Lessons learned from HLB as an immune-mediated plant disease

By Nian Wang

How the huanglongbing (HLB) pathogen *Candidatus* Liberibacter asiaticus (*C*Las) causes damage to infected citrus trees has been widely debated. A recent study demonstrates that HLB is an immune-mediated plant disease (Ma et al., 2022). It was discovered that *C*Las infection of citrus stimulates systemic and chronic immune response in phloem tissues, including reactive oxygen species (ROS) production, callose deposition and induction of immune-related genes.

Research has provided evidence that phloem cell death is the key for HLB symptom development, which primarily results from excessive and chronic immune response such as ROS production triggered by *C*Las. This discovery is supported by many other studies and grower observations, including the following:

- *C*Las lacks homologs of known pathogenicity factors that are directly responsible for causing HLB disease symptoms (Ma et al., 2022).
- Suppressing *C*Las-triggered ROS production with antioxidants (uric acid and rutin) and immunoregulators [such as gibberellic acid (GA)] mitigates phloem cell death and HLB symptoms.
- Some citrus cultivars, such as Valencia sweet orange, Vernia and Sugar Belle, demonstrate improved tolerance against HLB with horticultural approaches including optimized nutritional programs.
- HLB-tolerant cultivars such as Persian triploid lime contain higher levels of antioxidants and antioxidant enzyme activities than more susceptible cultivars such as Mexican lime (Sivager et al., 2021).

This work is consistent with the results of other University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) researchers.

The finding of citrus HLB as an immune-mediated plant disease provides useful information to guide the battle against this notorious disease. Both horticultural and genetic approaches that suppress ROS damages and promote plant growth can alleviate the harms of HLB to citrus trees.

INTEGRATED HORTICULTURAL APPROACHES

Antioxidants, nutrition and immunoregulators are commonly used to treat human immune-mediated diseases by halting or reducing ROS-mediated cell death. Optimized fertilization with macronutrients (nitrogen, phosphorus and potassium), micronutrients and irrigation, along with treatment with antioxidants and hormones can help alleviate HLB damage. For the integrated horticultural approaches, it is important to note that nutrition deficiency, salinity stress and drought cause more ROS production, which will further increase the ROS level triggered by CLas.

It is also important to note that a tradeoff relationship exists between growth and immunity in plants (e.g., growth usually suppresses immunity and vice versa). Consequently, horticultural approaches used to promote citrus growth suppress the harmful effects of CLas, thus alleviating HLB symptoms. This is indeed consistent with grower observations.

Additionally, it is probable to induce the activity of antioxidant enzymes via application of micronutrients [boron (B), iron (Fe), molybdenum (Mo), nickel (Ni) and zinc (Zn)] to reduce ROS damages. Micronutrients are critical for plant growth. Furthermore, B, copper, Fe, manganese (Mn), Mo, Ni, selenium or Zn at suitable concentrations activate endogenous antioxidative enzymes and non-oxidizing metabolism to mitigate ROS damage (Tavanti et al., 2021). Soil application of Mn and Zn, or magnesium (Mg) and B and foliar spray of Mn, Zn, Mg and Mo significantly alleviated HLB symptoms compared to negative controls (Shen et al., 2013; Atta et al., 2021; Zhou et al., 2021). However, the positive effect on HLB management seems to be limited once HLB causes severe damage to trees (Gottwald et al., 2012).

The micronutrients B, Fe, Mo, Ni and Zn have shown to be able to suppress cell death of citrus cells caused by ROS. The application doses and frequency need to be optimized based on scion/rootstock cultivars, local soil composition and environment. Plant growth hormones, such as gibberellin, can promote plant growth and reduce ROS damages. Gibberellic acid (GA) has been shown to inhibit ROS production.

Foliar sprays of HLB-positive *C. sinensis* trees with GA at both 5 milligrams per liter and 25 milligrams per liter or higher have reduced HLB symptoms and promoted citrus tree growth. Tripti Vashisth's UF/IFAS lab has demonstrated the positive effect of GA treatment on fruit production and tree health of HLB-positive trees (Singh et al., 2022).

It is also probable to suppress ROS damages using antioxidants, such as uric acid, which is yet to be labeled on citrus. Many of the horticultural applications are a double-edged sword that need to be tested in groves to minimize putative negative effect on fruit yield and quality.

As shown in a UF/IFAS field trial, trees have performed well in the presence of HLB with optimized fertilization and irrigation (Figure 1, page 19). No significant difference was observed between the different treatments and the non-treated control trees one year after the first treatment. As observed before, the treatment effect might take a longer time to show.

GENETIC IMPROVEMENT

UF/IFAS has been generating HLB-tolerant citrus cultivars by enhancing plant tolerance of ROS or reducing ROS production triggered by *C*Las using CRISPR and other modern technologies. The CRISPR genome-editing technology is, so far, the most promising technology that enables the generation of non-transgenic disease-resistant citrus plants (for example, non-transgenic canker-resistant Hamlin sweet orange).

Plant growth hormones, such as gibberellin, can promote plant growth and reduce ROS damages.

For commercialization, it is critical to make non-transgenic citrus cultivars to get regulatory approvals from the U.S. Department of Agriculture Animal and Plant Health Inspection Service (APHIS), the Environmental Protection Agency (EPA) or the Food and Drug Administration (FDA) and to address concerns of consumers. However, it is important to point out that there are still multiple hurdles.

Researchers need not only to knockout the genes to enable the edited trees to have improved HLB tolerance/resistance, but they also need to make sure the edits will not affect tree growth, fruit quality and yield. Some promising target genes have been identified. Researchers are generating genome-edited plants for the target genes and investigating their phenotypes.

