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

Quarter Ending 30 June 2018

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 is one ongoing project on controlling HLB using soil and tree microbes by Dr. N. Wang (15-043) and two ongoing projects on tree nutrition by Drs. J. Grosser's (15-013) and A. Schumann's (15-023).

Microbes. N. Wang (15-043) project (end date 9-30-18 + extension) is on "Control of citrus Huanglongbing using endophytic microbes from survivor trees" and is a greenhouse and field project to understand the role of endophytic microbes from survivor trees. Several phyla, such as Proteobacteria, Acidobacteria and Bacteroidetes were enriched in healthy root-associated microbiome. The challenge has been how to maintain a beneficial microbiome which is under study now. Uradyrhizobium and Burkholderia are the most abundant bacteria that have shown dramatic changes in HLB survivor and healthy trees. The two strains can successfully colonize the root surface and maintain a relative high population even seven months after inoculation. "Characterization of antimicrobial-producing beneficial bacteria isolated from HLB escape citrus trees" has been published by Frontiers in Microbiology. One more manuscript on the effect of induced systemic resistance against disease by rhizospheric bacteria has been accepted for publication by Phytopathology. Consortium of bacteria of different combinations are being used to test their effect on Las and ACP.ÐHLB impairs the rhizosphere-to-rhizoplane enrichment process of the citrus root-associated microbiome" has been published by Microbiome. We have requested a no cost extension for this project.

Nutrition. Dr J. Grosser's 15-013 project is on "Interactions of Rootstocks and Constant Nutrition to Enhance Profitability of Citrus Plantings in HLB-Endemic Areas". Obj. 1: In greenhouse studies, there was no significant differences in the N,P,K, Mg and Ca levels in any of the rootstock/fertilizer combinations. Plants growing in the Harrell's nursery mix treatment had

boron levels 5-6 folder higher and Mn levels were double in all rootstocks except the GFT+50-7 and X639. when compared to Swingle. There were no fertilizer effects on rate of infection from HLB positive bud wood but WGFT+ trifoliate orange 50-7 rootstocks with the St Helena mix exhibited higher CT values (lower bacterial titers) than the other rootstocks. This rootstock is also showing good HLB tolerance in several field plantings and is being considered for commercial release.

Obj. 3: To evaluate the effect of balanced constant nutrition on HLB-affected mature trees, fruit were harvested from both Arcadia and Fort Meade In March. There has been significant improvement in yield and fruit quality with the use of CRF and Tiger micronutrients (20%-50% higher rates on Manganese, Boron, and Iron) over the IFAS recommendation control treatment. Overall, the hurricane-affected yields seem to have improved with use of CRF and Tiger micronutrients. With the collection of year 3 data on yield and quality, we will have more confidence in results and be able to develop some guidelines for growers. The third year

application of fertilizer was completed in March and June 2018 and the year 3 tree health data has been collected. Everything is now going according to schedule.

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. Soil samples from all 3 regional sites were sampled and analysis within the neural network software. Based on leaf size and leaf nutrient values from broad range of locations around Florida, we identified the critical threshold values (CT) for deficiency of the leaf nutrient concentrations. These CTs will be useful in future to guide growers how much fertilizer to apply to specifically the HLB-affected groves. In a greenhouse study, three nutrient solutions were in use to make minor changes to the phosphorus/calcium amounts to accelerate root hair development and mycorrhizae proliferation in rootstocks. Leaf samples from all three locations have been analyzed and included Image J analysis, nutrition, as well as tree canopy measurements, leaf greenness, canopy height and volume. These data were added to our comprehensive database for analysis using the neural network software Easy-NN for any possible connection or correlation with HLB severity. Root hair development seems enhanced in the low P, high Ca environment and will be studied closer over the next few weeks, utilizing the microscopy lab to quantify and identify root hair development in each treatment. Project end date was extended to 12-31-18 to allow data analysis in hurricane damaged field sites.

Significant Meetings or Conferences:
A research update presentation on this leaf nutrition work by Schumann was given as part of CRDF's continuing communication seminars series following the July 24th CRDF BOD meeting.

