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

Quarter Ending December 31, 2017

1. *Candidatus* Liberibacter asiaticus PATHOGEN INTERVENTION

Project title: 1a. Bactericide Strategies

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

A. Candidate Bactericide and Application Technology Development

Subproject goals for this project area for the next year:

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

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

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

Narrative of Progress against Goals:

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

On December 31, 2017 project number 16-012C, a research service agreement for testing potential bactericides in a greenhouse assay, concluded. The final group of materials sampled are potential new biopesticides. The results of this testing was positive and CRDF project managers have discussed next steps in the development process with the company. However, this is an experimental pesticide and will require several years of development before product registration.

Chemicals tested in this greenhouse assay included potential biopesticides, new pesticidal molecules (early in development) and agricultural chemicals that may have bactericidal properties. Results from this assay are not publically available because the purpose of this assay was to facilitate development of new products and the companies require confidentiality agreements before materials could be tested. This project improved the understanding of how to test bactericides in a greenhouse assay and the information may be used downstream in new research projects.

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

Project managers attended the first steering committee meeting for project 16-026C, the CRDF/Bayer partnership. The milestones of this project are being reached as planned and the project is on track.

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

The project 17-005C is examining the effect of thermotherapy on bactericide uptake. This project is reported to be on track for completion by the end of quarter three.

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

Quarter Ending December 31, 2017

Candidatus Liberibacter asiaticus PATHOGEN INTERVENTION

Project title: 1a. Bactericide Strategies

B. Bactericide Field Testing

Subproject goals for this project area for the next year:

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

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

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

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

Narrative of Progress against Goals:

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

This is a study to provide a side-by-side comparison of five essential oil products as a preventative treatment and a therapy for HLB on young trees. Trees were selected in 2015 from two blocks, one with undetectable bacterial titers and the other with detectable titers. The impact of treatments on tree health, foliar nutrition, disease rating, and HLB status are being evaluated through June 2017. Products are being applied at the rates and with the adjuvants recommended by the product companies. This is a replicated complete block trial with six trees per block and four blocks per treatment (24 evaluated trees total per site). The trees were planted in April of 2013.

Materials for this trial are the following:

1. Thyme Guard, Agro Research International
2. Ecotrol, Keyplex
3. Onguard Eo, AgXplore
4. Xplode, AgXplore

Complete results will be provided at the conclusion of the trial, the following are the results from the 2017 harvest:

	Total fruit (harvest + fruit drop)			Yield (lb)		
Low Disease Site	Mean	Standard deviation (±)	Means Separation	Mean	Standard deviation (±)	Means Separation
Thyme Guard	205.33	105.19	B	21.89	17.96	B
Ecotrol	239.42	87.74	B	20.19	11.04	B
Onguard EO	271.21	81.58	B	25.10	17.01	AB
Xplode	240.58	91.91	B	23.55	13.39	AB
UTC	263.88	73.33	B	31.29	19.22	AB
Moderate Disease Site	Mean	Standard deviation (±)	Means Separation	Mean	Standard deviation (±)	Means Separation
Thyme Guard	339.63	68.46	A	28.19	11.15	AB
Ecotrol	366.00	87.20	A	36.09	15.46	A
Onguard EO	375.43	110.23	A	31.21	16.56	AB
Xplode	341.33	86.15	A	29.03	18.09	AB
UTC	352.38	76.60	A	32.35	15.19	AB
P=	<.0001			0.0001		

	Percent fruit drop			Disease Severity (0-40 0= no disease)		
Low Disease Site	Mean	Standard deviation (±)	Means Separation	Mean	Standard deviation (±)	Means Separation
Thyme Guard	62.42	16.11	A	17.63	2.76	CD
Ecotrol	65.70	14.26	A	18.38	2.84	C
Onguard EO	66.65	13.17	A	16.48	2.47	D
Xplode	62.04	16.02	A	18.71	2.93	C
UTC	59.34	17.15	A	18.29	2.46	CD
Moderate Disease Site	Mean	Standard deviation (±)	Means Separation	Mean	Standard deviation (±)	Means Separation
Thyme Guard	70.55	11.98	A	23.29	2.53	A
Ecotrol	66.05	10.90	A	20.79	2.28	B
Onguard EO	71.01	9.56	A	22.39	2.43	AB
Xplode	71.22	11.53	A	22.63	2.48	AB
UTC	69.96	9.85	A	22.67	1.83	AB
P=	0.03			<.0001		

Harvest data results: None of the treatments were significantly different from the untreated control.

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

Evaluations continue with this field trial. This is a Valencia trial and it will be harvested in the fourth quarter of this fiscal year. Results from year one of this trial will be available in the first quarter of the next fiscal year.

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

Project 15-037C, "T-sol antimicrobial for the management of citrus canker and HLB" was completed this quarter, a final report has been requested from the researcher.

