CRDF Commercial Product Delivery Sub-Project Progress Report FY 2016-17

Quarter Ending March 31, 2017

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: 1aI. 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.

CRDF is focusing on chemicals that can be available to growers in the near-term, although new active ingredients are tested in the pipeline when appropriate. This quarter two new companies were identified with materials to be evaluated for use against HLB. Information is being assembled on the feasibility of these materials to be used in plant agriculture.

CRDF and other stakeholders have been in contact with an agricultural chemical company to discuss the development of partnership. This company plans to develop a screening pipeline to test their chemicals against HLB. The chemicals to be tested will mainly be biopesticides and plant defense modulators. A decision will be made on this partnership in the next quarter.

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

No new materials were tested in the in vitro assay this quarter. New materials were identified for this assay and will be tested in the next quarter.

A pesticide with a citrus label was tested in the greenhouse assay. The results of this assay were promising, but may require changes to the label to be used against HLB. Further testing is necessary to confirm the effect and another round of testing is being planned. Testing will take place in the next quarter and results are expected in the first quarter of FY 2017/2018.

Subproject Title: 1aII. 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.

A project was approved last quarter to test new adjuvants for the introduction of chemicals into citrus. This project is ongoing, with results delivered to the company by the end of the fiscal year. These chemicals are in early stages of development, a time-to-market has not yet been established and will depend on efficacy. Project managers are working closely with this company to move this project forward efficiently.

A workshop will take place in the next quarter with researchers, growers and industry representatives to discuss trunk applications of pesticides. This workshop will define a pathway forward for trunk application research.

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

A project was approved this quarter to evaluate a new method for delivering bactericides to the canopy, the project will begin in the next quarter. This project aims to determine if the method significantly improves bactericide uptake. If this phase of the project is successful, phase two of the project will be developed and presented to the Commercial Product Delivery committee. The second phase of the project will evaluate the benefit to tree health of the increased level of bactericide uptake.

Another project funded in this quarter from a University of Florida researcher aims to evaluate the hypothesis that thermotherapy increases the uptake of bactericides. This study will evaluate uptake in small greenhouse plants after heat treatment using biochemical analysis. If uptake is improved, a field trial may be developed to test uptake in field trees to complement the grower field trial that was set-up this quarter in cooperation with CRDF and with a commercial thermotherapy application company. The field trial set-up this quarter is being evaluated by CRDF to determine if the combination of thermotherapy and bactericides improve the health of the trees.

A research project to evaluate methods of detecting bactericides in plant parts was initiated in July 2016 and is being conducted by a research group at the University of Central Florida. This project uses infrared spectroscopy techniques to detect and quantify both oxytetracycline and streptomycin in a citrus leaf. This goal of this project is to evaluate movement of bactericides in citrus to better understand the dynamics of bactericide movement within the tree to help develop better methods of formulation and delivery. The methods used can successfully detect streptomycin on and in the citrus leaf on a nanoscale. Next steps of the project will be to look at the absorption and movement of oxytetracycline and streptomycin on a whole plant scale. This project was scheduled to be completed by the end of the fiscal year, but because of unexpected delays, the project will be extended for three months.

A CRDF field trial was recently completed that evaluated the efficacy of trunk injection of oxytetracycline (OTC), streptomycin or a zinc-based bactericide compared to conventional foliar applications. The trial was initiated in February 2016 and ran for one year. The trees were injected two times, in March and September 2016, in 100ml of water with rates up to 0.5g of OTC or streptomycin. The Arborjet Tree I.V. kit (Arborjet, Woburn, MA) was used for trunk injections and materials were applied through four injection ports. Foliar applications were conducted three times, in March, June and September, at maximum labeled rates, at a volume of 100 gallons per acre.

The field trial design consisted of four replications of blocks of six trees (24 trees per treatment). The evaluations consisted of tree health measurements: canopy volume, trunk cross-sectional area, disease severity (disease index); canker evaluations, polymerase chain reaction to quantify bacterial titer, leaf nutrient analysis, fruit drop counts, fruit quality and yield.

After one year, after two trunk injections of bactericides, few evaluations showed significant improvement when compared with the controls. Unfortunately, the block was hedged, so canopy volume provides very little information. Trunk cross-sectional area was not significantly different between treatments. Disease severity did have some significant means separations, but this statistical variation is most likely not meaningful because this is a visual rating and a two-point value difference is within the human error range. The range of values are shown in table 1. One OTC trunk injection treatment did show significantly less canker compared with the water control, but canker was only evaluated at one time-point during the trial (Table 2, Figure 1). PCR results from February 2017 showed a significant reduction in bacterial titer in the OTC-2 trunk injections treatment (Table 3, Figure 2), but the PCR results do not follow a consistent pattern over time and because of this, do not provide much information on the individual treatments.

This trial has been completed and will not be continued for another year. CRDF continues to explore alternative application strategies, a workshop will take place in the next quarter with researchers, industry scientists, and citrus growers to discuss trunk application research and to develop objectives for next level research projects.

Evaluation Method		Range of Values
Trunk Cross-Sectional Area cm^2	Feb. 2016	52-59
Trunk Cross-Sectional Area cm^2	Feb. 2017	60-67
Canopy Volume m^3	Feb. 2016	46-58
Canopy Volume m^3	Feb. 2017	44-45
Disease Severity	Feb. 2016	16-18
Disease Severity	Feb. 2017	15-17
Percent Canker		5-12
Fruit Drop Percent		11-28
Kg Yield		41-60

Table 1. Minimum and maximum values for the pre-application and final evaluations

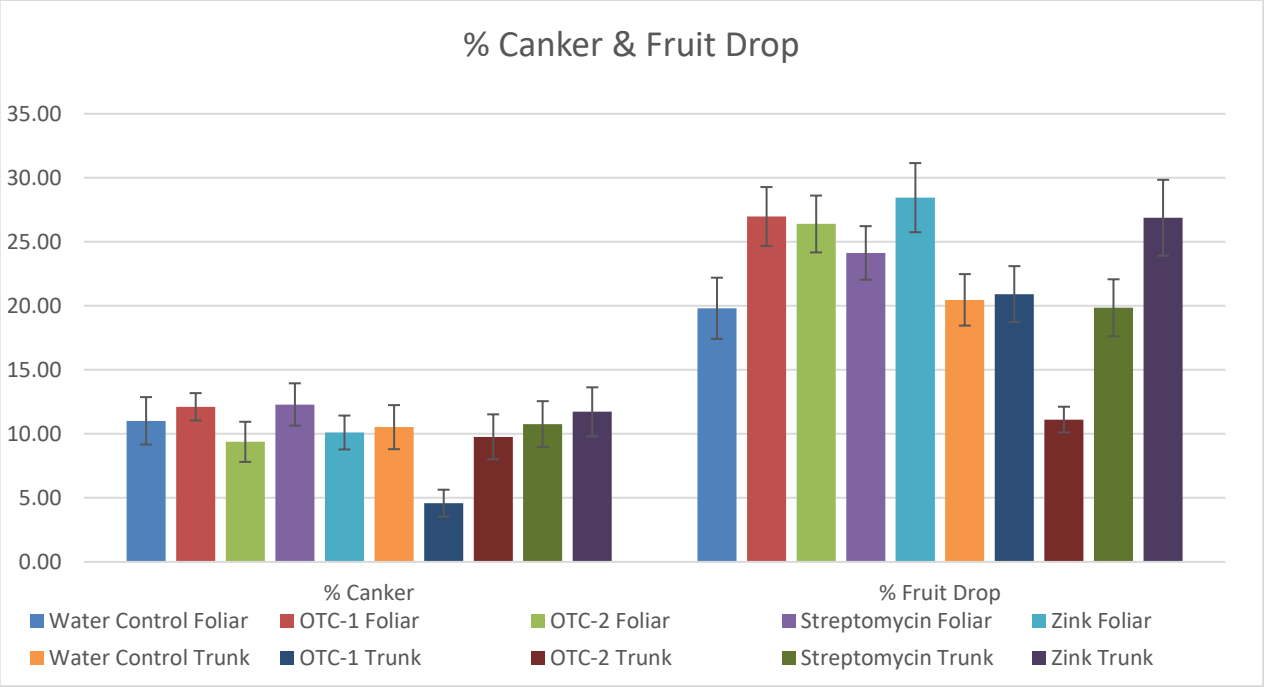

Figure 1. % canker and % fruit drop.

	% Canker				% Fruit Drop				Yield (kg)			
Treatment	**Mean**	**Std Error (±)**	**MS**	**p =**	**Mean**	**Std Error (±)**	**MS**	**p =**	**Mean**	**Std Error (±)**	**MS**	**p =**
Water Control Foliar	11	1.9	A	<.0001	20	2.4	AB	<.0001	60	5.1	A	<.0001
OTC-1 Foliar	12	1.1	A		27	2.3	A		51	4.6	AB	
OTC-2 Foliar	9	1.6	AB		26	2.2	A		44	3.6	AB	
Streptomycin Foliar	12	1.7	A		24	2.1	A		48	4.6	AB	
Zink Foliar	10	1.3	AB		28	2.7	A		47	5.1	AB	
Water Control Trunk	11	1.7	A		20	2.0	A		54	4.5	AB	
OTC-1 Trunk	5	1.1	B		21	2.2	A		47	4.6	AB	
OTC-2 Trunk	10	1.8	AB		11	1.0	B		56	3.8	AB	
Streptomycin Trunk	11	1.8	A		20	2.2	AB		50	3.8	AB	
Zink Trunk	12	1.9	A		27	3.0	A		41	3.9	B	