Grosser's yield data was presented at the OJ Break on May 15, 2018 and at SWFREC. Detailed results of years 1 and 2 of this experiment will be presented at Citrus Expo Aug 15, 2018. Results showing a therapeutic affect from overdoses of manganese against HLB and interactions with B are being presented at the annual ASHS meeting in August in Washington DC; a manuscript is in preparation.

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) 15-010 Gmitter – Project reports progress towards objectives, however, it continues to be a challenge to track projects with Broad objectives that pertain to the program and not specifically to individual projects
b) 15-025 – Dr. Stover has observed putative tolerance to HLB in some transgenic events and will continue to evaluate and make selections for field testing. It is unclear how plant introductions fit in with the project objectives.
c) 15-033c and 15-045c – The transformation laboratories ran by Drs Orbovic and Zale both developed transgenic plants for researchers. Various events have putative resistance or tolerance towards HLB and citrus canker. The laboratories confirm presence of transgenes and transfer the plants to the researchers for further testing.
d) 15-020 – Dr. Mou has made progress in molecular characterization on several rootstock and scion transgenic lines showing tolerance towards HLB. Recent reports include results on a number of selections that are being increased for a field trial.
e) 16-001 Li. The project is making slow progress towards objectives. Project managers should schedule a meeting with the PI to understand the challenges and report back to RMC.
f) 16-016c Eyrich – The progress towards objectives was slowed by the hurricane. However, it is important that the PM follow up with the PI to get a better sense of the project status.

Grower Field Trials – Assessing HLB tolerance in volunteer grapefruit Wescott Groves
Preparations to plant the Grapefruit grower trial with Wescott groves are underway. The experiment is broken into two sections. The composite grafted trees will be planted in August of 2018. The ungrafted grapefruit scion trial has been delayed by the slow growth of rooted cuttings and it is likely that the ungrafted scions will be planted in the spring of 2019.

Transgenic field trials

CRDF committees and Board continue to review proposals submitted for funding. Once those decisions are final, (September 2017) staff will determine how many new transgenic field trials will be planted. CPDC continues to fund the secure site at the USDA Picos farm, project 15-039c, which will end in December of 2018. The Committee will e=review a new proposal to fund the site for a new cycle.

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.

CRDF committees and Board held several meetings to consider coordinated research efforts with other funding agencies including sharing research topic ideas. These ideas may help build on current knowledge or stimulate new ideas and secure funding independently through separate entities. Discussions are ongoing.

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

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 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 June 2018, 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). Rootstock performance groupings for each variable can be separated by the best performing rootstocks in the following order US_942, UFR_4, US_812, Swingle, UFR_16, UFR_2, and UFR_3. Data collected and analyzed in this quarter indicate the tree growth has slowed remarkably in the last year.

Table 1. CRDF Duda site rootstock trial horticultural traits and HLB Disease index (DI) means ± standard error of the mean data collected in June 2018

Rootstock	Canopy Volume (m^3)	TCSA (cm^2)	Tree Height (cm)	HLB DI (max. 10)
Swingle	6.2 ± 0.3 B	42.7 ± 1.5 BCD	201.2 ± 3.6 BC	3.1 ± 0.2 C
UFR_16	5.0 ± 0.3 C	41.6 ± 1.5 CD	190.5 ± 3.6 C	4.1 ± 0.2 A
UFR_2	5.5 ± 0.3 BC	39.1 ± 1.6 D	191.4 ± 3.7 C	3.3 ± 0.2 BC
UFR_3	3.7 ± 0.3 D	30.1 ± 1.5 E	174.0 ± 3.6 D	4.1 ± 0.2 AB
UFR_4	7.4 ± 0.3 A	48.6 ± 1.5 AB	217.0 ± 3.6 A	3.7 ± 0.2 ABC
US_812	7.3 ± 0.3 A	47.9 ± 1.5 ABC	215.3 ± 3.6 AB	3.2 ± 0.2 C
US_942	8.2 ± 0.3 A	50.3 ± 1.5 A	226.1 ± 3.6 A	3.2 ± 0.2 C

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.