The following is the final report from project 16-025C:

Title of Project: Comparison of chemical uptake with laser ablation and conventional foliar application

Investigators:

Brad Booker, Pacific Ag Research
Greg Drouillard
Nian Wang, UF, IFAS, CREC

Situation Statement:

Bactericides are one of the few therapies available to citrus growers to improve the health of Huanglongbing diseased citrus trees. The efficacy of these materials is limited by the amount of the chemical that can be delivered through the leaves, bark or roots to the phloem where the bacteria reside. Due to a lack of absorption, a great proportion of materials applied to the foliage of the citrus tree is thought to be lost due to photodegredation, drift, runoff etc., reducing the economic and environmental sustainability of chemical use.

In laboratory culture *Liberibacter crescens*, a relative to *Candidatus* Liberibacter asiaticus (*C*Las) the causal agent of HLB, is very sensitive to a number of chemicals. This sensitivity is beneficial in identifying chemicals that may be used to treat *C*Las, but plant surface and phloem characteristics limit the number of chemicals that can successfully penetrate and move in the plant at effective concentrations. Adjuvants improve chemical uptake by improving contact between the chemical and plant surface, prolonging chemical activity and/or changing the permeability of the plant surface, but even with adjuvants, rates of chemical uptake of bactericides now in use appear to be low.

Alternative application strategies may be available that improve the uptake of bactericides, which may improve bactericide use against *C*Las. One method that may improve bactericide uptake is the laser ablation of a leaf surface prior to application creating an opening in the cuticle, which provides a pathway for movement of chemicals into the leaf. This method has been tested in the laboratory and in greenhouse trees and some preliminary work has been conducted in the field. Laboratory and greenhouse experiments have demonstrated a significant increase in chemical uptake in plant leaves after laser ablation and preliminary field experiments have shown a similar result. These experiments led to the development of a laser ablation machine that can be used in the field and may lead to the development of a full-scale field applicator. The next phases in the evaluation of this technology are to test the hypothesis that laser ablation leads to an increased uptake of chemicals in field trees, the increased uptake improves tree health over a season and the technology can reduce the amount of bactericide needed to adequately suppress HLB symptoms.

Objective:

The objective of this project was to determine if laser ablation improves uptake of a bactericide in field trees and quantifying the amount of uptake.

Narrative:

This experiment tested the hypothesis that laser ablation improves the uptake and movement of a chemical compared with a conventional foliar application. This experiment evaluated the uptake of FireLine with and without laser ablation by two methods over a period of five weeks.

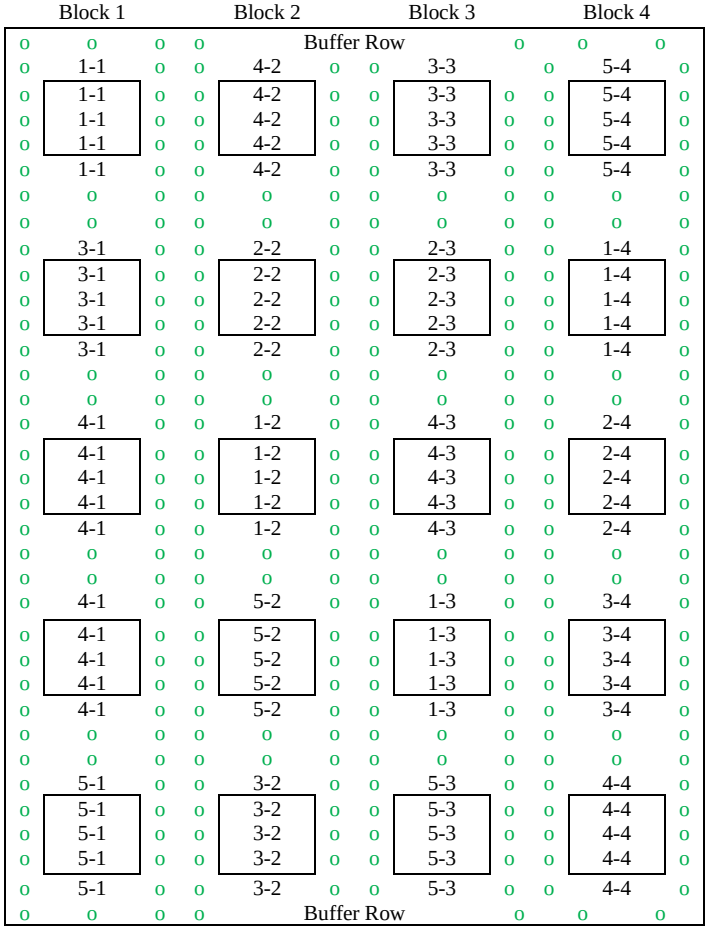
Trial Design:

Five year old Hamlin trees with a full canopy and few HLB symptoms were used for this trial. Treatments were replicated four times with five trees per replication. Three trees per treatment were sampled for analysis. Two buffer trees separated each replication and two rows separated each block. Trees on the edge of the grove block served as a buffer. The trial was set up in a randomized complete block design in the layout shown, the data were analyzed using ANOVA.

