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

Quarter Ending March 31, 2018

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.

No progress to report. Any new projects are expected to be submitted through RMC-18 or CPDC-18.

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

The milestones of this project are being completed as planned and the project is on track. A steering committee meeting took place in January to report on progress towards milestones. The next steering committee meeting will be scheduled in quarter one of the next fiscal year.

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

The project 17-005C examining the effect of thermotherapy on bactericide uptake was completed this quarter. The final report will be submitted next quarter and a presentation will take place on May 22.

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2. Asian Citrus Psyllid VECTOR INTERVENTION

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

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

16-020C, Dr. C. Vincent, UF. "Dyed kaolin to repel Asian citrus psyllid in field conditions." Preliminary results on one-year-old trees indicate that the low ACP counts of both kaolin treatments compare favorably with foliar insecticides, and red-dyed kaolin has been more effective than non-dyed kaolin. The kaolin effect continues to result in differences in CLas infection. A preliminary January 2018 sampling for qPCR assessment of CLas infection indicated that 30% of untreated control plants were infected, 25% of foliar insecticide-treated plants were infected (not significantly different from the untreated control), but only 10% of undyed kaolin plants were infected, and still no plants of the red-dyed kaolin plants were infected. Additionally, plants in both of the kaolin treatments had higher growth rates than the other two treatments and the red kaolin treatment had significantly more flowers than the others. White kaolin and foliar insecticide had significantly more flowers than the control. These results on one-year-old trees are indicative of differences in plant carbohydrate status, and a possible red-light enhancement effect on floral induction. Project end date is 11-30-19 and spending rates are on target as of May 2018.

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

There are no activities to report in this area.

Obj. 3 - Continue participation in pesticide stewardship activities

15-036C, Dr. M. Rogers, UF is entitled, "Correlating pesticide residue analysis with psyllid feeding to improve protection of young trees." The primary goals of objective 1 was met by determining how much of each of the three neonics is required to cause morality via contact exposure versus ingestion. It takes more neonicotinoid insecticide to kill via ingestion compared to contact exposure to the insecticide, which was the opposite of what we hypothesized. When we examined the field populations of psyllids at 4 locations around the state, we found that there were shifts in susceptibility to the neonics suggesting serious insecticide resistance development in the psyllid populations. The full story on understanding the spatial and temporal distribution of neonics within a tree over time under field conditions is underway (Objective 2). Our major contribution to citrus pest management from objective 3 is the realization that the rates for thiamethoxam are possibly only working in the smallest tree class based on the recommended rates by the registrant. Thus, any trees greater than 1 year of age are unlikely to benefit from thiamethoxam applications without going above the rate currently recommended for psyllid management. Project end date is 6-30-18 and spending rates are on target as of May 2018.

17-001C, Dr. L. Stelinski, UF. “Insecticide resistance management in Florida citrus production.” End date 6/30/2019. Continued evidence of insecticide resistance in isolated ACP populations to several insecticide chemistries (Imidacloprid, Bifenthrin, Dimethoate and Afidopyropen) during the winter months of 2017-2018. Therefore, rotation of five modes of action allows optimal reversion to susceptibility among ACP populations given their generation time (approx. 1 month from egg to adult) in Florida. Under laboratory conditions, Afidopyropen was lethal to egg, nymph, and adult stages of ACP. Furthermore, Afidopyropen reduced nymph to adult emergence, reduced adult host settling and feeding, and contributed to overall population decline in ACP developing on treated as compared with control citrus. The direct and indirect effects of Afidopyropen against ACP indicate that it should be a useful tool as part of an integrated management program for ACP and HLB management. Further field scale testing is needed to determine how to best incorporate this new insecticide into a comprehensive rotation in citrus management. Project end date is 6-30-19 and spending rates are on target as of May 2018.

Obj. 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.

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2. Asian Citrus Psyllid VECTOR INTERVENTION

Project Title: 2B. RNAi Molecules/Psyllid Shield

Narrative of Progress against Goals:

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

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

All in the field trees have tested negative for HLB**.** The permit for the RNAi field trial requires monitoring for the brown citrus aphid, a CTV vector, thus far, no aphids have been found and those reports have been submitted to regulatory agencies. A design for challenging plants with psyllids to evaluate the RNAi treatment response has been finalized to include caged and uncaged trees.

