CRDF Commercial Product Delivery Sub-Project Progress Report FY 2015-16
Quarter Ending December 31, 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.

One new company has been identified this quarter and materials are being tested *in vitro*. No new materials have been identified through suggestions submitted to the "solutions inbox" (solutions@citrusrdf.org).

CRDF-funded research projects by university researchers on bactericides are all on track. Of the research service agreements for the evaluation of bactericides, project 780C, "Evaluation of Soil-Based Antimicrobials as Control Agents Against HLB", has ended this quarter and will not be renewed. No bactericides have been identified from this assay to be moved into the assay pipeline. The remaining three assay RSA projects are on track.

The CRDF project 907, a novel zinc-based bactericide development, greenhouse screen and field evaluation, was set to expire this quarter, but this project was given a no-cost extension to complete crop destruction activities and payments.

A white paper will be available in February 2016 describing bactericide use in the past and present with an emphasis on materials used traditionally for 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 and will be available on the CRDF website and as a supplement in the Florida Citrus Mutual Triangle newsletter.

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. Almost two hundred materials were tested and all are from companies capable of producing a product for agriculture. 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 for testing in the next quarter 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 identified as systemically infected in mid-September. Preliminary tests began to validate the positive controls for each application method (root infusion, soil drench, foliar). Controls for the soil-drench application method have been validated and several bactericide candidates will be completed in the next quarter. Development of the foliar and infusion method controls continues. 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.

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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.

Adjuvants continue to be evaluated for use in assays. Several proprietary adjuvants are being used in the biopesticide/"minimum-risk" field trial that should increase the uptake of these types of materials.

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, the CPDC project (15-031C), examining the ability of a laser treatment to facilitate bactericide movement into the citrus phloem was initiated in July 2015. Greenhouse evaluations continue and new detection methods have been developed to evaluate the level of chemical uptake and movement in the plant.

Project 15-048C evaluating efficacy of trunk injection as a treatment method was approved in October 2015 and was initiated this quarter. The field site of young (4-5 year old) Hamlins was selected and plots have been laid out. Preliminary evaluations will take place in January and early February and treatments will begin in early March. The first treatment applications will be timed based on when root and vegetative flushing takes place. This timing is based on new data on root flush timing of HLB infected trees as well as the timing of full bloom.

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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 Board approved a bid for the biopesticide/minimum risk field trial in October 2015, a site was selected and PCR sampling was completed in this quarter. Blocks will be set-up, pretreatment evaluations will be completed in early February and bactericide applications will take place in mid-February.

The three AgroSource projects are on track, a quarterly report was received and the quarterly meeting was held. Year two of the projects runs through June 2016.

The Nufarm residue study is making progress; a report was submitted for CRDF project manager review.

Both of these company's products are part of a Section 18 (FIFRA) exemption application that was submitted to EPA in early December. This petition is for the use of oxytetracycline hydrochloride, oxytetracycline calcium and streptomycin sulfate on citrus in Florida. EPA is in the process of reviewing the application and a decision is expected in early March 2016. FDA and the CDC are also involved in the review of these materials.

Data from the Company C field trial continues to be collected. This trial was initiated in August 2014. Project managers are preparing data from the first year of the trial for analysis. This trial is scheduled to run for two years.

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.

New field trials will be developed as new bactericides are identified. EPA may require studies upon approval of the Section 18, these will be developed as necessary. Trials to improve grower recommendations will also be a priority upon approval of the Section 18 petition. New field trials will be developed in the first two quarters in 2016 as bactericides and techniques are identified as targets for study.

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 work plans. No other document has been provided this quarter, describing progress towards project goals.

The bactericide project manager presented information on the CRDF bactericide trials at the Gulf CHMA Workshop in Immokalee this quarter.

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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:

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 due 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 imidicaloprid 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 imidcaloprid 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.

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 10d 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.

<u>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</u>.
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.

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, and 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.
At this reporting period, 11 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. None of these trials are completed, but the results to date provide a glimpse of the variation of measures and tree responses. Significant additional data analyses will be available following the 2015-16 fruit harvest, providing yield, quality and other metrics.
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 11 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.
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 year one 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.
The 11 sites reported on in this period are all ongoing thermotherapy projects where tree responses to different thermotherapy conditions are being monitored. The different sites are of various aged trees and varieties. Most projects have recent post-treatment leaf samples awaiting PCR analyses. All data and observations should be considered preliminary, as monitoring tree status and data analysis are continuing.

BHG site.
Murcott /Cleo trees, planted 2007-2008, 7 to 8 years old. The 25 trees to be steam treated were hedged and topped to a height of 6 ft. prior to treatment, so they would fit inside the tent, while control trees were left at heights of 12-16 ft. Leaves for pre-treatment PCR were collected from all 30 trees on 7/29/14, and the 25 trees were treated at 126 F (52 C) for 30 seconds (sec). The 5 untreated control trees were not steamed. Leaves for post-treatment PCR were sampled from all 30 trees on 12/18/14 and 3/13/15. The average cycle threshold (CT) values from PCR were:
PCR (CT):

Treatment	7-29-2014	12-8-2014	3-13-2015
Steam (n=25)	23.5	32.0	30.2

Control (n=5)	36.6	36.8	36.1

There was an improvement in the thermally treated trees from HLB positive on 7/29/2014 to HLB negative on 12/18/2014. There apparently was some reinfection occurring by 3/13/2015 or elevation in Clas titer.

As of 12-22-15, tree height, canopy diameter, trunk circumference and DI Rating are being monitored and pictures taken. There is moderate pest pressure- Psyllids, eggs, nymphs present, citrus canker is present and the block is receiving conventional grove inputs and is generally in decent condition.

Scott

Scott Trial 3. Ray Ruby GF on Sour orange trees, 5 years old. Leaves for PCR were collected from 35 trees on 6/30/15 and 20 trees were steam treated on 7/20/15 at either at 130 F for 15 sec or at 130 F for 30 sec. There were 15 non-treated control trees, rounding out the 3 treatments in this trial.

