Annual Report
on CRDF Programmatic Activities
July 1, 2017 through June 30, 2018

This report is directed to the Florida /s ummarizing CRDF activities in pursuit of solutions to HLB and other challenges to the dustry. This report covers Fiscal Year 2017-18, bridging the period July 1, 2017 through June 30, 2018. The narrative covers activities managed by the Board of Directors via the Research Management; Commercial Product Delivery; and Industry Research Coordinating Committees.

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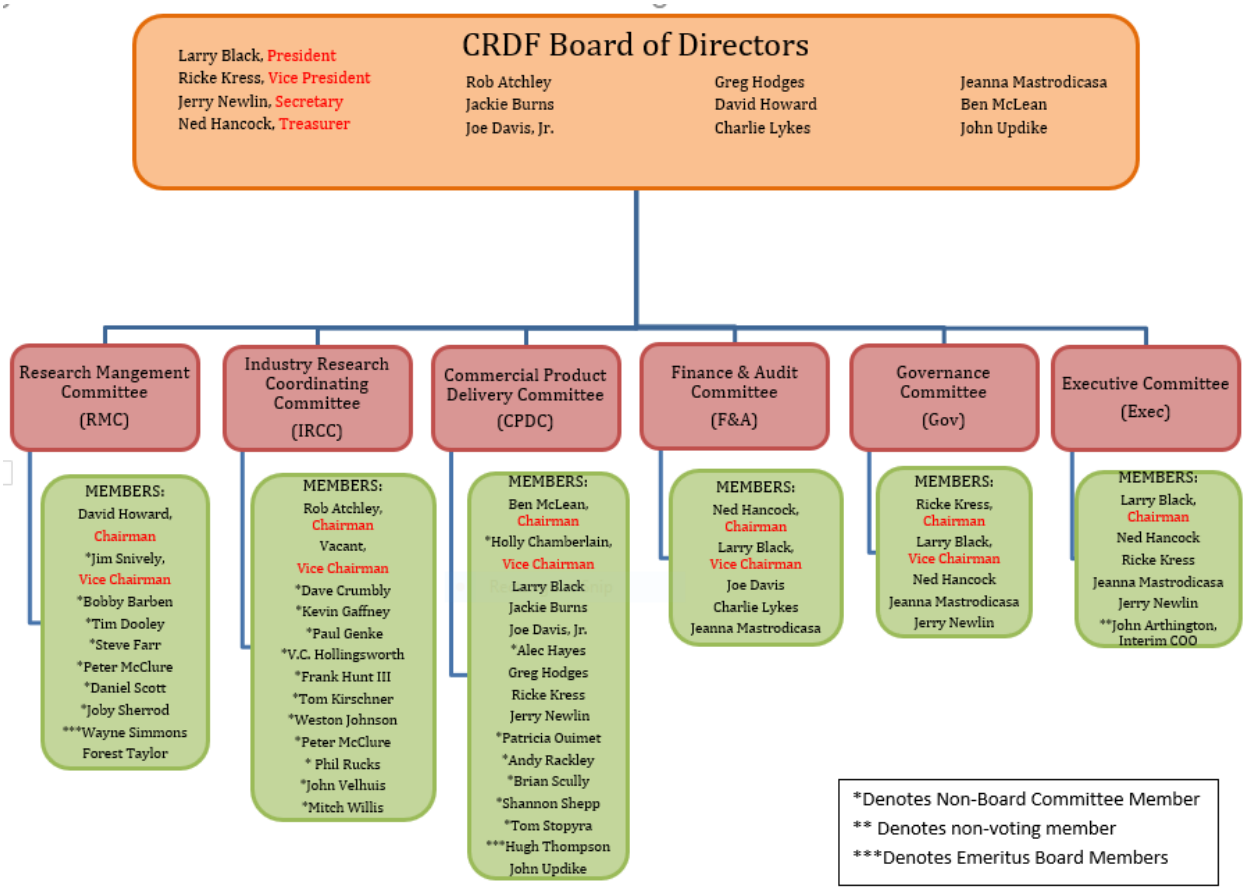
INTRODUCTION

Projects and activities highlighted in this report represent the ongoing research portfolio of CRDF. CRDF's Board of Directors is the governing body for the organization, exercising its mission to develop and deliver solutions to HLB. The content of this report summarizes the progress and accomplishments of projects funded by CRDF through guidance from the Research Management Committee (RMC) and the Commercial Product Delivery Committee (CPDC).

