This ***Grower Report*** will appear just ahead of the 2024 Florida Citrus Industry Annual Conference, a time when many of us will be together. I'm submitting it at this time so you will have an up-to-date report on CRDF's activities over the last year to facilitate our discussions of plans going forward.

Overall Research Strategy: After numerous meetings with growers in which CRDF discussed research priorities, it was decided that our highest three priorities should be squeezing the most we can out of oxytetracycline (OTC) usage; finding molecules or compounds that could take the place of OTC if the bacterium becomes resistant to OTC or the trees become damaged from multiple injections; and funding the development of the "tree of the future," a tree that is sufficiently tolerant to greening or perhaps even resistant. Since these priorities were adopted, CRDF has funded 32 projects addressing them. Here are examples:

Summary of Research Strategy Implementation: With growers clamoring for immediate help, the only thing we saw that could work as effectively as it was needed was injecting oxytetracycline (OTC), so we went all in on that therapy. We assisted in getting a product approved by regulators in record time and set about to fund the testing of it in every way imaginable so we could squeeze the most benefit out of its use. A second product came onto the market soon thereafter.

We also recognized the need to have backup products available in case the bacterium developed resistance to the OTC, or the tree responded badly to annual injections of products with such low pHs. So, we either solely funded the testing of additional compounds/molecules or assisted in funding them. We also continued our discussion with regulators and interested parties about registering other products to be used during the third or "off year" when OTC can't be used as restricted by the registration approval. These discussions are ongoing.

Finally, we shifted our primary breeding focus from conventional plant breeding to breeding involving gene editing because it may just be that the pathogen is too darn strong to be conquered with conventional plant breeding. If we are lucky, the gene editing can be done with a CRISPR methodology that doesn't result in the tree being deemed a GMO, but we should stay open to a GMO solution because that may be what is required.

We call the three research priorities outlined above our "three-legged research stool." In addition to the 32 projects under contract to implement it, we have 29 preproposals in hand that mostly seek to answer practical questions growers have raised from the use of OTC, so we are giving it everything we have.

We didn't forget that we are here to respond to other urgent citrus research needs, and one of those is Diaprepes. We funded two projects, one to provide short term help to growers and the other to end the problem once and for all if it works. The methodology has worked in other commodities, and there is no reason to think it won't work with citrus.

We also funded a project involving **blight**, which is pointing more and more towards a CRISPR solution, and have a project on **blackspot** under consideration. The blackspot project is one we sought after I spoke to the FCM board and was asked why we didn't have anything under contract regarding blackspot, which proves the value of meeting frequently.

All in all, we have 68 research projects under contract, which is an extremely heavy load for CRDF, but we know this is no time to leave any gas in the tank. Consequently, our funding is running low. There are two reasons for this. The first is because we knew how dire things were in the industry, so we saw no reason not to fire nearly all our ammo. The second is because we simply don't receive the amount of funding we used to receive. With reduced production, box tax revenues are down, and with programs such as CRAFT helping growers administer new therapies and get new trees in the ground, programs we support, there hasn't been as much for research. We aren't complaining and are meeting the most urgent research needs, but it has affected the amount of funding we have available. Finally, we lost three veteran board members because of term limits: David Howard, John Updike, and Rob Atchley. Between them they had 24 years on the board and service on committees. They were replaced by Matt Machata, Sarah Spinosa, and Trevor Murphy.

Detailed Report on Strategy Implementation:

Priority One: Maximizing the efficacy of injecting OTC

23-001 Stelinski, Kirsten - Effects of trunk-injected oxytetracycline on tree infection and health, psyllid pathogenicity, and vector population.

> This evaluates the utility of trunk-injected OTC for reducing CLas infection and transmission in citrus trees. The study will quantify CLas reduction and tree health in response to OTC, determine the effect of OTC injection on psyllid populations, and determine the effect of OTC injection on CLas transmission. Field trials will be conducted on grower cooperator farms in both seedling and mature citrus plantings.

23-002 Albrecht, Ute – Evaluating different rates of OTC administered by OTC injection in mature sweet orange trees.

> This compares different rates, injection sites, and annual use patterns of trunk-injected OTC on Valencia to determine their effects on health, yield, and fruit quality over a period of 3 years in a commercial citrus production environment.

