GROWER RESEARCH REPORT
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Pathway to a Sustainable Florida Citrus Industry

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This Grower Report is a condensed version of a CRDF document that Florida Citrus Mutual will present to the Legislature to aid in support of the legislative budget request for citrus research. Please feel free to let me know what you think.

It has been a long and difficult road, but many of the research building blocks necessary for a sustainable Florida citrus industry are in place. The projects CRDF and other entities have funded – and continue to fund – have brought us to this point. However, additional funding will be required to finish the task.

Research successes tend to be sequential in nature, meaning that one advancement sets up the next project until something useful is achieved, but we believe we are nearing the point where things will be in place for industry sustainability and growth. Assuming the pathway we envision is correct, the work remaining has been identified. It is not the kind of exploration that has marked much of the work over the last 12 years, work that was necessary since so little was known about the disease when we started. It is, instead, work that is focused on specific advancements, discoveries, and solutions.

It is important to understand that the specific package of work towards sustainability could potentially take 15 years to fully work. As discouraging as this timeframe might be, it is not as bad as it seems because this package also includes bridge projects that allow growers to span the 15 or so years with advancements that could potentially increase production by 50 percent. This is a bold claim, but there are data to support the prediction.

This two-pronged approach – bridge work that carries us to the point of eradicating HLB or making it functionally irrelevant – begins with treatments that will improve production and fruit quality, but the disease will still be prevalent. Ensuing breakthroughs will provide resistant trees that should make HLB irrelevant or perhaps even eradicated. The necessary technologies for these breakthroughs have been built and the remaining work is to carry them out and get the products into the hands of growers. CRDF will accelerate the work required to see that these projects get into the field as quickly as possible.

What will "curing" HLB look like? CRDF defines it as eradicating HLB or making it functionally irrelevant. Eradicating it would mean causing it to disappear with only occasional recurrences or outbreaks, much like smallpox or measles in humans. Making it functionally irrelevant would be something different, where it would still be with us but managed much as growers successfully manage other pathogens.

There are potential pitfalls that must be acknowledged. First, necessary regulatory approvals might not be forthcoming. We believe the risk of this is slight, but it exists. Second, the citrus industry as a whole must make a collective decision to accept what is proposed, something that has not yet occurred. Third, it is unclear if the genes that allow HLB to do its damage have been successfully identified. We believe there are reasons to think they have, but scientists performing this work are reticent to so declare without thorough testing. Fourth, plants have the genes they have because they need them, and if the genes that allow HLB to express itself are silenced there could be negative effects on the tree, including the possibility of tree failure. So, there could be obstacles, but these obstacles should be able to be overcome.

The approaches outlined herein revolve around the advancement of eight categories of products, production practices, technologies, or trees: peptides, citrus tristeza virus (CTV), tree injection, production practice improvements, rootstock and scion testing, transgenics, clustered regularly interspaced short palindromic repeats - referred to as "CRISPR," and other methods of plant breeding. The first categories are designed to work in combinations to reduce disease enough to allow the industry to survive until the more effective longer-term solutions are developed and amplified for widespread commercial use. These longer-term approaches will need continued funding for this period.

Antimicrobial peptides are being developed by many companies to provide near-term management of HLB. Just like they transformed pharmacology in human health, peptides have the potential to transform tree and plant health in agriculture.

Antimicrobial peptides are produced by all organisms as a first defense against invading pathogens, and many peptides strongly inhibit bacteria. We are investigating peptides that recently have been found to strongly inhibit CLas.

Peptides can be applied by spraying, injection, the CTV vector, or as genes permanently transferred into trees. Because of mode of action and cost, some peptides are more applicable to certain methods of application.

Also, in terms of production and timing, the applications can be overlapping. For example, before new nursery trees are produced, the peptides can be injected. CTV vector or transgenic trees from nursery production avoid the need for labor in continued applications by spraying or injection. However, the CTV vector normally produces the peptide for 7-10 years, so this could be followed by injection or sprays. Testing by spraying and injection on field trees is underway, too.

Another area of research of peptides is to find better peptides. If CLas were to become resistant to a peptide, we would need to have alternatives in hand.

CRDF has been working with three companies that have proprietary peptides: Elemental Enzymes, Invaio and Southern Gardens Citrus.

