CRDF Commercial Product Delivery Sub-Project Progress Report FY 2015-16

Quarter Ending September 30, 2015

1. *Candidatus Liberibacter asiaticus* PATHOGEN INTERVENTION

Project title: 1a. Bactericide Strategies

The goal of this project is to identify bactericides effective against Huanglongbing (HLB). Project managers will identify bactericides from various sources from products in the market to materials in early stages of development that are effective against HLB, and assist with formulation for effective delivery, provide regulatory guidance by engaging regulatory consultants and EPA and assist with commercialization if necessary. This is an ongoing project that will build on the development of an assay pipeline for screening bactericides and the *in vitro* screening of more than eight hundred compounds including material libraries from agriculture, biotech and pharmaceutical companies. Bactericides that have been identified by project managers, as potential short to long-term solutions will continue to be tested in assays and in field trials and steps will be taken to encourage commercialization of these materials to provide a solution to growers for HLB.

Subproject Title: 1a. Bactericide Strategies: Candidate Bactericide Testing

Narrative of Progress against Goals:

Obj. 1- Form relationships with companies with candidate bactericides for testing in the CRDF assay pipeline. Assemble data on potential bactericides to assist in prioritization.

The CRDF "Solutions inbox" (http://citrusrdf.org/grower-participation#more-7) is a method used for people to communicate ideas for HLB solutions to CRDF. Several emails were received during this quarter, these solutions were examined and responded to appropriately. No new materials were entered into the assay pipeline, but in most cases the products were nutrition or insecticide products, which are generally not appropriate for the bactericide assays.

No new companies have been identified with potential bactericides, but several meetings are scheduled for the next quarter. A meeting is scheduled with Company O, which is developing a bactericide for HLB that will be registered as a biopesticide. CRDF has been screening the components of this product *in vitro* with positive results and a meeting to discuss the next steps is scheduled in the next quarter to discuss further assay testing and field trials to be sponsored by this company.

CRDF-funded research projects by university researchers on bactericides are all on track. Of the five research service agreements for the evaluation of bactericides, one has ended this quarter and will not be renewed because a new bactericide assay has been developed that provides a greater level of information on activity within the plant. Another assay that evaluates soil-based antimicrobials will be concluded in the next quarter and no effective bactericides have been identified from this assay. The remaining three assay RSA projects are on track.

The other CRDF funded projects in the Commercial Product Delivery research area include a zinc-based bactericide development, greenhouse screen and field evaluation, a novel delivery method project, two new bactericide field trials and four ongoing bactericide field trials. These projects will continue to be monitored to ensure that efficacy data continue to be collected and product development or registration assistance occurs as necessary.

Four other projects are within the bactericide strategy category, one was concluded this quarter and the other three are on track.

A white paper is in development and will be completed in the next quarter describing bactericide use in the past and present with an emphasis on materials used traditionally for citrus greening and huanglongbing. This document will be used to address questions raised by growers and the public resulting from the recent compiling of a document requesting an emergency exemption from FIFRA for use of oxytetracycline and streptomycin on citrus for HLB.

Obj. 2- Move bactericide candidates through assay pipeline to identify promising materials for field trials.

Materials that have been tested in this quarter were from companies with the ability to formulate, manufacture and register a product. This includes all sizes of agricultural product companies. One hundred and thirty materials were tested and all but one are from companies capable of producing a product for agriculture. One material is presently available for citrus and three other materials are insecticides or fungicides labeled for citrus. The majority of the materials tested are microbial supernatants that may be developed into biopesticide products.

Five materials each from two companies are being moved forward to be tested in the greenhouse assay. These materials may be developed into biopesticide products and are from both a small and large agricultural company.

The greenhouse assay has been in development since early 2015. The project was approved in February 2015 and plants were delivered in March. The trees were inoculated with “hot” psyllids and a large percentage of the plants were determined to be systemically infected in mid-September. Preliminary tests began to validate the positive controls for each application method (root infusion, soil drench, foliar) and will continue during the next quarter. Testing of candidates will begin during the next quarter. This assay will continue to be developed and scale-up is being discussed. Materials that are to be tested in the greenhouse assay should result in candidates for field trials.

CRDF Commercial Product Delivery Sub-Project Progress Report FY 2015-16

Quarter Ending September 30, 2015

1. *Candidatus Liberibacter asiaticus* PATHOGEN INTERVENTION

Project title: 1a. Bactericide Strategies

Subproject Title: 1b. Bactericide Strategies: Bactericide Delivery

Narrative of Progress against Goals:

Obj. 1- Coordinate with researchers, companies and other institutions to define formulations and delivery methods for field trials with minimal regulatory requirements.

Several adjuvants have been examined for use with materials being tested in the greenhouse assay. Research will continue to identify the best adjuvants for the various bactericide chemistries.

Obj. 2- Track RMC and CPDC research projects relevant to the formulation and delivery of bactericides against HLB; integrate findings into project planning.

Towards the goal of developing novel delivery methods, a new CPDC project examining the ability of a laser treatment to facilitate bactericide movement into the citrus phloem was initiated this quarter.

A meeting is scheduled for the next quarter for the CRDF field staff, crop consultant and project managers to be trained on trunk injection methods. This training is in preparation for the trunk injection field trial that will be initiated in early 2016.

CRDF Commercial Product Delivery Sub-Project Progress Report FY 2015-16

Quarter Ending September 30, 2015

1. *Candidatus Liberibacter asiaticus* PATHOGEN INTERVENTION

Project title: 1a. Bactericide Strategies

Subproject Title: 1c. Bactericide Strategies: Bactericide Field Testing

Narrative of Progress against Goals:

Obj. 1- Managing existing field trials including analyzing data, refining treatments and reporting progress to CPDC.

The biopesticide/minimum risk field trial was concluded because pesticides were applied incorrectly. The field trial was modified so all of the data collection except yield-related data is the responsibility of the CRDF field technicians. The board will approve a bid for the new trial in October 2015. After approval, site selection will occur and PCR sampling will be completed to allow for block set-up. The first treatments will be applied in early February 2016.

The three AgroSource projects are on track, a quarterly report was received and the quarterly meeting was held. The Nufarm project is also on track; a report was submitted for review. Both of these company's products are part of a Section 18 (FIFRA) exemption application that has been submitted to the Florida Department of Agriculture & Consumer Services (FDACS). This application is for the use of oxytetracycline hydrochloride, oxytetracycline calcium and streptomycin sulfate. FDACS is in the process of reviewing the application and if they believe that a strong case has been made for an emergency exemption they will make a recommendation for sending the application to EPA to the Florida Agriculture Commissioner.

Data from the Company C field trial continues to be collected. This trial was initiated in August 2014. Project managers will begin analyzing year one data during the next quarter.

Project managers continue to meet with growers to discuss ongoing field trials not sponsored by CRDF. No new materials have been identified for future testing during this quarter.

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

A field trial to examine alternative application strategies was developed and approved by the Board this quarter. This field trial will examine the effectiveness and economics of trunk injections. The materials to be evaluated will be oxytetracycline hydrochloride, oxytetracycline calcium, streptomycin sulfate and a zinc-based bactericide. A trial site of Hamlin trees has been identified and applications will begin in early February.

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

This report describes the progress towards the goals laid out in the bactericide project workplans. No other document has been provided this quarter, describing progress towards project goals.

Significant Meetings or Conferences:

A presentation on CRDF bactericidal projects was given by Stephanie Slinski at the Citrus Expo in Fort Meyers on August 19th titled “Current progress of CRDF CPDC field trials for managing HLB and canker”.