23-005 Albrecht, Ute - Bac. Trial 1: Use of CRDF Rootstock Trial Locations for Testing OTC Injected into Trees.

> This will determine the large-scale efficacy of trunk injection of OTC on tree health, fruit quality, and yield in three existing CRDF rootstock trials. Tests will be conducted to

determine the efficacy of OTC injection in the rootstock trunk compared to the scion and determine whether OTC injection increases/restores root densities, as well as if there are interactions with the rootstock.

23-009 Curtis, John - Bac. Trial 2: Use of OTC in Combination with GA and 2,4-D (Plant Growth Regulator) (Separate from ongoing CRDF PGR Trials)

23-010 Curtis, John - Bac. Trial 3: Impact of OTC injections on improving tree health and root density.

23-011 Curtis, John - Bac. Trial 5: Alternative Injection Sites for OTC

23-012 Yonce, Henry - Bac. Trial 6: OTC Combined with Vismax™

23-013 Yonce, Henry - Bac. Trial 8: Yield Comparison Between OTC and Non-treated Control Blocks on Yield and Tree Health.

23-014 Batuman, Ozgur - Determining the effect of oxytetracycline when rotated with additional crop antimicrobials on phytotoxicity and CLas reduction.

This utilizes a relatively new excised-leaf assay to determine the phytotoxicity of antimicrobials compounds to provide faster results than other analytical methods. The project will also determine the effect of OTC and additional crop antimicrobials on CLas reduction and ACP mortality. Antimicrobials will be tested in young greenhouse seedlings and mature trees in the field. Additional studies will be conducted to determine the efficacy of OTC when exposed to degradation conditions (i.e. UV light) on CLas reduction and ACP mortality using excised-leaf assay.

23-019 Yonce, Henry - Tank mix 3-day test of 2 products

23-004 CRAFT, Inc. 2.0 – CRDF funds to CRAFT to help start existing therapies program.

23-026 CRAFT, Inc. - Large Scale Field Trials and Existing Tree Therapies Cycle V

23-036 Albrecht, Ute - RFP 2: OTC in a pH neutral solution

23-048 Yonce – Testing of OTC in the “off year”: Treated vs. Untreated Trees

Priority Two: Finding Substitutes for OTC

23-018 Mandadi, Kranthi – Injection and evaluation of novel anti-CLas chemistries and OTC combinations for Florida citrus and HLB disease management.

23-025 Minter-Yonce - Evaluation of PT 150, PT 159, and TPR 1 for ACP and HLB control in Florida Citrus.

23-027 Wang, Yu - Exploring the efficacy of natural antibacterial agents for CLas control via trunk injection.

> This will screen various natural compounds for their antibacterial effects against CLas. As natural products are identified as potential CLas control agents, these compounds will be further studied for synergistic effects in combination with OTC to broaden or lengthen CLas control.

23-035 Yonce, Henry - RFP 1: Efficacy of injecting Phosphoric acid and Copper Sulfate.

23-038 Grower Cooperator A - Field trial of molecules identified by Dr. Brian Scully for their ability to mitigate the effects of HLB on citrus trees.

23-039 Grower Cooperator B - Field trials of molecules identified by Dr. Brian Scully for their ability to mitigate the effects of HLB on citrus trees.

23-043 Grower Cooperator C - Field trials of molecules identified by Dr. Brian Scully for their ability to mitigate the effects of HLB on citrus trees.

23-046 Grower Cooperator D - Field trials of molecules identified by Dr. Brian Scully for their ability to mitigate the effects of HLB on citrus trees.

23-047 Grower Cooperator E - Field trials of molecules identified by Dr. Brian Scully for their ability to mitigate the effects of HLB on citrus trees.

Priority Three: Finding the HLB Resistant or Tolerant Tree of the Future

23-003 Ritenour, Mark - Evaluation of Potential HLB Tolerant Grapefruit Rootstock/Scion Combinations.

23-006 Deng, Zhanao - Evaluating Novel Gene-edited Duncan Grapefruit Mutants for Resistance to Huanglongbing (HLB).

