CRDF Commercial Product Delivery Sub-Project Work Plan FY 2017-18

Quarter Ending September 30, 2017

1. *Candidatus* Liberibacter asiaticus PATHOGEN INTERVENTION

Project title: 1a. Bactericide Strategies

The goal of this project is to deliver bactericides and application strategies that are effective against Huanglongbing (HLB) to Florida citrus growers. This will be addressed by the development of new projects through CRDF and by providing support to projects outside of CRDF with similar goals. Near-term solutions will be prioritized for project development.

A. Candidate Bactericide and Application Technology Development

Subproject goals for this project area for the next year:

Obj. 1. - Develop and support new projects to identify and evaluate new bactericides.

Obj. 2. - Develop and support new projects to identify and evaluate new bactericide application strategies.

Obj. 3. - Provide communication of progress towards project goals and results to the CPDC, BOD and growers.

Narrative of Progress against Goals:

Obj. 1a. - Work with companies and researchers to develop projects with potential bactericides. In FY 2017-18 project managers will continue working with companies and researchers developing therapies for HLB. New projects will be developed as potential bactericides are identified.

On July 31, 2017 project number 16-012C concluded. This project was a research service agreement for the *in vitro* assay testing of bactericides against *Liberibacter crescens*. This project was not renewed because no new chemical libraries are available for screening. If chemical libraries do become available, a new project may be developed for the library screening.

Only three chemicals were tested *in vitro* this quarter from a single company. These chemicals may be considered biopesticides by EPA if they are developed into a product, however development is in very early stages. These materials will be tested in the greenhouse assay in the next quarter.

Chemicals tested in the greenhouse assay, project 16-019C, include agricultural chemicals that may improve uptake of bactericides. These tests are ongoing and will provide preliminary data that can be used for further investigation. Project 16-019C will terminate at the end of the next quarter.

Obj. 1b. - Support the Bayer bactericide discovery project.

The Bayer/CRDF partnership, project 16-026C, was announced this quarter at the Citrus Expo in Fort Myers. CRDF project managers met with the Bayer project manager for this project in August and continue to work with the Bayer project team to facilitate the implementation of the project.

Obj. 2. Work with companies and researchers to develop new application strategies.

CRDF project managers are working with researchers to develop research project priorities for investigating trunk injections as an application strategy. Several projects have been conducted by CRDF and UF researchers investigating application strategies. Results from the CRDF trial can be found in the progress report from quarter three of 2017.

Project title: 1a. Bactericide Strategies

B. Bactericide Field Testing

Subproject goals for this project area for the next year:

Obj. 1- Manage existing field trials including analyzing data, refining treatments and reporting progress to CPDC. Track field trials conducted by researchers.

Obj. 2- Develop new field trials to test promising bactericidal therapies.

Obj. 3- Manage the resistance monitoring project required by the EPA

Obj. 4- Provide communication of progress towards project goals and results to the CPDC, BOD and growers.

Narrative of Progress against Goals:

Obj. 1a. - Manage Biopesticide/Minimum-Risk field trial

Material applications and evaluations will continue for this trial through June 2018. The trees will be harvested in the next quarter. Two years of data from this trial will be available at project termination. The results from the first year of evaluations can be found in the progress report from quarter three of 2017.

Obj. 1b. - Manage the grower field trial to evaluate thermotherapy coupled with bactericide applications.

Evaluations continue for this trial and data will be evaluated after harvest during the second half of the fiscal year. A supporting project, 17-005C, is evaluating the combination of thermotherapy and oxytetracycline applications in small trees. This project evaluates movement of bactericides and the effect of treatments on photosynthesis. This project will terminate in the fourth quarter.

Obj. 1c. - Track researcher field trials evaluating new bactericides and application methods.

Data continues to be collected on the laser ablations trial, 928.3C when all data has been collected, results will be analyzed.

Project 15-037C, "T-sol antimicrobial for the management of citrus canker and HLB", is on track for completion in the next quarter. Uptake studies are in progress to test the uptake and translocation of T-sol in citrus.

Project number 16-022C, "Large-scale lab/greenhouse/field trial evaluations", will be concluded early next quarter. Results have been presented at grower meetings and a final report will be given at a CRDF board of directors meeting in the second half of the fiscal year.

Obj. 1d. - Manage Grower Bactericide Trial Data.

In response to the availability of oxytetracycline HCl (Fireline™), oxytetracycline Ca (Mycoshield®) and streptomycin sulfate (Firewall™) and the lack of available data on use, nearly 50 field trials were set-up by CRDF field staff throughout the citrus growing regions of Florida in FY 2015-16. The CRDF field staff continue to collect data from these trial. An additional 30+ trials were set-up with the help of CRDF and CRDF field staff assist in collecting data from these trials. Harvest data collection will begin during the next quarter for early varieties and will continue through the end of the fiscal year.

Obj. 2. - Develop, implement and monitor new field trials based on identified bactericides.

New projects ideas are being discussed with growers and the Commercial Product Delivery committee. Project ideas may be incorporated into priorities for a call for proposals in the second half of the fiscal year.

Obj. 3. - Monitor progress of the off-target resistance monitoring program required by the US EPA for the use of oxytetracycline HCl (Fireline™), oxytetracycline Ca (Mycoshield®) and streptomycin sulfate (Firewall™) under the section 18.

CRDF project managers are monitoring the progress of these trials and collect data necessary for periodic EPA reporting.

Significant Meetings or Conferences:

Project managers attended the Citrus Expo in Fort Myers this quarter.

CRDF Commercial Product Delivery Sub-Project Progress Report FY 2017-18

Quarter Ending Sept 30, 2017

1. *Candidatus Liberibacter asiaticus* PATHOGEN INTERVENTION

Project Title: 1B. Thermal Therapy to Reduce *CLas* Titer in Infected Trees

Narrative of Progress against Goals:

Obj. 1 - Track ongoing research on thermal therapy and its role in HLB and tree health.
There have been several CRDF funded research projects to determine the potential impact of thermal therapy treatment on CLas acquisition by ACP and to evaluate HLB infected citrus trees before and after thermal therapy treatments. Earliest thermotherapy projects (#586C Ehsani) used low-cost solar thermal treatment under translucent plastic tents to determine in-grove reduction of Las / HLB inoculum in infected orange trees. Such tents were able to raise tree canopy temperature but temperatures and required durations were variable. More consistent steam-generated covered treatments at 55°C gave the better results regardless of the duration. For the trees treated at this temperature, there was an increase in phloem area 30 DAT, fruit set, fruit size, and leaf areas. PCR analysis showed a decrease in the number of Las copies on the leaves after treatment. Subsequent psyllid and leaf miner infestations were a problem which likely impacted the results. There was no reliable technique, however, for detecting live vs. dead DNA from CLas in the plant as heat treated trees were never free of HLB. Future work will use supplemental steam heat to treat both the canopy and the roots of the tree.