Average pre-treatment CT values showed that all trees were HLB+; trees have been resampled post-treatment PCR and await analysis. Canopy volumes (CV) were measured on 6/30/2015 and will be repeated in early 2016. Visible disease index (DI) was evaluated periodically from June to November 2015 and harvested fruit yields were measured on 12/14/15.

PCR (CT):

Treatment	**6-30-2015**
130F 15 seconds	21.6
130F 30 seconds	25.0
Control	23.1

Canopy Volume (m^3)

Treatment	**6-30-2015**
130F 15 seconds	8.6
130F 30 seconds	9.8
Control	9.2

Disease Index (DI):

Treatment	**6-30-2015**	**7-23-2015**	**9-8-2015**	**10-9-2015**	**11-16-2015**
130F 15 sec	17.6	18.8	15.8	18.1	20.5
130F 30 sec	17.3	18.2	14.3	16.8	18.4
Control	15.9	15.0	13.5	18.7	20.1

Yield (lbs of fruit):

Treatment	**12-24-2015**
130F 15 seconds	42.4
130F 30 seconds	57.8
Control	55.1

Visible DI values all improved (decreased) during the good growing conditions of September, 2015 and trees on the 30 second treatment duration appeared slightly better in October and November periods

than either the 15 second treatment or the untreated control. Fruit yield (FrYl 12-24) appeared to be increased by the 130F 30 second treatment. Fruit were analyzed for juice quality (brix, acid, pound solids per box, etc.). There were no significant treatment effects among the measured juice quality parameters.

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 120 seconds, 132 F for 1 second (turned off immediately when temperature in canopy reached 132 degrees, or 132 F for 10 seconds. 12 trees were left untreated as a control. Initial DIs (9/4) varied little but trees appeared worse from the treatment 1 month after treatment (10/9) but all improved by 11/2. There was a higher percentage leaf drop (pct_lfdr) and leaf bleaching (LfBle) in the 132 F treatments than in the 128 F treatment or control trees. Fruit drop (FrDr) was also increased by the treatments and became greater later in the season as the grapefruit matured even in the control trees. Initial Ct values from PCR before treatment revealed that all tees had HLB. We now have DNA concentration and copy number concentrations for these Ct values. Follow up post-treatment leaf samples have been submitted and await analysis.

PCR (CT):

Treatment	**9-08-2015**
Control	28.4
128F 120 seconds	27.4
132F 1 second	25.7
132 F10 seconds	25.9

Disease Index:

Treatment	**9-4-2015**	**10-9-2015**	**11-22-2015**
Control	13.8	20.3	17.1
128F 120 sec	13.8	20.7	17.6
132F 1 second	14.6	23.1	17.8
132 F10 seconds	15.7	24.1	19.3

Leaf Drop (%)

Treatment	**10-09-2015**
Control	0.0
128F 120 sec	0.4
132F 1 second	4.6
132 F10 seconds	4.6

Leaf Bleaching:

Treatment	**10-09-2015**	**11-12-2015**
Control	0.0	0.0
128F 120 seconds	4.2	0.0
132F 1 second	20.0	5.4
132 F10 seconds	24.2	6.3

Fruit Drop counts:

Treatment	10-09-2015	11-12-2015
Control	14.1	29.8
128F 120 sec	27.2	37.9
132F 1 second	25.2	30.5
132 F10 seconds	25.6	34.3

Gapway (cooperative with Dr. Ehsani)
Hamlin/ Swingle trees, 6 years old. 18 trees were steam treated at either 55 or 60 C on 6/24/2014 but leaves were not sampled for PCR until 12/17/14. It was only possible to sample leaves pre-treatment for PCR on 6 trees that were treated at 60C for 15 sec. Based on CT values, 5 of the 6 were initially HLB + prior to treatment and 3 trees became HLB- about 6 months after treatment. Two of the initially HLB + trees remained positive and the initially healthy tree remained HLB negative.
PCR (CT) for 6 treated trees:

6-24-2014	12-17-2014		
Pre Treatment CT	**Post Treatment CT**	**Pre-Post CT**	**Change**
22	35	-13	+ HLB to -
22	40	-18	+ HLB to -
23	40	-17	+ HLB to -
22	21	1	No Chg
24	22	3	No Chg
40	35	5	No Chg

Trees were harvested in December, 2014 and juice quality characteristics were not affected by treatment.

Davis
Valencia/Swingle trees 10 years old. 24 trees were sampled for PCR on 4/6/15 and leaves have been resampled for PCR in Jan 2016. All 24 trees were steam treated on 4/9/15 at 120 F for 30 seconds. Tree trunk and canopy growth, fruit drop, and visible disease index (DI) has been monitored monthly since April 2015. Average initial CT (28) indicated the trees were HLB+. Average visible DI was relatively high in April but began improving (lower DI) right after treatment as tree appearance continued to improve through September and remain improved in December, 2015. Fruit drop was high (41 per tree per month) after treatment, but declined in the late summer and fall.

Disease Index (DI) and Fruit Drop (FrDr) counts

Date	4-6-15	4-20-15	5-8-15	6-19-15	8-10-15	9-22-15	10-19-15	12-16-15
DI	30	28	24	20	20	19	20	22
Fr Dr		41	41	21	15	7	7	14

Lykes (cooperative with Dr. Ehsani)
Hamlin / X639 trees, 4 years old. 24 trees were steam treated on 10/6/15 at 55 C (131 F) for 30 seconds and 24 trees were left untreated. Leaves were sampled for initial CT from PCR on 10/5/15. Trees in this trial uniformly had HLB disease per the PCR results. Leaves have been sampled for post-treatment PCR and are awaiting analysis. Initial Disease index (DI) on 10/5/15 declined only slightly by 11/11. Treated trees initially dropped more fruit (FrDp10-6) but dropped less fruit than the control trees by 11/11.

Citrus canker was prevalent in most trees and likely contributed to fruit drop. Initial canopy volumes (CV) were similar. Initial percentage leaf drop on 10/6 (%LD10-6) was higher in the treated trees than in control trees but leaf drop declined by 11/11. Additional post-treatment DI, Fruit drop, canopy volume and percentage leaf drop will be resampled and monitored for treatment effects. Fruit samples were analyzed for juice quality on 12/24/15, and measured parameters (average fruit weight, % juice, brix, acid, etc.) were not significantly affected by the steam treatment.