These data reflect continued negative effects of hurricane Irma in September 2017. Although the grower pumped excess water from the field in less than 24hrs of the hurricane event, there were significant effects of the hurricane force winds on the trees that will take some time to overcome. The data show smaller canopies, shorter trees that may be explained by HLB infection as well as defoliation and added stresses of the hurricane. The trees will continue to grow out of the stresses in the environment and other physiologically stressful parameters such as the crop load. Additional data collected at harvest will inform on the effect of the environmental stressors on yield and juice and fruit quality. It is likely that it will take several years for the trees to overcome the hurricane effects that will likely continue to be exacerbated

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 June 2018 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 continues to reflect possible effects of hurricane Irma wind gusts. Although the trees at this location were not affected by flooding post hurricane, the reduced rate of growth is likely a function of the hurricane stress and HLB infection. CRDF will collect harvest yield and fruit quality data in Spring of 2019.

Table 2. CRDF Peace River site rootstock trial horticultural traits and HLB Disease index (DI) means ± standard error of the mean data collected in June 2018

Rootstock	Canopy Volume (m^3)	TCSA (cm^2)	Tree Height (cm)	HLB DI (max. 10)
Carrizo	3.6 ± 0.2 AB	30.3 ± 1.2 A	168.0 ± 4.2 A	3.3 ± 0.2 BC
UFR_16*	1.8 ± 0.2 D	13.6 ± 1.2 CD	139.7 ± 4.2 BC	4.6 ± 0.2 A
UFR_2	2.3 ± 0.2 CD	18.7 ± 1.2 BC	141.4 ± 4.2 BC	3.5 ± 0.2 BC
UFR_3	1.6 ± 0.2 D	13.1 ± 1.3 D	126.6 ± 4.2 C	4.1 ± 0.2 AB
UFR_4	3.4 ± 0.2 AB	28.6 ± 1.2 A	163.7 ± 4.2 A	3.0 ± 0.2 C
US_812	3.8 ± 0.2 AB	28.9 ± 1.2 A	173.4 ± 4.2 A	2.8 ± 0.2 C
US_897	3.0 ± 0.2 BC	22.5 ± 1.2 B	156.0 ± 4.2 AB	3.2 ± 0.2 C
US_942	4.1 ± 0.2 A	31.6 ± 1.2 A	171.4 ± 4.2 A	2.8 ± 0.2 C

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 in 2017. It is likely that the hurricane stress exacerbated the effects of HLB and that it is likely that the slowed growth will be observed for some time to come.

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 June 2018

Rootstock	Canopy Volume (m^3)	TCSA (cm^2)	Tree Height (cm)	HLB DI (max. 10)
Sour	2.5 ± 0.11 AB	22.3 ± 0.68 A	156.3 ± 2.93 A	4.2 ± 0.18 A
*UFR_16	1.2 ± 0.11 C	10.1 ± 0.68 D	129.8 ± 2.93 B	3.8 ± 0.18 AB
UFR_2	1.7 ± 0.11 C	13.8 ± 0.68 C	137.6 ± 2.93 B	3.6 ± 0.18 AB
UFR_3	1.4 ± 0.11 C	10.9 ± 0.68 D	132.1 ± 2.93 B	3.8 ± 0.18 AB
UFR_4	2.3 ± 0.11 B	17.8 ± 0.68 B	152.6 ± 2.93 A	3.7 ± 0.18 AB
US_812	2.9 ± 0.11 A	21.3 ± 0.68 A	162.9 ± 2.93 A	3.3 ± 0.18 BC
US_942	2.8 ± 0.11 AB	22.4 ± 0.68 A	157.1 ± 2.93 A	2.8 ± 0.18 C

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.

Significant Meetings or Conferences:

Citrus Grower Institute - April 2, 2018, Dr. Hatcher gave an update on CRDF's 2018 request for proposals (RFP 2018) process and topics.

Citrus Regulatory Working Group – Bi-weekly April – June 2018. Dr. Hatcher represented CRDF and Florida in this collaborative group working to understand regulations about citrus germplasm movement. The goal is to streamline regulations with the help of State and Federal agencies to accelerate research while protecting the Citrus Industry from unwanted pests and diseases.

HLB-MAC, Various dates April – June 2018. Dr, Hatcher represented CRDF at the HLB-MAC meetings where projects with possible shovel ready solutions to HLB were considered. HLB-MAC will update website information and send out notices to stakeholders to announce suggestions for the next round of funding next quarter.