Table 2. Table of statistics for % canker, % fruit drop and kg yield

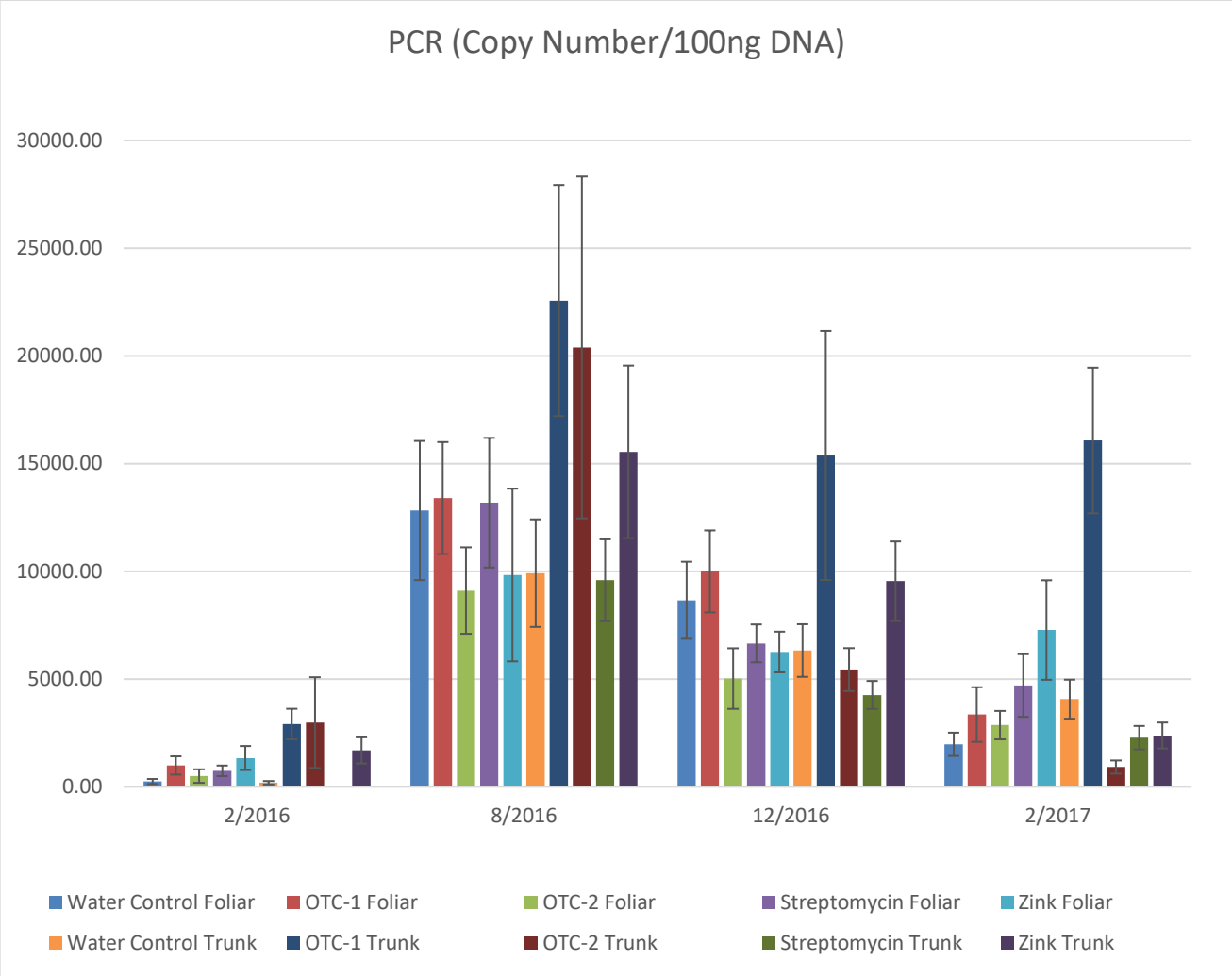

Figure 2. Copy number per 100ng tissue.

	PCR (Copy Number/100ng DNA) 2/2016				PCR (Copy Number/100ng DNA) 12/2016				PCR (Copy Number/100ng DNA) 2/2017			
Treatment	**Mean**	**Std Error (±)**	**MS**	**p =**	**Mean**	**Std Error (±)**	**MS**	**p =**	**Mean**	**Std Error (±)**	**MS**	**p =**
Water Control Foliar	249	115.7	BC	0.012	8661	1788.6	BCD	0.001	1975	540.4	BCD	<.0001
OTC-1 Foliar	992	423.7	BC		9999	1903.3	ABC		3355	1267.9	CD	
OTC-2 Foliar	499	313.2	BC		5025	1406.8	D		2863	659.8	BC	
Streptomycin Foliar	742	246.0	ABC		6659	877.9	AB		4702	1454.0	BC	
Zink Foliar	1336	557.7	ABC		6258	945.1	ABC		7278	2309.7	AB	
Water Control Trunk	189	79.8	BC		6326	1220.8	BCD		4068	905.7	ABC	
OTC-1 Trunk	2914	707.8	A		15379	5781.3	A		16075	3382.7	A	
OTC-2 Trunk	2980	2106.5	AB		5441	996.6	CD		918	305.6	D	
Streptomycin Trunk	1	0.5	C		4262	650.5	BCD		2282	542.7	BC	
Zink Trunk	1689	608.3	BC		9544	1845.5	BCD		2383	600.8	BCD	

Table 3 Table of statistics for copy number per 100ng tissue, treatments with significant models.

Subproject Title: 1aIII. 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.

Since March 2016, the bactericides Mycoshield, Fireline and Firewall have been available for use in Florida. CRDF has set-up nearly 70 field trials with growers to evaluate the efficacy of individual grower applications. Data being collected includes disease severity, bacterial titer/C_t values, fruit drop and yield. Initial PCR, disease severity, fruit drop and yield data has been collected on all Hamlins and grapefruit. Valencia harvest data collection will be completed in the next quarter. Data from the majority of the trials, including harvest data. The data analysis has shown that there is much variation in the data. Since these are non-replicated trials, few conclusions can be drawn from the trial results at this time. Data will continue to be analyzed as it is collected. All data is available to growers on the CRDF website under presentations or at http://citrusrdf.org/wp-content/uploads/2012/10/Update-GBT-Tables-4-24-17.pdf.

Registrant trials funded by CRDF continue for a third year. In 2016-2017, these trials focus on the use of alternating applications of oxytetracycline and streptomycin (three applications of each active ingredient) and a mixture of the two active ingredients for use in grapefruit. Application timing include applications at even intervals throughout the year and applications grouped in the spring and fall. This project is on track, objectives for this quarter have been completed. A presentation on this project is scheduled for the Florida Citrus Mutual Annual Conference.

The biopesticide field trial, project 15-049C, was set-up in late February on Hamlins. Data collection Was completed this quarter and data has been analyzed. The treatments in this trial were Thymeguard (Agro Research International, Ecotrol Plus (Keyplex), OnGuard EO, Xplode (AgXplore) and a research oil product. All products were applied at the recommended rates and with the recommended adjuvants by the registrants. Applications of these products were applied every 60 days (6 applications total)

Two sites were used for this trial, at the initiation of the trial the trees from one site tested PCR negative for HLB, although the trees were visually infected and the second site trees all tested positive for HLB. The field trial design consisted of four replications of blocks of six trees (24 trees per treatment). The evaluations consisted of tree health measurements: canopy volume, trunk cross-sectional area, disease severity (disease index); canker evaluations, polymerase chain reaction to quantify bacterial titer, leaf nutrient analysis, fruit drop counts, fruit quality and yield.

The results of this trial were not remarkable, the only evaluation parameter that showed a benefit of the treatment was PCR. In the PCR positive site, Ecotrol Plus had a significantly lower titer than the untreated control (Figure 3). This result however, was not seen in the PCR negative site (Figure 4). The range of values for this trial are summarized in table 4.

Evaluation Method	PCR Negative Site	PCR Positive Site
Disease Severity (0-40)	14	17-18
Canopy Volume	35-38 m^3	16-19 m^3
Tree Height	2.77-2.97 m	1.95-2.13
Fruit Drop	41-60%	50-69%
Yield	15-22 kg (33-48.5 lbs)	9-15 kg (20-33 lbs)

Table 4. Minimum and Maximum values by site, final evaluations

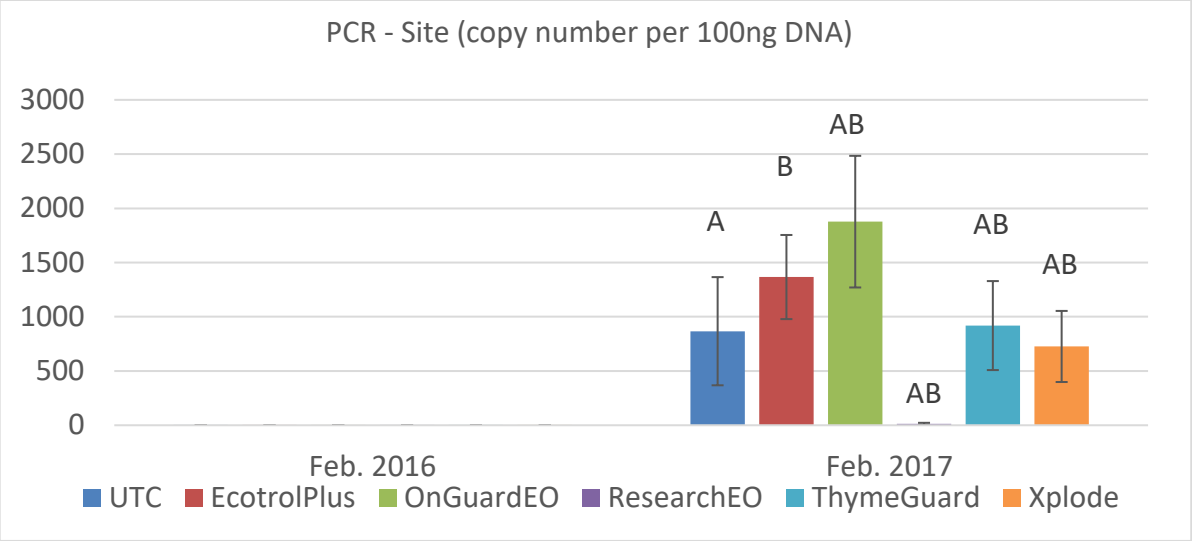

Figure 3. PCR (copy number per 100ng DNA), PCR negative site. Pre-application and post-applications sample dates.