PCR Results (CT)

Treatment	**10-5-2015**
55C 30 seconds	24.1
Control	24.8

Disease Index

Treatment	**10-5-2015**	**11-11-2015**
55C 30 sec	22.6	20.9
Control	20.0	19.6

Fruit Drop counts

Treatment	**10-6-2015**	**11-11-2015**
55C 30 sec	52.2	36.9
Control	48.1	43.6

Canopy Volume (m^3)

Treatment	**10-6-2015**
55C 30 sec	14.1
Control	15.1

Leaf Drop (%)

Treatment	**10-6-2015**	**11-11-2015**
55C 30 sec	12.3	5.0
Control	0.0	0.0

Premier, Wheeler

Valencia/Swingle, 4 years old. Wheeler's Lake Wales block was the site of the December 3, 2015 field day for growers. Leaves were collected for pretreatment PCR on 6/9/2015. 15 trees were steam-treated on 6/12/15 at 122-128 F for 20 seconds, with 15 trees serving as untreated as controls. Trees were resampled for post-treatment PCR on 9/15/15. All trees were HLB+ but the treated trees had elevated CT (less bacteria) by 10/15. It was clear that the grower chose the poorer appearing trees to treat as treated trees had a lower CT and had a lower tree height (TH) in 6/9 and 9/10 but treated trees grew more by 10/9 to the extent that they did not differ from control trees by 10/20. Treated trees had consistently smaller canopy volumes (CV) than control trees. Treated trees had a higher initial DI (DI6-9) but DIs became similar as trees responded to treatment and by 10/20, Treated trees had a lower DI than control trees. Fruit drop (FrD) was unaffected by treatment.

PCR results:

Date :	6-9-2015	10-15-2015
Treatment	**Pre-CT**	**Post-CT**
Control	26	25
126F 20 sec	20	23

Tree Height Measurement (cm; * & ** = significant difference)

Treatment	6-9-2015	9-10-2015	10-20-2015
Control	184	180	196
126F 20 sec	160*	162*	178 ns

Canopy Volume (m^3)

Treatment	6-9-2015	9-10-2015	10-20-2015
Control	6	6	8
126F 20 sec	4*	5*	6*

Disease Index

Treatment	6-9-2015	7-17-2015	9-10-2015	10-20-2015
Control	14	13	14	18
126F 20 sec	18*	13 ns	15 ns	16*

Fruit Drop counts:

Treatment	7-17-2015	9-10-2015	10-20-2015
Control	2	7	7
126F 20 sec	2 ns	5 ns	7 ns

Premier, Shinn

Valencia /Swingle, 3 years old, double set. Pretreatment leaves sampled for PCR 8/7/2015 18 trees treated 8/7/2015 at 122-127 for 30 sec. and 18 trees were untreated as controls.
Treatment trees were clearly HLB+ with an average CT=24 while control trees had somewhat higher CT in the HLB questionable range of 31. Leaves have been resampled for post-treatment PCR and await analyses. Control trees had greater tree heights (TH) initially and remained taller until 12/10/15. Treated trees had greater fruit drop than controls trees in Nov (FrD11_5) but control trees had greater fruit drop in Dec. Some leaf bleaching (LfBle) was visible shortly after treatment on 8/38. Fruit Drop Less than 1% leaf drop following treatment. Canopy volumes (CV) in Aug were similar.

PCR results:

Treatment	CT 8-7-2015	DNA Concentration
126F 30 seconds	24.6	112.8
Control	31.2	116.3

Tree Measures (height (TH, cm) and canopy volume (CV, m^3)

Treatment	TH 8-7-2015	TH 12-10-2015	CV 8-28-2015
126F 30 seconds	133	144	1.4
Control	136	148	1.9

Leaf Bleaching

Treatment	8-28-2015
126F 30 seconds	5.6
Control	0.0

Fruit Drop counts:

Treatment	11-5-2015	12-10-2015
126F 30 seconds	2.1	4.8
Control	1.8	7.5

Monitoring of Tree Height, Trunk Diameter, Canopy Diameter, DI and pictures are continuing.

Premier, Raley

Valencia / Carrizo, 5 years old. Pretreatment leaves sampled from 40 trees for PCR on 5/22/15. 30 trees were treated on 5/28/15 at 120 F for 20 sec. while 10 trees were untreated as controls. Initially, treatment trees were clearly HLB+ with an average CT=25 while control trees had significantly higher CT (32.8) in the HLB- range. Leaves have been resampled for post-treatment PCR and await analyses. Due to grower's interest in treating only the worst appearing trees while avoiding treating healthy trees, treated trees initially had significantly smaller canopy volumes (CV 5_22) and higher DIs in May. Control trees remained larger with higher DIs than treated trees through Dec. Leaf drop and fruit drop were initially higher in treated trees than control trees but leaf and fruit drop were not different between treatments by Dec. at harvest on 12/15. Total fruit drop for the season did not differ. Total fruit yield, fruit number and average fruit weight were all smaller in treated trees than in control trees. Juice brix however, was higher in treated fruit.

PCR Results:

Treatment	CT	DNA concentration
Control	32.8	125.6
120F 20 sec	25.1*	178.2*

Canopy Volume

Treatment	CV 5-2015	CV 6-2015	CV 10-2015	CV 12-2015
Control	11.53	13.61	15.10	15.56
120F 20 sec	8.53 *	9.57 **	10.97 **	11.33 **

Disease Index by date 2015 (*,** = significantly different)

Treatment	5-22-15	6-15-15	7-17-15	9-10-15	10-20-15	12-08-15	Avg DI 2015
Control	16	13	11	13	17	16	14.55
120F 20 sec	20 **	19 ***	16 ***	16 *	21 **	19 **	18.43 ***

Leaf Drop

Treatment	6-18-2015	7-17-2015	9-10-2015	10-20-2015	12-8-2015	Tot Lf Drop
Control	0	0	0	0	0	0
120F 20 sec	20.7***	5.7*	1 ns	6 ns	0 ns	32.8 ***