CRDF Commercial Product Delivery Sub-Project Progress Report FY 2015-16

Quarter Ending Sept 30, 2015

1. *Candidatus Liberibacter asiaticus* PATHOGEN INTERVENTION

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

Narrative of Progress by Project Goals:

1. Track ongoing research on thermal therapy and its role in HLB and tree health

2. Determine impact of thermal treatment on CLas acquisition by ACP. Ongoing CRDF-funded research at UF and USDA does not identify how thermal treatment affects availability of CLas to be acquired by ACP feeding on treated trees. Discussions occurred on the need for this to be included in the MAC funding proposal addressing thermal therapy scale-up and research. Overlay of CLas acquisition testing on current field trials was suggested as a simple way to accomplish this goal. A project plan was developed by Kirsten Pelz-Stelinski of UF, IFAS, CREC and has been approved by CRDF, and subsequently approved for funding through the USDA MAC HLB program. The one-year research project is underway and has the following update.

The objective of this project is to evaluate the effect of thermal therapy treatment on *Candidatus* Liberibacter asiaticus (Las) transmission by the Asian citrus psyllid (ACP). Since the initiation of this project, a citrus grove, located at the Citrus Research and Education Center, has been identified for conducting bioassays. Trees in this grove are 4-year-old Hamlin oranges. Of the 203 trees tested using quantitative PCR (qPCR), 50 trees have been identified with cycle threshold (Ct) values below 36, indicating the presence of Las.
In May 2015, bioassays were completed to quantify the rate of Candidatus Liberibacter asiaticus (Las) acquisition from infected citrus trees by Asian psyllids (ACP). These acquisition rates will serve as a baseline for acquisition prior to treating these trees with thermal therapy to reduce Las infection. Thirty newly-emerged adult ACP were enclosed on individual branches of Las-infected and uninfected (control) citrus trees using mesh sleeve cages. After one week, ACP were collected from the trees and placed in 80% ethanol. DNA from individual ACP was subsequently extracted and stored at -80oC. The rate of Las acquisition by psyllids will be quantified from these samples using nested quantitative polymerase chain reaction analysis (qPCR).

After initial acquisition bioassays were conducted, thermal therapy treatments were postponed until late June because equipment was under repair, and therefore unavailable. Treatment was postponed an additional week, because trees in the experimental plot were mistakenly treated with imidacloprid. Trees were immediately irrigated for a 24 h period to reduce uptake of imidacloprid. It is critical to this study that imidacloprid is not present in trees one month (4 weeks) after thermal treatments because this is the initial time point for determining the effect of thermal therapy on Las acquisition by ACP. One week after the imidacloprid treatment, data from tap sampling indicated that the ACP population in the experimental plot was not reduced. This suggests that irrigation prevented the uptake of imidacloprid into trees. It also indicates that there will not be a negative impact of imidacloprid on ACP survival during acquisition assays one month after thermal treatments are applied.

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 four weeks, the rate of Las acquisition by immature and adult ACP will be compared among infected trees receiving thermal therapy, untreated infected, and uninfected, untreated trees.

Following steam treatment of Las-infected trees in July, test trees were monitored for defoliation and re-emergence of flush. After new flush was evident (approximately 5 weeks after treatment), adult and immature psyllids were bagged on treated trees the CLas acquisition access periods, insect and leaf samples were collected. Samples were stored at -20oC for subsequent nucleic acid preparation. Analysis of these samples via quantitative real-time polymerase chain reaction (qPCR) is ongoing. Once qPCR assays are complete, data analysis will conducted to determine changes in plant CLas titer pre- and post-treatment, and to evaluate acquisition efficiency following steam applications. Analysis should be completed in November. The next acquisition assay is also scheduled for November.

Obstacles: As indicated above, the actual application of thermal therapy to test trees was delayed due to equipment issues, but the treatments have been applied and post-treatment evaluations are underway.

2. Refine requirements and environmental conditions for most effective thermal treatment. Field research by UF and IFAS is obtaining further detailed information on the performance requirements needed in thermal therapy to reduce CLas titer. Tenting and steam application experiments have incorporated data recording devices to document thermal conditions for correlation with tree response. Dr. Ehsani of UF reported that application of steam heat of 58 C (136 F) for 30 sec resulted minimum plant injury, reduced PCR titer and recovery of tree health for at least 18 months. Such guidelines are currently being used by 3 commercial steam companies and several growers who are establishing large scale and small thermal therapy trials. This will have to be followed and perhaps modified seasonally as tree conditions change throughout the year. 3. Encourage scale-up of individual tree, over-the row and root supplemental heat and evaluation of their performance in reducing disease and improving health of treated trees.
The USDA, APHIS MAC group was charged to manage the federal funding to put HLB solutions in the hands of growers. This group quickly identified thermal therapy as a “shovel-ready” project area and encouraged development of project ideas and mechanisms to attract and encourage solvers to come forward with plans for scale-up, and to propose how this funding could facilitate rapid scale-up.

USDA, APHIS responded with consideration of a mechanism that has been used by their agency previously in seeking solutions to challenges, and plans were established to solicit solvers for thermal therapy scale-up. Two Mac projects were approved to facilitate scale-up and both were in place at the end of this quarter. Evaluation of thermal therapy conducted by those involved in scale-up is being initiated by the CRDF evaluation team. Six enterprises are either field testing machines in Florida or will have machines ready for testing or will have them field-ready within the next couple of months. Those with capability are operating at multiple locations in Florida, and the evaluation team is in the field conducting the evaluations.
CRDF CPDC moved forward with plans to coordinate evaluation efforts of thermal therapy. Building on the methods used to evaluate effects of other treatments (antimicrobials, soil amendments, etc.) on CLas and/or HLB and tree response, a before and after protocol was developed to document tree and environmental the conditions surrounding thermal treatments and a data plan for follow-up so that individual trials will be evaluated similarly and treatments can be compared. This protocol has publicized on the CRDF web page so growers can do some self-assessments of their own thermal therapy

trials and been implemented on a small scale with grower and research trials. The protocol will become standard in the MAC funded CRDF project to evaluate thermal therapy scale-up described above. An overview of current field activity that the CRDF evaluation team is engaged in follows:

3. Encourage scale-up of individual tree, over-the row and root supplemental heat and evaluation of their performance in reducing disease and improving health of treated trees.

There have been 9 new thermotherapy (TT) field trials established in which the CRDF staff is participating in tree evaluations. Trails are located in private groves in St. Lucie and Hardee County (bedded) and Polk and Lake County (Ridge), and we have been invited by the grove owner's to evaluate the effects of the thermotherapy. Trees include Valencia, Rio Red Grapefruit, and Ray Ruby Grapefruit on a variety of rootstocks (sour, Swingle, Carrizo). Most trees being evaluated are in varying stages of the decline due to HLB; most are heavily managed for psyllid control, nutrient applications, root health, etc. One grove in Lake county, however, is under organic production practices and there is one grove using conventional production practices but is under managed for comparison of TT results.

Site 1 – Cliff Whitaker grove - Lake County. This trial was done in conjunction with Dr. Ehsani. There are 20 trees being evaluated in 4 classification: steam, thyme oil (bactericide), steam and thyme oil combined, and control. Each group has 5 trees. Measurements being taken are Disease index (DI), trunk circumference, height, canopy diameter, PCR, and fruit drop counts.

Site 2 – Larry Davis - Hardee County. Mr. Davis was contacted on 4/3/15 and set up the trial on 4/6/15. There are 24 trees being evaluated. All of the evaluation trees are steam treated, as the thermal applicator didn't want to leave any trees untreated. Measurements being taken are DI, trunk circumference, height, canopy diameter, PCR, and fruit drop counts.

