What can we learn from HLB survivor trees?

By Nian Wang and Fred Gmitter

With the rapid spread of HLB in Florida, the majority of citrus trees in the state are already infected with HLB. Some groves are no longer productive. Some apparently healthy citrus trees have been found in heavily HLB-diseased citrus groves (Figure 1); we refer to those trees as HLB survivor trees.

This phenomenon was observed earlier in the citrus groves in parts of China. Multiple causes have been proposed: genetic mutations of the trees so that they become HLB resistant or tolerant; specific soil properties, possibly beneficial microbes, making the trees HLB resistant or tolerant or repellent to the Asian citrus psyllids (ACP) that spread HLB; or natural differential feeding of psyllids on the citrus trees so that they are infected by *Candidatus* Liberibacter asiaticus (CLas) later than other trees. Here, we will briefly discuss the information behind each hypothesis and what we have learned so far from HLB survivor trees.

1. Genetic mutation to the trees so that they become HLB resistant or tolerant

It is possible that naturally occurring mutations have taken place in either the scion or rootstock that enable the trees to survive CLas infections, and thus remain healthy and productive. Certainly, such things have happened before in evolutionary history.

To test this possibility, we have collected scion budwood from some survivors and are now propagating these to test by inoculation either with infected budwood or through natural ACP inoculations. Previous work in the Guangdong and Guangxi provinces of China indicated that the propagated trees were not HLB resistant if grafted with affected budwood, though the original trees remained free of disease in the field. Nonetheless, we are pursuing this approach now in Florida; although it is a long shot, it is one opportunity to seek solutions to HLB that cannot be overlooked.

Mutations in the rootstock are another possibility. Further, given differences we have seen in field performance of rootstocks from the breeding programs, another possibility is that the rootstock arose as a zygotic seedling. We have been using DNA markers to fingerprint root systems of survivors, but until now, nearly all of them have

Figure 1 (all 3 photos). HLB survivor trees in heavily infected citrus groves in Florida.

been found to be true-to-type nucellars. As with scions, we have been collecting rootsprouts when possible, though it is more difficult to propagate the root systems for further testing in the field.

How might rootstocks affect the trees' responses to HLB disease? Could it simply be that they proliferate such tremendous numbers of roots that they outpace the decline as a result of infection? Or, are they more effective at mining nutrients from soils, either because of their sheer numbers or an inherently greater efficiency and superior adaptation to a given soil, so that they can keep the tree in a better condition? Are they antagonistic to CLas once it is transported to the roots, or do they send some signal to the scion to counteract infection there? These are researchable questions to which we plan to find answers.

2. Specific soil properties, possibly beneficial microbes, that make the trees HLB resistant or tolerant

Suppressive soils in which little or no disease occurs under conditions that are favorable for disease development have been identified for multiple soil-borne plant pathogens, including take-all on wheat, wilt of flax and other crops, potato scab, *Phytophthora* on apple, black root rot of tobacco, and plant-parasitic nematodes. Plant disease management has been achieved by mixing disease suppressive soils with disease conducive soils. Indigenous soil microbes including bacteria, mycorrhizal fungi and protozoa have been shown to be critical to plant disease suppression. Plant-associated beneficial microbes have been reported to show positive effects in stimulating plant growth, managing soil and plant health, and suppressing plant diseases. Soil fungi have also been reported to influence pathogen levels and play a significant role in improving plant health, e.g. *Trichoderma* spp. and mycorrhizal fungi. In addition, the physical and chemical characteristics of the soil, and rootstock adaptation to specific soil conditions as well, may also play important roles in suppressing plant diseases and promoting soil microbial communities.

This suppression interaction of soil-borne pathogens occurs in the rhizosphere, the zone surrounding the roots of plants in which complex relations exist among the plant, the soil microorganisms and the soil itself. Among all the soil microbes, plant-growth-promoting bacteria (PGPB) are associated with most of the plant species and are commonly present in most of the environments. The most widely studied group of PGPB are plant-growth-promoting rhizobacteria (PGPR). Some of these PGPR can also enter the root interior and establish endophytic populations. Beneficial microbes exert their positive effect on plants through multiple mechanisms including effective and competitive colonization, production of antibiotics that inhibit growth of pathogens, and induction of systemic resistance in the host plant that increases the resistance to a broad spectrum of pathogens.

In our previous study, we have found that the bacteria associated with healthy citrus roots include many bacteria showing similarity to some well-known PGPB, e.g. *Caulobacter, Burkholderia, Lysobacter, Pseudomonas, Bacillus*, and *Paenibacillus*. However, infection by CLas led to the loss of the detection of most of those bacteria while promoting the growth of bacteria such as *Methylobacterium* and *Sphingobacterium*. We are investigating whether application of beneficial microbes could promote plant growth and prevent or slow down the colonization by CLas.

3. Specific soil properties, mainly beneficial microbes, that make the trees HLB repellent to psyllids

Beneficial soil-borne microbes can induce an enhanced defensive capacity in above-ground plant parts to protect plants against insect herbivores. This induced systemic resistance (ISR) triggered by soil-borne microbes is often not associated with enhanced biosynthesis of plant hormones that are important for defense against insect herbivores, nor with massive changes in defense-related gene expression. Instead, beneficial soil-borne microbes prime the plants for enhanced defense that is characterized by a faster and stronger expression of defense responses activated upon insect attack, resulting in increased resistance to the insects. Soil-borne microbes can also induce the production of plant hormones such as salicylic acid, which plays a role in plant defense against insect herbivores with a piercing-sucking feeding mode, such as ACP. We are investigating whether the beneficial bacteria could affect ACP transmission of CLas.

4. Natural differential feeding of psyllids on the citrus trees so that they are infected later than other trees

It has been reported that ACP's distribution is not uniform. Instead, random spatial distribution was observed for eggs of ACP whereas aggregated spatial distribution was observed for nymphs and adults of ACP. Thus, we could not rule out the possibility of survivor trees resulting from non-uniform feeding behavior of ACP. In other words, there once was the first tree infected and showing symptoms, so at some point, there must be the last tree infected. While this is not the hoped-for conclusion, we cannot rule it out. But certainly, the appearance of HLB survivors in many different locations is an opportunity — a potential resource for a solution — that cannot be ignored as we leave no stone unturned in this fight for survival of the citrus industry. We hope useful information and products may result from the ongoing research projects, spearheaded by the Citrus Research and Development Foundation, that will contribute to our ultimate control of citrus HLB.

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