Fruit Drop counts

Treatment	6-18-2015	7-17-2015	9-10-2015	10-20-2015	12-8-2015	Tot Fr Dr
Control	5	6	22	8	28	70
120F 20 sec	13.6 ns	14 ns	10 **	23 **	54 ns	114 ns

Fruit Yield

Treatment	Frt Yld (kg)	Fruit No.	Avg Frt Wt	Brix
Control	61.59	361	0.171	8.45
120F 20 sec	29.35 **	173 **	0.158 **	9.33 *

Premier, Dunson

Hamlin/Swingle trees, 5 years old. Leaves were sampled on 30 trees for PCR analysis on 6/8/2015. 15 trees were steam treated 125 F for 30 seconds on 8/4/2015 and 15 remained as untreated controls. The average CT value for treatment trees 34 (HLB-) whereas the control trees were HLB+. Leaves have been resampled for post-treatment PCR and await analyses. Treatment trees appeared worse as they had consistently higher average DIs than control trees but only significantly so on 8/14. Fruit drop was consistently higher in the treatment trees than in control trees.

PCR Results (CT)

Treatment	6-8-2015
Control	23.6
125 F 30 sec	34.2*

Disease Index (*,** = significantly different)

Treatment		6-9-2015	8-14-2015	9-25-2015	10-28-2015	12-30-2015
Control	Control	19.0	16.9	20.9	19.5	21.2
125 F 30 sec	Treated	19.6	21.4*	21.0	20.5	22.0

Fruit Drop counts

Treatment	8-14-2015	9-25-2015	10-28-2015	12-30-2015
Control	3.1	2.6	36.3	3.7
125 F 30 sec	6.0*	10.8*	40.1	10.7*

Uncle Matts (cooperative with Dr. Ehsani)

Rio Red/Swingle. 1yr old and 5 yr old. Pretreatment leaves sampled from 30 trees for PCR on 8/13/15. Ten 1 year old trees and 10 five year old trees were steam treated at 133 F for 30 sec. on 6/29/15 while 5 trees of each age were left untreated as control trees. The average CT value for the larger 5 yr old treatment trees (25 to 26) was lower (HLB+) than the smaller 1 yr old trees (33 to 34, HLB-). There did not appear to be a treatment effect on HLB status. The older trees had a higher DI than the younger trees but treatment appeared to have little effect on DI from June through December 2015. Fruit drop (FD) was greatest in the untreated control 5 year old (ConLg) in September but there was little drop by December regardless of treatment. Trunk cross sectional area (TA) was little affected by treatment as average TA in control and their respective treatment trees had the same relationship before (6/29) and after (8/13) treatment. There appears to be only small differences in tree height (TH) attributable to the treatment. Leaf drop and canopy volume data have not been analyzed yet.

PCR Results:

Treatment	CT 8-13-2015	DNA Concentration	CN _2ul
133 F 30 sec. 1-yr-old	33.5	151.8	8956.1
133 F 30 sec. 5-yr-old	26.6	137.3	39764.3
Control 1-yr-old	34.8	164.0	12713.2
Control 5-yr-old	25.9	134.1	49437.9

Disease Index

Treatment	6-26-15	8-13-15	9-21-15	10-21-15	12-30-15
133 F 30 sec. 1-yr-old	6.7	8.2	9.8	8.6	9.1
133 F 30 sec. 5-yr-old	30.0	29.0	26.4	29.4	31.3
Control 1-yr-old	7.4	7.6	10.0	10.0	10.0
Control 5-yr-old	28.4	30.0	25.4	29.4	32.2

Tree Growth Measures (Canopy Volume (CV); Trunk Cross Sectional Area (TA, cm^2)

Treatment	CV (m^3)	TA 6-29-2015	TA 8-13-2015
133 F 30 sec. 1-yr-old	0.4	68.8	77.5
133 F 30 sec. 5-yr-old	4.7	430.7	432.2
Control 1-yr-old	0.3	37.6	45.9
Control 5-yr-old	5.3	472.1	482.2

Tree Height (cm)

Treatment	6-29-15	8-13-15	9-19-15	10-21-15	12-30-15
133 F 30 sec. 1-yr-old	107.0	105.0	106.0	103.0	103.0
133 F 30 sec. 5-yr-old	189.0	192.0	184.0	193.5	184.0
Control 1-yr-old	118.0	116.0	118.0	110.0	106.0
Control 5-yr-old	184.0	184.0	184.0	189.0	185.0

Tree Height, Trunk Diameter, Canopy Diameter, DI, Pictures, Leaf Bleaching, Leaf and Fruit Drop are continuing to be monitored.

Overall, 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. 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”.

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 sponsored Thermal Therapy Field Day scheduled for 3 Dec 2015 at the Wheeler 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.

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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:
The portfolio of CRDF projects was reviewed in December, 2015 by Project Managers, reviewing projects that are scheduled to continue past June 30, 2016 as well as projects which will mature before the end of the current fiscal year. Projects related to ACP management fall into both categories, and the team evaluated the need for additional work on many topics. The completion of field trials that compared metalized reflective mulch, organic mulch and insecticides has been completed, demonstrating the value of these tools. Recommendations from this review will be presented at January CRDF meetings.

Obj. 2- Engage registrants and regulatory entities in need for label modifications
During the latter portion of this quarter, progress was made in discussion of the need for extension of Section 18 use of Belay® clothianidin insecticide for suppression of ACP on young trees. Working with the registrants and FFVA, plans for submission of an extension request in early 2016 were made.

Obj. 3- Continue participation in pesticide stewardship activities
Progress reported during this period on the project to monitor for development of resistance in ACP populations subjected to insecticide applications (Stelinski 15-0240 indicates that the bottle assay for field monitoring of susceptibility of ACP to field applied materials is functioning well and will be deployed. The method is efficient and will allow the team to increase the number of field sites and local populations of ACP that can be monitored.

Obj. 4- Continue to support CHMA implementation of ACP and other HLB management tools
Activities continue to support CHMAs through the IFAS project, including data mapping from CHRP monitoring of ACP populations. The implementation of revised CHMAs in southwest Florida has been advanced during this quarter

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

Regular communication with CHMA coordinator at CHMA meetings.

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.

