Starting in the grove to find new HLB therapies

By Randall P. Niedz, Guilherme Locatelli, Nick Larson, Lorenzo Rossi, Ellen Cochrane and Michelle Heck

Scientists at the U.S. Department of Agriculture-Agricultural Research Service (USDA-ARS) developed an idea to bypass lab assays and test molecules directly in the field for their ability to solve HLB. As part of a USDA National Institute of Food and Agriculture (NIFA) grant (number 2020-70029-33176), they built a collaborative team of researchers at USDA-ARS, the University of Florida and other partners. The team is field-testing 88 different molecules to identify those that can rejuvenate HLB-infected trees back to economic productivity.

Figure 1. The FlexInject system from TJ BioTech LLC was used to inject trees in the experiment.

ONE TRUE TEST

By 2024, the research team plans to have more than 200 molecules in the field. None of these molecules have been tested using a laboratory assay. Why? Because it is unknown how useful lab assays are for accurately predicting how well a treatment will work in the field. There is only one assay known that can definitively determine if a molecule or treatment works, and that assay is treating an HLB-infected tree in the field and observing the horticultural rejuvenation of the tree.

Field testing of molecules, products and technologies is arguably the single-most valuable research resource for solving HLB. If a product or technology does not result in the horticultural rejuvenation of a tree, then it does not work. This is the grove-first approach.

The grove-first approach is a research framework that requires three conditions:

1. The objective is to screen a large number of molecules.
2. Large numbers of HLB-infected trees are readily available.
3. The objective is to screen only for large effects.

These three conditions are easily met in Florida, making the state's citrus industry a living laboratory ideally suited for molecule/treatment screening. The process is simple. First, screen molecules in a limited number of HLB-infected trees to identify those that will then move to a second phase of grower-scale replicated field trials. This makes grower cooperators integral to the process of identifying molecules that can solve HLB.

The process is like how plant breeders develop new varieties. Large numbers of progeny from hybrid crosses are planted in the field, superior individuals are selected, and those selected individuals are tested in replicated field-trials.

The importance of the growers in helping to solve the HLB problem was recognized by Mike

Sparks, former executive vice president of Florida Citrus Mutual, who conceptualized the Grower Replanting Initiative Program (GRIP) that evolved into the current grower-research Citrus Research and Field Trial program.

A MULTITUDE OF MOLECULES

The molecules being tested were identified from multiple sources, including substantial and ongoing contributions from Brian Scully, retired USDA-ARS laboratory director and plant breeder, who is scouring the literature and global regulatory records for molecules to test. Scully's molecule-sleuthing research is funded by the Citrus Research and Development Foundation and has resulted in a large and rich assortment of molecules. Currently, over 200 extremely diverse molecules have been identified and placed into a queue to test.

The grove-first approach is testing commercially available formulations of these molecules. Scully's research has uncovered quite literally hundreds of molecules that are suitable for grove-first testing. These molecules vary in their regulatory and public perception friendliness. They range from no to minimal regulatory requirements and positive public perception to those that would require higher levels of regulation similar to what is required for oxytetracycline (OTC) and other antibiotics used in agriculture. The objective is to broaden the number of molecules available to growers to treat HLB.

INJECT AND OBSERVE

The basic approach to test a molecule is to inject an HLB-infected tree with 100 milliliters of a solution containing the molecule using a FlexInject device (Figure 1, page 8). Horticultural rejuvenation is determined by improvements in the canopy, roots, and most importantly, fruit quality/size/number. Trunk damage is also being carefully assessed with photo documentation.

The improvements in tree health observed in the screening stage must be large for a molecule or molecule combination to be advanced to field trials. For foliage, this means effects that are visual and readily apparent. For a molecule to be selected for

further testing, its effects must be similar to or better than a commercially available formulation of OTC, which is being used as the baseline control.

A molecule's effect on tree horticulture is evaluated over the summer, fall, winter and spring phases of the phenological growth and fruiting cycle. Thus, at least one full season (one year) is required to determine if a molecule's effect is sufficiently large to justify more extensive field trials. The 88 molecules currently being tested were injected into 10-year-old Valencia trees from June to mid-July 2023. Canopy health was measured using the Page/Slinski Disease Index (DI) quadrant rating system. Before treatment, all trees were photographed, and DI ratings were taken. Thereafter, DI ratings were taken every 30 days. This created a visual rating and documentation system to assess effects.

