# **GROWER RESEARCH REPORT** APRIL 2021

#### Taking the Road Less Travelled for a Brighter Future by Rick Dantzler, COO of CRDF



"The ox is in the ditch," a Citrus Research and Development Foundation (CRDF) board member told me recently. "Growers need help now!"

Indeed, they do. Thankfully, fruit prices have rebounded some, but with fruit drop taking its toll and fruit quality on the edge this has been a trying period. Just two weeks ago I received a heartfelt message from a young grower about how he was running out of time because of his losses from fruit drop. It affected me deeply and I shared it with the CRDF board.

We hear you. We know that business as usual is not going to cut it. We must be willing to take the road less travelled, and we are. One of the roads less travelled is considering testing peptides/compounds/ molecules/elements that have not yet been registered to see if they work, skipping the normal and slow scientific process of which we are familiar. This would require the destruction of the fruit at harvest and is an idea first raised by a board member. Where this goes, we do not yet know, but it is an example of how we are trying to speed things along.

### REQUEST FOR PROPOSALS LOOKS AT SHORT- AND LONG-TERM SOLUTIONS

**FIRST:** The 2021 CRDF request for proposals (RFP) is live and pre-proposals are being sought. It is the best RFP since I have been here and can be found on the CRDF website. It addresses short-term needs like solutions to fruit drop and greasy spot, and long-term solutions such as peptides. It also pushes the envelope by challenging entrepreneurs to develop tree injection systems that are cost effective and efficacious. There are some who believe I am kidding myself when I promote tree injection, but this is a technology that I know will work and become less expensive over time. Just think of how expensive pocket calculators used to be and now they are given away free as promotional items. The same thing will happen with tree injection devices. In fact, anecdotally I hear it might already be cost effective, but let's see what is presented per the RFP. If we can just figure out what to inject, the technology will progress to the point where we will find a way to get it in the tree affordably, and think of the benefits that could follow. From HLB therapies to nutrition, the sky is the limit.

Likewise, the RFP challenges researchers to develop an assay that measures HLB therapies quickly. If we can develop a way to quickly measure if an HLB therapy is working - like within a year think of the speed we will save, and speed right now is key.

Something else that is new with this RFP is if we don't get satisfactory proposals to answer the questions growers need answered, the board has authorized CRDF staff to pull together research teams to get the proposals we need or even draft the proposals and put the services out to bid. This is a paradigm shift for a funding entity like CRDF, but one which is warranted considering the urgency of things.

#### PLANT BREEDING REFORMS ADDRESSED WITH STANDARDIZED DESIGNS

**SECOND:** Reforms needed in plant breeding actively addressed. CRDF has created a document that will standardize trial design and data collection. From this point on, any plant improvement project CRDF funds will be required to follow this document, which will result in a disciplined, systematic, and successful march toward HLB-tolerant and resistant rootstocks and scions. The data generated will also assist growers in making planting decisions.

As part of the reform, there is a good chance CRDF will assume the role of testing all Stage 3 (last stage) testing of scions and rootstocks. It is not a decision we made lightly because CRDF is primarily a research funding entity, and it will require the cooperation of our partners, but we thought the need was so great that we had no choice. And frankly, a model where breeders breed and an independent third party does the testing is not a bad model because it eliminates potential conflicts of interest. This is, indeed, how some other commodities do it.

What form these reforms ultimately take is unclear as this goes to print, but all the debate in the CRDF Select Committee on Plant Improvement and the CRDF board about this might have the effect of bringing additional resources — including perhaps from CRDF — to plant breeding programs. This is good because the Florida citrus industry deserves and needs the best programs possible.

#### EMBARKING ON A NEW ROUND OF TRIALS

**THIRD:** CRDF has embarked on a new round of rootstock and scion trials. In 2014, CRDF began implementing three trials rootstock trials of rootstocks recommended by the breeders we fund. The trees have been in the ground for a little over six years and the trials have been smashing successes. They were laid out properly, cared for by quality growers, and generated data that growers have relied upon.