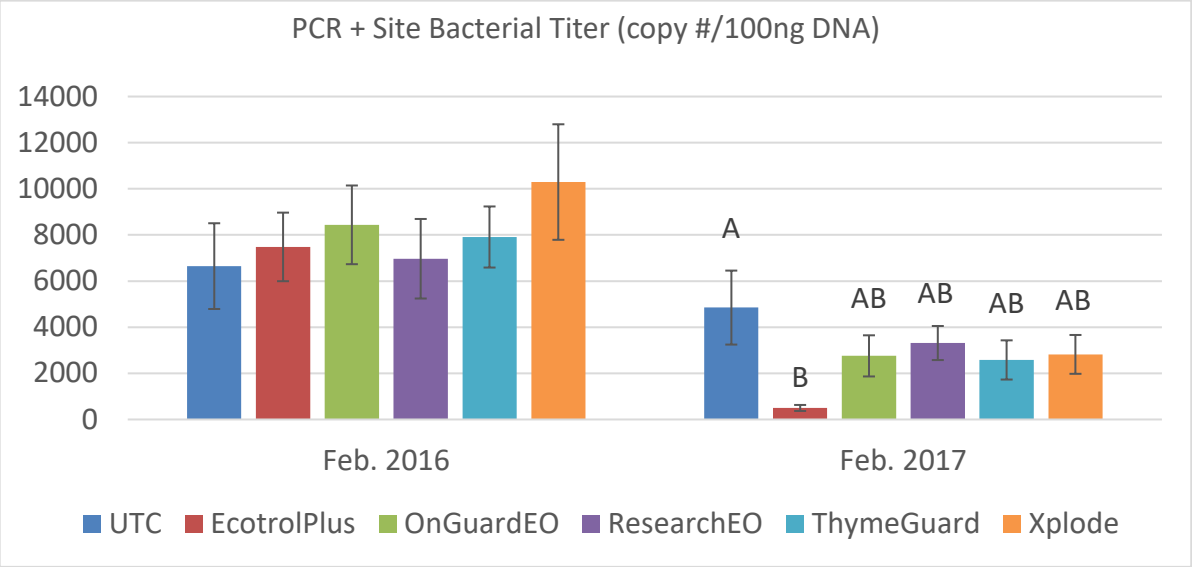

Figure 4. PCR (copy number per 100ng DNA), PCR positive site. Pre-application and post-applications sample dates.

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

No field trials on new bactericide therapies is presently in development.

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

The Tree Health Section 18 was recertified by the US EPA for use through December 31, 2017. This is for continued use of oxytetracycline and streptomycin in Florida in 2017. An interim report will be compiled in the next quarter, this is a requirement for the section 18. A resistance monitoring program is also required by the EPA. A protocol was developed by CRDF, FFVA and FDACS with the assistance of George Sundin, a researcher at the University of Michigan. This protocol was approved by EPA, researchers were recruited to conduct the work and grower blocks were identified for the sampling. The first round of sampling will begin next quarter.

Field trials are underway for Project 15-037C evaluating the use of the newly developed bactericide T-SOL™, to test several formulations. Laboratory tests are also being conducted to evaluate the mode-of-action of this bactericide. This project continues through the next quarter. The PI of this project, Dr. Santra, gave a presentation to the CPDC this quarter on this project and his Zinkicide Project.

Significant Meetings or Conferences:

- CRDF Project managers attended the Citrus Show January 25 & 26 to attend the education session, to discuss CRDF projects with growers and to discuss grower concerns.
- CRDF project managers attended the International research conference on HLB March 15-17th. This conference assembles several hundred HLB researchers from around the world. A talk on the bactericide CRDF projects was presented on the 15th of March.
- The CRDF bactericide project manager attended the American Society for Microbiology meeting on Innovative Microbial Ecology for the Mitigation of Antibiotic Resistance and Bacterial Diseases on March 22-25 in Crystal City, VA. This conference gathered key researchers from a variety of fields including agriculture and ecology to discuss current research in antibiotic resistance. This topic is relevant to the antibiotic use in Florida and will help CRDF address concerns from regulatory agencies, growers, industry and the public.

CRDF Commercial Product Delivery Sub-Project Progress Report FY 2016-17

Quarter Ending March 31, 2017

1. *Candidatus Liberibacter asiaticus* PATHOGEN INTERVENTION

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

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

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.
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 health of treated trees.
4. Continue outreach efforts to inform growers of optimized thermal treatments including CRDF sponsored field days to include thermal therapy researchers and active steaming commercial companies.

Narrative of Progress by Project Goals:

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 55^{o}C for 30s. In approximately 4 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 during the CLas acquisition access periods and insect and leaf samples were collected. Samples were stored at -20^{o}C 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 be 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.

As previously reported, trees were steam-treated as described in our research proposal during July 2015. Adults and nymphs were enclosed in mesh sleeves on trees for acquisition feeding approximately 5 weeks following treatments. Following acquisition feeding, insect and leaf samples were collected (45 d post-treatment) from trees and taken to the lab for subsequent nucleic acid extraction and analysis. Acquisition feeding assays were repeated approximately two months later, with samples collections beginning 114 d post-treatment. Nymphs were collected from plants after adult emergence, until no psyllids remained in the mesh sleeves. The titer of Candidatus Liberibacter asiaticus (CLas) in trees receiving steam treatment did not significantly differ from untreated trees on days 0, 45, or 114 post-treatment ($p = 0.99$, 0.11, and 0.81, respectively; Tukey's Honestly Significant Difference (HSD) test); however, CLas titers in treated and untreated trees were lower at 45 d post treatment as compared to days 0 and 114 post-treatment. This is likely due to naturally-occurring seasonal decreases in CLas titers. CLas titers were significantly higher in steam-treated trees than untreated trees on day 0 as compared to day 45. CLas acquisition by adult psyllids enclosed on trees receiving thermal treatments did not differ significantly from acquisition by adult psyllids on untreated trees. Samples from CLas acquisition feeding assays with psyllid nymphs are still being processed.

Based on these results, which indicated the thermal treatments applied during July 2015 did not reduce plant CLas 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 acquisition of CLas under controlled conditions. Two-year-old Valencia trees were inoculated with CLas by enclosing plants with CLas-infected psyllids for two weeks. Currently, plants are being held in a secure, insect-free greenhouse until they are determined to be positive for CLas. At that time, a controlled environmental chamber will be used to apply heat treatments to trees for use in subsequent acquisition experiments.

Obstacles: None for this period. All activities followed prescribed plans.

3. Refine requirements and environmental conditions for most effective thermal treatment.
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 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.
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. Evaluation of thermal therapy conducted by those involved in scale-up is ongoing by the CRDF evaluation team. Six enterprises are operating field thermotherapy machines in Florida. At least two other companies are supported by USDA, APHIS, MAC to deliver additional thermal therapy to Florida for field trials. Those with capability are operating at multiple locations in Florida, and the evaluation team is in the field conducting the evaluations.
In this reporting period, 14 trials are being evaluated, with varying intensity and with different machinery delivering a range of temperature/duration combinations. Since the trials continue to be set up as opportunities arise, we are providing the current data sets associated with trials currently being conducted. Results to date provide a glimpse of the variation of measures and tree responses and several of these trials can be considered completed. Significant additional data analyses comparing pre- and post-treatment tree status will be available providing yield, quality and other metrics.

All of these trials have been 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. Having 14 locations under evaluation is ahead of the plans, and we anticipate being able to be able to drop some sites as we fulfill the work plan and budget.

Additional trial evaluations have been established as thermal therapy providers are ready for evaluation of their machinery and treatments. Additional treatment sites have been established to evaluate the new generation machines from Dr. Ehsani (UF, IFAS), Premier Energy, and Daniel Scott.

Status at end of 24 months of the scale-up program. While this project does not control the tempo of innovation or the timetables for the various solvers who are commercializing thermal therapy for HLB-infected trees in Florida, there is significant progress being demonstrated on several fronts that is driving the evaluation component of this project. Several participants have revised designs in response to early evaluation results, and have deployed next generation machines.

There are now 14 sites reported on in this period and are all ongoing thermotherapy projects where tree responses to different thermotherapy conditions are being monitored. Most of the treated trees that displayed previous short-term responses to thermotherapy have now become not different from non-

treated control trees. The different sites are of various aged trees and varieties. Most projects have post-treatment leaf samples that have been analyzed by PCR in 2017. All data and observations should be considered preliminary, as monitoring tree status and data analysis are continuing.

Conserve Trial 1

On 2-26-16, 15 sets of paired, uniform of trees of Valencia on Swingle rootstock were selected for evaluation. Fifteen trees were non-steamed control trees and 15 trees were steamed at 131 F for 30 s. By 3-31-17, average canopy volumes (CV, m^3), tree height (TH, m), trunk cross sectional area (TCSA, cm^2), Disease index (DI, 0-40), average fruit drop (Fr Dr), Yield and total fruit number per tree were all not affected by the treatment. From PCR analysis on 2-3-17, leaf CT and copy number per DNA were also not affected by treatment. Thus, all trees have recovered from the previous treatment 10 months ago and any short-term differences have disappeared.

Treatment	CV (m3) 3_31_17	TH (cm) 3_31_17	TCSA (cm2)	DI 3_31_17	CT 2_3_2017	CN_DNA 2_3_2017	Fr Dr 1_7_17	Yield (kg) 3_31_17	Frt No. 3_31_17
Control	4.9	193.7	35.2	16.9	25.1	6858.9	10.3	11.7	76.9
131F30s	4.9	195.6	34.8	16.4	25.5	7068.3	7.6	13.1	87.4

Conserve Trial 2

On 3-2-16, 15 sets of paired, uniform of trees of Hamlin on Swingle rootstock were selected for evaluation. Fifteen trees were left as non-steamed control trees and 15 trees were steamed at 131 F for 30 s. On 1-6-17, fruit drop was not different and on 2-3-17 fruit yield, fruit number per tree, leaf CT and CN/DNA were not affected by treatment. On 3-31-17, Canopy Volumes (CV), tree height (TH), trunk cross sectional area (TCSA) and Disease index (DI) were not affected by the treatment. Thus, all trees have recovered from the previous treatment 1 year ago and any short-term differences have disappeared.

