Accelerating citrus breeding efforts

By John M. Chater

Professors Jude Grosser and Fred Gmitter have been breeding improved citrus varieties at the University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) Citrus Research and Education Center (CREC) for decades. Over time, they have released several of their advanced selections. Many of these selections are sweet oranges. There is a subset of these creations that appear to have some appreciable level of HLB tolerance under the right environmental and cultural conditions (Figure 1).

Today, there are even more promising materials in the breeding pipeline either under evaluation or awaiting field trials. Some of these are sweet oranges, and others are fresh market fruit selections. The program has also selected sweet orange-like hybrids that have brilliant color, high Brix and exceptional orange-like flavor.

From a breeder's standpoint, the program has been hugely successful in creating and identifying both scions and rootstocks of diverse and clever genetic backgrounds for Florida's growers. The list is long and full of potential winners. Currently, this program's successes have led to a healthy backlog of citrus material from Gmitter and Grosser's breeding program that needs thorough evaluation. Therefore, work is underway to characterize germplasm as quickly as possible.

HIGH-THROUGHPUT PHENOTYPING

In plant breeding, finding new and improved, rapid methods to phenotype (or characterize) plant material for important traits of value to industry is helpful because there can be thousands, even tens of thousands of individuals or more, to evaluate across multiple sites. Being able to determine important traits for HLB tolerance, such as canopy size and health status, of 10,000 or more trees in minutes with a drone can assist breeders in the selection process and in phenotyping during the course of field trials.

Figure 1. N13-32 Hamlin sweet orange displays good health in a commercial grove.

In horticulture and other disciplines, the act of collecting data quickly from a large population is called "high-throughput phenotyping." Some have defined high-throughput image-based phenotyping as characterizing minimally "hundreds of plants per day." The ability to characterize large populations allows for quick phenotyping or for the best individuals to be selected as parents and/or for more advanced field trial stages. It also opens the door to 21st century advanced breeding methods using genomics and other tools.

One of these methods, marker assisted selection, would allow a breeder to select individuals for desired traits based on molecular markers. This means he or she would be able to use the genetic or biochemical composition of the leaf

Figure 2. Jude Grosser stands in front of his OLL lines in St. Cloud, Florida.

of a seedling to determine its value downstream. These advanced methods could reduce the costs of phenotyping substantially. Imagine only having to phenotype 12 individuals for HLB tolerance, yield and pounds solids instead of 30,000 trees.

In most breeding programs, phenotyping is typically the most expensive and time-consuming process in the system. Hundreds of thousands to millions of dollars per year are required to support a premier tree-fruit breeding program. These costs involve personnel; space in the laboratory, greenhouse, and field; materials and supplies; vehicle upkeep and much more.

For a species like citrus, the costs are extraordinary. The space is on the acreage scale, the time required is long, and the path to success can be pushed at a moment's notice. Florida Citrus Hall of Fame Inductee and Professor Emeritus Bill Castle would say that a trial should last at least eight years at multiple sites. This timeline of eight years makes good sense and comes from a place of wisdom considering the challenges that the industry has faced with rootstock incompatibility. Such incompatibility can take years to manifest, as was seen in Roble on Swingle, Carrizo and C-35 citranges. Fruit disorders, like fruit split on Early Pride, and other undesirable outcomes can result from unfortunate scion and rootstock selection.

There are currently dozens of trials and other plantings that have trees with evaluations ongoing. Many of these trees are mature now and have several years of data collection. Other trials are newer, and some are just being budded this season. Data are continuously being compiled on a website (crec.ifas.ufl.edu/citrus-research/rootstock-trials) for the rootstock trials that has long been overseen by Castle. But with new faculty hires, the passing of the torch is underway and writing up the results of the trials in papers has been delegated.

EVALUATIONS VIA DRONE

To create a snapshot of the ongoing citrus trials and plantings, the Citrus Research and Development Foundation sponsored a project titled "Utilizing high-throughput phenotyping to screen germplasm and ongoing field trials for promising citrus accessions in HLB-endemic Florida" to evaluate all of the UF/IFAS citrus material in trials. The purpose of this project was not only to get a status update from the plant improvement team, but to create inventories and lists of top performers with potential to serve the industry.