Sites 3 -5 – Evaluations of thermal therapy applied by Premier Energy (Central Florida Ridge area)

Site 3 - Raley grove in Dundee – this trial was set up 5/21/15 and treatment was 5/28/15. There are 40 Valencia on Carrizo trees being evaluated, 10 control and 30 treated. Measurements being taken are DI, trunk circumference, height, canopy diameter, PCR, % leaf drop, and fruit drop counts.

Site 4 - Wheeler grove in Lake Wales – This trial has 15 control trees and 15 treated trees. The trail was set up on 6/9/15 the treatment was done on 6/12/15. Measurements being taken are DI, trunk circumference, height, canopy diameter, PCR, % leaf drop, and fruit drop counts.

Site 5 - Dunson grove in Haines City - this trial was set up on 6/8/15. Treatment has yet to be done. This grove is under a less intense management strategy. Measurements being taken are DI, trunk circumference, height, canopy diameter, PCR, % leaf drop, and fruit drop counts.

Sites 6-8 – Daniel Scott thermal therapy applicator – St. Lucie County (Indian River area)

Site 6 - Trial A - 36 trees (Red grapefruit on Sour) – this trial was a comparison of 3 different temps applied at different durations. The grower was trying to determine what combination of time and temperature worked best. Measurements being taken are DI, height, and PCR.

- The temp and times tested are : (time is total time, beginning to end)
- 125 degrees F for 35 sec, 40 sec, 45 sec, 50 sec, 60 sec
- 130 degrees F for 25 sec, 30 sec, 35 sec, 40 sec, 50 sec, 60 sec
- 135 degrees F for 60 sec

Site 7 - Trial B – 54 (Red grapefruit on Sour) – this trial was a comparison of 3 different temps applied at different durations. The grower was trying to determine what combination of time and temperature worked best. Measurements being taken are DI, height, and PCR.

- The temp and times tested are: (time begins when the temp is first met)
- 128 degrees F for 20 sec, 35 sec
- 130 degrees F for 10 sec, 25 sec
- 132 degrees F for 0 sec, 10 sec

Site 8 - Trail C – 35 trees (ray ruby on sour) 20 treated trees and 15 control trees. When I set up the trail on 6/30/15 all the trees were to be treated at 130 degrees F for 15 sec. The grove owner decided to treat some trees at 15 second and some at 30 seconds. I will adjust my trial data to reflect which trees were treated for 15 sec and 30 sec during my next visit. We will take post treatment PMA samples on 7/23/15. Measurements being taken are DI, trunk circumference, height, canopy diameter, PCR, % leaf drop, and fruit drop counts.

Site 9 – Uncle Matt's Eddy Block in Winter Garden. This Experiment is being conducted with thermal treatment by Dr. Reza Ehsani of If, IFAS, and is the only trial that is being conducted in an organic grove. Most of the trees are in poor condition. If thermotherapy works here, it will provide hope for the whole industry. There are 10 control trees and 20 treated trees. The study trees are split into 4 groups: Large treated trees, Small treated trees, Large control trees, and Small control trees. Measurements being taken are DI, trunk circumference, height, canopy diameter, PCR, % leaf drop, and fruit drop counts.

Summary: All of these trials will be subjected to the protocol for evaluation as outlined per the approved work plan. The CRDF evaluation team is working with commercial scale-up thermal treatment applicators, helping to lay out field trials, collecting pre-treatment PCR bacterial measures, and other parameters. According to the protocol, periodic data collection following treatments will assess the tree health response as well as the specific impact on CLas bacteria. In cases where there are no untreated controls, the test will likely be reduced to the "short version evaluation". Having 9 locations under evaluation is ahead of the plans, and we anticipate being able to conduct many additional evaluations beyond those estimated in the work plan and budget.

There are 6 different thermotherapy machines being used in the trials—all are making visible improvements in tree appearance. The first steam machine was constructed by a private grower, Larry Davis, from Wauchula and operates in a similar fashion to Dr. Ehsani's steam machine using 120 degrees F for 20 seconds based on successful visible results of treatments in his grove. The second hot water/steam machine is built on a 'goat' frame by Premier Energy. The water used is purified prior to being heated 122 – 128 F for 20 seconds. There are 3 levels of 100 psi nozzles inside an octagon-shaped over-the-tree canopy. Most of the time there is foliage dropped at the top of the tree following treatment. So far no fruit drop directly due the treatment has been observed. The 3rd machine is owned by the Scott Family on the east coast who specialize in grapefruit. Their machine is similar the goat-based, hot water/steam Premier Energy machine but the treatment water is not pre-purified prior to heating. The round over-tree canopy has a single row of four 1000 psi nozzles that are 14" from the ground that spray at a 45 degree angle that create a "vortex" inside the canopy. The current TT treatment is 130 F for 15 seconds. Although this machine does not cause leaf drop, visible temporary leaf bleaching is common. Nonetheless, this method of thermotherapy may be visually the most promising but quantitative tree evaluations are yet to be analyzed.

The ongoing quantitative tree evaluations compare treated and non-treated trees as well as before and after TT comparisons on the same trees. Comparisons include pretreatment photographs and PMA-PCR to separate short-term living vs dead CLas DNA. Follow up leaf qPCR samples will be re-evaluated in the winter months (December). Current and winter-time tree height, canopy diameters,

trunk circumference, fruit drop counts, % leaf drop, % leaf bleach and visible Disease Index will be measured. Summer time (July-August) leaf nutrient analysis will be evaluated along with fruit yield and juice quality testing where appropriate. Data analyses will focus on current and winter time tree comparisons to be followed annually to evaluate recovery and/or relapse.

4. Continue outreach efforts to inform growers of the availability of thermal treatment and to refine conditions that will lead to effective thermal treatment. The CRDF-initiated Thermal Therapy field days is being planned for 3 Dec 2015, along with the Premier Energy Team and Dr. Ehsani's group, to inform growers of this emerging tool, and also attract entrepreneurs and innovators along with at least 3 commercial companies, who are interested in helping to deliver thermal treatment to growers on a broader scale. This is an important step in moving from proof to use, and CRDF is strongly encouraging the partnerships that can develop economic analysis to encourage use. In addition, with recent federal funding being made available to bring HLB solutions to the field, CRDF and the industry are pursuing the opportunity that exists to partner with the USDA Multiagency Coordinating group to direct some of the federal funding to encourage scale-up of thermal therapy.
More information and the presentation contents can be viewed by going to thermal therapy page at citrusrdf.org, which highlights the presentations made during the field days, photo galleries from the field visits, and additional information related to thermal therapy and its use in treating HLB-infected trees.

Significant Meetings of Conferences:
CRDF Project reviews at Citrus Expo, August 19, 2015, Ft Myers, FL.
CRDF sponsored Thermal Therapy Field Day scheduled for 3 Dec 2015 at the Lake Wales site.

Obstacles Encountered and Breakthroughs:
The project is going well and the available thermal treatment companies that have equipment in the field are participating in the field trial evaluations. The limitation at present is that some of the MAC-funded scale-up projects have not yet initiated actual field testing in Florida, limiting our ability to initiate evaluation of those groups. This will be remedied as the other companies begin their field testing. As of the end of October, 2015, machinery from Ag Harvesters for the scale-up project supported by MAC remains under development, and to date, has not been available for evaluation by the CRDF/MAC evaluation team. It is not clear when first field trials will be available for this solver.