Treatment	Fr Dr 1_6_17	Yield (kg) 2_3_2017	Fruit No. 2_3_2017	CT 2_3_17	CN_DNA 2_3_17	CV m3) 3_31_17	TH (cm) 3_31_17	TCSA (cm2) 3_31_17	DI 3_31_17
Control	28.3	3.3	25.5	27.5	3445	4.4	201.9	33.7	17.5
131F30s	25.6	3.5	26.7	25.3	6915	4.3	197.3	31.9	18.1

Conserve Trial 3

On 2-26-16, 10 sets of 3 uniform trees of Valencia on Swingle rootstock were selected for evaluation. Ten trees were left as non-steamed control trees, 10 trees were steamed at 131 F for 30 s., and 10 steamed at 120 F for 40 s. By 1-6-17, there were no treatment effects on canopy volumes (CV), tree height (TH), trunk cross sectional area (TCSA) or Disease index and there was little change in tree growth or DI by 4-4-17. Leaves sampled on 2-3-17 were all HLB positive and there were no treatment effects on CT or copy number per DNA. Thus, all trees have recovered from the previous treatment 1 year ago and any short-term differences have disappeared.

Treatment	CV (m3) 1_6_17	CV (m3) 4_4_17	TH (cm) 1_6_17	TH (cm) 4_4_17	TCSA (cm2) 1_6_17	TCSA (cm2) 4_4_17	DI 1_6_17	DI 4_4_17	CT 2_3_17	CN_DNA 2_3_17
Control	7.1	7.2	218.6	226.2	47.1	49.1	19.9	20.3	27.6	876
131F30s	7.2	6.9	214.6	213.7	50.4	52.8	20.0	20.0	27.9	1170
120F40s	7.2	6.9	215.0	211.0	46.6	49.4	20.2	20.5	26.5	1205

Cutrale Trial 2

There were 10 sets of paired, uniform 2 year-old Valencia trees on Swingle rootstock that were selected for evaluation. Ten trees were left as non-steamed control trees and 10 trees were steamed at 127 F for 90 s. On 10-18-16, canopy volume (CV), tree height (TH), trunk cross sectional area (TCSA), fruit drop (Fr Dr) and DI were not affected by treatment. There were no treatment effects on 2-10-17 CT values or copy number per DNA. Thus, all trees have recovered from the previous stream treatment and any short-term differences have disappeared.

Treatment	CV (m3) 10_18_16	TH (cm) 10_18_16	TCSA (cm2) 10_18_16	DI 10_18_16	Fr Dr 10_18_16	CT 2_10_17	CN_DNA 2_10_17
Control	2.4	152.4	17.1	4.6	1.7	29.2	985
127F 90s	2.6	158.5	18.8	5.3	2.1	25.8	3108

Cutrale Trial 3

There were 10 sets of paired uniform trees of 4-year-old Hamlin on Swingle rootstock selected for evaluation. Ten trees were left non-steamed control trees and 10 trees were steamed at 127 F for 90 s. On 10-18-16, Canopy Volume (CV), tree height (TH), trunk cross sectional area (TCSA) and Disease index (DI) were not affected by treatment. Fruit yield and fruit number per tree on 12-7-16 were not affected by treatment. On 2-10-17, there were no treatment effects on CT or copy number per DNA.

Treatment	CV (m3) 10_18_16	TH (cm) 10_ 18_16	TCSA (cm2) 10_18_16	DI 10_18_16	Yield (kg) 12-7-16	Frt No. 12-7-16	CT 2_10_17	CN_DNA 2_10_17
Control	6.8	229.4	36.2	15.0	6.5	54.7	24.0	5680
127F 90s	7.0	229.6	36.5	14.3	8.9	80.4	26.0	2457

Blue Goose Trial 1

There were 10 pairs of uniform trees selected for evaluation, 10 steamed at 128F_30s and 10 non-steamed control trees. On 10-14-16, Canopy Volumes (CV), tree height (TH), Disease index (DI) and fruit drop (Fr Dr) were evaluated. Canopy volume and TH of the treatment trees were larger than the untreated control trees but DI and Fr Dr did not differ. On 2-16-17, CT values, copy number /DNA from PCR, yield, fruit number and fruit drop were all not affected by the previous treatment.

Blue Goose Trial 1	n=10								
Treatment	**CV (m^3) 10_14_16**	**TH (m) 10_14_16**	**DI 10_14_16**	**Fr Drop 10_14_16**	**CT 2_16_17**	**CN_DNA 2_16_17**	**Yield (Kg 2_16_17**	**Fruit No. 2_16_17**	**Fr Drop 2_16_17**
Control	7.6 b	2.2 b	17.7	22.4	32.7	826	30.5	88.1	35.5
128 F 30 s	9.2 a	2.4 a	16.8	28.9	34.9	573	41.7	107.5	21.0

Blue Goose Trial 2

There were 10 pairs of uniform trees selected for evaluation, 10 trees were steamed at 128F for 30 sec steamed and 10 were left as non-steamed control trees. On 10-14-16, there were no treatment effects on canopy volume (CV), tree height (TH), trunk cross sectional area (TCSA) or Disease index (DI). Leaves were sampled for PCR on 3-16-17 but there were no treatment effects on CT or copy number / DNA. Likewise, there were no treatment effects on yield, total fruit or fruit drop on 2-16-17.

Variable_Date	Control	128F_30S
CV_10_14_16	7.6	8.0
TH_10_14_16	212.5	213.2
TCSA_10_14_16	55.9	56.8
DI_10_14_16	23.2	21.7
CT_2_16_17	27.2	25.0
CN_DNA_2_16_17	2532	2321
Yield_Kg_2_16_17	18.1	16.5
Total_Fruit	62.2	59.5
FrDr_2_16_17	42.3	38.6

Blue Goose Trial 3

There were 10 pairs of uniform trees selected for evaluation. 10 trees were steamed at 128F for 30s and 10 were left as non-steamed control trees. On 10-14-16, there were no treatment effects on canopy volume (CV) or Disease index (DI). Tree height (TH) and trunk cross sectional area (TCSA) of treated trees was lower than control trees. Leaves were sampled for PCR on 2-16-17 but there were no treatment effects on CT or copy number / DNA. Likewise, there were no treatment effects on yield, total fruit or fruit drop on 2-16-17.

	TT Treatment	
Variable_Date	Control	128F_30s
CV_10_14_16	9.3	8.8
TH_10_14_16	230.8	213.8 *
TCSA_10_14_16	64.7	56.62 *
DI_10_14_16	23.3	22.8
CT_2_16_17	25.6	29.3
CN_DNA_2_16_17	1875	3076
Yield_Kg_2_16_17	7.7	11.1
Tot Fruit 2_16_17	25.1	38.8
Fr Dr_2_16_17	46.7	44.2

Scott Trial 3

Ray Ruby GF on Sour orange trees, 5 years old. On 6/30/15 and 20 trees were steam treated at either at 128 F for 15 s or at 128 F for 30 s. There were 15 non-treated control trees, resulting in the 3 treatments in this trial. On 10-11-16, there were no treatment effects on Canopy Volumes, (CV), tree height (TH), trunk cross sectional area TCSA), Disease index (DI) or Yield (kg). The total number of fruit per tree at harvest, however, was greater in the 128 F_30 s than on the control trees. Overall, all trees have recovered from the previous treatment 8 months ago and any short-term differences had disappeared. Leaves were sampled for PCR analysis 2-15-17 but there were no treatment effects on Cycle threshold (CT) or copy number per DNA.

			TT Treatment			
Variable_Date	Control		128F_15sec		128F_30sec	
CV_10_11_16	11.6		10.6		12.1	
TH_10_11_16	228.4		222.4		223.3	
TCSA_10_11_16	81.4		77.0		87.2	
DI_10_11_16	19.9		19.3		19.8	
Frt_Yld_kg_2016	56.4		52.6		68.4	
Total_fruit_2016	187.8	B	183.6	AB	251.7	A
CT_2_15_17	26.5		26.9		27.2	
CN DNA_2_15_17	3536		3414		3003	

Scott Trial 4

Ray Ruby Grapefruit on Sour orange trees, 6 years old. 12 trees each were steam treated on 9/8/15 at either 128 F for 20 s, 132 F for 1 s (turned off immediately when temperature in canopy reached 132 degrees, or at 132 F for 10 s. 12 trees were left untreated as a control. On 10-4-16, Canopy Volume (CV) of the untreated were larger than the 128 F_20 s treated but tree height (TH) was not affected by treatments. The untreated control trees had a lower Disease index (DI) than the 128 F_20 s treated trees. Leaves were sampled for PCR analysis 2-15-17 but there were no treatment effects on Cycle threshold (CT) or copy number per DNA.

		CV_10_4_16	Treatment		TH_10_4_16	Treatment			DI_10_4_16	Treatment
	A	24.3	untreated	A	284.4	untreated		A	23.8	128F_20sec
B	A	18.4	132F_10sec	A	261.7	128F_20sec	B	A	22.4	132F_1sec
B	A	18.2	132F_1sec	A	257.8	132F_1sec	B	A	22.3	132F_10sec
B		17.6	128F_20sec	A	257.7	132F_10sec	B		20.9	untreated

	CT_2_15_17	Treatment		DNA_2_15_17	Treatment
A	28.0	132F_10sec	A	3758	untreated
A	27.5	untreated	A	3384	132F_10sec
A	27.0	132F_1sec	A	3338	132F_1sec
A	26.6	128F_20sec	A	2978	128F_20sec

Davis

Valencia/Swingle trees 10 years old. All 24 trees were steam treated on 4/9/15 at 120 F for 30 s. Canopy growth, fruit drop, and visible disease index (DI) have been monitored monthly since April 2015. On 6-22-16, 12 trees were retreated with steam at 120 F for 30 s (2x 120 F 30 s) while 12 trees were left as a TT/control. By 2-3-17, there were no treatment effects on Cycle threshold (CT) or copy number per DNA. On 4-5-17, Canopy Volume (CV), tree height (TH), trunk cross sectional area (TCSA) and Disease index (DI) were not affected by treatments. Thus, 10 months after the second steam treatment, there were still no treatment effects.

Treatment	CV (m3) 4_5_17	TH (cm) 4_5_17	TCSA (cm2) 4_5_17	DI 4_5_17	CT 2_13_17	CN/DNA 2_13_17
TT/Control	20.7	257.0	124.8	22.8	27.8	3363
2x 120F_30s	21.1	248.8	125.8	21.1	29.2	2166

Shinn

Valencia /Swingle, 3 years old, double set. Pretreatment leaves were sampled for PCR on 8/7/2015. Eighteen trees were steam treated 8/7/2015 at 122-127 F (avg 125) for 30 s and 18 trees were untreated as controls. By 2-3-17, there were no treatment effects on cycle threshold (CT) or copy number per DNA. On 4-5-17, average Disease index (DI), Canopy Volume (CV), tree height (TH), trunk cross sectional area (TCSA) were unaffected by treatment. Fruit yield and fruit number / tree also were not different.