UF mathematics professor Dr. James Keesling (Project 932.1C) continues efforts to evaluate the Psyllid Shield concept by accurately modeling its performance over different spatial dimensions, neighboring psyllid and disease pressure, and RNAi performance. This model is being refined to bring the necessary precision to a field trial design. This two-year project ends 5/31/2016.

During the quarter efforts continued to refine the model for tree symptom development using survey data Provided by Southern Gardens (SG), and simulations run on the University of Florida's High Performance Computing system. The model assumes the inoculum accumulates at a rate proportional to the number of infected nymphs present in the citrus trees, and that trees begin to decline after a certain threshold. This threshold in particular continues to be refined using the SG data.

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

Dr. Keesling continued 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 continue to be 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 CRDF-funded project (618C), that ran from 4/2013 to 9/2015, tested 14 selected dsRNAs separately for activity against psyllids when expressed in plants using the CTV vector. The evaluation was conducted against adults and nymphs feeding on citrus in caged greenhouse experiments. The study found a number of sequences of certain psyllid genes that, when expressed using the CTV vector, greatly reduced the production of psyllid progeny in citrus seedlings. Going forward, this research is being funded by a USDA Specialty Crop Grant, which will continue to identify the most effective target sequences, test multiple sequences, and optimize delivery methods.

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 continuing to understand what might also be a worthwhile further improvements. It is therefore a priority to advance the most promising current candidates into field trials.

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

Dr. Keesling continued discussions with statisticians to address how many constructs can be used in a field trial while still differentiating among the constructs.

Simulations are being run to specify the size and 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.

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 nuPsyllid and RNAi research in combination with CTV vector development, and therefore cuts across several areas of Intellectual Property (IP). This is both a challenge and opportunity because it involves multiple stakeholders, IP owners and licensees.

Discussions with University of Florida Office of Technology Licensing (Dr. Byatt) 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 opportunities for return on investment.

CRDF continues to facilitate, accelerate and incentivize corporate action and is prepared to provide regulatory, commercial delivery and other support, as appropriate, to candidate partners.

Southern Gardens (SGC) has agreed to partner with CRDF to develop and implement plans for a Phase 1 field trial, subject to CPDC Approval. (See Obj. 6)

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

The science and modeling efforts have progressed to the point where it is feasible to proceed with field trials on RNAi for psyllid control in 2016. CTV is the most appropriate delivery "niche" for CRDF to pursue in terms of 2016 field trials. Five RNAi constructs were identified in Dawson, et.al research as particularly effective in killing psyllids in greenhouse trials, and are therefore recommended for further evaluation in the field trials.

At the December 2015 CPDC meeting, a proposal was presented by CPDC staff recommending a two phased approach to field trials. Phase 1 would be a small-scale trial, followed by a Phase 2 area-wide trial that captures the Phase 1 learning and experience from both a scientific and regulatory point of view and establishes relationships with regulatory agencies.
Southern Gardens (SGC) is a licensee of key CTV technology, has experience in conducting field trials involving CTV, has established relationships with regulatory agencies, and has in place tools and resources to facilitate conduct of trials. SGC indicated its willingness to participate in this effort, and would need to play a significant role in managing this project. CRDF could provide some level of financial and other to-be-defined support to SGC in the development and implementation of Phase 1.

At its December meeting, CPDC authorized SGC and CPDC staff to proceed to the next step, which is a presentation at the February CPDC meeting of a proposed Phase 1 field trial that would include a detailed plan and budget, as well as a proposed CRDF-SGC relationship to implement the project.

Significant Meetings or Conferences:

None.

Obstacles Encountered and Breakthroughs:

The key progress during the quarter were Southern Garden's agreement to work with CRDF on a proposal for a Phase 1 field trial for consideration by CPDC and CRDF Board; and CPDC authorization to proceed with a more detailed plan and budget for review at the February 2016 CPDC meeting.

Other Information:

Over the past two years, 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. Based on the results of the February CPDC review, appropriate decisions will be made as to the next steps to advance the Psyllid Shield concept to commercial use.

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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) 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**. The project's 2nd year anniversary will come up May/June 2016 after the second harvest. 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. 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.

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 are sampled annually to determine root density. Leaves were sampled across all treatments at the 3 sites in Aug, 2015 for nutrient analysis and compared to values from August 2014. These data were reported in the last report. This report will focus on disease index rating, canopy growth and fruit yield (2015 harvest).

Site Results to Date:

Ridge Site: Valencia/Swingle trees are 17 years old. At this site, area wide block variation in tree disease index (DI) and yield (Frt wt) was important as there were significant block effects on both DI and yield (Frt Wt). When + and – mulch blocks were combined (7x6, n=42). Blocks 1 & 3 had higher DIs and lower yields than blocks 2 & 4.

DI	Mean	Block
A	17.214	1
A	16.476	3
B	13.976	2
C	11.786	4

Frt Wt (lbs)	Mean	Block
A	181.58	4
A	173.51	2
B	140.55	1
B	137.57	3

There were no effects of treatment on fruit weight or DI with no mulch (n=24). With mulch (n=4), there were no effects of treatment on fruit weight. With mulch, the Aliette treatment had the lowest DI and Quantum had highest DI. Although all treatments had numerically slightly larger canopy volumes (CV, m^3), there was no treatment effect on CV even with + and – mulch treatments combined (n=28).

DI + mulch		Mean	N	Treatment
	A	19.25	4	Quantum
B	A	17.5	4	BioFlour
B	A	16.5	4	Serenade
B	A	15.25	4	Ecofrien
B	A	12.75	4	UTCon
B		11	4	Aliette

C V +-m	Mean	N	Treatment
A	31.357	28	BioFlour
A	30.8	28	Serenade
A	27.318	28	EcoFrCSA
A	26.42	28	Aliette
A	25.883	28	Quantum
A	25.096	28	UTCon

There was no effect of mulch (n=4) vs no mulch (n=24) on DI or fruit weight.

Fruit weight (lbs)	Mean	N	Trt Mulch
A (low DI)	208.60	4	Aliette y
A	195.10	4	Serenade y
A (high DI)	180.50	4	Quantum y
A	175.58	4	Ecofrien y
A	169.37	24	Serenade n
A	163.85	24	Quantum n
A	163.70	24	Biofluor n
A	157.61	24	Aliette n
A	157.60	4	Biofluor y
A	145.58	24	Ecofrien n
A	142.78	4	UTControl y
A	131.34	24	UTControl n

Root samples and leaves samples for PCR analysis have been collected and await analyses.