CRDF Commercial Product Delivery Sub-Project Progress Report FY 2015-16

Quarter Ending September 30, 2015

2. Asian Citrus Psyllid VECTOR INTERVENTION

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

Narrative of Progress against Goals:

Obj. 1- Pursue actions that will support expanded tools for ACP management

During the third quarter, CPD project 15-036 was initiated by Dr. Michael Rogers. This new effort grew from the former project looking at pesticide residues at citrus bloom, and which provided useful information to registrants whose products may be applied around the period of citrus bloom, coincident with spring flushes. This is a critical time for protecting emergent flush, particularly on young trees.

The new project focus is on better understanding the distribution of pesticide residues within plant tissues, particularly leaves, and how ACP feeding correlates with the active residues in the tissues. This project has not been in place long enough to provide a first-quarter report.

CRDF Research projects which support this goal also are focusing on the interaction of ACP with citrus plants, especially as it relates to better use of ACP treatments. Fine-tuning the dynamics of spring flush and flowering is being pursued to enhance the flowering model that currently predicts when bloom will occur. The modifications to this grower tool will assist in identifying the period of open flowers, an aid to decisions on treatment for ACP during this vulnerable time (Albrigo 850). As pesticide use specifications vary according to use during open bloom, this improvement will provide useful information to growers. During this quarter, the data resulting from the tagging studies during spring, 2015 bloom across a number of citrus varieties are being incorporated into the existing model database, with the goal of updating the ability to predict the bloom more precisely. Plans for the next quarter for Albrigo 850 will be to complete this analysis and prepare for the 2016 spring flush and bloom.

An additional element that determines ACP management success is the movement (dispersal) of psyllids. While there is a sense that ACP dispersal is affected by weather conditions, the project by Stelinski (15-024) was approved to gain more understanding of this situation. The project, just underway is testing ACP adults placed on citrus trees and held under a range of temperature and humidity conditions. Preliminary runs suggest that dispersal is highest at high ambient temperatures, but appears to be less affected by humidity. The work also is set up to establish the lower end of the activity range with regard to temperature.

Project 766 ended during the third quarter of 2015, completing a two-year timeline to evaluate the role of windbreaks on the distribution and numbers of ACP. The other objective of this research was to compare populations of ACP in young trees planted in solid plantings versus scattered resets within a mature grove environment. Dr Stelinski reported in his final progress report that numbers of ACP was lower along edges of groves surrounded by windbreaks compared to grove edges with no windbreak. This supports general observations that windbreaks could affect ACP distribution. Interestingly, the natural enemies associated with ACP did not seem to be affected by the presence/absence of windbreaks.

Another finding of the Stelinski 766 project is that ACP numbers were higher in solid planted young trees compared to same-aged trees in scattered reset configuration. This finding results from 4 field sites evaluated over two years.

Finally, this project confirmed under central Florida conditions that ACP does not use alternate plant species as an off-season refuge when citrus trees have minimal flush.

Finally, a research project being conducted in field sites in Southwest Florida and in the Indian River (Stansly 477) is nearing the end of its four-year life. Results of the three-year series of evaluations in a grove at the UF, IFAS SWFREC indicate that differences occur with regard to ACP populations when comparing white plastic ground cover with metalized reflective mulch (ground cover) in a young planting. Similarly, the growth parameters of the plants treated with the ground covers, and within sub-plots where organic mulch were applied varied by treatment. This project also proposed to look at the interaction with foliar nutrients, but no new information was presented in recent progress reports. A second site at Immokalee is being evaluated, as is a site in Vero Beach that was planted in 2014. This project is slated to continue into February, 2016.

Obj. 2- Engage registrants and regulatory entities in need for label modifications

Conversations continue with registrants towards label status on several fronts. The continued availability of neonicotinoid insecticides under emergency expanded use labels remains vital to protecting young trees. Registrants have indicated willingness to continue supporting these uses, and the annual renewals of SLN product labels will be pursued in the next quarter. During the third quarter, 2015, rumors that the US EPA would take action on the label for Dow's sulfoxaflor (Closer™), a material that is used for ACP suppression. At the close of the quarter, no definitive announcement has been made with regard to this product.

At this time, CRDF does not have any ongoing activities related to additional pesticide label modifications.

Obj. 3- Continue participation in pesticide stewardship activities

The primary stewardship activity is the ongoing effort to monitor status of pesticide resistance under field conditions in Florida citrus groves. During this quarter, project 15-038 (Stelinski) was initiated, continuing the 6 years of prior research and monitoring for ACP resistance development in Florida. The aggressive use of several classes of pesticides warrants close monitoring. As this project was established, a new team member with toxicology background was recruited to assist in development of a new method for monitoring field-level resistance levels in wild ACP. The goal is a more effective and economical assay system.

Preliminary evaluations during this quarter on project 15-038 indicated that like the previous cycles of monitoring, there is no evidence of significant increase in resistance to materials tested. This may be a benefit of rotation of modes of action in recommended ACP programs, and the role of CHMA coordinated sprays with similar active ingredients at key times in the ACP seasonal phenology. The goal for this aspect of the monitoring project is to expand the number of sites being monitored.

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

CHMAs continue to demonstrate the value of coordination in managing ACP. During this quarter, project 15-035 (Rogers) was initiated to continue the IFAS role in providing support to the 50 CHMAs in Florida and to provide data analysis and reporting on CHMA progress. This project supports Mr. Brandon Page as the IFAS liaison to the FDACS CHRP program and to growers engaged in the CHMA activities. A significant CHMA activity during this quarter is the organization of formal CHMAs in the Indian River region of Florida. While this region has followed the principles of coordinated treatments for ACP, the formalization will bring them into league with the other CHMAs, and allow more effective data management. A strength of the CHMA program is Florida is the concurrent commitment through the CHRP (Citrus Health Response Program) to provide per-cycle ACP counts within a large number of citrus blocks across the state. This support allows for the real-time analysis of hot spots where ACP numbers are rising or are well above surrounding areas. The depiction of this information allows growers within the CHMA to monitor not only their groves, but to see local trends, and to respond when groves in their area have elevated ACP counts.

An interesting opportunity occurred during the past 6 months to utilize ACP population information within CHMAs as the FDACS inoculum removal demonstration was deployed. ACP population suppression was not practiced as unmanaged groves were removed, resulting in movement of adult psyllids from these blocks into adjacent blocks. Data from the CHRP counts are being used to analyze the changes in ACP immediately following the tree removal. As phase II of the inoculum removal demonstration occurs, strong recommendations have been made to treat the unmanaged grove just prior to tree removal to avoid mass movement of psyllids. This conforms to IFAS recommendations to pre-treat against psyllids in groves where trees are being removed.

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

Field Day, A. Duda and Sons, June, 2015. Project 447 Stansly to share results of mulch/nutrition experiments.

Florida Citrus EXPO, August, 20, 2015, Fort Myers. Updates to growers on CHMAs and how they are impacting ACP, and progress in organizing the Indian River region into CHMAs.

Significant Meetings or Conferences:

During July-August, 2015, Harold Browning travelled to Parana and Sao Paulo, Brazil to discuss research and management activities of common interest to Florida and Brazil. During this visit, discussions with research project leaders and field site visits were incorporated into the agenda.

Among ACP management tools of interest is the field evaluation of border rows of orange jasmine, *Murraya* sp. to attract ACP adults moving into/out of the citrus block. These areas of *Murraya* border have been installed adjacent to existing (young) groves, but also as new trees are being planted. Results shared during the visit indicated that the impact of an attractive border was greatest when the plants were installed simultaneously to the planting of the citrus trees. Monitoring of ACP populations and infection levels of the nearby citrus trees will provide further evidence for the impact of border planting of this plant.