Treatment	CT 2_3_17	CN_DNA 2_3_17	DI 4_5_2017	CV (m3) 4_5_17	TH (cm) 4_5_17	TCSA (cm2) 4_5_17	Yield (kg) 4_6_17	Fruit No. 4_6_17
Control	24.8	4048	16.6	2.7	144.5	20.9	2.0	12.8
125F_30sec	23.7	4726	16.2	2.3	140.3	19.6	1.7	11.3

Lykes

Hamlin / X639 trees, 4 years old. 24 trees were steam treated on 10/6/15 at 131 F for 30 s and 24 trees were left as untreated control trees. Canopy volume (CV, m^3), tree height (m), trunk cross sectional area (TCSA, cm^2) and disease index (0-40) were measured on 12-7-16. Treated trees had a smaller CV and TCSA but DI did not differ. By 1-4-17, there were no treatment effects on cycle threshold (CT) or copy number per DNA not were there any differences in fruit yield or number of fruit per tree.

Treatment	CV (m3) 12_7_16	TH (cm) 12_7_16	TCSA (cm2) 12_7_16	DI 12_7_16	CT 1_4_17	DNA 1_4_17	Yield (kg) 1-4-17	Fruit No 1-4-17
Control	16.2	290.5	75.0	15.9	30.0	2274	22.6	250.0
131 F 30s	14.3 *	291.8	62.9 *	15.2	29.0	2955	16.4	194.2

Lee Jones

Based on initial PCR evaluations of HLB status, 22 uniform trees that were HLB positive and 22 uniform HLB negative were selected for evaluation. On 3-22-16, one tree in each pair was steam treated at 128 F for 30 s while the other paired tree was left as an untreated control. On 10-12-16, canopy volume (CV), tree height (TH), trunk cross sectional area (TCSA) and Disease index were evaluated and were apparently affected by treatment but data await statistical analyses. Trees were sampled for PCR and harvested in January 2017. Results of these analysis will reveal if the apparent negative treatment effects on yield and fruit number are significant.

HLB	Treatment	CV (m3) 10_12_16	TH (cm) 10_12_16	TCSA (cm2) 10_12_16	DI 10_12_16	CT 1_13_17	CN/DNA 1_13_17	Yield (kg) 1_19_17	Fruit No 1_19_17
neg	128F_30s	10.5	289.5	36.6	13.1	25.1	4438	6.1	43.0
pos	128F_30s	4.5	208.1	18.3	14.0	25.1	3193	2.9	18.4
neg	Control	10.4	289.7	33.8	11.5	27.4	5215	16.1	90.8
pos	Control	3.8	187.8	18.2	14.7	24.9	6636	7.6	43.1

CRDF Commercial Product Delivery Project Progress Report FY 2016-17

Quarter Ending March 31, 2017

2. Asian Citrus Psyllid VECTOR INTERVENTION

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

Narrative of Progress by Project Goals:

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

   The continuing CRDF ACP portfolio has a number of active projects. Among the CRDF projects are several that may help with understanding and response to the increase ACP populations in Florida that have escalated over the past 3 years. CHMA/CHRP ACP counts from recurrent survey are significantly higher again this spring, leading to discussion of a number of related factors, including:

   - Growers reducing numbers of suppression treatments to reduce input budgets;
   - Modes of action selected for use in ACP suppression may be based on cost rather than duration of control;
   - Potential for reduced susceptibility to one or more insecticides in ACP populations exposed to continued applications;
   - Timing disconnects as combination of enhanced irrigation/nutrition, consistent rainfall and HLB-induced phenology changes in flushing in infected trees. Effectiveness of sprays can be reduced as a result of less synchronous populations;
   - Increasing acreage of unmanaged citrus groves, and their continued flush resulting from consistent rainfall in recent years;
   - Loss of natural enemies from groves under intensive management, including challenges to introduced Tamarixia radiate.

   The portfolio review in January- February 2017 revealed a number of projects that are near end dates and thus may need to be considered for a new cycle. Recommendations from staff to Committees and Board include the following:

   a) CHMA support project (#15-035C) by IFAS, which fuels real-time posting of CHRP counts and updating of regional maps showing cycle changes in ACP populations. Dr. Rogers was invited by CRDF to develop a proposal to continue this project as appropriate. The current project remains in effect, providing support to growers who are managing ACP based on local population estimates. The March 2017 progress report indicated that cycles 94-98 CHMA counts had been processed and mapped, and the ranking of top ten CHMAs in terms of fewest ACP per scouted block remained somewhat stable. The results indicate that even with increasing ACP pressure in Florida, CHMAs are performing across a wide range in managing populations.

   b) Insecticide Resistance Monitoring (#15-038C Stelinski) will end during the 4th quarter of FY 2016-17, and this topic remains a priority. Dr. Stelinski was invited to submit a proposal for continued work, with a request to consider expansion of the scope and to ensure that best available techniques were being used to measure sensitive ACP population changes in susceptibility to various classes of active ingredients, and to extend the monitoring to more sites across the state.

The March progress report for this project highlighted ongoing work to evaluate the susceptibility of the different color morphs of ACP found in Florida to a range of modes of action represented by approved ACP pesticides. The three morphs showed variable levels of LD_{50} doses in response to exposure to one of four tested modes of action. The report also showed that levels of detoxifying enzymes present in the yellow/orange, blue/green, and dray/brown morphs were detectable, indicating a correlation among these subpopulation components in their response to pesticides. Monitoring of field populations continues in this project.

c) Results of earlier work by Sharma et al. (#860 Sharma) indicated that colored dyes, when added to a foliar substrate, could influence ACP attraction to the plant. The particle film containing specific spectral dyes showed repellency potential in limited experiments. In early 2017, IFAS submitted a requested proposal (#16-026C Vincent) to evaluate this potential under field conditions, comparing the impact on ACP populations and infection against standard kaolin clay (Surround ®) treatments and in comparison to other ACP management programs. The project was approved by CRDF and the March 2017 progress report indicated that mixing and adjuvant strategies are being worked out in anticipating of installing the trial in the field in April.
d) The project that is evaluating metalized reflective mulch (MRM) installed at planting of grapefruit trees in the Indian River (#16-011C Adair) continues to report differences in ACP numbers (eggs, nymphs, and adults), with fewer ACP in MRM plots versus those either treated with compost or with bare ground. The second season of fruit production is nearing end, with yield data expected as the project is completed. The project was extended for this past year to allow both yield data from the second year of production on the experimental trees as well as to evaluate economics of the MRM installation against early returns. The final report on this project is expected as the yield results are analyzed.

ACP research projects focused on developing knowledge towards interventions continued during this period as well. These projects included #15-021 Pelz-Stelinski, which has as an aim the correlation between CLas transmission and the ACP immune system status. Potential points of intervention may result from this work. Likewise, Bonning (#711) is searching for endotoxins from Bacullus thuringiensis (BT) bacteria with activity against ACP. Widely used against other insect groups, this approach has led to discovery of 2 endotoxins to which ACP appear to react. While this project is nearing its end, two circumstances indicate that this work will continue. First, Dr. Bonning has been hired by UF, IFAS, Department of Entomology into an Endowed Chair position, and thus will be more integrated into Florida-based work on HLB than from Iowa State. In addition, Dr. Bonning was awarded a USDA, NIFA, SCRI Citrus Disease Research Project in the most recent cycle, providing continuity to her current project from CRDF.

ACP Management Projects Transferring to USDA, APHIS HLB MAC Funding

A new HLB MAC project is underway to integrate ACP behavior-affecting strategies into field-deployable population disruption, attract and suppress, and monitoring tools, bringing together the results of a robust set of projects on ACP biology and behavior that have been supported going back to around 2000, following initial introduction of the ACP into Florida

Additional Effort by the USDA, APHIS HLB MAC program to support research to answer a fundamental grower question relating to the importance of super-infection (more than one ACP inoculation) on either establishment of CLas in healthy trees or the decline brought about by the disease cycle. The hypothesis that supplemental ACP inoculations of infected trees accelerate or

otherwise exacerbate HLB disease and tree decline has been investigated by several groups (Hall - ARS; Rogers – IFAS; and Killiny – IFAS). Preliminary indications from these greenhouse and field experiments indicate a collective increase in disease with additional infections, but the details remain elusive. During the current quarter, USDA, APHIS HLB MAC invited a team proposal from the core group, including representing entomologists from Texas Rio Grande Valley and from California. The group is preparing a work plan for appropriate field experiments in Florida and Texas to investigate single versus multiple or time series infection of citrus trees with CLas via vector transmission. Various disease conditions and tree ages will serve as starting points for these tests, and results of these experiments will assist in difficult ACP management decisions in Florida at present

2. Engage registrants and regulatory entities in need for label modifications
   Several discussions were held in the 2nd quarter FY 2016-17 regarding the potential for Aldicarb pesticide to be considered for use in Florida citrus. A registrant has resumed manufacture and has marketed the product in other states on other crops. Discussions with the registrant and distributor has identified a series of questions and issues that surround re-introduction of Aldicarb into Florida citrus. During the third quarter, registrants discussed field demonstrations in Florida of the impact of Aldicarb on citrus tree health in groves impacted with

   Current review of use of pyrethroids by EPA also is an issue of importance to continuing use of diverse classes of pesticides. Florida growers and support organizations are participating in discussions and information gathering in support of the continued need for diverse tools for ACP management.

   Reregistration of pesticides in the neonicotinoid group has commenced, with Imidachloprid being the first active ingredient being reviewed. Due to widespread use of this class of insecticides in agriclulture, landscape management and other arenas across the United States.

3. Continue participation in pesticide stewardship activities
   15-038C Resistance Monitoring: Dr. Stelinski continues to monitor at locations around the state for resistance development. Discussion among the researchers and growers have highlighted the importance of rotation of active ingredients. This topic is becoming more contested as growers are attempting to reduce ACP suppression costs and resort to lower cost spray materials.
   15-036C Distribution and behavior of pesticides targeting ACP: Correlating pesticide residue analysis with psyllid feeding to improve protection of young trees is providing results that will improve our understanding of the movement and retention of pesticides on/in targets and inform adjustments to spray recommendations, both timing and choice of materials. Portfolio review in Q3 will include identifying needs for moving this project into the next phase, and a broader approach has been suggested to meet the current needs.