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

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

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

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

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

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

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

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

During the first quarter of this fiscal year, there have been no outreach activities.

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

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

Significant Meetings or Conferences:

Obstacles Encountered and Breakthroughs:

Other Information:

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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 selections identified by a grower. The composite trees for the first field trial are growing well in the screen-house and propagation of rooted cuttings (Ungrafted) of three grapefruit scion selections for the second field trial began in April.

Trial Design: Composite trees, First Trial
The trial contains 9 grapefruit scion selections on two different rootstocks. The trial is a randomized complete block split plot design with 3 blocks replicated four times. Each split plot contains 4 trees of one scion/rootstock combination. The whole plot contains 8 trees total with one scion on two rootstocks.

Trial Design: Rooted cuttings (Ungrafted), Second Trial
The field trial design for the rooted cuttings is a randomized complete block (RCB), one block with 4 replications and 8 trees per replication.

The rooted cuttings field trial will be planted next to the composite tree field trial for evaluation and comparison of horticultural performance. This side-by-side comparison may provide clarity on putative HLB-tolerance of these volunteer scion selections because the mature volunteer trees are not budded to rootstocks. The volunteer trees in the commercial grove are ~25+ years old and are infected with HLB but show very low bacterial titers and copy number (PCR testing) compared to the commercial trees in the grove... The trees also produce few HLB-symptomatic fruit. A field map has been finalized and the grower expects to plant the trial in July 2018.

b) Transgenic field trials
Transgenic events derived for project 15-020 and 424 will be added to Dr.Stover's transgenic permit to allow for field evaluation. Propagation of materials for the field trial is ongoing. It remains to be determine how many of the selections will be part of a research or commercial product delivery trial depending on how much data has been accumulated to inform the PIs' decisions.

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

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

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

The following activities focus on objectives 2, 3 and 4.
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. These discussions led to a meeting of over 60 representatives of citrus breeding and genetics researchers, growers, government officials, process and other citrus industry stakeholders. The result of this discussion was the identification of four major barriers:

1. Scientific barriers regarding data standardization, collection and sharing in addition to other scientific and researcher-related issues.
2. Regulatory barriers regarding the interstate movement of citrus plant material for research purposes and the need for help in complying with biotechnology regulations.
3. Intellectual property and tech transfer barriers associated with scientists' host institutions, i.e., university or government entities.
4. Funding agency related barriers regarding their role in promoting better coordination and collaboration among scientists.

A major outcome of the meeting was the collaborative development of minimum requirements for experimental design and data collection procedures for plant improvement projects. Ten researchers representing Arizona, California, Florida and Texas drafted guidelines for greenhouse and early field trial evaluation of citrus. Guidelines for much larger trials were also drafted with input from the funding organizations. CRDF Committees and Board elected to adopt these data collection guidelines for all trials funded by CRDF beginning with the 2018-RFP.
Sub-groups are working with state and federal agencies to simplify regulatory requirements for inter-state movement of citrus to facilitate research whilst safeguarding the industry.
In addition, CRDF and HLB-MAC will work together to implement researcher regulatory education programs to provide support and speed up acquisition of permits, establishment of compliance SOPs and any other expert connections which further citrus research.

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

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 an approriate procedures 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 at 8.5 ft. between trees and 21.4 ft. between rows. 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.

Duda Yield and fruit quality data for the 2018 harvest season

The Duda rootstock trial was harvested by plot (70 trees/plot) excluding buffer trees and a subsample of approximately 18 lbs. was collected for maturity and juice quality analysis test at the University of Florida's pilot plant. Ten out of 35 plots did not pass the Valencia maturity test (total brix < 8 %). Data were analyzed using the GLM procedure of SAS® software (SAS Institute Inc, 2002 -2012). There were no statistically significant differences ($p < 0.05$) among the rootstocks or rootstock effect on the traits of interest. The traits measured empirically or by calculation were: sub-sampled juice per plot, calculated juice weight per box (lbs), acid, total brix, brix acid ratio, calculated total soluble solids per box (lbs), juice color, yield per plot (lbs) yield per plot in boxes (lbs), calculated yield yield per plot(boxes) and calculated total soluble solids per acre(lbs) (Table 1.). The total harvested trial (35 plots x 70 trees) yielded 156.6 bozex of fruit (raw data). This trial was impacted by hurricane Irma with hurricane force winds and flooding and a lot of fruit was lost as a result. The grower cooperator was able to pump water out of the grove very quickly , however, the stress on the trees likely had a direct impact on harvest and juice quality.