Upon recommendation from these committees, the CRDF Board acts to approve priorities and funding of specific projects to meet the goals of developing a balanced management system for sustainability and success of the Florida citrus industry in the presence of HLB. Greatest focus for much of the work is directed to growers and grove practices.

Board and committee members (Figure 1) continue to aggressively pursue all avenues towards important short-term solutions as well as long-term, durable solutions that will allow Florida to regain its place as a world leader in citrus production, quality and health.



CRDF Establishes Emeritus Status for Outgoing Board Members

The CRDF Board of Directors adopted an "Emeritus" status for those Board members who have completed two full terms on the Board and had an interest in retaining involvement and awareness of current Foundation activities and obtaining information available to Directors. Emeritus members have a one-term limit of up to two years and can continue to serve on committees but may not serve as Chairman or Vice-Chairman. Emeritus members possess active voting rights on committees but not at Board of Directors' meetings.
Three of the four board members leaving the Board in January 2018 requested emeritus status: Mr. Tom Jerkins, Mr. Wayne Simmons and Mr. Hugh Thompson. Mr. Bobby Barben left the board but did not request emeritus status.

NAS Study: A Review of the Citrus Greening Research and Development Efforts Supported by the Citrus Research and Development Foundation: Fighting a Ravaging Disease

Contracted by CRDF, the National Academies of Sciences, Engineering, and Medicine (NASEM) conducted this review of the foundation's research portfolio, with the goal of identifying ways to reconfigure HLB research to accelerate the development of tools and strategies to abate disease impacts and prevent the collapse of the Florida citrus industry. The review concluded that research supported by CRDF and other agencies has expanded knowledge of every aspect of HLB, yet there have been no breakthroughs in HLB management.

The reasons for the lack of breakthroughs in HLB management, despite the investments in research, are complex. The disease itself seems intractable for a variety of reasons, including the inability to culture the pathogen in a laboratory; the complexity of the pathogen, insect, and host interactions; the perennial nature of citrus; the lack of resistance in any citrus relative; and the lack of a good model system for understanding the disease. What's more, other than research on ACP in Florida, most of the available information on HLB prior to 2005 was primarily based on research performed outside the United States, so researchers faced a steep learning curve.

An analysis by the NASEM of HLB research outcomes revealed progress and pitfalls in major research areas, and recommended the following as critical to achieving progress towards a viable HLB solution:
• Building on knowledge generated through previous research.
• Supporting research on factors that influence adoption of management practices proven effective.
• Greater collaboration and more-frequent venues for information-sharing by scientists.

• Timely and systematic communication of research outcomes and evaluation of research progress.
• Increased research coordination by CRDF and other funders of HLB research.

Citrus growers, particularly in Florida, still need short-term solutions to sustain the industry while researchers continue to generate longer-term approaches for managing HLB. Thus, support of basic and applied, short- and long-term research is needed. Longer-term HLB solutions are likely to involve citrus variety improvement, derived primarily from new molecular techniques such as gene editing, focusing on targets that mediate molecular interactions among plant, bacteria, and vector.

Because a single breakthrough discovery for managing HLB in Florida is unlikely, funders should support the development of sets of management approaches that can be combined in different ways and optimized and validated for use in different locations and conditions. This approach, founded on the integrated pest management (IPM) strategy for long-term control of pests, would allow optimization of management for each grower.

Economic and sociological factors that impact decision-making and behaviors of growers, processors, and the public will influence the adoption and success of future HLB management efforts; hence, CRDF should consider funding these research areas and creating accessible databases to support sociological and economic modeling of HLB-related research outcomes and application projections.