Treatments:

FireLine (oxytetracycline HCl, AgroSource) was used for treatment 1-3 to test for changes in uptake and movement. The chemical was applied with the adjuvant LI 700 (Loveland Products) at 3 oz./50 gallons. The treatments are the following:

1. Laser (one side of tree) + FireLine at 112.25g/10g (Targeted spray on ablated area)
2. Laser (one side of tree) + FireLine at 114g/100g (Conventional foliar application)
3. Conventional Foliar application of FireLine at 114g/100g
4. Untreated control
5. Laser ablated, no FireLine (for observations of phytotoxicity only; leaf drop, discoloration, fruit drop etc.)



Phase I Field Experiment Layout

Treatment one was a targeted application of FireLine during laser ablation on one side of the canopy. Treatment two was a laser ablation treatment immediately followed by a conventional foliar application of FireLine and LI 700. Treatment three was a conventional foliar application of FireLine and LI 700, with no laser ablation. Treatment four received no FireLine or laser application and served as the untreated control. Treatment five was laser ablation and no FireLine, to allow for phytotoxicity observations. Phytotoxicity symptoms were recorded from all treatments one week and 14 days after treatment. Treatment five was used for observation only, no samples were collected from these trees.

Prior to treatment, branches from three canopy sections were labeled and each branch was covered with a plastic bag to shield from direct bactericide treatment. The branches were on the top of the canopy (labeled with the treatment number and “top”) and a section distal to the laser ablated area on either side of the canopy (labeled with tree number and cardinal direction e.g. “1-1N”) and had a minimum of thirty-five leaves of similar maturity. The bags were removed twelve hours after bactericide application, allowing the application to dry on the treated leaf surfaces prior to removal. The application took place late in the day to avoid heat injury of the bagged branches.

Sample Collection:

Material applications were put out on June 20, 2018. For sample collection, five leaves per sample were collected per branch from five separate locations on the branch 48 hours after treatment and weekly up to five weeks post-treatment (June 22, 28, July 5, 12, 19, 26). Samples were placed in plastic bags labeled with the branch label, stored in a cooler with ice, and delivered to the testing laboratory on the same day as they were collected.

Biochemical Sample Totals:

4 treatments x 12 trees = 48 trees total
48 x 3 samples/tree = 144 samples/time-point
120 x 6 time-points = 864 samples total

Biochemical Analysis:

Samples were delivered to the testing lab and analyzed using the methods described in Hu et al. 2016.

Hu, J., & Wang, N. (2016). Evaluation of the Spatiotemporal Dynamics of Oxytetracycline and Its Control Effect Against Citrus Huanglongbing via Trunk Injection. *Phytopathology*, *106*(12), 1495-1503.

Results:

No phytotoxicity was reported in treatment five, the laser only treatment. Some phytotoxicity was reported on treatment one, but was not quantified. The phytotoxicity was likely because of the high rate of FireLine applied. At time point one, two and three, oxytetracycline was detected in the control sample. The rate of oxytetracycline declined over time. The detected oxytetracycline in the untreated control, may be from drift post-application or pre-application or another source of contamination in the field. No oxytetracycline applications were applied sixty days prior to trial set-up.

The untreated control treatment had a significantly lower detection level all time points except at the initial time point (Table 1). Treatment one and three, the targeted spray and conventional application, were statistically similar at all the time points. The two laser treatments were not significantly different. In this study the laser treatments did not improve uptake of FireLine compared with conventional foliar applications (Figure 1).

Figure 1.

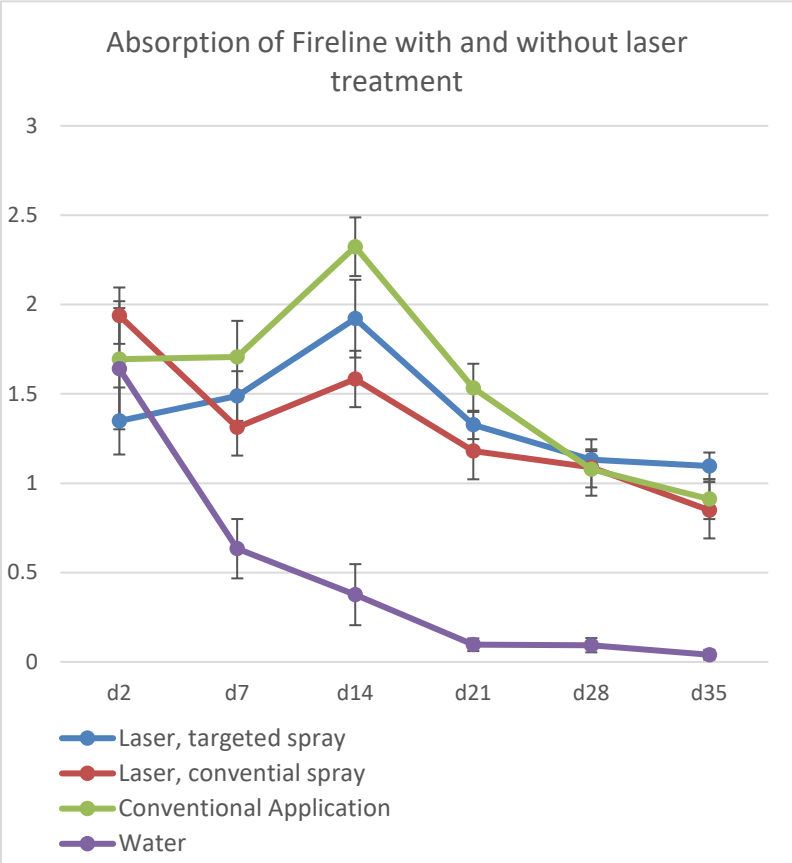


Table 1.

