By now everyone is well aware of the confusion that can exist in trying to distinguish visible symptoms of citrus greening disease (Huanglongbing, HLB) from nutrient deficiency symptoms. In fact, many papers describing the leaf symptoms of greening will often describe them as appearing nutrient deficient. In recent reviews on greening, Jose Bové and John da Graça both indicate that as the disease progresses in a tree, symptoms of zinc (Zn) deficiency will develop. However, visible Zn deficiency alone is not a good indicator of citrus greening infection since Zn deficiency may occur in uninfected trees and is quite distinguishable from the typical asymmetrical blotchy mot- 
	le of greening leaves associated with high leaf starch. This article discusses our current thinking on the citrus greening/leaf nutrition connection and the research being done to further our understanding of this topic.

Other micronutrient deficiencies, particularly boron (B), can also cause symptoms that are frequently seen on greening infected trees. In a paper from 1930, A.R.C. Haas described citrus trees with corking and splitting of leaf veins, abscission of leaves and accumulation of excessive amounts of carbohydrates in affected leaves. One could easily believe he was describing greening symptoms, but he was actually describing B deficiency.

The visible connection between nutrient deficiency and citrus greening is not new. During the 1970s, two separate studies showed that greening symptomatic leaves had lower levels of calcium (Ca), magnesium (Mg) and Zn compared to asymptomatic leaves. Recently, a number of University of Florida-IFAS researchers have confirmed these nutrient deficiencies in greening infected trees in Florida. However, many questions surrounding the nutrient deficiency/greening connection remain, among them:

• Do these nutrient deficiencies limit plant growth and yield (i.e., are they real or simply manifestations of greening infection)?
• Does greening infection restrict or limit nutrient uptake and or transport?
• And finally, can remedial applications of nutrients reduce yield loss and or prolong tree life even in the presence of greening?

We have been working to answer these and other questions and have been funded by the FCPRA box tax to continue these investigations. We have accumulated a substantial amount of information to answer the first question above, “Are these deficiencies real?”

Traditional leaf nutrient analysis reports nutrient levels on a percentage dry weight basis. This works well because we sample uniform, healthy 6-month-old leaves. However, this may not provide accurate data when analyzing leaves from greening infected trees. Since greening leaves accumulate large amounts of starch, their dry weight per unit of leaf area is naturally higher than a healthy leaf. Why does this matter? Starch is a carbohydrate and contains only carbon (C), hydrogen (H) and oxygen (O). Thus, when nutrient levels are examined on a dry weight basis, the high starch content dilutes the nutrient levels. For example, let’s say a healthy leaf has a dry weight of 1 gram. If that leaf has a normal level of Ca (3.5 percent), it would contain 0.035 grams of Ca (1 gram x 0.035). Now let’s say the same leaf accumulates starch and its dry weight increases to 1.25 grams. The leaf now has a greater thickness or density, and a greater dry weight per area. We haven’t added any Ca, only C, H and O, so the leaf still has only 0.035 grams of calcium, but that is only 2.8 percent Ca by dry weight (0.035/1.25), which is in the low range. Thus, the accumulation of starch causes our leaf to appear to have low Ca on a dry weight basis.

This “dry weight error” could be corrected for by analyzing the
CORRECTING WITH DRIS

Perhaps the best option for correcting for the dry weight change is to use DRIS analysis. DRIS, or Diagnosis and Recommendation Integrated System, was introduced to citrus growers in an article in last month’s issue of Citrus Industry. DRIS uses the ratio of one nutrient to another (e.g. N/P) to develop nutrient indices.

How does this help with the greening nutrition question? Let’s go back to the earlier example and consider another nutrient, say Mg. If we assume our 1 gram dry weight leaf started with a normal Mg level of 0.4 percent or 0.004 grams (1 gram x 0.004), then after the starch addition (+0.25 grams) Mg drops to 0.32 percent (0.004/1.25). You can see that if we look at the ratio of Ca to Mg, it remains the same (8.75) whether we use a leaf dry weight of 1 or 1.25 grams, before or after the addition of the starch, because the additional dry weight is only affecting the percentage of the nutrients, not the actual contents per leaf in grams.

So this raises the question, “Are the greening nutrient deficiencies based on percent dry weight real when examined by the DRIS method or on a leaf area basis?” It depends on the nutrient. For example, in a recent analysis comparing symptomatic (blotchy mottle) and asymptomatic leaves from greening infected PCR+ trees and healthy leaves from healthy trees (PCR−), changes in K, Mg, Ca and B were found to be consistent across analyses. Only K increased and the others decreased. Additionally, K and Ca showed intermediate changes in asymptomatic leaves, having levels midway between symptomatic and healthy leaves. Mg and B did not show this trend and were reduced by similar levels in both symptomatic and asymptomatic greening leaves compared to healthy leaves. The minor elements Manganese (Mn), Zn, Cu and Iron (Fe) were all significantly lower in greening infected leaves on a dry weight basis, but were not significantly different when analyzed on a leaf area basis or by DRIS.

Current research at the Citrus Research and Education Center (CREC) will look at these changes in more detail and try to separate out real nutrient deficiencies from greening symptoms by growing trees in hydroponics where nutrient levels can be easily monitored and altered. Additionally, research entomologist Antonios Tsagkarakis, working with Michael Rogers, will be studying how citrus leaf nutrient status affects psyllid feeding and reproduction.

To the final question posed earlier, “Can remedial applications of nutrients reduce yield loss or prolong the life of infected trees?” we still don’t have a clear answer. Maury Boyd, who has been making multiple foliar applications of micronutrients for some time now, has been very cooperative in sharing data with IFAS researchers and allowing us to monitor yields in his grove. Overall, the yields in Boyd’s block in Felda have increased, despite significant greening infection. When we compared the yield on infected (PCR+) and healthy (PCR−) Hamlin trees this past December, we found that the infected trees did have significantly lower yield. However, the infected trees also had a much smaller canopy than the healthy trees and when yields were corrected for canopy size, there was no difference. Several things may be happening. The micronutrient applications may be improving the yield of the healthy trees, which is counteracting the yield decline of the infected trees. Overall, the grove yields are being maintained. Also, the applications may be maintaining the yield of the infected trees, although they are growing less than healthy trees so their canopy size (and yield potential) may not be increasing. Answers to these questions will take several years of monitoring specific trees to know what is really happening.

Several nutrient decreases (e.g. Mg, Ca, B) associated with greening infection are real even after analyses are corrected for the increased dry weight from starch content of infected leaves. It is likely that these changes are from restrictions of nutrient uptake and/or transport caused by greening infection. However, it is unclear whether remedial foliar applications of these nutrients can reduce the effects of the disease. Our ongoing research will give us these answers, but it will take time.

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