4. Continue to support CHMA implementation of ACP and other HLB management tools
   CHMA meetings, further emphasis on APC suppression are continuing under leadership of Brandon Page and Dr. Rogers. These meetings addressed the increase in ACP populations in mid-summer cycles of CHRP scouting.

The third phase of FDACS abandoned citrus grove removal has proceeded with most targeted groves being removed. However, the current status of fire danger across Florida has prevented the burning of tree residue until rains start later in Spring.

CRDF plans to coordinate evaluation of herbicides for disabling unmanaged groves proceeded with treatment of two locations with variable volumes of Reglone spray. Follow-up evaluation is underway by the PIs (#16-027C) and reports of some regrowth have been provided. Regulatory considerations over this herbicide may limit its utility for managing abandoned citrus.

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

Significant Meetings and Conferences:

During the first quarter of 2017, the Fifth International Research Conference on HLB was held in Orlando. More than 40 presentations were made on the topic of ACP biology and management, with many CRDF-funded projects being highlighted. In addition, research being conducted in other countries and across US citrus states was presented. The UF, IFAS extension team is preparing presentations to summarize results presented during the HLB Conference. This meeting will be held in April at the University of Florida, IFAS, Citrus Research and Education Center.

Also during this quarter, the Indian River Citrus Show was held in the Indian River (January 2017) and the Florida Citrus Grower Institute is planned for April 4, 2017 at the Avon Park Campus of South Florida State College. Both of these meetings had scheduled updates on aspects of ACP management.

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2. ASIAN CITRUS PSYLLID VECTOR INTERVENTION

Project Title: 2b. RNAi Molecules/Psyllid Shield

Narrative of Progress against Goals:

Obj. 1 - Complete planning for and initiate field trials to begin in spring 2017

The principal goals of the 3- year field trials are as follows:

- Determine if selected target sequences that were found effective in a greenhouse environment against ACP when expressed in plants using the Citrus Tristeza Viral Vector (CTVvv) are effective in controlling ACP when delivered by CTVvv under field conditions
- Familiarize the regulatory agencies with the technologies and 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.
- Based on the results of this field trial, a decision will be made regarding a Phase 2 area-wide "Psyllid Shield" field trial.

During the quarter the following activities were performed:

- Insect Rearing: A Southern Gardens team visited labs of Dr. Hall and Dr. Dawson to observe and learn about how they are rearing ACP, and is setting up its insectary with the needed cages, etc. with a plan to begin rearing ACP in early to mid-April.
- Tree Inoculation: In February, 240 trees were inoculated with the CTVvv containing six constructs: JHE, FAOMT, Cathespin F, Rieske, Calcium Binding Protein and Osiris. Southern Gardens is currently managing the trees to promote the CTVvv to move systemically throughout the whole tree. Two of the constructs appear to be unstable. At this time, Southern Gardens believes it can redo one of the two genes, while the other is a question mark. Plans are to plant the other 4 constructs in early to mid-May and plant the others when they have trees with a stable insert/graft.
- Regulatory: Permits have been submitted and accepted by both APHIS and EPA. Due to changes in a few constructs, Southern Gardens is amending the permit to comply more accurately with the constructs. These amendments will not affect planting plans.

Obj. 2 - Continue outreach to other companies engaged in RNAi research and product development for potential collaborations.

This is an ongoing effort. Nothing new to report this quarter on communications with either Forrest Innovations or AUM LIfeTech. Program management is continuing to look for other companies that are conducting RNAi R&D and product development to contact for possible relevance to HLB.
Obj 3 - Continue to monitor ongoing RNAi research, including nuPsyllid project, for insights that may be applied to ACP intervention through Psyllid Shield.

At the November 2016 CRDF Board meeting, Dr. Turpen was requested to develop a white paper to communicate the expected outcomes from the nuPsyllid project as it comes to a conclusion in August, as well recommendations as how the progress will be continued. Dr. Turpen provided a draft of the report for review and comment by Committee in March. Based on feedback, he is making final revisions to the white paper.

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

This is an ongoing effort, and there is no new information to report on this front.
Commercial partners will be needed for follow-on work to the phase one field trial described above. This includes support for a Phase 2 area wide "Psyllid Shield" field trial, as well as supporting regulatory, product development and other work needed to bring products to market.
CRDF continues to be prepared to facilitate, accelerate and incentivize corporate action and is prepared to provide regulatory, commercial delivery and other support, as appropriate, to candidate partners.

Significant Meetings or Conferences:
None.

Obstacles Encountered and Breakthroughs:
During the quarter, Southern Gardens, CRDF, USDA were all engaged in discussions to reach alignment on ownership and management of the Intellectual Property associated with the 6 constructs that are planned for use in the RNAi field trial. After review with the CRDF Board, CRDF will be communicating its position on these issues in written communications to all stakeholders.

Other Information:
This project has effectively combined the results of RNAi research into psyllid control with Psyllid Shield modeling to create the information needed to develop the recommendation to proceed with a two-phased field trial approach. It is expected that enough data would be available by the end of year 2 to make some educated guesses as to the effectiveness of the RNAi constructs to begin planning for larger scale trials. The larger scale field trials would be designed to further validate the technology and to collect the data necessary for a full section 3 registration.

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3. Citrus Host Intervention

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

Project goal(s)

1. Track ongoing research on soil microbes and their role in HLB and tree health 2. Conduct field trials to test commercially available naturally occurring microbes 3. Provide communication on project goals, progress and results to CPDC, CRDF and growers.

Narrative of Progress by Project Goals:

2. Conduct field trials to test commercially available naturally occurring microbes.
The overall goal of the project is to screen candidate antimicrobials and deliver best performers through field trials to commercial use. This study is a side-by-side comparison of these 5 soil-applied commercially available products (+water control) as well as organic mulch as recommended by growers. We are testing the **hypothesis** that soil-applied products will mitigate the effects of HLB on tree health and yield. We expect that differentiation in tree health and disease status will appear in year 2, and after 3 years, we will have valid information on the true impact of these treatments on tree health, disease rating, HLB status, foliar nutrition, root density, yield and fruit quality.

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 depending on product label) 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**. All required field work at all 3 sites is on schedule and most of the data are complete.

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. Each of the 3 trials consists of the 6 treatments of 20 trees, 4 reps = 24 plots of 20 trees = 480 trees at each site plus. Sub-plots of 3 trees within each of the 24 plots = 72 trees mulched at each site. There are 6 unmulched sentinel trees in each treatment, replicated 4 times = 24 trees per treatment plus 1 mulched sentinel tree in each treatment replicated 4 times = 4 mulched trees per treatment. Thus, there are 28 sentinel trees times 6 treatments = 168 total measurement trees at each site.

Contracted crop consultants are applying product treatments plus mulch, monitoring canopy volume and Decline Index (DI), photographing sentinel trees and taking leaf samples for PCR and nutrient analysis. At harvest, total fruit weight fruit is evaluated and samples are taken for juice quality analysis. Soil cores were sampled in Dec 2014, 2015 and 2016 to determine root density. Soil amendment effects on root densities were not remarkable in 2014 and 2016 but were summarized in the June 2016 report. Root density data from 2016 have been analyzed and are summarized at the end of this report. This quarterly report (Mar 2017) follows almost 3 years of soil amendment treatments and focuses on final tree measurements, PCR evaluations, yield from the 2017 harvest and juice quality.

Site Results to Date:

Ridge Site, Balm FL: Valencia/Swingle trees are 17 years old. After 3 years of treatments, there were no effects of the mulch treatment so + an – mulch treatments were combined for n=28 trees per treatment. By 2017, there were no treatment effects on tree height, canopy volume, fruit yield (from the 4/13/17 harvest) or CT values as all trees were HLB positive with CT ranged from 24-27. Fruit yields (1.6-1.9 boxes per tree) were relatively low for mature trees with canopy volumes of 26-35 m^3. The Serenade treatment had a higher disease index (DI) than the untreated control but the other treatments had similar DIs.

TH_m_1_30_17			CV_m3 1_30_17			Yield Boxes 4_13_17	
TH	Treatment		CV	Treatment		Yield	Treatment
3.7	BioFlourish		35.2	BioFlourish		1.9	BioFlourish
3.7	Serenade		32.2	Serenade		1.8	Aliette
3.5	EcoFriendly		28.6	Quantum		1.8	Serenade
3.5	UT Cont		28.1	EcoFriendly		1.7	Quantum
3.4	Aliette		27.9	Aliette		1.6	UT Cont
3.4	Quantum		26.7	UT Cont		1.6	EcoFriendly

		DI_3_2_2017			CT_11_11_16			CT_1_13_17	
		DI	Treatment		CT	Treatment		CT	Treatment
	A	15.5	Serenade		25.4	UT Cont		27.8	UT Cont
B	A	13.6	BioFlourish		25.2	EcoFriendly		25.5	BioFlourish
B	A	13.5	Quantum		25.1	Serenade		25.0	Aliette
B	A	11.6	EcoFriendly		25.1	Quantum		24.9	EcoFriendly
B	A	11.2	Aliette		24.6	BioFlourish		24.8	Quantum
B		9.6	UT Cont		23.9	Aliette		24.4	Serenade

Juice quality was analyzed from the 4-13-17 harvested fruit samples using standard state test house methods and none of the measured variables were significantly affected by treatment.

Ridge site juice quality 2017

Treatment	n	AvgFtWt_l	% juice	Brix	% Acid	Ratio	LbSol_Box	Color
Bioflourish	8	0.34	62.66	10.82	0.91	11.99	6.10	38.84
Ecofriendly	8	0.35	63.28	10.96	0.91	12.14	6.24	38.29
Serenade	8	0.38	62.55	11.23	0.86	13.36	6.32	38.19
Aliette	8	0.35	62.67	11.06	0.88	12.71	6.24	38.18
Quantum	8	0.34	62.69	11.24	0.88	13.10	6.35	38.19
Wat Control	8	0.34	62.99	10.85	0.93	11.82	6.16	38.25

Soil cores from Dec 2016 were analyzed for root density and data are summarized at the end of this report.

