trees and the low-producing trees.

These results suggest that the leaf density of the tree canopy is very important to fruit production, regardless of how many bacteria are in the leaves. Measuring Ct value has little meaning in determining HLB severity levels. A better approach is measuring %INT, which is a relative measurement rather than an absolute one. Therefore, the %INT measurement is not dependent on the canopy volume or on the weather on the day of measurements and could be appropriate for field settings. Overall, the results indicate that aside from controlling bacteria, treatments to keep the canopy vigorous (for example, by enhanced nutrition) will have a strong positive effect on tree yield and should get more attention.

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