Obstacles Encountered and Breakthroughs:

All three CPD projects that address this topic were initiated during this quarter, limiting the results reporting. All project are up and running, and are on time according to the approved plans.

CRDF Commercial Product Delivery Sub-Project Progress Report FY 2015-16

Quarter Ending September 30, 2015

2. Asian Citrus Psyllid VECTOR INTERVENTION

Project Title: 2b. RNAi Molecules/Psyllid Shield

Narrative of Progress against Goals:

Obj. 1- Continue to refine the mathematical model with vector entomologists and epidemiologists.

During the quarter, modelling refinements focused on two areas:

a. Understanding the relationship between transmission of CLas and eventual development of HLB symptoms in trees.

After obtaining survey data from Southern Gardens, a model was developed for the appearance of symptoms. This model is based on the assumption that inoculum accumulates at a rate proportional to the number of infected nymphs present in the citrus trees, and that trees will begin to decline after a certain threshold is reached. Simulations were run on the University of Florida's High Performance Computing system. This threshold is being refined based on the results of these simulations, and comparing to Southern Garden's data.

b. Refining the psyllid movement and migration portion of the model.

Dr. Keesling continues to work with scientists at the USDA in Gainesville to understand how psyllid population density and citrus tree flush quality impact psyllid dispersal. These results are being incorporated into the model as refinements.

Obj. 2- Continue to experimentally evaluate candidate protective effects of selected RNAi in CTV inoculated plants.

Dr. Dawson's experiments have evaluated plant characterization of dsRNA effects on all psyllid life stages for each of the 5 dsRNA candidates. Those results have been incorporated into the model as refinements.

Obj. 3- Continue to evaluate new RNAi for improved activity with CTV vectors.

The search for new RNAi candidates continues through nuPsyllid and related research. The challenge is to advance what might be "good enough" into the regulatory pathway while we continue to understand what might also be a worthwhile improvement. Therefore it is a priority to advance the current candidates.

Obj. 4- Continue to model performance of best RNAi for field trials and complete scale-up feasibility analysis.

Simulations are being run to specify the size of the acreage and number of trees in the trial that can be expected to show a beneficial effect for each construct with high confidence. The size of the acreage required will dictate the CTV construct inoculum scale-up required for the number of trees in the field trial.

Simulations are also being run to inform on the number of different constructs that can be included in a single trial. Discussions have been held with statisticians on the design of field trials to answer these questions.

Obj. 5. Pursue a corporate partnership to carry this project forward toward field trials and Commercialization.

The Psyllid Shield idea is a spin-off of the nuPsyllid and RNAi research in combination with CTV vector development. Therefore, it 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 because it involves multiple stakeholders, IP owners and licensees. Our approach is to investigate and establish the impact of the science first while facilitating, accelerating and incentivizing corporate action.

CRDF can play a key role in regulatory and commercial delivery support. To date, discussions with Dr. John Byatt of the UF Office of Technology Licensing and outreach to potential partners suggests the most likely commercial partners will come from within the citrus industry due to the relatively limited size of the market and limited opportunities for return on investment.

Obj. 6- Make key decisions regarding initiation of field trials and regulatory approval process.

With the CTV vector protective parameters known and the uncertainty in those measurements estimated, the model will predict the size of the trial necessary to see a protective effect with a given confidence interval (95%). This information will be used by CRDF to communicate the potential benefits of the disease intervention to the EPA and to guide its investment decisions to continue with the trials in partnership with other corporate entities as registrants.

Significant Meetings or Conferences:

None

Obstacles Encountered and Breakthroughs:

Access to the Southern Gardens survey data and the increased scale of the simulations has provided valuable insights into the relationship between transmission of CLas and eventual development of HLB symptoms for incorporation into the model.

CRDF Commercial Product Delivery Sub-Project Progress Report FY 2015-16

Quarter Ending 30 September, 2015

3. Citrus Host Intervention

Project Title: 3a. Naturally Occurring Microbial Product Interactions with HLB

Narrative of Progress by Project Goals:

1. Track ongoing research on soil microbes and their role in HLB and tree health. No activity

2. Conduct field trials to test commercially available naturally occurring microbes Experimental protocols were developed to provide a sound scientific assessment of HLB effects of 5 commercially available microbial soil amendment products (BioFlourish, Ecofriendly, Serenade, Quantum and Aliette) plus a water treated control (UTC), in multiple applications per year as recommended. A subset of trees within each treatment was mulched with mature cow manure.

Ongoing treatments (quarterly or monthly) were began in May/June 2014 and are being applied with and without an organic mulch at the 3 Valencia/Swingle trial sites, Ridge, East Coast, Southwest Florida, using a professional crop consultant company at each site. All required field work at all 3 sites is on schedule and all the data has been submitted on time.

The Field Trial Project Manager, the Field Trial Administrator and Staff are monitoring the project activities. CRDF established data repositories for each project site so that all photos, data and treatment data are provided to CRDF as they are collected. New data this quarter included leaf nutritional effects from leaves sampled in August 2015 which are contrasted with the initial values from the same trees sampled in August 2014.

Ridge site: Valencia/Swingle trees are 17 years old. Leaf nutrition. Leaves were sampled across all treatments on Aug 11, 2015 for nutrient analysis and compared to values from August 2014. Regardless of treatments, most major nutrients (N,P,K,Mg) increased from 2014 to 2015 but overall, N remained a bit low (2.35%) in 2015. Leaf P, K, and Mg were in or slightly above the target optimum range (recommended by IFAS) in 2015; Ca declined to 2.46% in 2015, a bit low. Among the minor elements, Mn, Zn and Fe declined from 2014 to 2015 to levels below the optimum range. Leaf Cu also declined but remained high (26 ppm) while B changed little and remained in the optimum range. All these data have been shared with the crop consultant and production manager. Within the Aug 2015 nutritional data, the organic matter (OM) treatment had no significant effect on any nutritional values. The soil amendment treatments had no effects on leaf nutrients except in the overall low leaf Fe in that all treatments had higher leaf Fe than the lowest water control.

East coast, Indian River site: Valencia/Swingle trees are 6 years old. Leaves were sampled across all treatments on Aug 14, 2015 for nutrient analysis and compared to values from August 2014. Regardless of treatments, most major nutrients (N,P,K,Mg) declined from 2014 to 2015 such that all were in the deficiency range in 2015. For example, overall leaf N was 1.94% and leaf K was 1.10% in 2015. Ca, however, was increased in 2015 into the sufficiency range (3.6%). Most minor elements (Fe, Mn, Zn, Cu) all increased into the sufficiency range (Cu was very high: up to 500 ppm) from 2014 to 2015; B varied

little from 2014 to 2015 and was a bit low. All these data have been shared with the crop consultant and production manager. Within the Aug 2015 nutritional data, the organic matter treatment had no significant effect on any nutritional values except on leaf K which increased from 1.06% in the no OM to 1.15% in the OM treatment. The soil amendment treatments had no effects on any leaf nutrients.

SW FL site: Valencia/Swingle trees are 11 years old. Leaves were sampled across all treatments on Aug 26, 2015 for nutrient analysis and compared to values from August 2014. . Regardless of treatments, most leaf nutrients (N, P, K, Mg, Mn, Zn and Cu) increased from 2014 to 2015 an all remained within the optimum range except for leaf N, 2.95%, a bit high and Cu, quite high. Leaf Ca was within the optimum range and unchanged from 2014 to 2015. Leaf Fe was unchanged from 2014 to 2015 and a bit low. Leaf B decreased from 2014 to 2015 but remained within the optimum range. All these data have been shared with the crop consultant and production manager. Within the Aug 2015 nutritional data, the organic matter treatment significantly increased leaf N to 3.1% and OM also increased leaf Mn and Zn which remained within their optimum ranges. The mulch treatment did not affect other leaf nutrients. The soil amendment treatments had no effect on the uniformly high leaf N values. There were significant soil amendment treatment differences in leaf P, K, Cu and B but all treatments remained within the optimum ranges except leaf Cu which were uniformly high.

