

GROWER RESEARCH REPORT

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Pathway to a Sustainable Florida Citrus Industry and CRISPR

It has been quite a year. In the aftermath of hurricane and disheartening estimate, I know you have been working hard to keep things going, so this report will be rather brief. In fact, it will start with the substance of a briefing memorandum I provided to the Florida Citrus Commission (FCC) because I believe it does a good job of laying out CRDF's research strategy and includes a discussion of what many think will be the ultimate solution to HLB: a CRISPR tree that will be non-GMO and HLB resistant or sufficiently tolerant.

As you are aware, CRDF produced a document called Pathway to a Sustainable Florida Citrus Industry approximately a year and a half ago. It laid out a sequential approach to a pathway forward to revive the Florida citrus industry. The strategy was to provide a means to keep the industry viable until solutions could make HLB irrelevant.

I am pleased to report that either CRDF or the USDA funded all but one of the research topics identified, and for the one that was not funded (micronutrients), CRDF had funded numerous projects over the years trying to maximize micronutrient inputs. And the re-

search was working. Novel uses of plant growth regulators like 2,4-D, gibberellic acid and brassinosteroids was causing fruit to stick on trees better, lessening the effects of drop, color break was standardizing, fruit quality was improving, and fruit size was getting larger. Unfortunately, Hurricane Ian put much of the fruit from the current crop on the ground. Nevertheless, this and other research is helping to build a bridge of better production to allow the industry to hang on until resistant or sufficiently tolerant citrus varieties provide a permanent solution.

The Pathway document was not intended to be static, but to change as new products, technologies, or ideas were presented. One of these new products is to infuse trees with a particular bactericide through a proprietary systemic application device. CRDF is funding research on several of these products and devices and has worked with federal and state regulators on necessary approvals. This funding is demonstrating the effectiveness of this approach. Although the treatment is labor-intensive and the product expensive, we are confident it can improve production until the next technology is in place. Still, the industry badly needs another competitor in the marketplace to help keep prices down, and there are other companies working to get products approved. CRDF

Inventory of Citrus Tree Creations

CRDF recently funded a project with Dr. John Chater of UF/IFAS and Dr. Matt Mattia of USDA/ARS to create inventories of all trees created by the citrus breeders we help fund. CRDF's goal is to have these inventories in one place that can ultimately be accessed by other researchers and growers. There are valid concerns regarding security and intellectual property rights that must be respected, but CRDF is confident that, working with the institutions, we can make public enough data for growers to know what they are helping to pay for while still protecting valid concerns of the institutions.

A comprehensive report from these researchers is due in March of 2023. Once it is received, CRDF will review the report and consider next steps for thoroughly evaluating promising germplasm identified.

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stands ready to help these companies so long as they have data which shows the product is safe and will work.

The next technology outlined in the Pathway document is the ability to utilize CTV to be crafted into a vector to continuously produce antimicrobial peptides in citrus trees to substantially reduce CLAs's ability to cause disease. This technology was developed with CRDF funding. The advantage of this technology is that it requires no labor and cost once the viral vector is inoculated into trees. Although the production of the antimicrobial peptides in infected trees is not permanent, it lasts for years, estimated to be 7-10 years, which is sufficient to provide protection against HLB until the next strategy is in place. CRDF is also in discussions with partners regarding getting this technology and the latest spinach defensin genes approved by the necessary regulatory bodies.

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) is a relatively new technology that is revolutionizing all areas of biology and medicine. It is based on a process that bacteria use to destroy other bacteria in which an enzyme – guided by an RNA – can cut DNA in a particular area. For plants, this process can knock out a gene by removing one or a few nucleotides, making it non-functional. Thus, CRISPR modifications of citrus that simply remove a few nucleotides from a gene provide a result identical to X-ray mutations that have routinely been used by citrus breeders. Examples include pink and red grapefruit produced by Texas A&M, which are in production in Florida. The difference is that CRISPR targets a specific gene whereas X-ray mutations are random. CRISPR mutations so designed insert no foreign sequences into citrus and thus should be considered non-GMO.