Project #834 (Duan) sought to optimize heat treatment in the field and to understand the molecular mechanism underlying the success of thermotherapy for the control of citrus HLB. Effects of thermotherapy heat stress on Las, associated prophages and those genes involved in the phage lytic cycle were monitored in both HLB-affected and healthy periwinkle (heat-treated at 40°C) and citrus (at 42°C) to heat stress. Reverse-transcription PCR has confirmed the results of the RNA-seq analysis and has suggested a possible mode of action behind the successful elimination of Las seen in thermotherapy. There were 31 consistent up-regulated genes and 47 down-regulated genes in the citrus trees treated with exposure to 4 hours for 4 days of 40°C, 85 % relative humidity (similar to heat exposure in field setting) in a controlled greenhouse study. Four thermotherapy experiments resulted in data that clearly demonstrates heat exposure affects healthy and diseased citrus gene expression during the actual treatment and changes in expression are still present a month later when the new flush is produced. The greatest effect was present at 40°C, 41°C, and 42°C for 5 to 7 hours in 3 out of 4 test plots. Solar thermotherapy, using plastic tents to cover trees for several days to supply heat from the sun, also reduced Las titer and increased tree vigor for a short period of time but heat levels and results were variable.

Juice quality taste panels could distinguish between heat treated and no treatment juice sampled said that heat-treated HLB juice was sweeter, less acidic, less bitter, and had more flavor and body than the juice produced from unheated trees. Two genes were affected by heat stress and continued work will lead the way towards deciphering the molecular mechanisms for thermotherapy on roots as well as the foliage. Bark-paint therapeutic efficacy of selected chemical treatments combined with thermotherapy at 45 °C, was much greater than these chemical treatments coupled with thermotherapy at 40 °C or 42 °C.

The influence of thermal therapy on transmission of Candidatus Liberibacter asiaticus (Clas) was studied to determine how heat treatment methods may influence acquisition and transmission of Clas by the Asian citrus psyllid and its nymphs in # 941C (Pelz-Stelinski). Thermal therapy was applied to Las-infected trees in the experimental plot during the second week of July. Using a steam-generating machine, trees were heated to 55ºC for 30s. In approximately 4 weeks, leaf CLas titers were not significantly reduced in infected plants receiving thermal treatment as compared to untreated plants, although numerical reductions in the CLas titers of infected plants occurred during the first sample period following thermal treatment. Similarly, CLas acquisition from infected, thermal-treated trees was not significantly different as compared to acquisition from infected, untreated trees in the field study. These results suggest that treating the aboveground portion of infected citrus trees at 55ºC for 30 seconds is not adequate to reduce acquisition of CLas by psyllids. In a separate greenhouse study, whole tree application of thermal therapy to potted citrus tree resulted in no acquisition from thermal-treated trees compared with 75% of psyllids acquiring CLas from untreated, CLas-positive trees. Further studies are needed to confirm the efficacy of whole tree thermal therapy on psyllid acquisition.

Project #943C (CRDF) evaluated the impact of thermotherapy treatments at 8-14 Florida sites on various tree ages, rootstocks and scions. Thermotherapy trials used a variety of stem heat treatments from different machinery delivering a range of temperature/duration combinations in support of potential scale-up to commercial thermal therapy treatments. Citrus tree health, foliar nutrition, disease rating, HLB status, fruit drop, yield and fruit quality were evaluated before and after thermotherapy heat treatments over periods of 4 months to 2 years. Overall, results have been non-remarkable as thermotherapy treatments only achieved short-term improvements in tree appearance and titer reductions that lasted from 2 to several months. Reductions in fruit drop or increases in yield generally did not occur post-treatment. Some treatments had a negative impact on canopy size and yield. Based on disappointing results, several participants revised designs in response to early evaluations and deployed next-generation machines. Overall participation has slowed as most potential solvers have not participated. Most of the treated trees that displayed previous short-term responses to thermotherapy have now become not different from non-treated control trees. CRDF has thus discontinued monitoring tree status and data analysis as there was no evidence of any positive lasting effects of thermotherapy on HLB.

Obj. 2 - Determine the impact of thermal treatment on CLas acquisition by ACP.
Overall, Las acquisition by adult psyllids enclosed on trees receiving thermal treatments did not differ significantly from acquisition by adult psyllids on untreated trees. Based on these results, which indicated the thermal treatments applied during July 2015 did not reduce plant Las titer or psyllid acquisition, a second thermal treatment was applied during late November 2015. In early January 2016, adults and nymphs were enclosed in mesh sleeves on trees for acquisition feeding approximately 5 weeks following treatments. Insect and leaf samples were collected after 10 d of acquisition feeding or upon adult emergence to assess adult and nymph acquisition, respectively. In addition, we have initiated a complementary laboratory study to evaluate the effect of thermal therapy on the acquisition of Las under controlled conditions. Two-year-old Valencia trees were inoculated with Las by enclosing plants with Las-infected psyllids for two weeks. Untreated or thermal-treated, acquired significantly more Las than insects collected from uninfected trees for sampling period 3. No other significant differences were observed in nymph acquisition assays. Nymph acquisition from Las-positive trees did not differ significantly between trees that were untreated or treated with thermal therapy. Nymph acquisition was significantly correlated with Las titer in leaves. These data suggest that treating the above-ground portion of citrus trees at 55ºC for 30 seconds is not adequate to replicate the efficacy of previously observed in whole plant thermotherapy in potted trials.