Day	Treatment	Mean*	SE (±)	
2	1	1.35	0.19	A
	2	1.94	0.32	A
	3	1.69	0.20	A
	4	1.64	0.34	A
7	1	1.49	0.14	A
	2	1.31	0.20	A
	3	1.71	0.22	A
	4	0.63	0.17	B
14	1	1.92	0.22	AB
	2	1.58	0.16	B
	3	2.32	0.25	A
	4	0.38	0.17	C
21	1	1.33	0.08	AB
	2	1.18	0.13	B
	3	1.53	0.12	A
	4	0.10	0.04	C
28	1	1.13	0.06	A
	2	1.09	0.10	A
	3	1.08	0.09	A
	4	0.09	0.04	B
35	1	1.10	0.08	A
	2	0.85	0.11	A
	3	0.91	0.11	A
	4	0.04	0.03	B

*ppm per gram of tissue

Treatments:

1. Laser (one side of tree) + FireLine at 112.25g/10g (Targeted spray on ablated area)
2. Laser (one side of tree) + FireLine at 114g/100g (Conventional foliar application)
3. Conventional Foliar application of FireLine at 114g/100g
4. Untreated control
5. Laser ablated, no FireLine

Obj. 1d. Manage Grower Bactericide Trial Data.

In response to the availability of oxytetracycline HCL (Fireline™), oxytetracycline Ca (Mycoshield®) and streptomycin sulfate (Firewall™) and the lack of available data on use, nearly 50 field trials were set-up by CRDF field staff throughout the citrus growing regions of Florida in FY 2015-16. The CRDF field staff continue to collect data from these trial. Harvest data has been completed on the early varieties and Valencia data will be collected in the subsequent quarters of this fiscal year.

The following are data collected from the early variety trials:

Hamlin	Control Mean	Mean Separation	Treatment Mean	Mean Separation	P>F	Treatment tree effect
Hamlin 13						
% Fruit Drop	53.08	A	48.03	A	0.1511	
Yield_Kg	70.32	B	91.17	A	0.0017	Higher yield
Total_Fruit	1048.99	B	1197.47	A	0.0467	More fruit per tree
Hamlin 12						
% Fruit Drop	58.19592	A	51.594785	B	0.0353	Less fruit drop
Yield_Kg	69.445	A	76.02	A	0.2615	
Total_Fruit	1106.91	A	1019.99	A	0.1581	
Hamlin 14						
% Fruit Drop	62.43	A	56.77	B	0.0553	Less fruit drop
Yield_Kg	58.13	B	73.57	A	0.0204	Higher yield
Total_Fruit	1121.41	A	1166.23	A	0.4885	
Hamlin 17						
% Fruit Drop	66.27536	A	68.202888	A	0.5802	
Yield_Kg	57.58889	A	50.52	A	0.2472	
Total_Fruit	1145.33	A	1000.59	B	0.0387	Less fruit (negative effect)
Hamlin 15						
% Fruit Drop	73.79	A	67.62	B	0.028	Less drop
Yield_Kg	6.88	A	4.65	A	0.502	
FD + Total Fruit Harvested	631.39	A	490.58	B	0.001	Less fruit (negative effect)
Post-Irma Fruit Drop	277.75	A	198.3	B	<0.0001	Less fruit drop post-Irma
Hamlin 4						
% Fruit Drop	32.82	B	39.98	A		Less drop
Yield_Kg	106.44	A	90.6	A		
Total_Fruit	1063.64	A	1005.67	A		

Midsweet	Control Mean	Mean Separation	Treatment Mean	Mean Separation	P>F	Treatment tree effect
Midsweet 4						
% Fruit Drop	58.76	A	58.07	A	0.0265	
Yield_Kg	26.4	A	30.37	A	0.2133	
Total_Fruit	386.93	A	464.01	A	0.1273	
Post-Irma FruitDrop	307.73	A	300.14	A	0.8733	
Grapefruit	**Control Mean**	**Mean Separation**	**Treatment Mean**	**Mean Separation**	**P>F**	**Treatment tree effect**
Grapefruit 7						
% Fruit Drop	25.03	A	19.735446	B	0.0173	Less Drop
Yield_Kg	95.95	B	113.035	A	0.0495	Higher Yield
Total_Fruit	438.34	A	491.99741	A	0.1749	
Grapefruit 1						
% Fruit Drop	56.33	A	53.67	A	0.4414	
Yield_Kg	39.29	A	39.15	A	0.975	
Total_Fruit	231.04	A	229.45	A	0.9177	
Grapefruit 3						
% Fruit Drop	64.46	A	64.18	A	0.9478	
Yield_Kg	25.61	A	22.07	A	0.293	
Total_Fruit	222.12	A	210.51	A	0.3789	
Grapefruit 9						
% Fruit Drop	76.9	A	80.03	A	0.288	
Yield_Kg	31.5	A	20.54	A	0.8858	
Total_Fruit	488.89	B	494.47	A	0.035	More fruit per tree
Post-Irma FruitDrop	258.95	A	271.4	A	0.6818	