CRDF funded the initial work in several labs developing the CRISPR technology for citrus that allowed these labs to obtain larger funds from federal sources. There are several labs now working on several different approaches to creating resistant or tolerant trees. One lab has already created canker-resistant trees and is in the process of moving these trees into commercial production. CRDF also funded the work that led to identification of potential “susceptibility” genes that allow CLAs to infect citrus that are the CRISPR targets of Soilcea, a local biotech company commercially developing HLB-resistant citrus. This is a good example of how public sector research works. Here, CRDF provided funding to UF/IFAS to develop the technology and product, and the private sector acquired what had been developed and commercialized it. Currently, CRDF is discussing with Soilcea ways to expedite getting resistant trees into the field.

A problem with CRISPR and traditional citrus breeding is that the plants initially have a juvenile phase that

can last years. Another CRDF project is the use of the CTV vector to quickly induce maturity. Expression of flowering genes by CTV can induce conversion to maturity in a matter of months, after which the viral vector can be quickly removed by alternating heat therapy.

CRDF is supporting several parallel approaches. One is a modification of production of transgenic citrus called “cisgenics,” which is a process where all the sequences inserted into the tree are from citrus or citrus relatives that can be crossed into commercial citrus. The result is a tree that could have been produced by traditional crosses but produced in a shorter time. One such project is the insertion of the finger lime peptide into U.S. 942, Kuharske, and Hamlin. This should not be considered a GMO and should be much faster and less costly to pass regulations required for commercial use.

CRDF is also funding the production of transgenics that could provide resistance to HLB. Although these trees would be GMOs and require normal USDA and EPA regulations, until CRISPR or cisgenic trees are proven to be effective against HLB, we should pursue this strategy as a backup.

Diaprepes

Because HLB weakens the roots of infected trees, these trees become especially vulnerable to secondary afflictions, including infestation by Diaprepes root weevil (DRW). Prior to HLB, DRW and the sudden decline of groves caused by weevil damage to citrus root systems were primary concerns in Florida citrus production. In the face of the existential threat posed by HLB, however, this faded as a research priority.

From 2008 through 2018, DRW seemed to have maintained a relatively low population level, probably because of the intense spraying that was done during this period to control psyllid populations. However, spraying intensity targeting psyllids has been relaxed across the state of late, and Diaprepes infestations appear to have become more common. And when trees which are already infected with CLAs are attacked by DRW, the result is particularly challenging for growers.

Consequently, CRDF is funding research to combat DRW by evaluating currently available registered insecticides and by learning what we can of DRW dispersal, which should lower control costs for growers. CRDF is also maintaining a dialogue with a company that manufactures a nematode that controls DRW larvae; in fact, the CRDF COO and several CRDF committee members visited the manufacturing facility for this product in Georgia to learn more about it.

Effects of Hurricane Ian and the USDA Crop Estimate

In the aftermath of the estimate and storm, CRDF met in Executive Session to regroup and review our research portfolio.

First, we reviewed the progress we had made in implementing the document Pathway to a Sustainable Florida Citrus Industry, described above.

Second, we discussed a possible Request for Proposals (RFP) that we called "Game Changers," an RFP where we would solicit proposals which, if successful, had the potential to save the industry and not just provide incremental benefit. Frankly, new therapies that provide only incremental benefit are probably not going to cut it until the industry substantially recovers.

Next, we reviewed the budget to see what we had available to provide immediate help to growers if delivering bactericides through a systemic delivery device was approved.

Finally, we discussed the state of CRISPR technology and what had been done to date. While not a short-term fix, it is likely the long-term solution to whipping this disease once and for all.

After a great deal of discussion, the Executive Committee decided that our highest priority needed to be to assist growers in getting the bactericide into trees if the state approved its use. Three days after our meeting, state regulators did just that, and a label was issued.

Consequently, we are discussing with other industry partners how best to assist growers, and we know that time is of the essence. There are legitimate research questions that would help growers in using the product, questions such as whether delivering the bactericide in this fashion will work just as well with or without psyllid control or will putting the product into the rootstock instead of the scion help or hurt with phytophthora control. CRDF exists to serve the Florida citrus industry, so we will do whatever maximizes additional financial assistance to growers, which we believe we can do while still answering sincere questions growers have regarding its use.