Duda Horticultural Trait Data

There were significant differences ($p < 0.05$) among rootstocks for canopy volume (m^3), trunk cross-sectional area (TCSA) (cm^2) and tree height (cm) at this location (Table 2). 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). Canopy volume data corroborates the trait correlation data that where trees with larger canopies tended to have higher yield.

Table 1 CRDF Duda rootstock trial average rootstock effect on yield and juice quality ± standard error of the mean, plots harvested in April 2018

Rootstock	***Sub-sample Juice Weight Per Plot (lbs.)***	***Calculated Juice Weight Per Box (lbs.)***	***Acid***	***Total Brix***	***Brix Acid Ratio***	***Calculated Total Soluble Solids Per Box (lbs.)***	***Juice Color***	***Yield Per Plot (lbs.)***	***Yield Per Plot (Boxes)***	***Calculated Yield Per Acre (Boxes)***	***Calculated Total Soluble Solids Per Acre (lbs.)***
Swingle	*9.82 ± 0.45*	*48.9 ± 1.63*	*0.59 ± 0.02*	*8.29 ± 0.22*	*14 ± 0.36*	*4.06 ± 0.21*	*36.68 ± 0.33*	*453.38 ± 101.76*	*5.04 ± 1.13*	*14.65 ± 3.29*	*62.4 ± 13.25*
UFR_16	*8.9 ± 0.45*	*46.58 ± 1.63*	*0.61 ± 0.02*	*8.14 ± 0.22*	*13.26 ± 0.36*	*3.79 ± 0.21*	*36.34 ± 0.33*	*334.13 ± 101.76*	*3.71 ± 1.13*	*10.8 ± 3.29*	*42.69 ± 13.25*
UFR_2	*10.66 ± 0.45*	*50.77 ± 1.63*	*0.62 ± 0.02*	*8.5 ± 0.22*	*13.83 ± 0.36*	*4.32 ± 0.21*	*36.68 ± 0.33*	*416.77 ± 101.76*	*4.63 ± 1.13*	*13.47 ± 3.29*	*56.25 ± 13.25*
UFR_3	*8.85 ± 0.45*	*45.42 ± 1.63*	*0.55 ± 0.02*	*7.93 ± 0.22*	*14.37 ± 0.36*	*3.62 ± 0.21*	*36.06 ± 0.33*	*309.05 ± 101.76*	*3.43 ± 1.13*	*9.99 ± 3.29*	*35.18 ± 13.25*
UFR_4	*9.97 ± 0.45*	*48.88 ± 1.63*	*0.63 ± 0.02*	*8.34 ± 0.22*	*13.29 ± 0.36*	*4.08 ± 0.21*	*36.58 ± 0.33*	*383.17 ± 101.76*	*4.26 ± 1.13*	*12.38 ± 3.29*	*50.4 ± 13.25*
US_812	*8.99 ± 0.45*	*45.94 ± 1.63*	*0.61 ± 0.02*	*7.97 ± 0.22*	*13.13 ± 0.36*	*3.68 ± 0.21*	*35.82 ± 0.33*	*432.02 ± 101.76*	*4.8 ± 1.13*	*13.96 ± 3.29*	*51.98 ± 13.25*
US_942	*9 ± 0.45*	*44.61 ± 1.63*	*0.56 ± 0.02*	*7.83 ± 0.22*	*14.1 ± 0.36*	*3.51 ± 0.21*	*35.36 ± 0.33*	*491.36 ± 101.76*	*5.46 ± 1.13*	*15.88 ± 3.29*	*54.47 ± 13.25*