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

New projects ideas are being discussed with growers and the Commercial Product Delivery committee. Project ideas may be incorporated into priorities for a call for proposals in April 2018.

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

CRDF project managers are monitoring progress of these trials and collect data necessary for periodic EPA reporting. Samples have been collected from two of the sites and the remaining two sites will be sampled in the fourth quarter. A report will be developed for the EPA in June 2018.

Significant Meetings or Conferences:

A project managers attended the Biocontrol East conference. This conference help talks on biopesticide and biostimulant products and held a session on citrus and a talk on fire blight disease control.

A project manager attended the Materials Innovation for Sustainable Agriculture (MISA) conference at the University of Central Florida. Presentations at this conference covered many projects examining new therapies for citrus diseases.

CRDF Commercial Product Delivery Project Progress Report FY 2017-18
Quarter Ending December 31, 2017
2. Asian Citrus Psyllid VECTOR INTERVENTION

Project Title 2a. CHMAs and Asian Citrus Psyllid Management

This report covers the second quarter of FY 2017-18, during which CRDF had 49 active projects in the portfolio of research and delivery projects. Nine of these projects address management of the vector, Asian citrus psyllid. Within the group of CRDF ACP projects, there are three that focus on RNAi and thus are covered in another section of the Quarterly Report. Please see section 2b for CRDF projects relating to RNAi field trials (#16-016C Eyrich), immune system priming to affect bacterial infection (#15-021 Pelz-Stelinski) and the NIFA nuPsyllid project managed by CRDF.

None of the ACP topical projects ended during this quarter, but #16-011C Adair and #15-017 Killiny will end in January, 2018. In addition, it worth mentioning that CRDF does not have any self-directed projects within this topical area.

A project that aims to disrupt the transmission of CLas by ACP (Killiny #15-017) is closing at end of January 2018, and the final report will be due by February 15. No progress report for this period has been received. The goal of this project was to manage CLas *in planta* through use of quorum sensing signal disruption.

Narrative of Progress by Project Goals:

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

Project #16-020C (Christopher Vincent) to evaluate the additive value of ACP-repelling dye in applications of kaolin clay treatments was initiated in early 2017 and continued through the end of 2017. While still preliminary Dr. Vincent reports that repeated applications of dyed and undyed kaolin clay are rivaling and in this quarter, surpassing conventional pesticide treatments.

He states that "The two kaolin treatments (dyed and undyed) have demonstrated excellent control, and we have found a statistically significant effect of the red-dyed kaolin as compared to the undyed kaolin. The mean number of ACP per plant across all counts for the year was 1.669 for untreated control, 0.973 in foliar insecticide treatment, 0.046 in the non-dyed kaolin, and 0.031 in the red-dyed kaolin, with all means being significantly different from each other. The kaolin effect has resulted in differences in CLas infection." He cautions that these are early results and the true impact will emerge through further monitoring of this field trial.

Project #16-011C (Robert Adair) requested of CRDF and was granted a six-month extension in July of 2017 to allow for the harvest of a second grapefruit season in the trial. With approval, the horticultural and pest management treatments were continued per fresh grapefruit guidelines, and irrigation and

fertilization programs monitored and recorded. The three treatments in this trial (bare ground, organic mulch, and metalized reflective mulch (MRM)) received identical horticultural care and applications. Cost information on cultural practices were tracked.

Because this project is ending in January, no quarterly report was provided, and the comprehensive final report will be available following the project end.

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

Registrants of Aldicarb continue to pursue the opportunity to bring this treatment back to Florida citrus. The registrant, AgLogic, has conducted consultations with state and federal officials regarding the pathways for potential re-registration of Aldicarb for citrus use.

CRDF is planning for a consultation with US EPA in January, and the status of registration of ACP materials will be among the topics discussed at this meeting.

3. Continue participation in pesticide stewardship activities

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

The project shifted from field to lab during this quarter and laboratory comparisons of field populations identified as showing lower sensitivity to neonicotinoid insecticides indicated that in successive generations of pesticide-free lab colonies the resistance declined back towards baseline, a good result for development of resistant management practices.

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

CRDF is no longer directly engaged in supporting CHMA level ACP management, as Dr. Rogers has secured additional external resources to address regional implementation of ACP management. The environment that fosters regional ACP suppression applications has eroded as more growers are focused on horticultural practices to maintain tree health and on average, are investing less in ACP management.