3. Provide communication on project goals, progress and results to CPDC, CRDF and growers

Significant Meetings of Conferences:
CRDF Project reviews at Citrus Expo, August 19, 2015, Ft Myers, FL

CRDF Commercial Product Delivery Sub-Project Progress Report FY 2015-16

Quarter Ending 30 Sept 2015

3. Citrus Host Intervention

Project Title: 3b. Plant Growth Regulator Interactions with HLB. Final Report.

Narrative of Progress by Project Goals:

1. Track RMC research projects evaluating the effects of PGR application on plant physiological processes and on pre-harvest fruit drop. No Activity

2. Track and report on the portfolio of CPD field trials that have been completed or are underway during 2014-15.

Two years of contracted field trials on effects of a single application of 2,4-D on mitigating preharvest fruit drop in Valencia oranges were completed in June 2015. A manuscript will be published soon in the Proceedings of the FL St Hort Society. Title: A Single Application of 2,4-D Can Decrease Preharvest Fruit Drop in HLB-Affected 'Valencia' Orange Trees, by J.P. Syvertsen, Tom Minter, Henry Yonce, and W. Bandaranayake.

The goal of these field trials was to determine if a single spray of 2,4-D in late December could mitigate 'Valencia' preharvest fruit drop from HLB affected trees. An on-label application of 2, 4-D (Citrus Fix) was applied to mature 'Valencia' trees at four different sites in the central Florida ridge area in Dec 2013 and repeated using different trees at the same sites in Dec 2014. All trees had visible HLB symptoms. At each site, there were four reps of 6 trees treated along with 4 reps of 6 untreated control (UTC) trees. Fruit drop counts in 2014 and 2015 came from the 4 middle trees in each rep so there were 16 trees in the treated and UTC at each site. The grove floor under each tree was raked prior to treatment and approximately every two weeks thereafter for fruit drop counts until harvest in March or April. The percentage of fruit dropped was calculated after counting the remaining on-tree fruit at harvest and dividing the number of fruit dropped on each sampling day by the total number of fruit dropped + fruit harvested. At three sites in 2014, average total fruit drop ranged from 24 % to 76 % and there was no significant effect of the 2, 4-D treatment on fruit drop. At a forth site, fruit drop was significantly decreased by 28% from 47 % drop in the UTC to 19 % drop the 2,4-D treatment. In 2015, at 2 sites where disease symptoms were visible in 37-38 % of each tree canopy, fruit drop was significantly decreased by 5% and 18%, respectively, in the 2,4-D treated trees compared to the UTC. There was no significant effect of the 2,4-D treatment on fruit drop at a third site where symptoms were visible in 55 % of the canopy. Combining the % fruit drop results from the 7 trials at 4 sites over the 2 years, % fruit drop was significantly reduced from 5-28 % by the single 2, 4-D treatment in 3 of the 7 trials. The site with the highest visible disease index, however, did not have the highest total % fruit dropped so disease index was not a good predictor of % fruit drop**.**

PGR Field Trials Conducted by Dr. Gene Albrigo:

In the 2014-15 harvest season, more Hamlin trials were included and larger plots used to

include a greater number of replicate trees so that tree health (decline) status could be replicated more times in each plot. Since at least one test of 2, 4-D applied 2 years ago in December gave a significant reduction in pre-harvest fruit drop in Valencia, four PGR tests were started in December 2014 including a 2, 4-D treatment on pre-harvest drop of Valencia. One test was applied near Frostproof, another test was applied in Auburndale and a third test was installed in Lake Alfred. Two of these tests included Headline. A fourth test, in Auburndale, was grower applied to 5 acre plots and included 2, 4-D and Headline, a strobilurin fungicide. These tests were harvested in April 2015 and data are being analyzed.

In the fall of 2014, six PGR tests using 6 to 10 tree replicates were applied to Hamlin blocks and two tests were applied by growers using single 5 or 4 acre sprayed plots without replication. In these grower tests, matching sprayed plot trees and adjacent control trees (24 trees/plot in four 6 tree groups) were compared for percent fruit drop. Additionally, a white grapefruit test comparing GA, 2, 4-D and Headline was applied to double-row plots in the Indian River.

In three of five Hamlin trials, GA3 plus 2, 4-D showed some reduction in pre-harvest fruit drop compared to the controls. There were two of four tests that yielded reduction in fruit drop in Headline-treated plots. For both materials, the reduction sometimes was only in healthier or more declined trees and not both. In the grapefruit trial GA3 reduced fruit drop as did GA plus 2, 4-D, but the latter combination only reduced fruit drop in healthier trees. In Valencia trials, a combination of three primary PGRs reduced drop slightly for healthier trees in one trial and in another Headline and 2, 4-D reduced drop in healthier trees only. Other Valencia trials are not yet completed.

3. Communicate to CPDC, Board and growers on the project progress, results and interpretation
No activity

Significant Meetings of Conferences:
CRDF Project reviews at Citrus Expo, August 19, 2015, Ft Myers, FL.
Fruit drop results from the Albrigo trials and the CPDC field trials were presented at the annual Florida State Horticultural Society meetings in June 2015 and will be published as formal manuscripts in the subsequent Proceedings of FSHS.
See also the summary in: Albrigo, L.G. and J.P Syvertsen. 2015. Status of citrus fruit drop in relationship to HLB. Citrus Industry (July): 14-17.

Obstacles Encountered and Breakthroughs:
Tree to tree variation with HLB status is a reoccurring problem. It does appear, however, that trees with less severe HLB symptoms may respond better to PGR treatments by decreasing the percentage of pre-harvest fruit drop more than HLB declining trees. A single application of 2, 4-D can decrease preharvest fruit drop in HLB-affected 'Valencia' orange trees—but not always.

CRDF Commercial Product Delivery Sub-Project Progress Report FY 2015-16

Quarter Ending 30 Sept 2015

3. HOST PLANT INTERVENTION

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

Narrative of Progress for Project Goals:

1. Track ongoing research projects evaluating emerging scion and rootstock genotypes for tolerance or resistance to HLB, citrus canker and other diseases. No Activity.

2. Encourage early release of new commercial rootstocks and other strategies to make these rootstocks available to growers

Progress in development of techniques for nursery management of new citrus rootstocks emerging from UF and USDA breeding programs is reported here from the project being conducted by Dr. Richard Beeson of the UF, IFAS MFREC, Apopka. CRDF approved funding to Dr. Beeson to investigate barriers to propagation of new rootstocks and supported the construction of an addition to existing facilities at MFREC. Construction of the facility is complete and experiments are underway to evaluate seed germination, sanitation relating to seed contamination with citrus canker, and propagation methods for recalcitrant rootstocks. The new facility is full of cuttings established to expand the rootstocks where limited seed are available, and appropriate propagation and evaluation trials are underway.

All objectives of this project are being pursued, and methods are being modified as necessary.

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

The advancement of Phase II of the rootstock trials has been hindered by limited availability of propagative materials from the breeding programs and nursery operations. Further planning in the next quarter will determine if Phase II is feasible.

4. Evaluate ongoing grower plantings of candidate rootstocks at 3 different sites: 2 on Central Ridge and one in Southwest Florida. Sour, KCarrizo, Swingle) for comparison at individual sites.