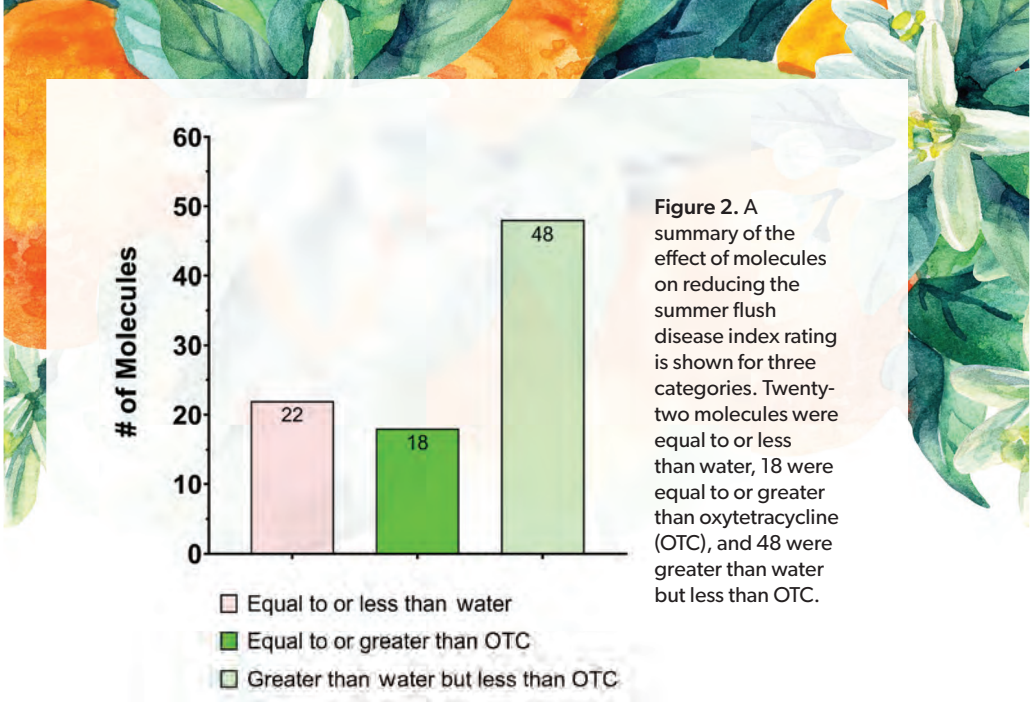

Figure 2. A summary of the effect of molecules on reducing the summer flush disease index rating is shown for three categories. Twenty-two molecules were equal to or less than water, 18 were equal to or greater than oxytetracycline (OTC), and 48 were greater than water but less than OTC.

RESULTS TO DATE

At the time of this article, the 90-day DI ratings for the effects on summer flush have been completed and over 8,000 images have been taken to document the effects. The effect of the 88 molecules on reducing the DI over 90 days is shown in Figure 2. Eighteen molecules reduced the DI equal to or greater than what is being seen with OTC, and there were 48 molecules with effects greater than the water-injected control but less than the OTC treatment. Trees with improved foliage resulted in a lower 90-day DI rating and exhibited various forms that ranged from an overall thickening of the canopy to a profusion of large leaves (Figure 3, page 11). These effects were often observed on the injection side of the tree.

The upcoming winter flush phase will determine how well the molecules

Figure 3. Top: A large effect was observed on Valencia tree leaves 90 days after injection with molecule #10. Bottom: Size comparison of a newly formed leaf to an older leaf from the same tree. Shoot flushes are long and have large leaves on the injected side of the tree.

maintain any improved summer flush. Because cooler weather exacerbates HLB symptoms, the winter flush DI ratings are expected to be particularly useful for selecting promising molecules. Fruit will be harvested and measured in the final spring phase.

Though various aspects of this NIFA project will eventually be published in refereed journals, there is a significant amount of information that might be of immediate value to growers. If you are a grower and this project interests you enough where you want to try any of the molecules that are showing effects, contact Randall Niedz (randall.niedz@usda.gov) or Michelle Heck (michelle.cilia@usda.gov) to be added to a list of grower cooperators. The team will also evaluate molecules or combinations of molecules for interested parties with the next set of molecules to be tested. Those experiments are expected to begin in February or March 2024.

Randall P. Niedz, Michelle Heck, Nick Larson and Ellen Cochrane are scientists with USDA-ARS. Lorenzo Rossi and Guilherme Locatelli are researchers with the University of Florida Institute of Food and Agricultural Sciences.

New Year Priorities

By Rick Dantzler, CRDF chief operating officer

The Citrus Research and Development Foundation (CRDF) board makeup is statutorily prescribed. Of the 13 members, 10 must be growers, two must be from the University of Florida, and one must be from the Florida Department of Agriculture and Consumer Services. Of the 10 growers, five are nominated by Florida Citrus Mutual and five by the Florida Department of Citrus (FDOC).

At the beginning of the year, CRDF will be losing three outstanding grower board members: David Howard, Rob Atchley and John Updike. Howard and Atchley are former presidents, and each brought something special. Howard had a strong focus on plant breeding and ushered in the advent of "directed research," a method of funding research that is quicker and more targeted. Atchley presided with a business-like approach, making sure the research we funded was not just an academic exercise. He also saw the entire field when deliberating. Updike chaired the Research Management Committee — the heart and soul of CRDF's work. He read everything and was an encyclopedia of knowledge. He was always one of the smartest people in the room. I'm going to miss these gentlemen.

Taking their places will be Matt Machata, Sarah Spinosa and Trevor Murphy. Machata is a grower from the Lake Wales area and has an engineering background. Spinosa is involved with her family's citrus operations and was once with the FDOC. Murphy is chief operating officer of Kahn Citrus Management out of Sebring. Each has a skill set that will fit nicely with research projects we will be considering.

So, what does the new year look like for CRDF? We have three high priorities. The first is to continue researching ways to squeeze the most we can out of oxytetracycline (OTC) usage, either by funding research on ways to get more product into the phloem or combining it with other compounds that maximize OTC's efficacy or help the tree fight *C*Las or other pathogens. The second is finding molecules or compounds that could take the place of OTC if the bacterium becomes resistant to OTC or the trees become damaged from multiple injections. The third is funding the development of the "tree of the future," a tree that is sufficiently tolerant to greening or perhaps even resistant. CRDF is funding many projects on these priorities and is considering numerous others.

A question I sometimes hear growers ponder is: Are we down to funding priorities one and two to hopefully buy us enough time for the tree of the future to solve the problem of HLB, and nothing more? While I understand the sentiment given the toll the disease has taken, I believe it would be a mistake to settle into that way of thinking. Science and technology are moving too fast to put all our eggs into just three baskets. Scientific advancements we haven't even imagined are right around the corner, so CRDF will continue looking for and funding the latest ideas as we push bravely into the new year.

CRDF Grower Dollars at work for you

Column sponsored by the Citrus Research and Development Foundation