In the field study, CLas acquisition from treated trees did not differ from untreated trees. Furthermore, psyllids acquired Las from a symptomless tree that tested positive for Las via qPCR. These "uninfected trees" later became qPCR-positive. Psyllid acquisition rates were positively correlated with leaf Las titers, underscoring the importance of maintaining psyllid control even when Las titers are low.

Obj. 3 - Evaluate HLB infected citrus trees before and after thermal therapy treatments to encourage scale-up of individual tree, over-the-row and root supplemental heat and evaluation of their performance in reducing disease and improving the health of treated trees.

Trees that have been evaluated were in varying stages of the decline due to HLB; most are heavily managed for psyllid control, nutrient applications, root health, etc. Evaluation of thermal therapy by the CRDF evaluation team has been completed. There are several enterprises operating field thermotherapy machines in Florida and at least two companies have been supported by USDA, APHIS, and MAC to deliver additional thermal therapy to Florida for field trials. From 11 to 14 field trials have been evaluated with varying intensity and over varying periods of time. Different machines have been used delivering a range of temperature/duration combinations. Current data sets associated with these trials have been summarized in previous quarterly reports over the past two years. Results to date provide a description of the variation of measures and tree responses and all of these trials can be considered completed. Data analyses comparing pre- and post-treatment tree status have been provided including yield, quality and other metrics. Overall, results have been non-remarkable as thermotherapy treatments only achieved short-term improvements in tree appearance and titer reductions that lasted from 2 to several months. Reductions in fruit drop or increases in yield did not occur post-treatment.

All of these trials were subjected to the protocol for evaluation as outlined per the approved work plan. The CRDF evaluation team also collected pre-treatment PCR bacterial measures, and other parameters. According to the protocol, periodic data collection following treatments assessed tree health responses as well as the specific impact on Las bacteria. Having 14 locations under evaluation allowed us to drop some sites as we fulfilled the work plan and budget.

Status at end of 24 months of the scale-up program.

It was hoped that project results could be used to encourage scale-up of individual tree, over-the-row and root supplemental heat and evaluation of their performance in reducing disease and improving the health of treated trees. While this project did not control the tempo of innovation or the timetables for the various solvers who expressed a willingness to commercialize thermal therapy for HLB-infected trees in Florida. Based on disappointing results, several participants revised designs in response to early evaluations and deployed next-generation machines. Overall participation has slowed as most potential solvers have not participated.

There were 14 sites reported on in the last June 2017 period and all of the treated trees that displayed previous short-term responses to thermotherapy have now become similar to non-treated control trees. All trials had post-treatment leaf samples that have been analyzed by PCR in 2017. CRDF has thus discontinued monitoring tree status and data analysis.

Obj. 4 - Outreach efforts to inform growers of optimized thermal treatments including CRDF sponsored field days have concluded. These previous efforts included thermal therapy researchers and active steaming commercial companies.

CRDF Commercial Product Delivery Project Progress Report FY 2017-18

Quarter Ending September 30, 2017

2. Asian Citrus Psyllid VECTOR INTERVENTION

Project Title: 2a. Asian Citrus Psyllid Management and Citrus Health Management Areas (CHMAs)

This report covers the first quarter of FY 2017-18, during which CRDF had 49 active projects in the portfolio of research and delivery projects. Nine of these projects address management of the vector, Asian citrus psyllid. Within the group of CRDF ACP projects, there are three that focus on RNAi and thus are covered in another section of the Quarterly Report. Please see section 2b for CRDF projects relating to RNAi field trials (#16-016C Eyrich), immune system priming to affect bacterial infection (#15-021 Pelz-Stelinski) and the NIFA nuPsyllid project managed by CRDF. Bryce Falk is well into his third year of a follow-on to the nuPsyllid project, making further progress in identifying insect viruses that might be manipulated to influence CLas survival within ACP. This 3-year NIFA project includes objectives to evaluate RNAi and virus disruption in greenhouse and field settings, and will continue into 2018.
A project that aims to disrupt the transmission of CLas by ACP (Killiny #15-017) remains active and has moved project objectives forward during this period. The report for this project indicates progress in expressing candidate genes using the CTV vector and evaluating the impacts of gene expression in various tissues. A method for more sensitive quantification of the target lactones (AHL) in treated plants is being developed and tested. The goal of this project is to manage CLas *in planta* through use of quorum sensing signal disruption.

The CRDF project led by Bryony Bonning (#711) searching for endotoxins from Bacillus thuringiensis (BT) bacteria with activity against ACP was completed during 2017 and a new NIFA SCRI Citrus Disease Research and Education project was approved to continue this work. As reported last quarter, Dr. Bonning has relocated to the UF, IFAS, Department of Entomology into an Endowed Chair faculty position, and has integrated into Florida-based work on HLB with new in-state colleagues. The NIFA, SCRI Citrus Disease Research Project led by Dr. Bonning continues the effort to develop “Bt toxin-based strategies for management of Diaphorina citri and citrus greening”. This 3-year project was awarded \$2.5 million to identify and evaluate Bt toxins, strategies to deliver candidates for ACP suppression, and to include economic considerations and outreach to affected industries. While this topic has graduated beyond funding by CRDF, it illustrates the value of the foundation of science that CRDF has supported since its inception, and which now is leveraging additional resources to continue promising leads. Another NIFA SCRI project addressing ACP intervention should be mentioned here as well, as this project complements CRDF projects in this important area. Michele Cilia Heck was successful during cycle 2 of the NIFA SCRI program in obtaining funding for further work on ACP disruption, and her project is entitled “Harnessing variation in transmission of CLas by ACP to develop novel Huanglongbing control strategies. This three-year project was approved at \$1.9 million and has reported promising early results.

Narrative of Progress by Project Goals:

1. <u>Pursue actions that will support expanded tools for ACP management</u>

Project #16-020C (Christopher Vincent) to evaluate the additive value of ACP-repelling dye in applications of kaolin clay treatments was initiated in early 2017 and continued into the fall. Dr. Vincent reports that repeated applications of dyed and undyed kaolin clay are rivaling conventional pesticide treatments during the summer months, with ACP populations very low in treated blocks. Following Hurricane Irma in September, ACP numbers dropped in the area, but the field trial continues with little disruption. Dr. Vincent also reports that a photo-stable dye has emerged from his work and will allow potential modifications to the schedule of treatments given the more stable formulated product. This project has reported interesting results within the first year of effort.