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

Quarter Ending Dec. 31, 2017

2. Asian Citrus Psyllid VECTOR INTERVENTION

Project Title: 2B. RNAi Molecules/Psyllid Shield (16-010C)

Narrative of Progress against Goals:

1. Complete first year of 3 year CRDF-funded RNAi field trial conducted by Southern Gardens and continue follow-on Phase 2 Psyllid Shield planning. Below is a synopsis of the latest quarterly report from the PI.

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

      Challenge of RNAi expressing trees with ACP was set back from the original timeframe of Jan-Feb to Mar-April by the hurricane. Canker mitigation by removal of infected leaves and control of leafminer by pesticide treatments has been implemented. No brown aphids have been detected.

      Additional Valencia scions grafted with bud chips carrying the CTVvv RNAi constructs will be kept at the Southern Gardens nursery until late spring or early summer, when they can be planted.

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

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

      Sentinel trees tested negative for the presence of CTVvv RNAi using rtPCR. A USDA inspection for permit compliance was conducted Nov. 28 2017 at Southern Gardens; all requirements were met.

      A white paper on the health and ecological risks of RNAi as a pesticide is available by internet search. This may be useful as a strategic roadmap to the questions regulators have and the assessments they would be most interested in. A link to the pdf is included http://www.thecre.com/premium/wp-content/uploads/2012/04/RNAi-White-Paper.pdf

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

Double stranded RNA compositions for reducing asian citrus psyllid infestation and methods of use: US20170211082A1 Hunter, Gonzalez, and Andrade
Application that focuses on trehalase of D. citri as a target for silencing.

3. Continue to identify companies engaged in RNAi product development.

This interim four small companies working in trigger design, RNA production, and RNAi insect control were identified. Of the four, Trillium's technology may be used either as a topical application or as part of a plant incorporated protectant, similar to the current approach of 16-010C.

Forrest Innovations – mosquito reversal of pyrethroid resistance

Trillium Ag – stinkbugs and palmer amaranth weeds, topical RNA, in planta RNA, novel trigger design

Greenlight – insect and virus pests, cell free synthesis of RNA, Syngenta Ventures is an investor

Apse – proprietary and cost effective production of RNAi molecules for direct applications

Production of RNAi in planta (GMO) – The big four ag companies primarily interested in row crops : Syngenta, BASF, Corteva, Bayer, all have the capability to produce RNAi PIPs (plant incorporated protectants). The challenge is to create a value proposition to do so. In addition Okanagan Specialty Fruits recently began marketing the arctic apple, which silences a plant gene involved in browning. This is a Canadian company, and may be a potential collaboration partner at some point in the future – obviously they have been able to develop and market the fruit, and might be approached for a collaboration during the development phase of an ACP or HLB solution.

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

Quarter Ending December 31st, 2017

3. HOST PLANT INTERVENTION

Project Title: 3c. 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) Grower Field Trials – Assessing HLB tolerance in volunteer grapefruit
Dr. Hatcher has been working with a grower to plan a scion field experiment to assess HLB tolerance in volunteer grapefruit identified by a grower. Due to the disruption by hurricane Irma, some rootstocks were budded later than planned which impacted bud-take and bud break. This issue only affects a small number of trees and recommendation

of Dr. Richard Beeson; supplemental lighting has been added to help break dormancy and speed up bud break.

b) Transgenic field trials

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

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

The following activities focus on objectives 2 and 3.
CRDF, NIFA staff and CRB staff and some CRB board representatives began discussions ways to facilitate better collaboration among agencies directed at citrus research and specifically solving HLB. The funding agencies also plan to engage stakeholders and other organizations in other citrus growing regions such as Arizona and Texas.
The agencies goal is to facilitate collaboration among researchers to speed up research progress. The strategy will at first target projects in plant improvement as they are long-term and have the highest likelihood of benefiting from aspects such as data sharing, project updates (success and challenges), navigating state and federal regulations on interstate movement of germplasm between states. Plans have been made for a meeting for the National Citrus Breeding collaboration in Denver, Colorado. Among those invited to attend are plant improvement researchers using conventional and biotechnological approaches to plant improvement, intellectual property and technology transfer experts at IFAS and USDA, patent attorneys, administrators from institutions, citrus processors and representatives from each funding organization. The meeting is scheduled for February 27th and 28th. Issues relating to collaboration to facilitate research have far-reaching implications, which could have a positive impact on many areas of investigation and not just plant improvement. Dr. Hatcher will give a full report to the CRDF Board on the ideas and proposed next steps after this meeting.

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

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 Trial Evaluation for Horticultural Traits.

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

Data Analysis and Results

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

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

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

Horticultural Trait Data

For data collected at the Duda rootstock trial in December 2017, there were significant differences ($p < 0.05$) among rootstocks for canopy volume (m^3), trunk cross-sectional area (TCSA) (cm^2), tree height (cm) and HLB disease index (HLB DI) (Table 1).