This work was commissioned

around the time of the Donaldson selection, and stakeholders wished to know if there is a similarly healthy selection in the UF/IFAS citrus breeding germplasm. Drones were flown over 91 sites comprised of plantings and trials. Some of these were replicated trials of over 70 acres with dozens of rootstock-scion combinations. Others were germplasm blocks, and some were smaller plantings, many on growers' land.

Due to the timeliness of work, it was determined by stakeholders that larger, fruit-bearing trees should be evaluated and not young trees. An interim report has been submitted that identified top performers among all trials (such as some of the OLL lines, Figure 2, page 10). The report provides stakeholders with some baselines for future comparisons.

Before this drone work even started, some selections were already being described by experienced growers as HLB tolerant. These selections are actively being planted by growers as resets or in solid blocks.

For example, there is some promise in N13-32, a somaclone of Hamlin, as a healthier alternative scion for early sweet oranges. At one site in Lake Alfred, juice quality and yield have been rather similar to Hamlin. Tree health seems to be going strong at some sites and trials. However, more data are needed to know if this selection is a good choice for resets or solid blocks as some, including the breeder, would say cropping may be delayed by about one season. Whether there is a tradeoff between this reported delay (notedly unsubstantiated by data) in cropping versus the building of biomass over time to make a large tree that can bear more boxes of fruit versus conventional Hamlin 1-4-1 remains to be seen. There are trees in some groves that are performing quite well for health. At other sites, trees are of typical health for Hamlin in HLB-endemic Florida. An important next step is discovering why there are differences among sites for this and other selections. Such a research direction is being pursued for funding. As Castle would say, "The beat goes on."

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Making the Most Impact

By Rick Dantzler, CRDF chief operating officer

July 1 is the first day of the Citrus Research and Development Foundation's (CRDF) fiscal year, so we have been spending a lot of time on our budget. We are grateful to growers and the Florida Legislature for funding support. With production down, box tax revenues for research are down. So, like growers, we are having to do more with less. Legislative funding has been critical.

As we head into the new fiscal year, we have 42 research projects under contract. Of these, many will end during the next 12 months. What should take their places? Stated another way, what is the research space that CRDF should occupy?

First, an editorial comment from me, not my board: I believe those of us with agencies or governmental entities must be about rebuilding the industry with our funds. It's why CRDF gave the Citrus Research and Field Trial Foundation (CRAFT) $1.2 million in uncommitted research funds recently. Unless we help growers get new trees in the ground and find ways to heal older sick trees, everything else we do may prove to be purely academic. With that paradigm in mind, here's a research space I see for CRDF.

CRDF can help provide tools for growers to hang on until a sufficiently greening-tolerant or -resistant tree arrives. The most likely tool is injecting antimicrobials to be used in conjunction with or in place of oxytetracycline (OTC). OTC as a therapy will likely have a limited shelf life, so something must take its place if the tree we are looking for hasn't arrived. While growers are increasing production and fruit quality with other therapies, nothing is as final as killing *C*Las, and that's what antimicrobials do. We have waited a long time for an effective antimicrobial and a means of getting it into the phloem. Now that we have this, CRDF will help find other antimicrobials and improve their efficacy.

CRDF can help fund CRISPR and the genetic engineering of trees to get HLB behind us. While I believe it would be imprudent to abandon conventional plant breeding entirely, it is time for us to turn more of our attention to CRISPR and genetically engineered trees. I wish that were not the case, but I'm afraid it is.

CRDF committees can evaluate germplasm in the plant breeding pipeline and turn those with sufficient resumes over to the Florida Department of Citrus for mass propagation.

CRDF can take on select non-HLB related topics, like diaprepes.

CRDF and CRAFT are developing a synergistic relationship. CRDF will fund basic research, novel ideas and projects that require a level of scientific involvement beyond what growers are able or willing to put in, and CRAFT will test these therapies and products on a large scale.

There you have it. Developing antimicrobials and the trees of the future, taking on select non-HLB topics like diaprepes, and continuing the synergy with CRAFT are research spaces I see for CRDF. It's an agenda that is limited compared to what CRDF has done in the past, but it is where I believe we can be the most impactful for growers.

CRDF Grower Dollars at work for you

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