Project #16-011C (Robert Adair) requested of CRDF and was granted a six-month extension in July of 2017 to allow for the harvest of a second grapefruit season in the trial. With approval, the horticultural and pest management treatments were continued per fresh grapefruit guidelines, and irrigation and fertilization programs monitored and recorded. The three treatments in this trial (bare ground, organic mulch, and metalized reflective mulch (MRM)) received identical horticultural care and applications. Cost information on cultural practices were tracked.

Observations from the field trial indicate that, at 3.5 years of age, canopies of the trees in the MRM treatment are shading out a larger portion of the reflective surface and as a result, fewer differences in the numbers of adult of ACPs were observed between the treatments. However, fewer nymphs and eggs were observed in the MRM treatment based on weekly scouting of 20 trees per treatment. Hurricane Irma (9/9/17 -9/10/17) locally produced high winds approaching gusts up to 100 mph and dropped 12 inches of rain at the trial site. However, the trial block suffered only minor tree damage, and storm water up to and above the trunks receded after 72 hours with minimal root damage. Post-hurricane fruit counts indicated that considerably more fruit remained on MRM plots compared to organic mulch and bare ground, and percentage of fruit drop also was lower. Subsequent fruit harvest in the next quarter should further clarify the impact of treatment on crop, even with the hurricane disruption. The MRM material was not damaged in any way by the impacts of the hurricane. This project will end during Q3 of FY 2017-18.

Project #15-024 (Lukasz Stelinski) completed it timeline at the end of July, 2017, addressing the details of ACP movement and how movement can be incorporated into suppression strategies, including pesticide application programs. Earlier reports highlighted the association of barometric pressure and relative humidity on ACP activity, and thus are not reported again here. The final report from Dr. Stelinski highlights how windbreaks and the associated modified environment reduced ACP populations near grove edges, but did not negatively impact ACP natural enemies in groves with/without windbreaks. His report also provides further evidence that ACP populations were higher in solid planted new groves as compared to same-aged trees planted as resets into established groves. This was observed over four locations where the field trials were conducted. Findings of this work should be considered with other ACP management strategies.

2. <u>Engage registrants and regulatory entities in need for label modifications</u>

Registrants of Aldicarb continue to pursue the opportunity to bring this treatment back to Florida citrus. The registrant, AgLogic, has conducted consultations with state and federal officials regarding the pathways for potential re-registration of Aldicarb for citrus use.
Renewal of the special local needs label for Clothianidin neonicotinoid soil drench pesticide has been approved which will allow growers to use this tool on young trees in spring. The conditions for the use of this product remain the same as in 2017.

3. Continue participation in pesticide stewardship activities

Dr Lukasz Stelinski completed work on the resistance monitoring project current phase (#15-038C) during this reporting period, and the final report provides useful insight. The project has evolved a relatively easy, reliable assay that can be used to evaluate the relative susceptibility of field ACP populations to any contact insecticide in comparison to a susceptible laboratory colony. This system has allowed the team to monitor susceptibility of ACP field populations over time to a range of commonly used materials, and to report on any changes showing up during testing and across seasons. Dr. Stelinski reports that during 2016, data supported the continued trends in reversal towards susceptibility which had been the case in his monitoring since 2014. However, as reported at numerous grower meetings, the data for 2017 from the field showed increased occurrence of loss of susceptibility to several modes of action in various parts of the state. Notable was a reduction in susceptibility to neonicotinoids in Central Florida in their monitoring locations. Discussions continue on how to utilize this emerging information to adjust recommendations for ACP management.

In late April, 2017, CRDF approved the next phase of the insecticide monitoring research (#17-001C Stelinski) and the project started July 1, 2017. The objectives of this project build on the former work and support greater diversity and number of field sample sites. This work also proposes to look further into cases where field loss of sensitivity to pesticide materials is observed, bringing ACP populations into the laboratory and maintaining generations free of pesticide exposure. This will approximate the stability of the observed loss of sensitivity in subsequent generations of ACP not exposed to the materials. A final goal of the project is to further investigate the mechanisms of resistance in ACP populations.

Progress in this quarter suggests that ACP collected from eight field sites demonstrates varying levels of loss of sensitivity to several insecticides, including LD50 resistance ratios for three active ingredients of greater than 7. However, for dimethoate, chlorpyrifos, fenpropathrin, bifenthrin, carbaryl, flupyradifurone, and spinetoram there was no or a very low level of insecticide resistance in D. citri field populations sampled at this time. Dr. Stelinski reports that the neonicotinoid insecticide resistance ratio observed in field populations may not be stable, as laboratory follow-up on successive generations from field populations indicates a slight reduction in resistance ratio compared to laboratory susceptible populations.

4. Continue to support CHMA implementation of ACP and other HLB management tools

The current project supported by CRDF focused on providing recommendations and support to CHMAs in Florida (#15-035C Michael Rogers) ended September 30, 2017. Increased disease decline and lower returns have growers contemplating the value of ACP suppression strategies, and this has led to decreased participation in CHMA programs for area-wide coordinated treatments. While a final report on the CHMA support project is not available, Dr. Rogers has secured additional funds to implement ACP management recommendations under the current environment via efforts with Citrus Health Management Areas (CHMAs)

Important supporting research to better understand the relationship between pesticide uptake in treated citrus and ACP feeding (#15-036c) continues. This work can provide information that underpins grower ability to use materials and timing to best advantage in a challenging ACP pressure and cost-limited environment for pest management. No report is available at this time on the details of this project for the current quarter.

5. Communicate progress and results of project to CPDC, CRDF and growers

Significant Meetings and Conferences:

During this quarter, the Citrus Expo was held in Ft. Myers, and presentations on several aspects of ACP management were presented, including a summary of Dr. Stelinski's resistance monitoring project.

CRDF Commercial Product Delivery Sub-Project Progress Report FY 2017-18

Quarter Ending September 30, 2017

2. Asian Citrus Psyllid VECTOR INTERVENTION

Project Title: 2B. RNAi Molecules/Psyllid Shield

Narrative of Progress against Goals:

Obj. 1 - Complete first year of 3 year CRDF-funded RNAi field trial conducted by Southern Gardens and continue follow-on Phase 2 Psyllid Shield planning.

a. Begin data collection toward an assessment of the efficacy of selected target sequences in controlling ACP when delivered by CTVvv under field conditions.