ELEMENTAL ENZYMES Elemental Enzymes (EE) is a midwestern company that contacted CRDF four years ago, saying they had a peptide they thought would work on HLB. We suggested they do field trials in Florida to get a sense for how the peptide would do in a commercial growing environment, which they did, and about a year and a half later brought us field data. We were impressed enough to connect them with other grower-cooperators.

Now, after more than 40 field trials in Florida, the results are impressive. Yield is up, on average, 20% for sweet oranges and grapefruit; drop is reduced by approximately 39%; and 82-87% of growers using the product have had a positive outcome. And the great thing about this product is that it appears to work equally well if sprayed or injected, although spraying must be done annually, whereas injecting must be done only every other year.

Regarding the EE product, an application for an EPA Section 3 approval is pending. Our goal should be to get the decision in time for the product to be applied during the spring flush of 2022. CRDF has been in touch with the acting director of the biological pesticide division within EPA, encouraging him to cause the EPA to make its decision quickly.

CRDF is funding research additional research to test the EE peptide product on pathogens below the ground, as well, hoping that it will help with controlling phytophthora and the root rot it causes. Testing of the product on canker, blackspot, and blight (Project 20-015) is underway, too.

Chronology: If EPA acts efficiently, Spring 2022.

INVAIO Invaio is a company that is developing the finger lime peptide – a peptide from the finger lime, which is a resistant relative to citrus - to attack the HLB bacterium in two different ways: to directly inhibit its multiplication and to induce the plant defense against the disease.

On the day the news broke that this peptide might effectively suppress CLas in infected citrus trees, I contacted Dr. Hailing Jin, the lead scientist, to find out what CRDF could do to accelerate her work. Within three weeks, she had made two presentations to CRDF staff and committees.

What we learned was that the peptide had not been applied to many trees - and only trees in the greenhouse - so we knew we had to assist in getting the peptide into the field to test it on HLB infected trees of fruit-bearing age. Since Invaio, a biotech company from Massachusetts, owned the license to utilize this peptide and was developing it as a product, CRDF allowed it to make a presentation to the Commercial Product Development Committee. As part of the presentation, CRDF was asked to become part of a funding consortium to investigate the peptide further. The amount CRDF was being asked to contribute was substantial (several million), so while CRDF chose not to become part of the consortium, we did agree to pay to have the peptide tested against the hairy root assay with a commitment to do more depending on the results.

I have maintained contact with the Invaio officials since then and am told they have a dozen field trials in Florida. Also, CRDF recently purchased $200,000 worth of peptide for Drs. Jin, Megan Dewdney, and Greg McCollum to perform trials; at least one of these trials is on Hamlin trees of fruit-bearing age. Dr. Dewdney has applied her supply to 25 Hamlin trees of fruit-bearing age. Research is examining the results of stem injection vs. spraying, too. This peptide could also be produced by the CTV vector or by integration into the citrus genome.

Chronology: Invaio claims that its product will be on the market in 2023.

SOUTHERN GARDENS CITRUS Southern Gardens Citrus, a major player in the Florida citrus industry, has numerous peptides they have been testing, and is also using the CTV vector to continuously apply spinach defensins to citrus trees to reduce losses due to HLB.

Southern Gardens Citrus has been field testing the CTV vector to insert spinach defensins into citrus trees for more than a dozen years and is approaching approvals by EPA and FDA for this biological pesticide. The CTV vector was developed with funding from citrus growers under the Citrus Box Tax, and screening for effective peptides which could be inserted (technically, the peptide gene or sequence is inserted) into the CTV vector was funded by CRDF, as well.

A value of CTV vectoring is that, once a tree obtains the vector expressing the defensin, the defensin is continuously produced in the tree with no further labor input. This product can be produced in nursery trees or grafted into trees already in the field. It is expected that spinach defensins and peptides inserted into a tree in this fashion would maintain efficacy for 7-10 years, so it should be pursued with all haste since it would buy sufficient time for the development of a greening resistant or tolerant tree through conventional breeding or genetic manipulation.

Peptides to which Southern Gardens Citrus owns the legal rights to are now in their second generation and are being aggressively tested. Several appear to be highly effective at killing Liberibacter and have been inserted into trees by CTV vectoring. CRDF will likely be assisting Southern Gardens in the approval process to get this approach to the field sooner.