East coast, Indian River site: Valencia/Swingle trees are 6 years old.
This site was relatively uniform as block effects were not significant. There were few significant differences when contrasting mulch vs. no mulch effects so selected data are summarized here after combining + and – mulch (n=28) treatments. When mulch and no mulch soil treatments were combined, the BioFluourish treatment had the lowest average DI (appeared the best), the Ecofriendly treatment appeared the worst while the untreated control (UT Cntrl) was intermediate. There was no significant effect of treatment on average fruit yields (Frt Wt).

DI		Mean	N	Treatment
worst	A	7.9	28	Ecofrnd
B	A	7.3	28	UT Cntrl
B	A	6.7	28	Aliette
B	A	6.5	28	Quantum
B	A	6.4	28	Serenade
B	best	6.1	28	BioFluour

Frt Wt (lbs)	Mean	N	Treatment
A	114.7	28	Serenade
A	113.1	28	UT Cntrl
A	111.9	28	Aliette
A	111.6	28	Ecofrnd
A	111.5	28	Quantum
A	109.6	28	BioFluor

Can Vol (m^3)			Mean	N	Treatmt
Lg	A		6.9	28	BioFluor
Lg	A		6.7	28	UT Cntrl
B	A		6.5	28	Serenade
B	A	C	6.3	28	Ecofrnd
B		C	5.6	28	Quantum
Sm		C	5.5	28	Aliette

Treatments did have a mostly negative significant effect on average tree growth as evaluated by canopy volume (Can Vol, m^3). Only the BioFluorish trees were as large as the untreated control trees whereas all the other treatments reduced tree growth. The Quantum and Aliette treatments had significantly smaller canopies than the untreated control trees.
Root samples and leaf samples for PCR analysis have been collected and await analyses.

SW FL site: Valencia/Swingle trees are 11 years old.
Block effects were not significant. The Biofluorish treatment had the greatest yield per tree (405 lbs) followed by the Aliette treatment (378 lbs/tree) which were both greater than the UTC (327 lbs/tree). The ranking of treatment effects on tree canopy size (CV) was similar to the ranking of average fruit yield as larger trees produced more fruit. Thus, Biofluorish and Aliette treated trees had the largest average canopy volumes (29 and 28 m^3, respectively) whereas Quantum and Ecofriendly treated trees were intermediate whereas and Serenade trees did not differ from the UTC at 17 m^3.

Frt Wt (lbs)			Mean	N	Treatmt
	A		405.5	28	Biofluor
B	A		378.1	28	Aliette
B	A	C	364.8	28	Quantum
B		C	348.2	28	Ecofriend
		C	327.3	28	UT Cont
		C	316.5	28	Serenade

C V (m^3)	Mean	N	Treatmt
A	29.8	28	Biofluor
A	28.1	28	Aliette
B	22.9	28	Quantum
B	21.4	28	Ecofrind
C	17.4	28	Serenade
C	16.8	28	UTCont

There was no mulch effect on average disease index (DI) and there was no treatment effect on DI. Average DI data for the no mulch trees (n=24) are below.

DI	Mean	N	Treatment
A	20.0	24	BioFlour
A	19.2	24	Quantum
A	19.1	24	Aliette
A	18.8	24	Serenade
A	18.5	24	Ecofriend
A	17.7	24	UT Con

Root samples and leaves samples for PCR analysis have been collected and await analyses

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

Significant Meetings of Conferences:

Obstacles Encountered and Breakthroughs:

Results of these field sites are being regularly communicated to the Florida citrus industry by CRDF through written reports, a quarterly progress report to the Committees and Board of CRDF which is posted to the CRDF website, and through presentation at grower meeting as indicated below. Completion of the fruit harvest data collection and analysis will allow a comprehensive view of the value of these treatments over the first two years of the trial. Valencia crop harvest will being in February, and these plots will be harvested per the site location harvesting plan. A field day will be planned for 1st half 2016 to highlight treatment effects as the second year of this trial comes to a close.

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3. HOST PLANT INTERVENTION

Project Title: 3c. Deployment of Disease Resistant or Tolerant Citrus Rootstocks and Scions
Project goal(s) for this project:

1. Track ongoing research projects evaluating emerging scion and rootstock genotypes for tolerance or resistance to HLB, citrus canker and other diseases. 2. Cooperate in in-depth evaluation and planning exercises related to Florida (and US) citrus breeding to better focus on HLB solutions and rapid evaluation and deployment of rootstocks and scions 3. Develop and implement plans for expanded management of tolerant and resistant citrus 4. Facilitate identification of best performing candidate rootstocks that appear to have HLB tolerance or resistance from Florida (and other) breeding programs 5. Implement and evaluate Phase I and II grower field trials of most promising candidate HLB tolerant rootstocks using standard varieties as scions. 6. Communicate progress and results of evaluation of rootstocks to industry

Narrative of Progress for Project Goals:
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 underway and plans are in place to evaluate seed germination, sanitation relating to seed contamination with citrus canker, and propagation methods for recalcitrant rootstocks.

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

Nine candidate rootstocks were selected and propagated in large numbers: 5 experimental rootstocks: 4 from the UF breeding program (Orange 4 (UFR-2), Orange 15 (UFR-3), Orange 19 (UFR-4), 46 x 31-02-13 (UFR-16) and 1 from the USDA breeding program (US 942), along with 4 standard rootstocks (US812,

4. Evaluate ongoing grower plantings of candidate rootstocks at 3 different sites: 2 on Central Ridge and one in Southwest Florida. Sour, Carrizo, 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 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 November 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 included tree height and trunk diameter; Digital Photographs and Disease Index which are summarized below for each of the 3 trial sites. Leaf nutrition was evaluated August 2015 and data were summarized in the September 2015 report. Leaves for qPCR have recently been sampled and await analysis.

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; mminus 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.