Because of the need for more such trials, CRDF is putting in not just rootstock trials of the newest creations, but scions, as well. The board has approved new CRDF Stage 3 (last stage) field trials of 24 scions and 16 rootstocks which are believed to offer enough hope to warrant testing.

We have even considered putting in several small interstocking or inarching trials but have been dissuaded by the plant breeders as of now. It is something we are interested in, however, because it is an intriguing concept. We will continue to analyze it.

#### PEPTIDES RIPE WITH POSSIBILITIES ≡

**FOURTH:** CRDF is pushing as hard as it can on peptide research, which we have identified as an area ripe with possibilities to assist growers. Several companies are approaching HLB management by using peptides, so CRDF has reached out to these and other companies that we are aware of that have peptides that might help and asked what we can do to speed their work along. Our first goal is to determine which peptides work. Next, of those that work, what modality (spray, tree injection, citrus tristeza virus transfer or transgenic) is required. Let's not rule anything out because of costs at this point. The important thing is to identify if it works and then how it works. Finally, we will identify what we can do to assist in the commercialization of peptides that require regulatory approval. Here is a primer on peptides that helps me keep this work on track:

Peptides are small groups of linked amino acids. They are like proteins in that they have defined amino acid structure and shape but are much smaller in size.

Peptides have many functions, from signaling between different cells and species to having direct action on pathogens. Perhaps the most famous peptide is insulin, which binds to peptide-binding proteins called "receptors" to tell your body to pull sugar from your blood into your cells. Peptides have been growing in use in pharmacology because of the wide array of effects they can have and their ability to perform specific actions. They also have an excellent safety profile because, upon ingestion, they break down into amino acids, one of the safest and well-known chemistries. Agriculture is now looking to peptides for plant health just as pharmacology companies looked at them for human health.

In agriculture, some peptides work by binding specific receptors on plant cells and cause specific plant responses, such as an immune response, which can result in tolerance to diseases. A benefit of these peptides is they can be applied through foliar spraying because they can attach to receptors near the leaf surface.

A second category is antimicrobial peptides (AMPs), which bind to pathogens (like liberibacter). In the right concentration, AMPs poke holes in pathogens to kill them. To be effective against HLB, AMPs must get into the phloem, the thin part of the plant's vascular system just beneath the bark where liberibacter lives. This is difficult because the size of most peptides is too large for them to penetrate from the surface of the leaf to the phloem. For that reason, technologies such as tree injection, laser etching, Citrus tristeza virus (CTV), and genetic transformation are being developed.

Another category is peptides that bind to insect receptors and act as insecticides. Here, the peptide is delivered into the gut of the psyllid where it attaches to bacteria, causing the death of the psyllid.

A homerun would be a peptide that could be applied by spraying, could stand up to the heat of a Florida summer, would induce HLB tolerance in the plant, would directly kill liberibacter and would not be toxic or allergenic to humans.

Most peptides would have to be routinely applied by spray or stem injection. However, genetic approaches such as a viral vector like CTV or genetic transformation could apply a peptide constantly and economically. With transformation, the gene containing the peptide would be inserted into the genetic code of new citrus trees, resulting in the peptide being there for the life of the tree. The negative of this approach is that it takes years to amplify the trees and to obtain regulatory approvals. A much quicker way would be to use the CTV vector to produce peptide in trees. This is done by inserting the peptide into the virus and producing infected trees. The problem is that the CTV will likely lose the inserted peptide within 7-10 years and no longer work. Nevertheless, using CTV would be an effective and economical interim approach.

Although transgenic trees would likely be considered a genetically modified organism (GMO), newly developed technologies such as one referred to a CRISPR CAS could possibly create such trees that would not be considered GMOs. One example would be production of the peptide from finger lime reported recently from the University of California Riverside. It may be that oranges and grapefruit contain the exact same peptide sequence as that found in finger lime, but within a large protein instead of being processed into the small peptide. If this is correct, it may be that CRISPR CAS could be used to alter similar proteins in oranges and grapefruit to produce a peptide that is identical to that from finger lime. Since this technology does not insert new genes into citrus, these trees are expected not to be regulated as GMOs. Unfortunately, this new technology is difficult and citrus labs are just learning how it works. However, UF researchers are working on these difficulties, and progress is being made.