Table 2 CRDF Duda site rootstock trial horticultural traits, HLB Disease index (DI) and PCR Cycle Threshold means ± standard error of the mean data collected in spring 2018

Rootstock	**Canopy Volume (m^3)**	**TCSA (cm^2)**	**Tree Height (cm)**	**HLB DI[a]**	**PCR Cycle Threshold**
Swingle	5.77 ± 0.3 BC	40.45 ± 1.49 BC	200.50 ± 3.92 C	3.45 ± 0.21 B	31.39 ± 0.78
UFR_16	4.83 ± 0.3 C	40.67 ± 1.49 BC	194.63 ± 3.92 C	3.63 ± 0.21 B	34.33 ± 0.78
UFR_2	5.94 ± 0.3 BC	37.02 ± 1.51 C	202.85 ± 3.97 BC	3.51 ± 0.22 B	31.91 ± 0.78
UFR_3	3.51 ± 0.3 D	27.36 ± 1.51 D	174.44 ± 3.97 D	4.56 ± 0.22 A	34.31 ± 0.78
UFR_4	6.98 ± 0.3 AB	44.58 ± 1.49 AB	223.18 ± 3.92 A	3.78 ± 0.21 AB	31.65 ± 0.78
US_812	7.27 ± 0.3 A	47.50 ± 1.49 A	219.08 ± 3.92 AB	2.55 ± 0.21 C	31.52 ± 0.78
US_942	7.45 ± 0.3 A	48.49 ± 1.49 A	229.48 ± 3.92 A	3.33 ± 0.21 BC	31.86 ± 0.78

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

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

The trial 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. The trees are planted at spacing of 8 ft. between trees and 18 ft. between rows. Eight sentinel trees were randomly assigned to each plot at planting for data collection.

Peace River Yield and fruit quality data for the 2018 harvest season

The Peace River rootstock trial was harvested by plot (64 trees/plot) excluding buffer trees and a subsample of approximately 18 lbs. was collected for maturity and juice quality analysis test at the University of Florida's pilot plant. 6 out of 40 plots did not pass the Valencia maturity test (total brix < 8 %). Data were analyzed using the GLM procedure of SAS® software (SAS Institute Inc, 2002 - 2012). There were statistically significant differences ($p < 0.05$) among the rootstocks or rootstock effect on, acid, brix acid ratio, juice color, yield per plot (lbs) yield per plot in boxes (lbs), calculated yield yield per plot(boxes) and calculated total soluble solids per acre(lbs) (Table 3). US 812 had the highest yield though not statistically significantly different from US 942, US 897, Carrizo and UFR 4 respectively.
The total harvested trial (40 plots x 64 trees) yielded 377.5 boxes of fruit (raw data). This trial was impacted by hurricane Irma with hurricane force winds alot of fruit was lost as a result and the stress likely had a physiological effect on the trees

Peace River Horticultural Trait Data

There were significant differences ($p < 0.05$) for horticultural traits reported in this period at the peace river location for canopy volume (m^3), trunk crossectional area (cm^2), and tree height (cm) (Table 4). The highest variation of performance among rootstocks was in canopy volume, TCSA and tree height where US 942, US 812, UFR 4, Carrizo and US 897, respectively, had better performance than UFR 2 and UFR 3.

Table 3 CRDF Peace River site rootstock trial average rootstock effect on yield and juice quality ± standard error of the mean, plots harvested in March 2018