Tree height (TH in cm) in Mar 2015 ranged from 69-77 cm (27-30 in) from the nursery: trees on UFR3 were the tallest, Swingle and US812 were the shortest. By May, US812 was still shorter and US942 taller than the other rootstocks but there were few significant differences.

TH 3-23-15		Mean	N	Rootstock
	A	76.7	40	UFR3
	B	73.8	40	US942
C	B	72.5	40	UFR2
C	B	72.0	40	UFR4
C	B	71.8	40	UFR16
C	D	70.5	40	Swingle
	D	69.0	40	US812

TH 5-26-15			Mean	N	Rootstock
	A		85.1	40	US942
B	A		84.6	40	UFR16
B	A	C	83.5	40	UFR2
B	A	C	83.1	40	UFR3
B	A	C	82.1	40	UFR4
B		C	81.3	40	Swingle
		C	80.5	40	US812

Tree Height in Oct 2015 ranged from 97-107 cm (38-42 in) 6 months after planting: US942 was the tallest, UFR3 the shortest. By Jan 16, UFR3 and UFR16 were the shortest and US942 remained the tallest.

TH 10-1-15		Mean	N	Rootstock
	A	106.9	40	US942
	B	101.8	40	Swingle
C	B	100.9	40	UFR4

TH 1-4-16		Mean	N	Rootstock
	A	124.7	40	US942
B	A	120.2	40	US812
B	A	119.3	40	Swingle

C	B	100.8	40	UFR16
C	B	100.3	40	UFR2
C	B	100.1	40	US812
C		96.8	40	UFR3

B	A	119.1	40	UFR4
B		115.7	40	UFR2
	C	109.0	40	UFR16
	D	100.8	40	UFR3

Trunk caliper (from trunk cross sectional area, TCSA in cm^2) ranged from 6-10 mm from the nursery in Mar 15. US942 largest caliper while Swingle had the smallest caliper from the nursery. Trees grew rapidly so by 4 Jan 16, US942 and US812 had the largest caliper while UFR3 had the smallest caliper. As canopies develop, future measurements will include canopy volume.

TCSA 3-23-15			**Mean**	**N**	**Rootstock**
	A		45.6	40	US942
B	A		42.2	40	UFR4
B	A		42.2	40	UFR2
B	A	C	40.7	40	UFR3
B		C	37.8	40	US812
	D	C	35.9	40	UFR16
	D		32.8	40	Swingle

TCSA 1-4-16		**Mean**	**N**	**Rootstock**
	A	765.3	40	US942
	B	654.1	40	US812
	C	588.1	40	UFR4
D	C	555.4	40	Swingle
D		524.3	40	UFR2
D		520.8	40	UFR16
	E	401.7	40	UFR3

Disease Index (DI) rating (scale 0 to10) in Oct 15; 0, no visible disease, 10 = all leaves with symptoms. Dis ranged from 0.02-0.3. So, very little or no HLB symptoms were visible in Oct 15 (data not shown). Leaves have been sampled for PCR and await analysis.

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. Leaf samples for nutrition were analyzed in August 2015 and were discussed in the last (Sept) quarterly report. This report will focus on tree height and trunk caliper (Trunk cross sectional area, TCSA).

After planting in May (5/22) from the nursery, trees on UFR_2 had the tallest tree height (Tr Ht in cm). Trees on US_812 were initially shorter than the others. UFR_3 trees were not all ready yet from the nursery (2 reps missing n=24) so were the shortest. By 6/22/15, the trees on all caught up with UFR2 as there were no differences in tree height; all Tr Ht were not different from Carrizo (CARR).

Tr Ht 5-12-15		**Mean**	**N**	**RootStock**
tallest	A	75.2	40	UFR_2
B	A	74.5	40	US_942
B	A	74.5	40	UFR_4
B	A	73.8	40	US_897
B	A	72.4	40	CARR
B	C	71.5	40	US_812
shortest	C	68.9	24	UFR_3

Tr Ht 6-22-15	**Mean**	**N**	**RootStock**
A	82.5	40	UFR_2
A	82.2	40	US_942
A	81.9	40	UFR_4
A	80.7	40	CARR
A	80.7	40	US_897
A	80.4	24	UFR_3
A	79.6	40	US_812

By 9/22, all trees were numerically taller than Carrizo but all Tr Ht were not different. All trees on UFR_3 were now planted but remained shorter than the rest. By 12/29, trees on US_897, US_942, US_812 were the tallest, followed by UFR_4, CARR and UFR_2 while UFR_3 remained the shortest.

Tr Ht 9-22-15	Mean	N	RootStock
A	91.9	40	US_897
A	91.5	40	US_942
A	90.3	40	UFR_2
A	89.9	40	US_812
A	89.5	40	UFR_4
A	89.1	40	CARR
B	82.9	40	UFR_3

Tr Ht 12-29-15	Mean	N	RootStock
A	99.8	40	US_897
A	99.7	40	US_942
A	99.0	40	US_812
B	94.2	40	UFR_4
B	93.4	40	CARR
B	93.1	40	UFR_2
C	88.0	40	UFR_3

Initially on 5/14, trees on UFR_4 and US_812 had the largest TCSA (cm^2), followed by CARR, UFR_2, and UFR_3, while US_942 and US_897 had the smallest caliper. After 7 months of growth, on 12/29 trees on the 3 US_ rootstocks had the largest caliper, followed by UFR_4, CARR and UFR_2 while trees on UFR_3 were the smallest.

TCSA 5-14-15		Mean	N	Rootstock
	A	44.9	40	UFR_4
	A	44.5	40	US_812
B	A	41.7	40	CARR
B		38.7	40	UFR_2
B		38.7	40	UFR_3
	C	33.4	40	US_942
	D	25.0	40	US_897

TCSA 12-29-15		Mean	N	Rootstock
	A	316.3	40	US_942
	B	262.6	40	US_897
C	B	257.1	40	US_812
C	B	248.1	40	UFR_4
C	D	231.8	40	CARR
	D	216.8	40	UFR_2
	E	134.3	40	UFR_3

We are continuing to monitor tree growth and health. As canopies develop, future measurements will include canopy volume. Leaves have been sampled for PCR and await analysis.