   Trees were sampled to determine expression levels (high, low, none) of RNAi constructs and tagged. Trees were selected and transported from nursery on August 22nd, and planted on August 23rd by Southern Gardens Groves team members with continuous oversight by a research team member. Trees were initially enclosed in Tree Defender covers. Covers were removed prior to the hurricane, and scouted for aphids/treated with Admire as per permit requirements, and then re-covered Sept. 14. Testing expected in Jan/Feb time frame.

b. Continue follow-on Phase 2 Psyllid Shield planning, using insights gained from Phase 1 trial.

   Approximately 1/3 of the constructs tested to date are unstable, and initial testing will determine if the remainder have potential for further optimization in terms of both efficacy and regulatory considerations.

c. Continue to support efforts to work with the regulatory agencies to help establish the field testing conditions for trials with RNAi. This will enable the industry to help develop the testing protocols and permit conditions for testing in conjunction with the agencies instead of having the conditions established completely by the agencies or by others.

   Continued open dialog on this and other considerations is ongoing with appropriate officials**.**

Obj. 2 - Monitor and report on research activities, including CRDF-funded projects, related to RNAi and CTV delivery for insights and potential applicability for ACP control. Look for new candidate gene targets and alternate delivery methods.

The Psyllid Shield idea cuts across several areas of IP on both RNAi and CTV and potentially additional new PIPS (plant incorporated protectants), if combined with *CLas*-targeted constructs like AMPs (spinach defensins and the like). This is both a challenge and an opportunity.

Concurrent with the work outlined above, we expect that new RNAi activities and delivery methods will continue to be discovered. The challenge is to understand the regulatory pathway in order to initiate work with that eventual aim in mind, and to advance what might be "good enough" into the regulatory pathway while we continue to understand what might also be a worthwhile.

Future review of public information on other PIP RNAi molecules that have been deregulated may provide insights into future direction for regulatory considerations and planning for the next steps in this project.

Obj. 3 - Continue outreach to companies engaged in RNAi product development for potential collaborations.

During the first quarter of this fiscal year, there have been no outreach activities. A list of companies with some background information should be developed and brought forward for committee and board consideration.

Obj. 4 - Continue to explore potential candidates for long-term commercialization of RNAi solutions for ACP intervention.

Currently there is considerable consolidation occurring in the ag sector. The major ag companies (Dow/Dupont, Bayer/Monsanto, Chem China/Syngenta, and BASF) have or have had efforts in this area, while there are a number of smaller companies (notably Greenlight and Forrest) that are also working in this area. Companies with market caps of less than a billion dollars are likely to be more approachable, although their regulatory expertise may not be as deep.

Significant Meetings or Conferences:

Obstacles Encountered and Breakthroughs:

Other Information:

CRDF Commercial Product Delivery Sub-Project Progress Report FY 2017-18

Quarter Ending September 30, 2017

3. Citrus Host Intervention

Project Title: 3a. Deployment of Disease Resistant or Tolerant Citrus Rootstocks and Scions

Narrative of Progress against Goals:

<u>Obj. 1 - Track ongoing research projects evaluating emerging scion and rootstock genotypes for tolerance or resistance to HLB, citrus canker, and other diseases.</u>

a) Project 940c: Propagation of Rootstock Tree Production in Greenhouses by Seed, Stem Cuttings, and Tissue Culture to Accelerate Budded Tree Production for Out plantin
Dr. Beeson has published two research articles on citrus propagation timing and procedures and a third on tissue culture is in process.

1. Beeson Jr., R. C. and D. Silva. 2017. Development of a Procedure to Maximize Production of Hardy rootstocks of Citrus Using Stem Cuttings. Am. J. Plant Sci. 8: 2837-2846.

2. Beeson Jr., R.C. and D. Silva 2017. Propagation of Citrus Rootstock Cuttings Success Depends on Season. J. Horticulture. Vol. 4, No. 4. 1000213 p.5.

b) Grower Field Trials – Assessing HLB tolerance in volunteer grapefruit
Dr. Hatcher has been working with a grower to plan a scion field experiment to assess HLB tolerance in volunteer grapefruit identified by a grower. Scions and the scion/rootstock combination entry lists and field map have been finalized.
The field trial planned is a split-plot randomized complete block with four replications per scion/rootstock combination. The trees are being propagated under FDACS DPI permit for grower propagation of citrus escape trees and will be evaluated in the field trial under the same permit. There are 9 grapefruit mother scion trees and two rootstocks (Sour Orange and US 942) selected. The rootstock varieties were selected for the heavier soil type of the Flatwoods and grower preference. Buffer trees around the trial are also grower selections and will have grapefruit/various rootstocks for observations. Buffer selections will not impact the trial. Budwood was collected from nine grapefruit scion mother trees and three rootstock selections (Sour Orange, and US 942) have been budded. The trial requires a final 48 trees of each scion/rootstock combination. An additional 20% rootstock liners were budded to account for possible poor bud take as budwood was collected from field sources and harsh conditions of a screen house compared to controlled greenhouse conditions.
The trees are being propagated under FDACS DPI permit for grower propagation of citrus escape trees and will be evaluated in the field trial under the same permit.

c) Transgenic field trials

Obj. 2 - Cooperate in in-depth evaluation and planning exercises related to Florida (and the US) citrus breeding to better focus on HLB solutions and rapid evaluation and deployment of rootstocks and scions.

Dr. Hatcher will continue to visit field trials with researchers and have discussions on the most efficient method to identify citrus varieties which continue to perform well with HLB infection. During this quarter several sites with field trials at various stages and ages were visited with IFAS researchers.

Obj. 3 - Develop and implement plans for expanded management of tolerant and resistant citrus

Obj. 4 - Facilitate identification of best performing candidate rootstocks that appear to have HLB tolerance or resistance from Florida (and other) breeding programs

There are ongoing discussions between CRDF and IFAS to develop and execute a memorandum of understanding outlining each organization's expectations and responsibilities to facilitate data field trial data collection as well as resource allocation for other support such as central data repository and grower education field days.