Genome editing is a long process. It takes more than a year to complete the editing of single cells and regenerating into trees. Finally, the time needed for approval is even longer. For example, even though non-transgenic canker-resistant Hamlin lines have

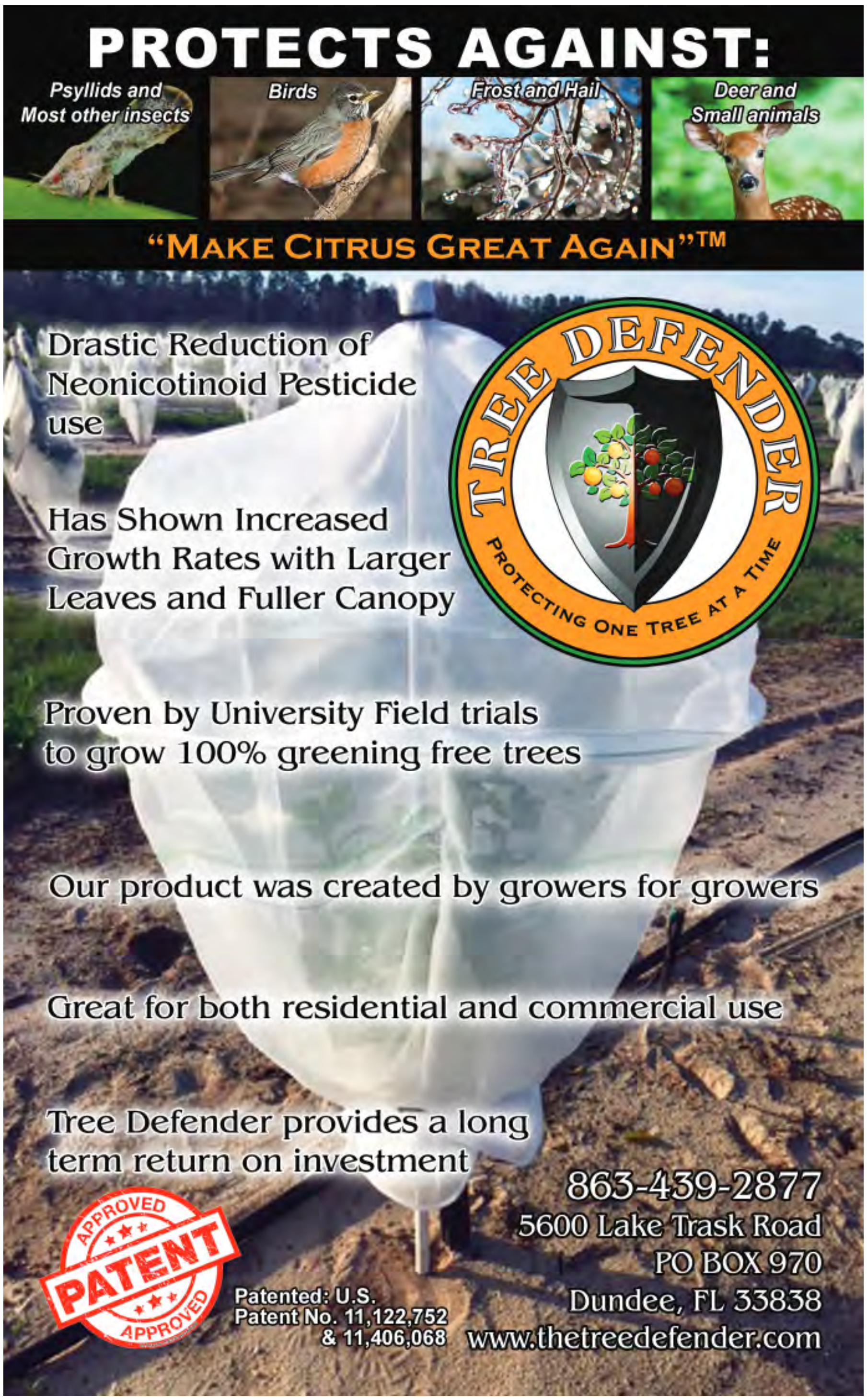

Figure 1. A field trial of micronutrients, gibberellic acid (GA) and uric acid was conducted on HLB-symptomatic trees. The trial took place in a commercial citrus grove of OLL-8 on US-942 trees, which were planted in April 2018. The treatments include micronutrients alone, micronutrients + GA, micronutrients + uric acid and micronutrients + GA + uric acid. Non-spray was used as the negative control (Treatment 13). The picture was taken in December 2022. The field trial was under an optimal irrigation and fertilization program. There were no significant differences between different treatments.

been generated, it was estimated that at least three to five years are needed to go through the Florida citrus budwood program, get approval for new citrus cultivar release and obtain federal approvals by APHIS, FDA and EPA.

SUMMARY

In summary, citrus HLB is an immune-mediated disease. Mitigating ROS and promoting new growth can reduce cell death of phloem tissues, thus controlling HLB. Growth hormones (e.g., GA), nutritional modulation (e.g., micronutrients) and antioxidants (e.g., uric acid) have potential to alleviate ROS damages triggered by *C*Las to reduce cell death of the phloem tissue to mitigate HLB symptoms. Growth hormones and nutrients, by promoting new growth, decrease the proportion of dead cells in phloem tissue, further mitigating HLB symptoms. Growers can test these strategies out before HLB resistant/tolerant citrus cultivars are available.

Genetic improvements that enhance plant tolerance of ROS, prevent overproduction of ROS or evade recognition of *C*Las are likely to generate HLB resistant/tolerant citrus varieties. However, this is a challenging and lengthy process that calls for collaborative efforts by all stakeholders.

Nian Wang (nianwang@ufl.edu) is a professor at the UF/IFAS Citrus Research and Education Center in Lake Alfred.