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 8-18-15. Trees on UFR16 will not be ready from the nursery until Spring 2016.

Shortly after planting from the nursery (7/29/15), there were no significant differences in tree height (TH in cm) among the trees on different rootstocks. Average height of trees on all rootstocks tended to be taller than trees on sour orange. Two months later, there still were no differences in tree height except that the newly planted trees on UFR3 were smaller than the rest. By 11/17/15, the trees on US812 and US942 grew slightly more than those on Sour but there still were no significant differences.

TH 7-29-15	Mean	N	Rootstk
A	66.8	39	US942
A	66.3	40	UFR4
A	65.9	40	US812
A	65.5	40	UFR2
A	64.9	40	Sour

TH 9-2-15	Mean	N	Rootstk
A	86.9	40	US942
A	86.8	40	US812
A	85.0	40	UFR4
A	84.7	40	Sour
A	84.0	40	UFR2
B	67.6	40	UFR3

TH 11-17-15	Mean	N	Rootstk
A	90.1	39	US812
A	89.6	40	US942
A	89.1	40	Sour
A	88.4	40	UFR4
A	87.5	40	UFR2
B	77.4	40	UFR3

Trees on UFR4 had the largest trunk caliper (from Trunk cross sectional area, TCSA in cm^2) on 7/29/15

while UFR2 and US942 had the smallest caliper. By 11/17/15, trees on US812 had the largest caliper and UFR2 the smallest of the older trees ignoring the younger smaller trees on UFR3.

TCSA 7-29-15	Mean	N	Rootstock
A	49.2	40	UFR4
B	42.8	40	US812
B	42.6	40	Sour
C	36.7	40	UFR2
C	35.9	40	US942

TCSA 11-17-15		Mean	N	Rootstock
	A	107.1	39	US812
	B	99.7	40	US942
	B	94.4	40	UFR4
C	B	92.9	40	Sour
C		86.4	40	UFR2
	D	50.3	40	UFR3

We are continuing to monitor tree growth and health. As canopies develop, future measurements will include canopy volume. Leaves have been sampled for PCR and await analysis.

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 of Conferences:

Obstacles Encountered and Breakthroughs: Availability of sufficient numbers of contracted trees to be ready to plant continues to be a delaying factor.

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Quarter Ending December 31, 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:

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.
During the quarter, the Mature Citrus Transformation Facility continued to produce transgenic events for its clients. Priorities included increasing the number of high quality genetic constructs used in transformations, and improving transformation efficiencies.

Dr. Zale continued the facility's outreach to additional research institutions and industry to acquire high quality genetic constructs for transformation. They have also been encouraged to submit vectors with all plant sequences and no pest sequences, which might lessen regulatory hurdles.

The transgenic plants produced during the quarter were for were Drs. Dutt (UF), Wang (UF) and McNellis (Penn State). Dr. McNellis recently obtained a USDA APHIS permit for transporting transgenic citrus to Penn State, and MCTF is in the process of obtaining certification to ship transgenic material.

Productivity has significantly improved since the facility began using vectors with linked reporter genes in July 2015. Since that time, forty-five transgenics have been produced. These transgenics have completed shoot production, micro-grafting and secondary grafting.

Another productivity improvement is the facility's ability to supply duplicate or triplicate micro-grafted transgenics to the scientists, which can serve as replicates for their continued testing.

In an effort to further increase transformation efficiencies, MCTF signed an MTA with Syngenta authorizing the use of a proprietary binary vector for Agrobacterium transformation and a proprietary bioloistics vector for research purposes only. Both vectors contain the PMI selectable marker, an alternative to the previously used nptII. PMI has never previously been used in mature citrus.

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%.

To improve efficiency and lessen potential micro-grafting incompatibilities, sweet orange is being micro-grafted onto sweet orange rootstock. Micro-grafting losses in mature rootstock have significantly decreased when young shoots are micro-grafted onto rootstock grown in high sucrose solution.

The facility is evaluating whether it can avoid the micro-grafting step altogether by growing the explants with developing shoots in bioreactors. If the shoots grow large enough, it may be possible to use them directly in secondary grafting, which is 100% effective.

Obj. 3- Continue plant propagation and budding events.
The facility is in the process of introducing new breeder lines in which to produce transgenics. These sweet orange varieties and one grapefruit are being introduced via shoot-tip grafting. Once plants are established, they will be used in budding mature citrus onto rootstock to obtain budstick for transformations.
Currently all transgenic events are being transferred to scientists directly without secondary grafting or propagation, unless otherwise requested.
All of Dr. Mou's primary transgenics have been transferred to Dr. Dawson's group, and some budded or rooted vegetative progeny originally intended for field tests are expected to be transferred to Dr. Dawson's psyllid house early in 1Q2016.

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 is waiting for the USDA SCRI citrus call for proposals which is expected in Spring 2016 to see which areas the federal government has given priority for funding.

Obj. 5- Biolistics transformations
Transgenics can now be produced in MCTF by two complementary methods: Agrobacterium and biolistics. Biolistic transformations are being pursued as time and resources permit. During the quarter, the facility continued to successfully transform both immature and mature citrus with biolistics and demonstrated that the protocol is reproducible. As a result, the lab can now offer plant production using biolistics as a transformation alternative.

Significant Meetings/Conferences/Publications

Dr. Zale, et.al. continue to collect molecular data for a biolistics of immature citrus manuscript which is expected to be completed in 1Q2016.

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.

Breakthroughs:

Biolistics transformation of immature or mature citrus has not previously been reported. This will be an important technology to transform citrus without pest sequences, which might lessen regulatory hurdles.

Other Information:

Funding for MCTF is provided in Project 15-045C, which expires 6/20/2016. Its mission is to develop protocols for mature transformation of citrus that can be used to incorporate genes of interest, when available, into Florida cultivars. Through MCTF, CREC will generate the first mature sweet orange transformants with development protocols adjusted in the lab and in the growth room for Valencia and Hamlin.

MCTF remains an important element of the overall HLB-tolerant rootstock pipeline. This overall pipeline encompasses 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 will be tied to further deliberations of the CRDF knowledge mapping exercise, and associated efforts to develop side-by-side field testing of the most promising candidates and delivery to Florida growers.