Obj. 5 - Implement and evaluate Phase I and II grower field trials of most promising candidate HLB tolerant rootstocks using standard varieties as scions.

Phase I field trials: Rootstock Trial Project 927c

Hurricane Irma

Hurricane Irma passed through Florida in September with strong winds and excessive amounts of rainfall. The 927c rootstock trials are located on the Ridge and in South West Florida. CRDF staff evaluated all three trials as soon as roads were passable to assess damage to the trees. High rainfall amounts were a concern for the South West location at Duda due to the site characteristics (heavier soil type, poor drainage and high water table). Fortunately, the grower was able to pump excess water out of the grove within a short period of time and standing water did not have much impact on the trial. Standing water was not a great concern at the ridge locations. The wind had an effect on all three locations and there was some damage to the trees ranging from broken limbs, sandblasted leaves, some defoliation and fruit drop. Although the damage sustained to the field trials was minimal, we will continue to evaluate the sites as the trees recover from the hurricane event.

Field Trial Evaluation for Horticultural Traits.

Field evaluations of field trials are ongoing using standardized CRDF protocols for evaluation and data collection of HLB disease incidence and horticultural traits. During the third quarter of 2017 horticultural data tree height (cm), canopy volume (m^3) and trunk cross-sectional area (cm^2) were collected and analyzed for rootstock differences within each site. HLB disease index (DI) was rated on a maximum scale of 0 to 5 on two sides of the crown, with 0 denoting no visual symptoms and 5 severe tree decline on more than 80% of the canopy. The maximum possible score for DI in these trials is 10.

Data Analysis and Results

All sites are planted in a completely randomized design (CRD) with 5 replications per rootstock. Data were analyzed using a mixed model analysis procedure GLM using SAS® software (SAS Institute Inc, 2002 -2012) with the appropriate comparisons to test for differences among rootstock means when it is appropriate.
All the rootstock data collected is currently analyzed within each site and not compared across all sites. It will be important to compare rootstock performance across sites as the trials mature, especially when yield and fruit quality data become available. Current results suggest it is too early to make such a comparison, although one can be made retrospectively later.
Results for the two ridge sites (BHG and Peace River) are presented for all rootstocks for informational purposes. However, UFR-16 was planted late at both locations and cannot be fairly compared to the other rootstocks at this time. Although there two planting dates of UFR-3 at the ridge sites, inclusion or exclusion from data sets did not affect the results.

Results for previously unreported (new) data are presented by location.

CRDF DUDA Rootstock Trial, Felda, FL (Southwest)
The trial is planted in a completely randomized design (CRD) with five replications of each rootstock budded with '1-14-19 Valencia' for straight comparison of rootstock performance. All trees were planted on March 18, 19, 2015. The rootstocks were US-812, US-942, UFR-2, UFR-3, UFR-4, UFR-16 and Swingle (as a standard). Eight sentinel trees were randomly assigned to each plot at planting for data collection.

Horticultural Trait Data

There were significant differences ($p < 0.05$) among rootstocks for canopy volume (m^3), trunk cross-sectional area (TCSA) (cm^2) and tree height (cm) at this location (Table 2).

Table 2 CRDF Duda site rootstock trial horticultural traits and HLB Disease index (DI) means ± standard error of the mean data collected in September 2017

Rootstock	Canopy Volume (m^3)	TCSA (cm^2)	Tree Height (cm)	HLB DI[a]
Swingle	6.81 ± 0.32 AB	37.50 ± 1.45 B	204.71 ± 4.21 AB	2.55 ± 0.14 B
UFR_16	5.02 ± 0.31 C	32.89 ± 1.41 B	195.58 ± 4.11 B	3.20 ± 0.13 A
UFR_2	5.86 ± 0.31 BC	35.19 ± 1.43 B	194.77 ± 4.16 B	2.56 ± 0.13 B
UFR_3	3.42 ± 0.31 D	24.68 ± 1.41 C	167.95 ± 4.11 C	3.15 ± 0.13 A
UFR_4	6.96 ± 0.31 AB	38.65 ± 1.41 AB	216.33 ± 4.11 A	2.45 ± 0.13 B
US_812	6.71 ± 0.31 AB	38.40 ± 1.41 AB	208.03 ± 4.11 AB	2.43 ± 0.13 B
US_942	7.87 ± 0.31 A	44.15 ± 1.41 B	220.25 ± 4.11 A	2.33 ± 0.13 B

Values represent the mean ± standard error. Means were analyzed with a one-way ANOVA, and letter groupings were obtained using the Tukey-Kramer method. Values followed by the same letter do not differ significantly at the 5% level.

Rootstock groupings for each variable can be separated by the best performing rootstocks in order US_942, US_812, UFR_4, Swingle,UFR_16, UFR_2, and (UFR_3). There was no significant increase in visual disease symptoms and HLB DI ratings at this location in the third quarter of 2017 are similar to those recorded in the second quarter.

Peace River CRDF Rootstock Trial, Babson Park, FL (Ridge)
The trial is planted in a completely randomized design (CRD) with five replications of each rootstock budded with '1-14-19 Valencia' for straight comparison of rootstock performance.Valencia trees on seven of eight rootstocks (US-897, US-942, US-812, UFR-2, UFR-4, UFR-3 (short half of the trees), & Carrizo (as a

standard) were planted in April 2015. Planting of UFR-3 trees was completed in September 2015. Trees on UFR-16 were planted in August 2016. Eight sentinel trees were randomly assigned to each plot at planting for data collection.

Horticultural Trait Data

There were significant differences ($p < 0.05$) for horticultural traits reported in this period at the peace river location for canopy volume (m^3), trunk crossectional area (cm^2), and tree height (cm) (Table 3). UFR_16 means are presented for information and should not be directly compared to other rootstocks because it was planted 11 months later at this site.