East coast, Indian River site: Valencia/Swingle trees are 6 years old. After 3 years of treatments, there were no effects of the mulch treatment so + an – mulch treatments were combined for n=28 trees per treatment. By 2017, there were no treatment effects on tree canopy volume (9-11 m3) or yield (1.5-1.8 boxes per tree. The BioFlourish treated trees had a higher DI rating than the Serenade trees but none of the treatment DIs differed from the Untreated Control trees. All trees were HLB positive having a CT less than 31 (23-28). Quantum treated trees had a higher CT than Ecofriendly trees but none of the treatment CTs differed from the Untreated Control trees.

CV (m^3) 2_1_17			Yield (boxes) 2_21_17					DI 12_16_16					CT 1_13_17	
CV	Treatment		Yield	Treatment				DI	Treatment				CT	Treatment
11.6	BioFlourish		1.8	Aliette			A	16.1	BioFlourish			A	28.3	Quantum
10.8	EcoFriendly		1.8	Quantum		B	A	15.8	Quantum		B	A	26.4	Unt Cont
10.4	Aliette		1.6	BioFlourish		B	A	14.7	Unt Cont		B	A	24.9	Serenade
10.3	Quantum		1.6	EcoFriendly		B	A	14.6	Aliette		B	A	24.9	Aliette
10.0	Serenade		1.6	Serenade		B	A	14.4	EcoFriendly		B	A	24.3	BioFlourish
9.2	Unt Cont		1.5	Unt Cont		B		12.5	Serenade		B		23.6	EcoFriendly

Juice quality was analyzed from the 2-21-17 harvested fruit samples using standard state test house methods and none of the measured variables were significantly affected by treatment.

East Coast, Feb 2017									
2_21_17			2_27_17						
Avg Frt Wt	Treatment		%Juice	Treatment		LbSol / box	Treatment		Juice Color	Treatment
0.38	Aliette		0.62	Serenade		5.57	Aliette		37.5	Ecofriendly
0.37	Unt Cont		0.62	Ecofriendly		5.50	Serenade		37.5	Aliette
0.36	Ecofriendly		0.62	Aliette		5.49	Ecofriendly		37.2	Bioflourish
0.36	Serenade		0.61	Bioflourish		5.46	Bioflourish		37.1	Untrt Control
0.34	Bioflourish		0.61	Quantum		5.46	Quantum		37.0	Serenade
0.34	Quantum		0.60	Untrt Control		5.35	Untrt Control		36.9	Quantum

Brix	Treatment		% Acid	Treatment		Ratio	Treatment
10.06	Aliette		1.04	Quantum		10.9	Aliette
9.94	Serenade		0.95	Bioflourish		10.7	Untrt Control
9.93	Quantum		0.94	Serenade		10.7	Ecofriendly
9.91	Bioflourish		0.94	Untrt Control		10.6	Serenade
9.89	Ecofriendly		0.94	Aliette		10.5	Bioflourish
9.86	Untrt Control		0.93	Ecofriendly		9.6	Quantum

Soil cores from Dec 2016 were analyzed for root density and data are summarized at the end of this report.

A grower field day was held on Dec 6, 2016 to highlight non-significant treatment effects on leaf nutrition, canopy volume and fruit yield at the East coast (Indian River) site. There were 42 people in attendance from all over the State.

SW FL Duda site: Valencia/Swingle trees are 11 years old. Current canopy (CV) volume measurements and disease index (DI) ratings will not be completed until late April 2017 so CV and DI data from the previous quarterly report (Dec 2016) have been added for completeness. There was no significant treatment or mulch effect on DI (20.2-21.6) on 12-28-16. On 3-30-16, Bioflorish and Aliette treated trees had the largest canopy volumes whereas the water treated control and Serenade trees were the smallest.

Canopy volume (CV, m^3) 3_30_16

DMRT	CV	N	Trtment
A	37.6	28	Biofluor
A	36.4	28	Aliette
B	31.7	28	Quantum
B	28.5	28	Ecofriend
C	23.6	28	WatCont
C	23.5	28	Serenade

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3. HOST PLANT INTERVENTION

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

Narrative of Progress against Goals:

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

a) Project 940c: Propagation of Rootstock Tree Production in Greenhouses by Seed, Stem Cuttings, and Tissue Culture to Accelerate Budded Tree Production for Out planting

Reports indicate that guidelines for the production of several commercially available rootstock genotypes through seed, cuttings or tissue culture have been successfully developed.
The project has been successful in propagating many rootstock selections for use in research and commercial nurseries. A summary of the work will be developed and distributed to the industry. The nursery industry will communicate further questions to CRDF for the development of a follow-up proposal addressing current issues with propagation techniques, propagation efficiency, and genotype specific protocols.

A short project to determine the most efficient method to propagate mature scions as a pre-cursor to a small project assessing HLB tolerance in grapefruit volunteer seedlings has begun. The purpose is to support production of starting material for a field trial of selected grapefruit volunteer seedling trees to assess HLB tolerance in a grower trial

b) Grower Field Trials

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 have been finalized, and the field map is under review. Plans are in place to collect leaf tissue samples and HLB disease index data on selected scions to have a baseline measure of HLB incidence and severity in the field.

c) Transgenic field trials

Researchers involved with projects 754 and 15-020 are collaborating with others to develop a pre-proposal for a field trial with side by side comparisons of transgenic scions to assess field performance and HLB tolerance at the USDA-ARS secure permitted site in Ft. Pierce, Fl. The project is in the planning stages to propagate plants, acquire necessary permits and outline work plans for data collection.

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.

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

The Research Management Committee communicated to researchers involved in plant improvement at the USDA/ARS (Florida) and the University of Florida the need for data pertaining to field trials that or ongoing to facilitate nest steps in identifying field trials containing advanced HLB tolerant or resistant germplasm with potential for commercialization. Following receipt of this information, Dr. Hatcher will coordinate with rootstock and scion teams to analyze the information available.

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

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 first 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 per side of the crown, with 0 denoting no visual symptoms and 5 severe decline on more than 80% of the canopy. The maximum possible score for DI in these trials is 10. Leaf tissue samples were collected for PCR analysis or bacterial titer.

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 GLIMMIX of SAS (SAS Institute Inc, 2004) with the appropriate comparisons to test for differences among rootstock means.

All the rootstock data collected was 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.
Data analysis for the two ridge sites (BHG and Peace River) exclude UFR-16 which was planted late at both locations and cannot be fairly compared to the other rootstocks. Despite the two planting dates of UFR-3 inclusion or exclusion from data sets did not affect the results and so it was left in the data sets for analysis.

Results for data that has previously not been reported 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 in 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 1). Differences in HLD disease index (HLB DI) and PCR cycle threshold were not statistically significant. Rootstock groupings for each variable can be separated by the best performing rootstocks in order US_942, US_812, UFR_4, Swingle, UFR_2, and poorer performance (UFR_3). Differences in HLD disease index (HLB DI) and PCR cycle threshold were not statistically significant.

Table 1 CRDF Duda site rootstock trial horticultural traits, HLB Disease index (DI) and PCR Cycle Threshold means ± standard error of the mean data collected in Spring 2017

Rootstock	Canopy Volume (m^3)	TCSA (cm^2)	Tree Height (cm)	HLB DI[a]	PCR Cycle Threshold
Swingle	4.30 ± 0.56 ABC	25.70 ± 2.24 BC	183.25 ± 9.41 AB	1.51 ± 0.69	36.65 ± 2.79
UFR_16	3.68 ± 0.68 BC	25.21 ± 3.78 CD	173.5 ± 9.26 BC	1.28 ± 0.57	38.29 ± 1.84
UFR_2	3.85 ± 0.55 BC	23.70 ± 1.76 CD	173.35 ± 7.62 BC	1.3 ± 1.31	35.86 ± 3.06
UFR_3	2.86 ± 0.66 C	18.93 ± 3.21 D	155.6 ± 26.276 C	1.98 ± 0.73	36.23 ± 2.96
UFR_4	5.26 ± 0.5 AB	28.11 ± 2.43 ABC	197.58 ± 11.78 AB	1.08 ± 0.67	36.69 ± 2.53
US_812	5.25 ± 0.72 AB	32.10 ± 2.61 AB	192.68 ± 8.62 AB	1.03 ± 0.59	37.56 ± 2.10
US_942	5.78 ± 0.76 A	34.26 ± 2.94 A	204.2 ± 10.28 A	1.75 ± 0.60	36.66 ± 2.49

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.

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 2). It should be noted that UFR_16 means are presented for information only and should not be directly compared to other rootstocks because it was planted 11 months later at this site. Similar to the other sites there are generally two groupings of rootstock performance with US_942, US_812, US_897, and Carrizo outperforming UFR_2 and UFR_3. HLB disease index and PCR cycle threshold (CT) were not statically significant among rootstocks. The lower PCR CT detected in UFR16 compared to the other rootstocks is likely due to the later planting date.

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

Rootstock	Canopy Volume (m^3)	TCSA (cm^2)	Tree Height (cm)	HLB DI[a]	PCR Cycle Threshold
Carrizo	2.19 ± 0.32 BC	14.93 ± 1.83 AB	149.53 ± 7.76 AB	0.90 ± 0.18	28.22 ± 1.38
UFR_16	0.40 ± 0.05 E	3.09 ± 0.15 E	101.93 ± 3.04 D	0.70 ± 0.28	39.74 ± 0.17
UFR_2	1.74 ± 0.18 CD	9.89 ± 0.73 CD	135.48 ± 4.22 BC	1.05 ± 0.17	29.29 ± 0.57
UFR_3	0.91 ± 0.27 DE	6.35 ± 1.76 DE	116.08 ± 8.86 CD	1.48 ± 0.41	30.15 ± 1.04
UFR_4	2.38 ± 0.15 BC	14.06 ± 0.77 BC	146.95 ± 2.88 AB	1.78 ± 0.65	26.29 ± 0.79
US_812	3.11 ± 0.16 AB	16.17 ± 0.40 AB	164.43 ± 3.91 A	1.37 ± 0.34	25.23 ± 1.13
US_897	2.48 ± 0.19 ABC	13.71 ± 0.91 CB	156.15 ± 4.26 AB	1.15 ± 0.36	29.77 ± 2.24
US_942	3.48 ± 0.33 A	19.36 ± 0.39 A	170.20 ± 2.54 A	1.03 ± 0.39	27.54 ± 1.29

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.