Rootstock	Sub-sample Juice Weight Per Plot (lbs.)	Calculated Juice Weight Per Box (lbs.)	Acid	Total Brix	Brix Acid Ratio	Calculated Total Soluble Solids Per Box (lbs.)	Juice Color	Yield Per Plot[#] (lbs.)	Yield Per Plot (Boxes)	Calculated Yield Per Acre (Boxes)	Calculated Total Soluble Solids Per Acre (lbs.)
Carrizo	9.48 ± 0.38	48.23 ± 0.98	0.56 ± 0.02 B	8.31 ± 0.21	14.81 ± 0.35 AB	4.02 ± 0.17	37.1 ± 0.2 B	1216.6 ± 174.1 AB	13.5 ± 1.9 AB	63.9 ± 9.1 AB	261.5 ± 39.5 AB
UFR_2	10.1 ± 0.38	51.50 ± 0.98	0.58 ± 0.02 AB	8.52 ± 0.21	14.83 ± 0.35 AB	4.39 ± 0.17	37.6 ± 0.2 AB	636.1 ± 174.1 BC	7.1 ± 1.9 BC	33.4 ± 9.1 BC	147 ± 39.5 BC
UFR_3	9.69 ± 0.38	50.34 ± 0.98	0.58 ± 0.02 AB	8.93 ± 0.21	15.42 ± 0.35 A	4.5 ± 0.17	38 ± 0.2 AB	352.4 ± 174.1 C	3.9 ± 1.9 C	18.5 ± 9.1 C	80.5 ± 39.5 C
UFR_4	9.89 ± 0.38	52.39 ± 0.98	0.64 ± 0.02 AB	8.98 ± 0.21	14.02 ± 0.35 AB	4.71 ± 0.17	37.8 ± 0.2 AB	845.6 ± 174.1 ABC	9.4 ± 1.9 ABC	44.4 ± 9.1ABC	205.1 ± 39.5 ABC
UFR_16*	8.95	45.13	0.60	7.96	13.41	3.61	37.40	37.8	0.42	1.98	7.4
US_812	10.06 ± 0.38	52.22 ± 0.98	0.63 ± 0.02 AB	8.76 ± 0.21	13.98 ± 0.35 AB	4.58 ± 0.17	38.2 ± 0.2 A	1458 ± 174.1 A	16.2 ± 1.9 A	76.6 ± 9.1 A	347.6 ± 39.5 A
US_897	10.36 ± 0.38	51.54 ± 0.98	0.65 ± 0.02 A	8.76 ± 0.21	13.60 ± 0.35 B	4.52 ± 0.17	37.8 ± 0.2 AB	931.6 ± 174.1 ABC	10.4 ± 1.9 ABC	48.9 ± 9.1ABC	220.8 ± 39.5 ABC
US_942	9.77 ± 0.38	50.09 ± 0.98	0.60 ± 0.02 AB	8.43 ± 0.21	13.99 ± 0.35 AB	4.22 ± 0.17	37.6 ± 0.2 AB	1317.4 ± 174.1 AB	14.6 ± 1.9 AB	69.2 ± 9.1 AB	290.6 ± 39.5 AB

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

*UFR-16 planted late, results presented for information purposes not comparison, excluded from main analysis.

Table 4 CRDF Peace River site rootstock trial horticultural traits, HLB Disease index (DI) and PCR Cycle Threshold means ± standard error of the mean data collected in spring 2018

Rootstock	Canopy Volume (m^3)	TCSA (cm^2)	Tree Height (cm)	HLB DI[a]	PCR Cycle Threshold
Carrizo	3.02 ± 0.17 B	26.18 ± 1.03 AB	163.95 ± 3.91 A	3.65 ± 0.21 A	30.36 ± 0.66 B
***UFR_16**	1.42 ± 0.17 DE	10.99 ± 1.03 E	133.93 ± 3.91 CD	3.25 ± 0.21 AB	35 00 ± 0.66 A
UFR_2	2.12 ± 0.17 CD	16.47 ± 1.03 D	142.08 ± 3.91 BC	3.33 ± 0.21 AB	30.02 ± 0.66 B
UFR_3	1.38 ± 0.17 E	11.54 ± 1.03 E	121.80 ± 3.91 D	3.63 ± 0.21 A	30.80 ± 0.66 B
UFR_4	3.26 ± 0.17 AB	24.95 ± 1.03 BC	160.55 ± 3.91 A	2.85 ± 0.21 AB	29.85 ± 0.66 B
US_812	3.41 ± 0.17 AB	26.27 ± 1.03 AB	169.80 ± 3.91 A	2.72 ± 0.21 B	29.92 ± 0.66 B
US_897	2.82 ± 0.17 BC	21.10 ± 1.03 C	157.35 ± 3.91 AB	3.13 ± 0.21 AB	30.13 ± 0.66 B
US_942	3.77 ± 0.17 A	29.44 ± 1.03 A	171.63 ± 3.91 AB	3.25 ± 0.21 AB	30.44 ± 0.66 B

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

*UFR-16 planted late, results presented for information purposes not comparison.