Table 1. CRDF Peace River site rootstock trial horticultural traits and HLB Disease index (DI) means ± standard error of the mean data collected in September, 2017

Rootstock	Canopy Volume (m^3)	TCSA (cm^2)	Tree Height (cm)	HLB DI[a]
Carrizo	3.08 ± 0.17 B	23.14 ± 0.9 AB	163.18 ± 3.97 AB	2.18 ± 0.11
UFR_16	1.41 ± 0.17 C	8.98 ± 0.9 E	136.40 ± 3.97 D	2.28 ± 0.11
UFR_2	2.03 ± 0.17 C	14.99 ± 0.9 D	141.38 ± 3.97 CD	2.18 ± 0.11
UFR_3	1.39 ± 0.17 C	9.64 ± 0.9 E	124.93 ± 3.97 D	2.45 ± 0.11
UFR_4	3.03 ± 0.17 B	22.07 ± 0.9 BC	157.80 ± 3.97 BC	2.40 ± 0.11
US_812	3.35 ± 0.17 AB	23.48 ± 0.9 AB	170.95 ± 3.97 AB	2.18 ± 0.11
US_897	2.81 ± 0.17 B	18.73 ± 0.9 CD	158.00 ± 3.97 ABC	2.15 ± 0.11
US_942	3.88 ± 0.17 A	26.33 ± 0.9 A	174.95 ± 3.97 A	2.33 ± 0.11

Values represent the mean ± standard error. Means were analyzed with a one-way ANOVA, and letter groupings were obtained using the Tukey-Kramer method. Values followed by the same letter do not differ significantly at the 5% level.

Rootstock did not differ significantly for in HLB disease index at the Peace River site in this quarter. However, rootstock influence on the other horticultural traits is significant. US-942, Carrizo, US_812 and UFR_4 have larger trees compared to the other rootstocks.

BHG CRDF Rootstock Trial, Venus, FL (Ridge).

The trial is planted in a completely randomized design (CRD) with five replications of each rootstock budded with '1-14-19 Valencia' for straight comparison of rootstock performance. Eight sentinel trees were randomly assigned to each plot at planting for data collection. Valencia trees on 5 of 7 rootstocks were planted July 2015. Only trees on 5 rootstocks were initially planted: UFR-2, UFR-4, US-942, US-812 and Sour orange as a standard. Trees on UFR-3 were planted in September 2015 and trees on UFR-16 were planted in June 2016.

Horticultural Trait Data

There were significant differences ($P < 0.05$) in rootstock performance for canopy volume (m^3), TCSA (cm^2), tree height (cm) and HLB disease index (Table 4). UFR_16 was planted ten months later at this site so performance data is provided for information purposes and may not be used in direct comparison with other rootstocks at this time. Trees on US_942, US_812, Sour, and UFR_4 are larger and taller than those on UFR_2 and 3 which follows the trend at the other two locations. Of note, id that the HLB disease ratings at this location are much higher during this quarter than those reported in the second quarter. Part of the reason for this jump in values is that trees which previously had DI values of zero in the second quarter had symptoms in this quarter. Secondly, there was an effect of hurricane force winds on the trees and the additional stress likely exacerbated the disease symptoms. Staff will continue to monitor the trees.

Table 2. CRDF BHG site rootstock trial horticultural traits, HLB Disease index (DI) and PCR Cycle Threshold means ± standard error of the mean data collected in September 2017

Rootstock	Canopy Volume (m^3)	TCSA (cm^2)	Tree Height (cm)	HLB DI[a]
Sour	2.14 ± 0.1 BC	16.45 ± 0.6 AB	142.73 ± 2.53 B	2.43 ± 0.11 AB
UFR_16	0.70 ± 0.1 F	6.12 ± 0.6 D	113.70 ± 2.53 D	2.53 ± 0.11 A
UFR_2	1.44 ± 0.1 DE	10.32 ± 0.6 C	132.95 ± 2.53 BC	2.38 ± 0.11 ABC
UFR_3	1.03 ± 0.1 EF	8.31 ± 0.6 CD	123.53 ± 2.53 CD	2.38 ± 0.11 ABC
UFR_4	1.84 ± 0.1 CD	14.11 ± 0.6 B	142.26 ± 2.57 B	1.92 ± 0.11 C
US_812	2.47 ± 0.1 AB	14.92 ± 0.6 AB	153.70 ± 2.53 A	2.48 ± 0.11 AB
US_942	2.66 ± 0.1 A	17.20 ± 0.6 A	156.03 ± 2.53 A	2.05 ± 0.11 BC

Values represent the mean ± standard error. Means were analyzed with a one-way ANOVA, and letter groupings were obtained using the Tukey-Kramer method. Values followed by the same letter do not differ significantly at the 5% level.

Significant Meetings or Conferences:

Obstacles Encountered and Breakthroughs:

Other Information:

CRDF Commercial Product Delivery Sub-Project Progress Report FY 2017-18

Quarter Ending September 30, 2017

3. Citrus Host Intervention

Project Title: 3B. Horticultural Practices and Impact on HLB

Project goal(s) for this project area for the next year:

For FY 2017-18, measurable progress is defined as:

Narrative of Progress against Goals:

The Horticultural Practices projects focus on the potential impacts of five objectives on HLB status in existing trees. These include thermotherapy, plant growth regulators, soil microbial amendments, nutritional treatments and overall grower practices.

Obj. 1 - Thermotherapy.
Overall, field results have been non-remarkable as thermotherapy treatments only achieved short-term improvements in tree appearance and titer reductions that lasted from 2 to several months. Reductions in fruit drop or increases in yield did not occur post-treatment. In some cases, growth yield of thermotherapy treated trees was decreased below that of non-treated trees.
This project did not control the tempo of innovation or the timetables for the various solvers who expressed a willingness to commercialize thermal therapy for HLB-infected trees in Florida. Based on disappointing results, several participants revised designs in response to early evaluations and deployed next-generation machines. Overall participation has slowed as most potential solvers have not participated. There were 14 sites reported on in the last period and most of the treated trees that displayed previous short-term responses to thermotherapy have now become not different from non-treated control trees. All trials had post-treatment leaf samples that have been analyzed by PCR in 2017. CRDF has thus discontinued monitoring tree status and data analysis.