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 PCR cycle threshold but not HLB disease index (Table 3). UFR_16 was planted ten months later at this site so performance data is provided for information purposes and should not be used in direct comparison with other rootstocks. US_942, US_812, Sour and UFR_4 had similar canopy volume performance while UFR_2 and UFR_3 smaller canopy volumes. Rootstock TCSA split into three groups with US_942, Sour and US_812 had the largest TCSA and UFR_3 the lowest. Tree height rootstock performance followed a similar pattern except UFR_4 having comparable tree height to US_942, US_812, and SOUR. There were no remarkable differences in HLB DI symptoms or PCR cycle threshold although all rootstocks displayed some degree of infection.

Table 3. CRDF BHG site rootstock trial horticultural traits, HLB Disease index (DI) and PCR Cycle Threshold means ± standard error of the mean data collected in Spring 2017

Rootstock	Canopy Volume (m^3)	TCSA (cm^2)	Tree Height (cm)	HLB DI[a]	PCR Cycle Threshold
Sour	1.45 ± 0.22 BC	11.09 ± 1.69 A	130.45 ± 9.71 AB	0.58 ± 0.36	35.63 ± 3.17 AB
UFR_16	0.31 ± 0.03 E	2.51 ± 0.19 C	96.30 ± 2.92 D	0.30 ± 0.47	39.25 ± 1.63 A
UFR_2	1.01 ± 0.16 D	6.68 ± 0.49 B	119.75 ± 5.65 BC	0.48 ± 0.14	36.58 ± 2.37 AB
UFR_3	0.68 ± 0.08 D	4.15 ± 0.28 C	111.53 ± 2.917 C	0.70 ± 0.38	39.15 ± 1.16 A
UFR_4	1.38 ± 0.15 C	7.76 ± 0.74 B	131.25 ± 6.84 AB	0.68 ± 0.34	36.89 ± 2.36 AB
US_812	1.76 ± 0.38 AB	10.97 ± 1.08 A	137.35 ± 8.847 A	1.00 ± 0.45	33.71 ± 2.09 B
US_942	1.84 ± 0.20 A	12.03 ± 0.48 A	140.78 ± 5.792 A	0.60 ± 0.39	35.34 ± 2.76 AB

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.

Obj. 6- Communicate progress and results of evaluation of rootstocks to industry

Significant Meetings or Conferences:

Dr. Hatcher gave a presentation at the IRCHLB meeting in March outlining CRDF's mission to support and facilitate the development and delivery of HLB tolerant or resistance scions and rootstocks to the industry.

Obstacles Encountered and Breakthroughs:

None

Other Information:

None

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3. Citrus Host Intervention

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

Project Goals for FY2016-2017

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.

Narrative of Progress Against Goals:

Obj. 1a – Continue Agrobacterium and biolistic transformation with genes to confer disease tolerance to HLB and canker as a service

In the last quarterly report, we described the significant decrease in productivity with the move to the packing house while the AC in the lab was being repaired.

With the move back into the lab, the contamination experienced in the packing house has decreased, but a new issue has surfaced in the growth facility. All mature scions and rootstocks were synchronized in flowering and the trees flowered for three months. This decreased the quality of the budwood because the tissues were too old. This is the first time the lab has experienced this issue.

After consulting with Drs. Albrigo and Castle, high analysis N fertilizer was applied, and mother trees were pruned and watered to encourage vegetative growth. Dr. Pena said the flowering in spring was normal and suggested re-budding all experimental material to reinvigorate it. He also recommended renewing the mother trees every 5 years. This has been completed and the lab now has new, more vigorous mother trees and experimental material.

Because of this delay, the lab is only screening high quality putatively transformed shoots at this time. Approximately 65 mature citrus transgenics have been produced this fiscal year to date.

Approximately 47 immature citrus transgenics were produced last quarter with a gene intended to increase organogenesis of mature citrus. Mature buds will be budded onto the immature transgenics in hopes of further reinvigorating mature tissue.

Obj. 1b – Biolistics: progress will be made in optimizations for mature citrus scion

During the quarter, approximately 30 immature transgenics were produced using PMI selectable marker and biolistics. PMI is an alternative to antibiotic selection. It also significantly decreased the number of non-transgenic escaped shoots. This technology is ready to be applied should the demand arise.

Obj. 1c – Determine which of the micro-grafting steps can be bypassed altogether by growing explants in bioreactors for elongation of shoots and secondary grafting

During the quarter, efforts continued to shorten the time involved in transgenic plant production. The goal remains to achieve this objective by June 2018.

Obj. 1d – Compare genes thought to enhance shoot production/transformation efficiencies and apply pre-treatments to increase organogenesis in mature rootstock

In the last quarterly report, we reported that the lab identified a cDNA that dramatically increases mature scion transformation efficiencies and began a process to investigate whether it will increase efficiencies in all cultivars. During the quarter, the in-house expression vector designed to increase efficiencies has been completed and is being tested in mature scion transformation.

The lab received 3 stable transformation vectors from Dr. Yi Li. Dr. Li (Project #16-001) has been testing the effects of proposed genes on both stable and transient transformation efficiencies of both juvenile and adult citrus tissues. One vector will hopefully increase transformation efficiencies of mature scions and the other 2 might increase micrografting successes. The immature transformation lab (Orbovic) will also be given these vectors to see if they increase his transformation efficiencies. Transient expression vectors will not integrate into the genome, thereby avoiding the addition of unnecessary genes.

The lab has already obtained 12 immature transgenics with the 2 constructs designed to increase micrografting efficiencies of mature shoots

Obj. 1e – Determine efficiencies of PMI selection in biolistics and Agrobacterium-mediated transformation compared to nptII.

MCTF continued its investigation of whether Phosphomannose isomerase (PMI) selectable marker will be useful for mature citrus transformations. The focus was on manipulating mannose concentrations to determine impact on shoot regeneration. Discussion of Objective 1b describes transgenics produced using PMI selectable marker and biolistics.

Obj. 2 – Test a more sensitive, non-destructive screening process to increase throughput

The current process uses a colorimetric substrate (GUS) histochemical assay that is labor intensive, tedious and destructive to tissue, and produces a visible blue stain as a marker. The lab has been evaluating a new screen that is more sensitive and less destructive, using fluorescent MUG as an alternative substrate to GUS for fluorometric detection.

The lab has set a goal of June 2017 to complete evaluation to determine if shoots survive the MUG application and subsequent grafting steps, and whether there will be auto-fluorescence in non-transformed shoots, i.e. false positives.

Obj. 3. Test new breeder lines using standard tissue culture protocols to determine whether optimizations are necessary.

The facility continues the process of introducing new breeder lines in which to produce transgenics. During the quarter, Dr. Grosser's new breeder lines were introduced as disease-free shoot tip grafts (STGs) and were being trialed in transformations. However, the OLL8 line will need to be re-transformed due to some misinformation being provided that led to the wrong tissue culture protocol being used the first time.

The above builds on work already accomplished in providing Kurhaski, which is similar to Carrizzo but with some nematode tolerance; and Glen Naval sweet orange cultivar, which is pollen sterile. to Drs. Grosser and Dutt. Mandarin and pummelo were also introduced for Dr. Wang.

Obj. 4 – Increase throughput of budded plants in the growth room

This remains a major team effort. Measures are being pursued such as increasing planting density using citrus pots where possible, and, after budding, leaving the bud stick attached to scion to accelerate growth.

Significant Meetings/Conferences/Publications

A manuscript (25% funded by CRDF and 75& by CRB was accepted for publication in Plant Cell, Tissue and Organ Culture (PCTOC). Y. Acanda, M. Canton, H. Wu, and J. Zale. Kanamycin selection in temporary immersion bioreactors allows visual selection of transgenic citrus shoots.

Obstacles Encountered and Breakthroughs

The contamination issues have been resolved with the move of the lab back into its quarters. The synchronized flowering issue described in 1a. was addressed during the quarter.

Other Information

FDACS has collected samples from the labs new mother trees for annual disease indexing. It will take months to receive the results.

MCTF remains an important element of the overall pipeline encompassing both conventional breeding and genetic transformation, from inception, to field testing, to scale-up and delivery to growers. MCTF's role in this overall process is tied to CRDF efforts address the overall process for HLB host resistance and tolerance, including side-by-side field testing of the most promising candidates and delivery to Florida growers.

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4. Other Citrus Diseases

Project title: 4a. Post-Bloom Fruit Drop

Narrative of Progress against Goals:

Obj. 1 - Summarize grower experiences in suppressing PFD during 2016 epidemic year

A survey for data collection was developed to evaluate severity of PFD in groves and CRDF has since surveyed twenty-one blocks. Data was collected from twenty trees per site. Fruit and residual fruit calyx buttons within a 0.5 square meter frame was counted twice on each side of the tree (4x total) and information on PFD treatments was collected from the growers. The goal of this survey was to detect trends that led to more or less PFD in specific groves and identify effective treatments. In the end, no effective treatment could be identified because not enough data could be collected. A final report is in progress.

Obj. 2 - Evaluate PFD management tactics under field conditions

The ongoing project titles “Enhancement of postbloom fruit drop control measures” was initiated in March 2016. This project is evaluating the efficacy and economics of PFD treatments, evaluating the period of efficacy of Luna Sensation during flowering, and determining if the flowering period can be narrowed using plant growth regulators, to eliminate offseason bloom. Applications were made in the 2016 season and will continue in 2017.

Results: In 2016 the navel field trial results show that all treatments are better than the untreated control. Topsin M did not perform better than the strobilurin containing fungicides and Ferbam is best when mixed with other fungicides. Applications were made on the PFD model on March 16 and 22nd.

Valencia trials had fungicides applied on March 17, 23 and April 1st based on the PFD model. All treatments were better than the untreated control. Topguard with Ferbam improved performance and the tree best treatments all had a mixture of strobilurin and DMI fungicides.

This data was presented by Megan Dewdney at the Postbloom Fruit Drop and Disease Management Seminar on February 8th in Arcadia, FL. Other researchers who gave talks at this event were Dr. Geraldo Silva & Dr. Franklin Gehlau from Fundecitrus, Araaraquara Brazil and Dr. Natalia Peres UF/IFAS.

The PGR trials have been initiated, this objective was added in the second year of this project.