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. The trees are planted at spacing of 8.25 ft. between trees and 22 ft. between rows.

BHG Yield and fruit quality data for the 2018 harvest season

The BHG rootstock trial was harvested by plot (64 trees/plot) excluding buffer trees and a subsample of approximately 18 lbs. was collected for maturity and juice quality analysis test at the University of Florida's pilot plant.28 out of 35 plots did not pass the Valencia maturity test (total brix < 8 %). Data were analyzed using the GLM procedure of SAS® software (SAS Institute Inc, 2002 -2012). There were no statistically significant differences ($p < 0.05$) among the rootstocks or rootstock effect on the traits of interest. The traits measured empirically or by calculation were: sub-sampled juice per plot, calculated juice weight per box (lbs), acid, total brix, brix acid ratio, calculated total soluble solids per box (lbs), juice color, yield per plot (lbs) yield per plot in boxes (lbs), calculated yield yield per plot(boxes) and calculated total soluble solids per acre(lbs) (Table 5.). The total harvested trial (35 plots x 64 trees) yielded 57.4 boxes of fruit (raw data). As reported last quarter, this trial was impacted by hurricane Irma with sustained hurricane force winds and sand damage to the canopy which exacerbated the HLB-DI collected in the fall of 2017 and also had a significant effect on fruit quality and retention. .

BHG 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 6). 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 and the higher HLB-DI values and low canopy volume are an indicator of the possible higher stress level effect on tree physiology at this location. The trees continue to grow out of this stress and it this time it is not advisable to draw large conclusions on rootstock horticultural performance given the confounding environmental effects.

Table 5 BHG rootstock trial average rootstock effect on yield and juice quality ± standard error of the mean, plots harvested in March 2018

Rootstock	Sub-sample Juice Weight per Plot (lbs.)	Calculated Juice Weight Per Box (lbs.)	Acid	Total Brix	Brix Acid Ratio	Calculated Total Soluble Solids Per Box (lbs.)	Juice Color	Yield Per Plot (lbs.)	Yield Per Plot (Boxes)	Calculated Yield Per Acre (Boxes)	Calculated Total Soluble Solids Per Acre (lbs.)
SOUR	10.42 ± 0.23	50.64 ± 0.75	0.58 ± 0.03	7.83 ± 0.12	13.53 ± 0.59	3.97 ± 0.1	37.42 ± 0.25	193.61 ± 18.29	2.15 ± 0.2	8.07 ± 0.76	32.02 ± 2.84
*UFR_16	7.9 ± 0.26	42.25 ± 0.84	0.73 ± 0.03	7.31 ± 0.14	10.29 ± 0.66	3.09 ± 0.11	35.88 ± 0.28	24.55 ± 20.45	0.27 ± 0.23	1.02 ± 0.85	3.15 ± 3.17
UFR_2	9.59 ± 0.23	48.17 ± 0.75	0.54 ± 0.03	7.62 ± 0.12	14.13 ± 0.59	3.67 ± 0.1	37 ± 0.25	90.61 ± 18.29	1.01 ± 0.2	3.78 ± 0.76	13.89 ± 2.84
UFR_3	8.84 ± 0.23	45.59 ± 0.75	0.55 ± 0.03	8 ± 0.12	14.7 ± 0.59	3.66 ± 0.1	37.3 ± 0.25	53.95 ± 18.29	0.6 ± 0.2	2.25 ± 0.76	8.36 ± 2.84
UFR_4	9.75 ± 0.23	49.22 ± 0.75	0.64 ± 0.03	7.93 ± 0.12	12.65 ± 0.59	3.9 ± 0.1	36.98 ± 0.25	104.45 ± 18.29	1.16 ± 0.2	4.35 ± 0.76	17.07 ± 2.84
US_812	9.47 ± 0.23	47.81 ± 0.75	0.57 ± 0.03	7.4 ± 0.12	13.12 ± 0.59	3.54 ± 0.1	37.2 ± 0.25	211.33 ± 18.29	2.35 ± 0.2	8.81 ± 0.76	31.18 ± 2.84
US_942	8.33 ± 0.23	42.98 ± 0.75	0.53 ± 0.03	7.11 ± 0.12	13.42 ± 0.59	3.06 ± 0.1	37.14 ± 0.25	359.92 ± 18.29	4 ± 0.2	15 ± 0.76	45.78 ± 2.84