Obj. 2 - Plant growth regulators (PGRs).
Overall, PGRs did not demonstrate an ability to reduce preharvest fruit drop. A single application of 2, 4-D only rarely decreased preharvest fruit drop in HLB affected 'Valencia' orange trees (2015 Proc. Fla. State Hort. Soc. 128: 70-72). Strigolactone (SL) applications on HLB-affected trees consistently resulted in vegetative and reproductive flush following foliar treatment (RMC 899 Etxeberria). Fruit set and retention were both enhanced by SL treatments. No effect was noted when SL were applied to healthy HLB- trees. SL-enhanced vegetative growth was accompanied by substantial increases in vascular tissue as evidenced by the growth in thickness and health of phloem elements. In addition, SL can regulate root architecture by increased formation of primary roots, lateral roots, and elongation of root hairs. Presently SL are relatively expensive, yet costs have declined substantially (reduced in half) during the experimental period. Although effective in improving health to HLB-affected trees, the cost remains prohibited at the moment. Further reduction in costs may offer an opportunity to integrate SL with present grove care practices.

Obj. 3 - Soil microbial amendments.

Survivor trees in the field and greenhouse tests suggest that some soil microbes delay the HLB symptom development (Wang (#15-042). Ongoing research by Wang seeks to confirm whether the endophytic microbes from survivor trees can diminish effects of HLB, improve plant defenses and affect their attractiveness to psyllids. The hypothesis that commercially available soil-applied microbial products can mitigate the effects of HLB on citrus tree health and yield was tested in three separate field trials over 3 years. There were six soil amendment treatments: BioFlourish, EcoFriendly, Serenade, Quantum, and Aliette plus an untreated control (Untrt Cont) applied to Valencia/Swingle trees. There were no consistent treatment effects on Overall, there were no persistent treatment effects on HLB status, fruit yield. Root density in the soil or on leaf mineral nutrition as all nutrient values were within optimum ranges. Thus, there were no positive effects of these soil microbial amendments on tree health and yield of HLB-affected trees. The bacterial survival in soil and effects of treatments on the soil bacterial community were determined and results suggested that the application of these soil amendment products did not affect the citrus rhizosphere bacterial community because the additional bacteria products did not survive in the soil (FSHS 2017, in Press)

Obj. 4 - Nutritional treatments.

The complex interaction of nutritional mixes with citrus tree health is complicated by variable growing conditions and exposure to other biotic and abiotic stresses. Ongoing research (Schumann #15-023 seeks to understand the variability in response to enhanced nutritional programs and to define optimal nutritional therapies to maintain root growth and target leaf levels of nutrients. It is likely that in the presence of HLB, the range of nutritional requirements for optimal yield is narrowed and/or shifted by HLB infection and the general susceptibility to stress, possibly including the stress of psyllid feeding. Interactions between psyllid management and nutritional treatments on growth and productivity in field plots revealed that aggressive psyllid management should continue even after all trees are HLB positive. Supplemental foliar nutrition was shown to increase flowering and yields especially in Flatwoods citrus trees with compromised root systems because of elevated water tables from poor water management. Foliar applications of nutrients could reduce the effects of HLB in trees with declining root systems. This allowed the development of the therapeutic concept of "spoon-feeding" nutrients in small frequent doses regardless of application mode- fertigation, of controlled release or foliar. There are differences in absorption efficiency of both macro- and micro-nutrients by roots and leaves so the mode of application (foliar vs. soil) continues to be controversial depending on soil pH or leaf permeability.

Obj. 5 - Grower practices (forensics).

An interview committee of CRDF managers, IFAS personnel and growers was formed to determine successful management practices for living with HLB. There was a free exchange of information between the committee and four growers statewide. In addition, the current NAS national committee conducted similar interviews of 6 additional growers via a webinar focused on best management practices for dealing with HLB. These efforts accumulated a good representation of what successful FL citrus growers are doing in an HLB environment. Clearly, there were common practices among these growers that included block-specific management of irrigation, nutrition and pest control. No silver bullets or magical practices were discovered. All growers felt that they were better, more intensive managers now than they were before HLB. Discussion focused on irrigation, nutrition and ACP management to avoid stress in HLB affected trees. A common denominator was a completes balanced nutrient program of frequent split applications—"spoon feeding" of irrigation and nutrient application by frequent applications of fertigation, controlled release fertilizer, foliar or even granular nutrients applied to the soil. All had a balanced program of psyllid control using alternating mode of action pecticides applied with ground sprayers or aerially and some systemics. Parasitic wasp applications were used as much as possible. There was a question as to how effective these programs were with rising psyllid populations. Neonic soil drenches were used on young trees and resets. Some used Ridomil™ annually. All had tried bactericides especially on young trees with variable inconsistent results. Some uncertainty about effectiveness. Frequent light hedging and topping was discussed to maintain root to shoot balance.

Significant Meetings or Conferences:

Project manager attended the Citrus Expo in Ft. Myers FL (August 2017) where various presentations on citrus varieties and horticultural practices were presented by IFAS researchers to growers.

Obstacles Encountered and Breakthroughs:

Other Information:

CRDF Commercial Product Delivery Sub-Project Work Plan FY 2017-18

Quarter Ending September 30, 2017

4. OTHER PATHOGENS

Project title: 4a. Other Pathogens

Project goals for this project area for the next year:

Obj 1. - Track progress of the CRDF funded post-bloom fruit drop research project.

Obj 2. - Track progress of the CRDF funded black spot research project.

Obj 3. -Develop new projects or project objectives, as needed to study non-HLB diseases.

Obj 4. -Provide communication of progress towards project goals and results to the CPDC, BOD and growers.

Narrative of Progress against Goals:

Obj. 1. - Track progress of the CRDF funded post-bloom fruit drop research project.

A two-year project, 16-010C, was funded 3/1/2016 entitled “Enhancement of post-bloom fruit drop control measures”. Project managers are tracking progress off this project and the project is reported to be on track towards completing the objectives within the project timeline.

Obj. 2. - Track progress of the CRDF funded citrus black spot research project.

A three-year project, 15-005, was funded 7/1/2015 entitled “Asexual inoculum production of *Guignardia citricarpa*, the causal agent of citrus black spot”. Project managers are tracking progress off this project and the project is reported to be on track towards completing the objectives within the project timeline.

Obj. 3. - Develop new projects or project elements, as needed to study non-HLB diseases.

No new projects have been discussed for development of research projects in this quarter.

Significant Meetings or Conferences:

CRDF project managers attended a workshop at the SWFREC in Immokalee this quarter to discuss the current progress of citrus black spot research and the needs of the industry.