

Doubling down on CRISPR research

By Michael E. Rogers

We have all been waiting (and waiting some more) for new varieties to be developed that are resistant to HLB. Ten years ago, researchers thought they were really close to having new HLB-resistant varieties developed using gene-editing CRISPR technology. But as science goes, the story was much more complicated than first believed. A single edit to the genome of citrus wasn't enough to shut down the citrus plant's response to HLB.

We have learned there are many pathways within citrus that are affected by the HLB-causing bacterium, thus leading to disease symptoms. It's similar to driving down the highway. If one road is closed leading to your destination, you take a detour. With HLB, it's pretty much the same. If only one gene is silenced, there's still other routes for disease symptom expression in citrus. HLB disease is a very complex system with no easy answer!

MAKING PROGRESS

The University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) multi-pronged approach to the battle against HLB has produced some short-term successes while the longer-term solutions develop. While we're hopeful that our efforts in this area

will lead to solutions for HLB that our industry desperately needs, we shouldn't place all our hope on just one approach. We have made progress in many directions, and we think setting up as many solutions as possible is the way to get some wins for the citrus industry.

Over the past decade, UF/IFAS scientists have been hard at work to better understand how HLB disease works, and more importantly, how to fine-tune CRISPR to make the multiple necessary edits needed to reach our ultimate goal: an HLB-resistant citrus tree. We've come a long way and have fine-tuned CRISPR to be able to edit multiple genes simultaneously in citrus. We're at the point now where we have multiple lines of citrus that have been edited using CRISPR, but it will still take time to grow these plants to a size that is suitable for field testing. It will also take time in the field to know whether these edited plants will indeed hold up against HLB infection.

INVESTING IN INFRASTRUCTURE

We're placing a lot of our emphasis going forward on CRISPR research, not only because we think it holds promise, but because that's what the citrus industry has

asked us to do. UF/IFAS is making additional investments in its CRISPR research program as we believe this new technology will ultimately provide many solutions — not only for HLB, but for other pest and disease issues the industry may face in the future.

To begin with, we are expanding the footprint of our laboratories at the Citrus Research and Education Center (CREC) in Lake Alfred dedicated to CRISPR research. This includes adding additional tissue culture rooms to greatly expand the number of citrus lines that can be edited and grown up for subsequent testing against HLB.

UF/IFAS has established a new 10-acre field site approved by the Animal and Plant Health Inspection Service for testing both gene-edited and transgenic plants.

We are also increasing our capacity to screen a greater number of gene-edited citrus plants against HLB by renovating screenhouses and greenhouses to accommodate even more plants once they are ready to move out of the lab. While this won't necessarily shave time off the early stages of the gene-editing process, it will allow us to move more plants through the first HLB screening process in the greenhouse to identify more potential candidates for field trials.

In addition, UF/IFAS is investing in the construction of a new citrus under protective screen (CUPS) structure at Lake Alfred dedicated to advancing CRISPR research. Concurrent with the movement of gene-edited plants to the greenhouse for the first HLB screening, trees from each line will also be planted in this CUPS structure to serve as a source of budwood for future field trials. We're planting these trees before we know how they hold up against HLB, but we'll ensure we'll have the right material on hand to move

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quickly to the field trials once winners are identified.

UF/IFAS has established a new 10-acre field site approved by the Animal and Plant Health Inspection Service for testing both gene-edited and transgenic plants. We've included transgenics as part of our evaluations because many of the created transgenic citrus lines that are part of the CRISPR research look promising. These lines could potentially be available to growers sooner than other non-transgenic varieties still in development.

While UF/IFAS is making a considerable investment in the infrastructure needed to expand the CRISPR research program, there are still shortfalls in funding for scientists and technicians as well as supplies to get the breadth of the work accomplished in a timely manner. Potential funding for those needs in the CRISPR research could come from the Citrus Research and Development Foundation, as it has recently indicated strong support for continuing the work on citrus gene editing.

ACTIONS TO TAKE NOW

CRISPR is a promising tool for developing new disease-resistant citrus varieties for the future of the industry. With nearly two decades of HLB research in Florida accomplished, we've learned a lot about this disease and have many additional tools that can be used now. We have a number of new citrus rootstock and scion varieties released by UF/IFAS that not only hold up well against HLB, but also have improved fruit/juice quality.

There are also management practices (nutrient and hormone applications) that can be used in the field now to reduce the severity of HLB disease symptoms. These applications can have beneficial effects on the very same pathways that influence plant defense and stress responses that are also the targets of CRISPR research!

UF/IFAS researchers will be talking more about each of these areas where action should be taken now at the upcoming Citrus & Specialty Crop Expo at the Tampa State Fairgrounds on Aug. 16–17. See you there! 🍊

Michael E. Rogers is the director of the UF/IFAS CREC in Lake Alfred.

Dog Days To-Do List

By Rick Dantzler, CRDF chief operating officer



The “dog days of summer” are from July 3 to August 11, a time of particularly hot and humid weather. This period coincides with the early morning rising of Sirius, known as the “Dog Star,” the brightest star in the night sky. Sirius’ early rising was believed by many in ancient Greece, Egypt and Rome to contribute to the extreme weather of the season.

This period is also a slower time of the year. But for citrus growers, there is still a lot to do. Water removal systems — from pump maintenance to canal cleaning — in the flatwoods and Indian River areas need to be ready to handle excess water from heavy summer rains or hurricanes.

Soil and leaf samples have just been collected or are in the process of being collected to aid in planning next year’s nutritional programs. Mapping out the nutritional program is likely underway for the fall fertilizer program that would begin in mid or late September, depending on weather conditions.

Growers with fresh fruit will be watching for citrus canker.

Irrigation systems and pumps will be serviced to make sure they are operational before heading into the fall period when soil conditions can become dry.

Processors will surely be estimating this year’s crop to help determine fruit contracts.

And this year, growers will be monitoring tree health and crop development after injecting oxytetracycline this past spring. The good news is that most growers are seeing a positive response in trees from this treatment. The proof will be in the pudding this season when the harvest comes in. However, the full benefit of this therapy will likely take several seasons to realize.

Growers are possibly considering the use of 2,4-D products to reduce pre-harvest fruit drop because there is data showing a positive effect. The Citrus Research and Development Foundation’s (CRDF) Brandon Page recently compiled data from an enlightening CRDF-funded trial. It can be found on the CRDF website (citrusrdf.org).

This same report includes data on gibberellic acid testing. It, too, showed improvements compared to the standards, although not as universal or as significant as 2,4-D. Please realize these results are from just one trial. Tripti Vashisth, University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) associate professor, is the expert and has much more data on these therapies.

Fernando Alferez, UF/IFAS assistant professor, has studies showing brassinosteroid use increased Brix levels by more than a point approximately 30 days after application. If calibrated with harvesting schedules, this could be huge.

CRDF has additional projects underway to test other antimicrobials and products that hold just as much promise. Watch this space for the latest news.

Of course, these therapies — those current and those to come — cost money, and growers can’t do it all. So, the dog days are a good time to plan caretaking budgets. And when you need a break, think of cooler weather and football season, both of which are right around the corner.



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