Table 6. CRDF BHG site rootstock trial horticultural traits, HLB Disease index (DI) and PCR Cycle Threshold means ± standard error of the mean data collected in spring 2018

Rootstock	Canopy Volume (m^3)	TCSA (cm^2)	Tree Height (cm)	HLB DI (max. 10)	PCR Cycle Threshold
Sour	2.31 ± 0.11 BC	20.34 ± 0.66 A	147.8 ± 2.79 AB	4.08 ± 0.23 BC	30.26 ± 0.67 B
*UFR_16	0.95 ± 0.11 E	7.64 ± 0.66 D	120.0 ± 2.79 D	3.80 ± 0.23 BC	34.39 ± 0.68 A
UFR_2	1.57 ± 0.11 D	12.23 ± 0.66 C	130.8 ± 2.7 CD9	4.25 ± 0.23 B	29.97 ± 0.67 B
UFR_3	1.10 ± 0.11 E	9.49 ± 0.66 CD	128.1 ± 2.79 D	5.48 ± 0.23 A	31.87 ± 0.67 AB
UFR_4	2.09 ± 0.11 C	15.76 ± 0.66 B	141.3 ± 2.79 BC	4.38 ± 0.23 B	31.40± 0.67 B
US_812	2.72 ± 0.11 AB	19.32 ± 0.66 A	155.5 ± 2.79 A	3.28 ± 0.23 C	29.83 ± 0.67 B
US_942	2.83 ± 0.11 A	20.51 ± 0.66 A	155.0 ± 2.79 A	3.45 ± 0.23 BC	29.68 ± 0.67 B

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

*UFR-16 planted late, results presented for information purposes not comparison

Additional analyses correlating horticultural traits, HLB DI, PCR cycle threshold on yield and fruit quality

Data mining to determine whether there is any correlation between HLB-DI and PCR cycle threshold, yield and fruit quality traits is ongoing. Preliminary results indicate that HLB infection is moderately negatively correlated (-50% ≥ r ≤ -60%) and that canopy volume has a weak to moderate (30% ≥ r ≤ 60%) positive effect on yield (boxes/plot) at all locations. Data mining will continue.

Significant Meetings or Conferences:
The project manager attended the citrus show in January 2018.

Obstacles Encountered and Breakthroughs:

Other Information:

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

Quarter Ending March 31, 2018

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 of management practices 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.

Nutrition. Grosser's 15-013 project (end date 3-31-19) 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. <u>Progress</u>: 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. Year 2 yield and fruit quality data collection will begin this quarter. Spending rate is on target through Dec 2017.

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. <u>Progress</u>: In November and Early December, soil samples from all 3 regional sites were sampled and analysis within the neural network software is in progress. Measurements of permanent wilting point (PWP) on the first two years of soil data have been completed. 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. Three nutrient solutions are in use 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). Project end date was extended to 12-31-18; spending rates are on target as of May 2018.

Significant Meetings or Conferences:

Obstacles Encountered and Breakthroughs:

Other Information:

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

Quarter Ending March 31, 2018

4. OTHER PATHOGENS

Project title: 4a. Other Pathogens

Project goals for this project area for the next year:

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

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

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

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

Narrative of Progress against Goals:

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

A two-year project, 16-010C, was funded 3/1/2016 entitled "Enhancement of post-bloom fruit drop control measures". An annual report was submitted reporting progress through December 2017. From this report, the progress was on track through the quarter ending on December 31, 2017. The researcher is delinquent in reporting on this project for the quarter ending in March 31, 2018, 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 both annual reporting and March quarterly 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.

Non-HLB citrus diseases are still of significance to the Florida citrus industry and RMC-18 and CPDC-18 research priorities for the include studies on diseases such as PFD, citrus canker and citrus black spot.

Significant Meetings or Conferences:

Project managers attended the Citrus Show in Fort Pierce in January.