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

Rootstock	Canopy Volume (m^3)	TCSA (cm^2)	Tree Height (cm)	HLB DI (max. 10)
Swingle	6.27 ±0.24 BC	37.74 ± 1.14 BC	206.75 ± 3.57 BC	3.28 ± 0.13 AB
UFR_16	5.05 ±0.32 D	35.77 ± 2.08 1C	195.13 ± 4.42 C	3.78 ± 0.20 AB
UFR_2	5.75 ±0.23 CD	35.53 ± 1.09 C	202.63 ± 4.12 C	3.13 ± 0.13 B
UFR_3	3.76 ±0.26 E	27.01 ± 1.54 D	176.23 ± 4.73 D	4.03 ± 0.18 A
UFR_4	7.23 ±0.28 B	42.27 ± 1.20 AB	226.15 ± 3.29 A	3.78 ± 0.20 AB
US_812	7.26 ±0.27 AB	43.64 ± 1.57 A	221.47 ± 3.27 AB	3.05 ± 0.14 B
US_942	8.01 ±0.29 A	47.25 ± 1.19 A	230.18 ± 3.12 A	3.60 ± 0.25 B

Values represent the mean ± standard error and letter groupings were obtained using the Tukey-Kramer method. Values followed by the same letter do not differ significantly at the 5% level.

Rootstock groupings for each variable can be separated by the best performing rootstocks in order US_942, US_812, UFR_4, Swingle,UFR_16, UFR_2, and UFR_3. Data collected and analyzed in this quarter reflect possible effects of hurricane Irma wind gusts and flooding although the grower cooperator pumped water out of the site within 24 hrs. of the hurricane event. However, there was defoliation reflected by the

canopy volume, tree height, and HLB DI. The data show smaller canopies, shorter trees and higher disease index which may be explained by HLB infection as well as defoliation and added stresses of the hurricane flooding.

Peace River CRDF Rootstock Trial, Babson Park, FL (Ridge)

The trial was 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 December 2017 at the peace river location for canopy volume (m^3), TCSA (cm^2), tree height (cm) and HLB DI (Table 2). Rootstock groupings for each variable can be separated by the best performing rootstocks in order US_942, US_812, UFR_4, Swingle,UFR_16, UFR_2, and UFR_3. Data collected and analyzed in this quarter reflect possible effects of hurricane Irma wind gusts. There was some defoliation reflected by the canopy volume, tree height, and HLB DI. Data from December 2017 do not indicate as severe an impact as at Duda. The lower than expected canopy volume, tree height, and higher HLB disease index values reflect additional stress on the trees.

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

Rootstock	Canopy Volume (m^3)	TCSA (cm^2)	Tree Height (cm)	HLB DI (max. 10)
Carrizo	3.25 ± 0.26 BC	26.91 ± 1.7 AB	164.25 ± 6.0 A	3.22 ± 0.20 BC
UFR_16*	1.37 ± 0.11 E	11.47 ± 0.8 DE	136.10 ± 6.0 B	3.72 ± 0.19 AB
UFR_2	2.19 ± 0.12 D	16.02 ± 0.8 D	140.93 ± 2.5 B	3.60 ± 0.20 AB
UFR_3	1.32 ± 0.16 E	11.27 ± 1.2 E	125.60 ± 2.5 B	3.45 ± 0.22 ABC
UFR_4	3.14 ± 0.18 BC	24.83 ± 1.2 BC	162.60 ± 3.8 A	4.12 ± 0.21 A
US_812	3.69 ± 1.3 AB	26.56 ± 0.9 AB	173.32 ± 4.2 A	2.72 ± 0.17 C
US_897	2.83 ± 0.93 CD	21.21 ± 0.8 C	161.05 ± 2.9 A	2.87 ± 0.19 BC
US_942	4.01 ± 0.23 A	29.74 ± 1.1 A	173.20 ± 4.3 A	3.47 ± 0.20 ABC

Values represent the mean ± standard error and letter groupings were obtained using the Tukey-Kramer method. Values followed by the same letter do not differ significantly at the 5% level.

*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.

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

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

Horticultural Trait Data

There were significant differences ($P < 0.05$) in rootstock performance for canopy volume (m^3), TCSA (cm^2), tree height (cm) and HLB disease index (Table 3). Rootstock groupings for each variable can be separated by the best performing rootstocks in order US_942, US_812,Sour, UFR_3, UFR2 and UFR_16 respectively. The trial at BHG was impacted by high-velocity winds during hurricane Irma as reported last quarter. Data from December 2017 support that observation with lower canopy volume and tree height than expected after three months of growth as well as high HLB disease index.