CRDF is pursuing all these leads. I am personally keen on CTV vectoring as an interim fix, and if the industry agrees, there will come a time when support for it with EPA will be needed.

#### **ODDS & ENDS**

The Parson Brown orange has had a checkered past. Emanating from the Lake Weir area, it spread across the citrus landscape, growing in popularity because of its good flavor, excellent color and early-season maturity. It was seedy,

though, and had more than an average amount of peel oil. So, it fell into less favor than other varieties such as Hamlin. Still, it hung on, and more than a few growers continued to grow it.

When HLB hit, it was noticed that Parson Brown seemed to be faring better than other genotypes. In fact, one grower claims to continue to pick upwards of 600 boxes per acre from his Parson Brown grove. This caught the attention of University of Florida Institute of Food and Agricultural Sciences emeritus Extension agent Gary England. He aims to document Parson Brown's true status and determine which clones are best.

England is working with CRDF's Brandon Page and Manjul Dutt of UF/IFAS, who presented CRDF with a research proposal to determine why Parson Brown seems to stand up to HLB better than many cultivars. It is suspected that Parson Brown has higher levels of the PR1 and PR2 defense genes, which are the molecular markers of systemic acquired resistance, than Hamlin. The research project will determine if this is the case.

But what about the high peel oil and seed content qualities that caused Parson Brown to fall into disfavor with processors? With an orange crop estimate of not quite 52 million boxes in Florida, processors are in such need of fruit that factors such as high peel oil and seeds are of lesser concern than the need for fruit. Additionally, plant breeders have indicated an ability to breed the less desirable characteristics out of the fruit over time.

Some of the earlier and perhaps best Parson Brown clones are few in number, so a priority is to preserve the gene sequence for those that are considered the best. That work, perhaps with the assistance of a very supportive corporate partner, will be considered, as well.

Wouldn't it be something if part of the solution to HLB has been with us all along, and the good ol' Parson Brown is part of the answer? We will find out.

We are researching if gibberellic acid application can prevent multiple fall blooms of early maturing fruit, and if zinc application can prevent drop. We also drafted a survey, which Florida Citrus Mutual circulated, to gather grower data on what is

#### FRUIT DROP

helping and what is not. We will get the data at about the time this goes to print. We will collate the results and post on the citrusrdf.org website as soon as possible.

#### **BLACK SPOT**

With the threat of HLB, we sometimes forget there are other maladies that need attention. One of those is black spot.

At CRDF, we recently began thinking of going at it in a different way. Instead of eradicating the disease, what if we pursued

research that had the effect of assisting growers in getting out from underneath the quarantine? We have approached state regulatory partners who are receptive, and they have been in touch with their federal counterparts. Future meetings are planned and hope springs eternal.

Something interesting was recently observed having to do with the possibility that tree covers could help prevent or reduce sting nematode and Diaprepes root weevil severity. CRDF intends to find out.

## STING NEMATODE & DIAPREPES ROOT WEEVIL

#### A FINAL NOTE WITH A LOOK AT THE FUTURE

Several weeks ago, I spoke to a group of UF Blue Key students at the Adams Ranch in Ft. Pierce. Mike Adams, one of my fraternity brothers, was there, so it was good to see him again. Adam Putnam was there, too. When I was in the Florida House of Representatives, I took Adam to Tallahassee with me for a week during a legislative session when he was in the ninth grade, which is when I believe he really switched onto government, so I've always felt I had a hand in Adam's development for which I am proud.

I mention this not to talk about Mike or Adam, but the students. Few had agricultural roots, but all were keenly interested and supportive. I say this because I believe the future is in good hands. Usually it is bad kids who make the news, but there are far more good ones than bad, and the good ones are special. Their grasp of technology and willingness to work together assures me that everything will be fine. And no one is against farmers. Our industry has widespread support. That doesn't mean we won't have to modify practices at times, but no one wants us gone. Our job at CRDF is to fund the science that gives you an opportunity to grow a large crop of high-quality fruit. If we do, the political support will be there for you to thrive.

We will keep at it. There are reasons to be hopeful.

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