> This attempts to develop HLB resistant or tolerant citrus using CRISPR by knocking out two potential genes that might be involved in allowing pathogens to cause diseases in plants. Previously, these genes were knocked out of Duncan grapefruit using a combination of CRISPR and transformation and found each to provide resistance to citrus canker. In this project, they are testing whether these plants also might be resistant or tolerant to HLB. Since these plants are transgenic and GMOs, if they are resistant to HLB these researchers will knock out these genes in other commercial citrus varieties using only CRISPR to produce non-GMO HLB-resistant trees.

23-020 Grower-cooperator project for CRDF Trials of Next Generation Rootstocks.

23-030 Mou, Zhonglin - Evaluate new transgenic rootstocks for HLB tolerance.

Dr. Mou has shown that high levels of expression of the NPR1 protein from Arabidopsis in citrus results in a relatively high level of tolerance against both canker and HLB in greenhouse experiments. Hamlin and Duncan transgenic scions with high levels of NPR1 are already in field tests at Fort Pierce and are showing tolerance to HLB. They are under application for necessary regulatory approvals. This project expands the field test to determine whether rootstocks expressing high levels of NPR1 can improve tolerance to HLB in scions in different combinations. Transgenic or non-transgenic scions will be tested on transgenic rootstocks. The transgenic rootstocks are already produced and are ready to be used.

23-031 Johnson, Weston, TCCC - Accelerate Establishment of Stage 2 Citrus Trials to Combat Citrus Greening Disease.

This is a cooperative project between Coca Cola, CRDF, UF/IFAS and USDA/ARS. From UF/IFAS and USDA/ARS breeding programs, approximately 500 accessions from multiple field trial locations will be evaluated. From these 500, 50 of the top performers will be planted in at least 2 replicated field trials. The review and selection of the accessions to be budded will be completed by May 2024 and immediately budded onto 3 or 4 rootstocks intended for planting by late spring or early summer of 2025.

23-034 Messina, Charles - Taking aim at Citrus Greening: Activating the IFAS Crop Transformation Center (ICTC) to implement an idea to product framework.

This is aimed at increasing the number of genes tolerant to HLB and diversify the modes of action. Also, it will develop efficient experimental designs and predictive methods to enable the citrus industry to make the best choices of genes and manage the portfolio of genes to combat HLB. Finally, it will characterize barriers to adoption of HLB resistant citrus and devise strategies to overcome these barriers.

23-029 Chater, John - Consolidation of citrus breeding plant material to vacate space for Stage I and Stage II field trials and to exploit tolerant germplasm for gene editing strategies.

This project will evaluate all citrus plant material located on approximately 127 acres at various CREC field locations. All declining plant material with no direct use to the citrus industry will be destroyed to make space for future Stage I and Stage II trials. Plant material with desirable characteristics will be conserved, moved, consolidated, propagated and planted at a centralized location at CREC.

22-011 Chater, John – IFAS drone flights and data collection.

With remaining funds from his 2022 drone project, Dr. Chater will re-fly the areas affected by the latest hurricane to confirm results from the first drone flight.

23-037 Mattia – USDA-ARS drone re-fly and data collection.

> Dr. Mattia will re-fly the areas affected by the latest hurricane to confirm results from the first drone flight.

CRDF is not ignoring other high priority research needs of the industry or unique opportunities, to wit:

Diaprepes: Projects 23-040 Bonning and 23-041 Duncan - We recently funded two projects on Diaprepes, one by Drs. Bonning, Killiny, Dutt and Stelinski which will screen for bacterial pesticidal proteins, including those produced by Bacillus thuringiensis, and screen for gene silencing RNAs, and the other with Drs. Duncan and Diepenbrock, which focuses on short-term treatment of Diaprepes. If the Bonning project works, it will solve the problem of Diaprepes once and for all.

Blackspot: Under consideration is a unique project which will document the epidemiology of the disease and whether protections growers are paying for are necessary.

23-028 Duan, Yongping - Exploring the HLB control potential of a new citrus-infecting virus, NMV-M/CFL and its expression system.

> This quantifies if and how much a nectarine marafivirus reduces symptoms caused by HLB, as well as its ability to be reproduced. Duan is also characterizing the virus to provide information that will be required by regulators to allow this approach to be used in Florida.