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 December 2017

Rootstock	Canopy Volume (m^3)	TCSA (cm^2)	Tree Height (cm)	HLB DI (max. 10)
Sour	2.12 ± 0.10 AB	19.94 ± 0.89 A	144.28 ± 3.16 AB	3.15 ± 0.23 B
*UFR_16	0.78 ± 0.05 D	7.89 ± 0.42 D	115.18 ± 2.53 E	4.10 ± 0.26 A
UFR_2	1.34 ± 0.06 CD	12.22 ± 0.45 C	129.3 ± 2.02 CD	3.23 ± 0.24 AB
UFR_3	1.37 ± 0.33 CD	9.76 ± 0.51 CD	122.68 ± 2.67 DE	2.93 ± 0.18 B
UFR_4	1.86 ± 0.09 C	15.96 ± 0.55 B	139.59 ± 2.53 BC	2.95 ± 0.24 B
US_812	2.45 ± 0.13 AB	19.31 ± 0.77 A	152.38 ± 2.38 A	3.23 ± 0.24 AB
US_942	2.58 ± 0.14 A	20.60 ± 0.74 A	151.15 ± 3.11 A	2.53 ± 0.14 B

Values represent the mean ± standard error and letter groupings were obtained using the Tukey-Kramer method. Values followed by the same letter do not differ significantly at the 5% level.

*UFR_16 means are presented for information only and should not be directly compared to other rootstocks because it was planted 10 months later at this site.

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

Quarter Ending 31 December, 2017

3. CITRUS HOST INTERVENTION

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

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

1. Track ongoing research on horticultural aspects of HLB and tree health
2. Provide communication on project goals, progress and results to CPDC, CRDF and growers

Narrative of Progress against Goals:

The Horticultural Practices projects focus on the potential impacts on HLB status in existing trees. These have included thermotherapy, plant growth regulators, soil microbial amendments, nutritional treatments and overall grower practices. Most projects have been completed and reported in previous reports. There are two ongoing projects on tree nutrition. Dr. J. Grosser's 15-013 project and Dr. A. Schumann's 15-023 project.

Grosser's 15-013 project is on "the Interaction of Rootstocks and Constant Nutrition to Enhance the Establishment, Longevity and Profitability of Citrus Plantings in HLB-Endemic Areas" is a greenhouse study to determine if combined overdoses pf TigerSul manganese and Florikan poly-coated sodium borate are phytotoxic or able improve HLB-infected tree health and impact Liberibacter titers in roots and shoots across multiple rootstocks. Progress: Nutritional profile as well as infection status of all grafted plants were analyzed in the past quarter. qPCR values indicated that there were no significant differences in the infection rate amongst the different treatments at this time. The Harrell's nursery mix supplemented with Boron had lower overall infection rates as estimated by higher Ct values in all the rootstocks except UFR15, where the nursery mix supplemented with Manganese performed better. It is still too early to see much treatment and rootstock differences. A second objective is to evaluate the effect of complete, balanced and constant nutrition of HLB-infected mature trees (combinations, delivery, economics). The total 2nd year fertilizer applications were completed. Year 2 yield and fruit quality data collection will begin this quarter.

The goal of Dr. A. Schumann's 15-023 project on "Citrus nutrition studies for improved survival of HLB-affected trees" is to find the reasons for inconsistent responses of HLB-affected citrus to Enhanced Nutrient (EN) programs and to develop feasible and economical remedies that can consistently replicate successful HLB mitigation with ENs in all Florida groves. Data will be used to determine optimum soil conditions and to establish nutrient sufficiency guidelines for leaf tissues of HLB-affected trees that have successfully responded to enhanced nutritional programs. Progress: In November and Early December, soil samples from all 3 regional sites were sampled and analysis within the neural network software is in progress. We have completed the

measurements of permanent wilting point (PWP) on the first two years of soil data. Soils from the South Florida area will be included into the data set in November and will be measured for PWP, organic matter content and color analysis. We are utilizing three nutrient solutions to enable us to make minor changes to the phosphorus/calcium amounts to accelerate root hair development and mycorrhizae proliferation. November leaf samples from all three locations are still being analyzed but included ImageJ analysis, nutrition, as well as tree canopy measurements, leaf greeness, canopy height and volume. These data will be added to our comprehensive database for analysis using the neural network software Easy-NN for any possible connection or correlation with HLB severity. Results will be delivered to the Florida citrus industry through extension / outreach to all stakeholders (growers, contractors, supporting industries).

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

Quarter Ending December 31, 2017

OTHER PATHOGENS

Project title: 5. Other Pathogens

Project goals for this project area for the next year:

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

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

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

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

Narrative of Progress against Goals:

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

A two-year project, 16-010C, was funded 3/1/2016 entitled "Enhancement of post-bloom fruit drop control measures". The researcher is delinquent in reporting on this project, therefor progress towards completion cannot be assessed.

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

A three-year project, 15-005, was funded 7/1/2015 entitled "Asexual inoculum production of *Guignardia citricarpa*, the causal agent of citrus black spot". The researcher is delinquent in reporting on this project, therefor progress towards completion cannot be assessed.

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

Research priority areas are being discussed by members of the citrus industry and researchers for the 2018 call for proposals. Non-HLB citrus diseases are still of significance to the Florida citrus industry and research priorities will likely include studies on diseases such as PFD, citrus canker and citrus black spot.