Chronology: Completion of registration by EPA should occur in 2024

TREE INJECTION

CRDF has obtained a novel type of injection device that makes it economical to inject antimicrobials into the phloem and xylem of citrus trees. Since a major obstacle to finding ways to treat trees against HLB has been finding methods to get products into the phloem of the tree, where CLas resides, this device offers promise and should greatly increase the effectiveness of antimicrobials. CRDF initiated this research with assistance from UF/IFAS and testing by the Florida Department of Citrus.

This technology could make it economical to apply antibiotics such as oxytetracycline, and since the target of this apparatus is the location of CLas in infected trees, it is also ideal for delivery of peptides and other antimicrobials like those being produced by Bayer Crop Science. CRDF will work with several companies to develop products to be delivered by this approach.

Chronology: Immediate.

PRODUCTION PRACTICE IMPROVEMENTS

Emerging improvements in production practices are becoming more evident and offer good examples of how research builds upon itself, with one project leading to an achievement, which leads to a new project and a new achievement until a beneficial practice is confirmed. Here are four which CRDF has either had a hand in or is considering for funding:

PLANT GROWTH REGULATORS GIBBERELLIC ACID (GA): CRDF funded early work involving gibberellic acid (GA), including using GA to reduce and synchronize flowering. This was used by Dr. Tripti Vashisth to pursue new research funded by Citrus Initiative funds, resulting in initial results showing, on average 34% more yield, which translates to a 15-40% increase in profit for growers.

BRASSINOSTEROIDS (HBR): A novel group of naturally occurring plant growth regulators (PGRs), HBrs were discovered to significantly delay HLB disease development, protecting trees for several months after application, as compared to non-treated trees. HBrs reduced ACP colonization in new flushes and reduced incidence of other pests such as rust mites in fruit bearing trees.

INDIVIDUAL PROTECTIVE COVERS (IPC) In an ongoing CRDF-funded research (project 18-032C), IPCs were demonstrated to effectively protect newly planted citrus trees from HLB and promote better fruit quality and yield as the tree enters bearing age. Trees covered with IPCs remained free from HLB for 30 months and had negligible fruit drop, whereas non-covered trees dropped 60% of their fruit. Fruit quality was also dramatically improved by use of IPCs; Brix was 10.9 in IPC trees whereas in non-covered trees Brix was 7.5.

ZINC AND NUTRITIONALS HLB causes increased oxidated metabolism, which is a hallmark of plant damage. Encouraging work with zinc, used by itself and in combination with specialized nutritionals, is on the verge of providing significant help to growers.

CRDF will fast-track the advancement of these and other tools to increase yield, reduce drop, and improve fruit quality.

Chronology: Immediate

BAYER CRDF and public sector partners engaged Bayer Crop Science in 2017 to begin working on the development of a plant defense modulator (PDM) and an antimicrobial to combat HLB. The development of a PDM is quite far along. While the discovery of a suitable antimicrobial is not as mature, approximately 150,000 have been screened and several are in the field being tested. This project is so encouraging that it was awarded a federal NIFA grant, bringing nearly $15,000,000 to bear over three years and relieving CRDF and other funding partners of further financial obligation.

Chronology: Intermediate to long-term

FIELD TESTING NEW ROOTSTOCKS & SCIONS

CRDF has led a grower effort to revise the citrus breeding programs and has begun independently field-testing new rootstocks and scions. Emerging from these meetings will be: 1. More disciplined testing in Stages 1 and 2 of the plant breeding pipeline coupled with more rigor in terms of which new cultivars are advanced. 2. Greater scientific rigor in Stage 3 (final stage) trial design, data collection and data distribution. From this point forward, all programs will abide by these guidelines, or they won't be funded by CRDF. What's more, for the UF/IFAS program, IFAS has hired a citrus horticulturist with a breeding background to make sure that Stages 1 and 2 of the plant breeding pipeline will meet these enhanced breeding requirements.

These guidelines and requirements also established a neutral third party (CRDF) as the entity doing the data collection of cultivars that have the potential to be released to growers. This is a big undertaking for CRDF, but we have a track record of doing this well and can do it.