Trees were budded with '1-14-19 Valencia' for scion uniformity and most have been planted at the 3 sites: Southwest Florida (Duda, LaBelle) site in March 2015, ridge site 1. (Peace River, Babson Park) at the end of April, at the 2nd ridge site (BHG, Venus) in July 2015. Trees on UFR16, were not large to be planted in the 2 ridge plantings in Spr/Sum 2015 so gaps have been left to be planted this fall or spring 2016.

Grove site evaluations include soil type, soil and water pH, and cultural practices including irrigation scheduling, fertility programs and pest/psyllid control. Best management practices will be determined by the individual cooperator and will be uniformly applied to all trees at each site. Cultural practices include:

• Aggressive psyllid management according to current CHMA recommendations or equivalent for young trees and early mature trees. Active participation in a CHMA or cooperative treatment area is encouraged as relevant.

• Irrigation, nutrition and grove floor management consistent with best management current practices to promote root health and growth in the presence of HLB

• Freeze protection should be a component of the planting plan.

Record-keeping on the field trial plantings has begun and includes dates, materials, rates and application methods for all practices. Grower cooperators and CRDF coordinate data collection on these field trials and will share information gained from the trials. CRDF and the local SW Extension agent has scheduled the first CRDF Rootstock Field Day at the Duda SW FL site for 10 Nov 2015. This site has a full complement of the 7 rootstocks in the March 2015 planting; the field day also will include 3 additional ongoing rootstock trials by Dr. Grosser at the same site.

Standardized CRDF protocols for tree evaluation: Each tree has been assigned a unique treatment and replicate number. Tree evaluations initially include tree height and trunk diameter; Digital Photographs, Disease Index and Leaves for qPCR, on a subset of measurement trees will follow after 1 year. Leaf nutrition was evaluated August 2015 and data are briefly summarized below.

CRDF DUDA Rootstock Trial. SW FL (Flatwoods)

Valencia planted March 18,19, 2015 at 10' x 21.4 ft. 7 rootstocks (UFR3, US942, UFR2, UFR4, UFR16, Swingle & US812) in 5 reps of 126 trees (7 x 18) in each rootstock plot = 630 trees; minus buffers: 5 x 14 = 70 evaluation trees in each rootstock plot. CRDF has 8 measurement trees in each plot: 8 Trees in each of 5 reps measured so N = 40 trees of each rootstock. All these data are useful as a baseline and have been communicated to the production manager.
Tree Height in Apr 2015 ranged from 69-77 cm (27-30 in) from the nursery: **UFR3 was the tallest, Swingle and US812 were the shortest**.
Trunk caliper ranged from 6-10 mm from the nursery in Mar 15; calculated Trunk Cross Section Area. **US942 largest, Swingle had the smallest caliper from the nursery.**

LEAF NUTRITIONAL VALUES. Leaves were sampled for analysis of 5 major and 5 minor leaf nutrients on Aug 6, 2015 So, trees were only 5 months old. Leaves sampled from 8 trees & pooled in each of 5 rootstock reps (N= 5).
Leaf N (Optimum range 2.5-2.7 % from IFAS for bearing trees) ranged 2.6 to 2.8 % dw. **There were no rootstock differences in leaf N and all were within the optimum range.**
Leaf P (Opt = .17-.3 %) UFR_3 highest P (.23 %) and US_942 & US_812 had the lowest P(.17%) but **all leaf P were within optimum range.**
Leaf K (Opt 1.2-1.7 %) UFR_2 & Swingle had the highest K (2.1 %) and UFR_4, UFR_16 & US_942

the lowest K (1.8%) but **all leaf K were in the high range.**
Leaf Ca (Opt 3- 4.9%) **UFR_16, US_812, US_942 & Swingle were all within the optimum range (3.3-3.4%) but UFR_2, 4, & 3 were in the low range (2.3-2.9%).**
Leaf Mg (Opt 0.3 -.49 %) **UFR_4 had the highest Mg (.42%); Swingle & US_942 had low Ca (.28%) below the optimum range.**
Leaf Fe (Opt 60-120 ppm) ranged from 70-84 ppm **No rootstock differences, all leaf Fe were within the optimum range.**
Leaf Mn (Opt 25-100 ppm) ranged from 20-32 ppm. **All in optimum range except US_942 had the lowest Mn (20 ppm) below the optimum range.**
Leaf Zn (Opt 25-100 ppm) UFR_4 highest leaf Zn (43 ppm) & US_942 (32 ppm) and UFR_2 (30 ppm) had the lowest Zn but **all leaf Zn were within the optimum range.**
Leaf Cu (opt 5-16 ppm for nutrition) ranged from 159-272 ppm. So, leaves on **all rootstocks had very high Cu** probably from canker sprays.
Leaf B (Opt 36-100 ppm) UFR_3 had the highest B (97 ppm) and US_942 & UFR_2 the lowest B (72 ppm) but **all leaf B were within the optimum range.**

Peace River CRDF Rootstock Trial, Babson Park, FL (Ridge). Valencia trees on 7 of 8 rootstocks (US897, US942, UFR3, UFR2, UFR4, US812 & Carrizo (Missing UFR 16 [all 5 reps] and missing 2 reps of UFR 3 missing so n=3) planted on Apr 27, 2015. Initial tree measurements are not available yet. On August 11, 2015 when trees were 3 months old, leaves were sampled for analysis of 5 major and 5 minor leaf nutrients from 8 trees & pooled in each of 5 rootstock reps (N= 5, except UFR 3 where n=3). All these leaf nutrient data are useful as a baseline and have been communicated to the production manager.

Average Tree Height on May 12, 2015 ranged from 71.5 to 75.2 cm (28.1-29.6 in) from the nursery. There were no rootstock differences. Average tree height on June 22, 2015 ranged from 79.7 to 82.2 cm (31.4-32.4 in). The average growth difference (Tr Ht in Jun – Tr Ht May) was 6.9 to 8.3 cm (2.7-3.3 in) and again, there were no differences among rootstocks. Trunk caliper ranged from 5-11 mm (0.2 –0.43 in) May 14, 2015.
Leaf N (Optimum range for bearing trees = 2.5-2.7 %) varied from 2.4 -2.9 % Dr wt. US897 & US942 had the highest leaf N at 2.9 % and Carrizo had the lowest at 2.4%. **All leaf N were in the optimum range.**
Leaf P (Opt .12-.16 %) UFR3 had the highest leaf P (0.35 %) and US812 & Carrizo had the lowest P at 0.27 % so **all leaf P were high.**
Leaf K (Opt 1.2-1.7%) UFR3, US812 & US897 had the highest leaf K (1.8-1.9 %) and UFR4 had the lowest K (1.5%) but **all leaf K were within the optimum range.**
Leaf Ca (Opt 3 - 4.9%) varied from the lowest of 1.9% for UFR3 to the highest 2.6% for US812 so **all leaf Ca were below the optimum range.**
Leaf Mg (Opt 0.3-.49 %) UFR3 had lowest Mg at 0.44 % but **all leaf Mg in the optimum range.**
Leaf Fe (Opt 60-120 ppm) varied from 84 to 107 ppm so **all leaf Fe were in the optimum range.**
Leaf Mn (Opt 25-100 ppm) varied from 54 ppm (for UFR3) to 96 ppm for UFR4 **so all leaf Mn were within the optimum range.**
Leaf Zn (Opt 25-100 ppm) varied from the lowest of 143 ppm for Carrizo to the highest of 189 ppm for UFR4 so **all leaf Zn were a bit high**.