23-032 Triplett, Eric - Proof of concept for phage therapy in the reduction CLas titer and HLB symptoms in citrus

23-021 Brlansky, Ron - Improved diagnostics and screening of the presence of a viral RNA protein to detect the presence of a Para retrovirus thought to be associated with blight.

Citrus Plant Breeding: In a keynote address, Dr. Leandro Pena of Spain made the comment that there is no such thing as “tolerance” to HLB, only different levels of sensitivity. Semantics? Perhaps, but it does help clarify that our goal should be true resistance, where the tree is totally unaffected by HLB. True resistance will likely require gene editing, and citrus breeders have already identified numerous genes which, when expressed or turned off, affect the ability of the HLB pathogen to hurt the tree. Because of these advances, I believe this should be our primary mode of attack. If we are lucky, genes can be edited through a CRISPR methodology that does not result in a tree being deemed a GMO, but even if the resistant tree is a GMO I believe

enough consumer acceptance will result, especially if the alternative is no orange juice or juice that is too expensive for the average consumer.

Conventional plant breeding should not be abandoned entirely, though. Conventional breeding creates greater genetic diversity, especially by crossing with hybrids and citrus relatives, made more possible by the movement to redefine the orange as something other than *Citrus sinensis*. Genetic diversity is nature's way of dealing with maladies. With enough time, conventional breeding would likely solve the problem of HLB, as it has for diseases in other commodities, but time isn't on our side, and conventional breeding takes decades.

Citrus breeders which CRDF has helped to fund have created tens of thousands of new cultivars. At the very least, we should thoroughly evaluate this germplasm to see if enough "tolerance" exists to take the pressure off bactericides and other therapies and buy growers enough time until a truly resistant tree is available.

OTC and Alternatives: Will injecting OTC save the industry until a resistant tree solves the problem? Are we correct in pinning so much of our hope on this one therapy? I have heard growers say in public forums that it is, hands down, the most effective and promising therapy of anything they have tried. I realize it didn't bail us out in one year, but it was never going to. It is a two-to-three-year proposition at best. Regardless, here's what we've seen: Root mass is being restored. Trials show substantially better fruit quality and stabilization of yield when compared to untreated trees. There is reduced *C*Las acquisition in the gut of psyllids, which hopefully will lower the rate of infection in trees. Positive results are being seen on all rootstocks and scions. Plus, growers are learning how to get more out of the OTC therapy every day.

Will growers have something to inject during the "off year" from OTC? I believe the answer will be yes. We have had discussions with regulators who are reviewing a list of molecules/compounds, some of which should have no regulatory implications whatsoever.

Finally, will regulators approve using OTC and streptomycin in combination, which kills 99% of the bacteria that causes HLB? It's too early to tell, but as an injectable (as opposed to a spray) it is placed into a closed system, so it does not hit non-target species. This virtually eliminates any environmental or worker concerns. Discussions with interested parties are starting, but where this will land, I do not know.

Coming Next: CRDF has received 29 preproposals in response to an RFP which primarily revolves around practical questions growers have regarding the use of OTC as an injectable. We are also going to be looking at projects on injecting micro-nutrients, which could be a way to finally get levels of these micro-nutrients in the tree as high as several researchers have been advocating.

Closing: I know how dire things are with the industry, and I know many growers have been disappointed in the first year's results with oxytetracycline. However, it was never going to be a one-year fix; it was always going to take 2-3 years to see the full effect of its benefit. Also, when compared to non-treated trees, we are seeing significant positive differences in all our trials. On top of that, I have not given up on the idea of getting the use of streptomycin approved for use with OTC because we know that combination kills 99 percent of the *CLas* bacteria in the tree. If we can get that approved, it will be off to the races.

I sometimes get calls from growers asking why they should stay in the citrus business. I do not believe it is my place to try to convince them one way or another, I simply tell them the reasons I see to be hopeful and describe promising research with commercial applicability that is in the pipeline. And there are reasons to be hopeful. I believe we will see a marked increase in the condition of mature trees after a second year of OTC, and gene-edited trees are in the ground that may be what we have been seeking. Also, now that we have shown that injecting trees can be done cost-effectively, a whole new world of novel therapies have opened, including injecting micro-nutrients, which might be a way to get levels as high as several researchers have been advocating.

We will discuss these and other topics at the Grower Conference. I hope to see you there.