CRDF has also decided to put in three new Stage 3 field trials for the 12 most promising new rootstocks and 20 of the most promising fruit scions. All of these rootstocks and scions were conventionally bred.

These aren't the only field trials of rootstocks and scions. Much more research on the development of tolerant and resistant sweet oranges and grapefruit is ongoing.

Chronology: Breeders have indicated that they believe HLB-tolerant trees that perform well enough have already been developed and are simply waiting testing. If this is true, these trees could be available to growers within five years.

TRANSGENIC CITRUS

Transgenic citrus is being field tested for resistance or tolerance to HLB. A number of transgenic scions and rootstocks that showed tolerance or resistance to HLB are now in the field. Some of these trees are expressing a gene to increase plant defenses and others produce a peptide against CLas.

These transgenic trees, which would be considered GMOs, are expected to provide resistance or tolerance to HLB for their lifetime without any extra labor input. Genetically modified Duncan grapefruit have already been produced and the same technology is being applied to sweet oranges. As GMOs, there will likely be several years of regulatory process required for approval by USDA, EPA, and FDA. These transgenics could identify desirable traits against HLB that could be duplicated in a non-GMO manner.

Chronology: Continued field testing and the requirements for USDA, EPA, and FDA approvals could take 20 years.

THE MISSION OF THE CITRUS RESEARCH AND DEVELOPMENT FOUNDATION IS TO

"Advance disease and production research and product development activities to insure the survival and competitiveness of Florida's citrus growers through innovation."

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CRISPR

CRDF has funded groups that have developed new technologies that now allow the development of citrus varieties resistant or tolerant to HLB without being considered GMOs.

A new technology – clustered regularly interspaced short palindromic repeats - referred to as "CRISPR," allows the introduction of small deletions in the genes needed for CLas infection. The value of this process is that it does not insert foreign nucleotides into the tree, resulting in an HLB-resistant tree that will not be a GMO.

Several citrus research labs are making considerable progress. With CRDF funding, one lab has already made Valencia trees that are resistant to citrus canker. These trees are still small juvenile plants but are going through propagation for field trials.

Two breakthroughs can expedite this process. The first breakthrough is identifying genes that, when knocked out by CRISPR, result in HLB resistance. However, the major limitation is finding genes that are necessary for CLas infection, spread, and induction of disease. If a potential gene is identified, the CTV vector can be used to turn off that gene to determine whether it is worthwhile to go to the effort of using CRISPR to knock it out.

However, another lab is bypassing the ambiguity of what gene to shut off with CRISPR by duplicating the phenotype of a transgenic tree that has been shown to be tolerant to HLB. By understanding the relationship between the multiple genes involved in plant defense, they are knocking out a gene that blocks the induction of tolerance to HLB (USDA grant). CRDF is funding the field trial testing of these plants.

The second breakthrough is to use the CTV vector to express flowering genes to quickly break juvenility after which the virus can rapidly be removed from the mature budwood. This would result in breaking juvenility in months, not years, and greatly reduce the time required to get a new CRISPR plant to the field and in production.

It has taken time and effort to get all of these processes developed and working at the pace required for commercialization, but this has been accomplished. It is only a matter of time before the genes that allow HLB to express itself are either cut out through CRISPR or a new gene is inserted that takes out or neutralizes CLas.

Chronology: The technological obstacles have been removed and the pathway is straightforward, but there is still a lot to do and much depends on the rate of growth of citrus. Protoplasts must generate into small plantlets, then be grafted onto rootstocks, then induced to flower by CTV, then remove CTV, grow plants to size for greenhouse tests, and then field tests, followed by nursery production. The whole process is likely 15 years or more.

IN SUMMARY

The first new products to become available for growers to increase productivity in the presence of HLB will likely result from different companies producing peptide products to attack CLas in trees. CRDF is assisting where it can to accelerate these products to the market.

The development of new techniques for economically injecting trees gives an additional tool for maintaining the sustainability of the citrus industry, as does smarter use of hormones, plant growth regulators and individual protective covers.

Better rootstocks and scions will increase productivity. However, more economical and lasting approaches are ongoing with CRISPR and speed breeding, and CRDF will continue its funding support to bring durable solutions to the industry.