Leaf Cu (Opt 5-16 ppm for nutrition) varied from 329 – 425 ppm) so **all leaf Cu are very high** probably from canker sprays.
Leaf B (Opt 36-100 ppm) varied from the lowest of 43 ppm for Carrizo to the highest of 57 ppm for US812 so **all were within the optimum range.**

The missing trees on UFR3 became available and were planted 9-17-15.

BHG CRDF Rootstock Trial, Venus, FL (Ridge). Valencia trees on 5 (of 7) rootstocks were planted July16-20, 2015, in 12 double set rows = 24 rows X 6 = 144 trees of each rootstock in each plot. 144 trees/plot X 7 rootstock X 5 reps = 5040 total trees. Only trees on 5 rootstocks were planted: UFR-2, UFR-4, US942, US812 and Sour orange. There were missing trees on UFR3 but became available and were planted 9-18-15. Trees on UFR16 will not be ready from the nursery until spring 2016. Tree height measurements were taken on the other 5 rootstocks 7-29 (just after planting) and again on 9-28-15. There were no significant height differences among rootstocks; trees averaged 65.8 and 85.3 cm (25.9 and 33.6 in) in height in July and Aug so they grew an average of 19.5 cm (7.7 in) during that 1 month period. No leaves were sampled for nutritional analysis since the trees were only planted in July.

5. Communicate progress and results of evaluation of rootstocks to industry
CRDF has scheduled the first Rootstock Field Day at the Duda SW FL site for 10 Nov 2015.

Significant Meetings or Conferences:
CRDF Project reviews at Citrus Expo, August 19, 2015, Ft Myers, FL

Obstacles Encountered and Breakthroughs:

Work with citrus seed has been delayed due to microbial contamination of seed sources, and thus, Objective 2.

Availability of sufficient numbers of contracted trees to be ready to plant continues to be a delaying factor in advancing objective 4. Latest estimates are for the final plants for the two ridge plantings to be available for field planting in February-March, 2016.

CRDF Commercial Product Delivery Sub-Project Progress Report FY 2015-16

Quarter Ending September 30, 2015

3. CITRUS HOST INTERVENTION

Project Title: 3d. Genetic technology (MCTF): Deploying Canker-Resistant Genes

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

1. Make measurable progress toward producing transgenic citrus lines from mature tissue transformation of commercially available cultivars for the Florida citrus growers. These citrus lines will have disease resistance to citrus canker and HLB, and will flower and bear fruit in a short time period. For FY 2015-2016, measurable progress is defined as follows:

Narrative of Progress Against Goals:

Obj. 1- Continue *Agrobacterium*-mediated genetic transformation of mature citrus rootstocks and scions to confer tolerance to HLB and canker, and conduct molecular analyses to show transgene expression and copy number.

Priorities include, increasing the number of high quality genetic constructs used in transformations and improving transformation efficiencies.

With support from the MCTF Steering Committee, Dr. Zale stepped up the facility's outreach to research institutions and industry to acquire high quality genetic constructs for transformation. During the quarter, the facility produced 35 mature transgenics using vectors with reporter genes. (Reporter genes significantly increase mature citrus transgenic production.) Transgenics were produced for Drs. Dutt (UF), McNellis (Penn State) and Wang (UF). During the quarter, additional scientists from research institutions and industry also expressed interest in the facility's mature transformation services.

During the quarter, a number of initiatives were undertaken to increase transformation efficiencies.

- The facility introduced a vacuum infiltration and sonication protocol. These treatments significantly increased transformation efficiencies in immature citrus.
- Growth of *Agrobacterium* in minimal media was another parameter identified for further testing.
- The facility acquired a selectable marker from Syngenta for testing in mature citrus transformation. This selectable marker been shown to increase transformation efficiencies in immature citrus. This is important because the most widely used selectable marker, *npt*II, does not function well in mature citrus transformation.

Obj. 2- Increase micro-grafting efficiencies or root mature citrus scion.

One of the reasons transformation efficiencies were low is that ~40-60% of transformed shoots were lost due to micro-grafting failures. Despite adding a dedicated micro-grafting station in the growth room upon Dr. Zale's arrival, the percentage of successful micro-grafts of scion is still relatively low at ~60 -~65%. During the quarter, this issue was addressed on a number of fronts:

- Protocol changes: During the quarter, the facility continued to evaluate the impact of standard protocol changes, e.g. shortening the rootstock stems.
- Practice: the most important step is to practice. A former employee of Dr. Pena was hired to train the staff in micro-grafting techniques, and this activity is ongoing.
- A kinetin spray increased micro-grafting efficiencies in immature citrus. Its effect on mature citrus will be tested.
- Efforts to date suggest that if the shoots are young, they are more amenable to micro-grafting and greater than 65% survive.
- Another area identified to avoid loss of positive shoots is to root the mature scion directly and conduct secondary grafting later in the growth room when shoots are larger. However, rooting mature scions is still problematic, while rooting mature rootstocks is highly effective.
- Losses of transgenic scions might be reduced by micro-grafting scions onto sweet orange rather than rootstock.

Obj. 3- Continue plant propagation and budding events.

During the quarter, immature wild-type Carrizo rootstocks were budded with different transgenic scion events (Drs. Mou and Wang genetic constructs) and detailed budding records maintained. Transgenic mature rootstock cuttings were also propagated. There are enough entries for 3 replicates at one location.

The facility also performed qPCR in support of the UF Immature Transformation Lab to identify high expressing *NPR1* immature rootstock transgenics. Seventy immature transgenic rootstocks were screened to identify 26 high expressing lines. qPCR was also conducted to identify high expressing mature citrus transgenic scions. The UF Immature Transformation lab will propagate and bud their immature rootstock with scion in different combinations next spring.

There was continued focus on increased capacity by switching to citrus pots in the growth room. This allows for higher planting densities, which might double the current capacity. It was also discovered that mature scions grow significantly faster after budding if the rootstock is not cut off after the buds break.

Obj. 4- Streamline operations, reduce expenses and secure additional external funding.

High operating expenses for staff, materials, equipment and facilities are being addressed, in part, through external funding sources. One method is to increase revenues through paid service charges from customers. In addition, Dr. Zale applied for an NSF exploratory grant for biolistics, but was not successful in securing funding.

Obj. 5- Biolistics transformations

This objective is being pursued as time and resources permit. During the quarter, the facility successfully transformed both immature and mature citrus with biolistics and demonstrated that the protocol is reproducible. This has never been reported, and manuscripts are being prepared. As a result, the lab can now supplement plant production using biolistics.

Significant Meetings or Conferences:

Hao Wu, Yosvanis Acanda, and Janice Zale (2015) Mature Transformation to Combat Diseases in Florida. American Society of Horticultural Science, New Orleans, LA, August 4-7.

Obstacles Encountered:

As the facility moves forward there are a number of issues and challenges that have been identified and are being addressed with support from the MCTF Steering Committee:

- Continue to increase the number of high quality genetic constructs for evaluation by the facility. This will require outreach strategies to identify and evaluate potential candidates.
- Leverage the knowledge and experience of Dr. Pena (IVIA Spain) to continue to increase transformation efficiencies of the facility.
- Take measures to ensure a stable supply of healthy, viable rootstocks, including a steady supply of disease free rootstock seed.
- Participate in the discussion of the broader challenge of moving plants forward for subsequent field testing. This topic is being addressed as part of the CRDF Knowledge Mapping exercise.

Breakthroughs:

Biolistics of immature or mature citrus have never been reported. This will be an important technology to transform citrus without pest sequences, which might lessen regulatory hurdles.

Other Information:

None