The review found inconsistency in laboratory and field experimental designs and sampling methods. Because inconsistency limits the comparison of findings across teams and institutions and the use of previous research.to inform further exploration, the development of community-accepted standards to conduct, evaluate, and assess research is recommended. Improved consistency in reporting research outcomes is also needed to reduce constraints in reviewing research progress and delays in applying new information to HLB solutions. CRDF should develop a standardized format and procedure, and set a timeline for mandatory reporting of project progress and final reports, to include publications and presentations, outcomes, practical applications, and impacts.

Despite commendable efforts of multiple agencies to coordinate funding and encourage appropriate inter-state, inter-agency, and inter-disciplinary collaborations, decisions about research funding priorities and allocations occur largely within the domain of each agency. CRDF and other agencies should work together to create an overarching HLB research advisory panel to develop a fresh, systems approach to HLB research prioritization and the strategic distribution of resources for research leading to effective HLB management.

CRDF has made a list of these recommendations and is not ignoring them. Even if CRDF ultimately disagrees with a recommendation, it intends to seriously consider it and not reject it out-of-hand.

Link to NAS Press Release of April 10, 2018:
http://www8.nationalacademies.org/onpinews/newsitem.aspx?RecordID=25026

Link to NAS Report Brief (2 pages):
https://www.nap.edu/resource/25026/Citrus%20Greening%20Report%20highlights-final.pdf

Link to NAS online Full Report .pdf (184 pages): http://nap.edu/25026

CRDF representatives participated in the National Citrus Breeding Collaboration meeting on February 27, 2018 in Dever, Colorado. A team of plant improvement researchers from Arizona, California, Florida and Texas adopted a document outlining minimum guidelines for r new greenhouse experiments and preliminary (early stage) field trials.

To bring reliability to the research and provide consistent useful data for growers and researchers alike, an Appendix was included with the 2018 RFP instructions to guide PIs. This appendix may be found following the RFP details later in this report.

Section 1 details guidelines for proposals seeking funding to collect data on existing germplasm evaluation trials. This included experimental design and data collection guidelines for Greenhouse Experiments, Early-stage Field Trials and Existing Pre-commercial or large-scale field trials.

Section 2 provides Proposal guidelines for new Pre-commercial citrus field trials, including Description of genotypes nominated for pre-commercial citrus field trials, Final candidate determination, General Pre-commercial field trial design minimum requirements, and Pre-commercial field trial evaluations (of Environment, Horticulture performance, Diseases and Pests) for both rootstock and scion field trials.

COMMUNICATIONS AND OUTREACH TO GROWERS

Citrus Research and Development Foundation (CRDF)

Communications Strategy

Objective: To better inform the Citrus Industry of CRDF's research, education outcomes and current investments aimed at improving the future of the Florida Citrus Industry.

Background: Since 2009, the CRDF has supported multiple research and education activities aimed at helping the Citrus Industry survive multiple threats, most notably citrus greening. To date, over 400 projects have been supported. Efforts to communicate its efforts included educational outreach to the public we serve.

Efforts highlighted eight major themes:

1. **Website:** The CRDF currently hosts a website and database, housing reports from nearly all its funded research. Through a new host, the website is currently being updated, with a focus on improving the search function of the report database. This effort will assist the public in obtaining written documents explaining the funded research and associated outcomes.

2. **Written Educational Documents:** The CRDF hosted monthly articles and columns in industry trade magazines. In addition, a quarterly newsletter highlighted on our website and shared electronically to all clientele contacts.

3. **Research Summaries:** On a quarterly interval, the CRDF scientific staff created an executive summary of current CRDF-funded research based on inputs derived from the PIs' quarterly reports. In addition, research summary documents were created throughout the year. These articles highlighted past and current research associated with a common theme (i.e. antibiotics, micronutrients, mulch, etc.). These documents are featured on our website and advertised broadly to our clientele.

4. **Board Meeting Venue and Committee Involvement:** The CRDF Board normally meets 11 times annually. To date, these meetings were at the UF/IFAS Citrus Research and Education Center in Lake Alfred, FL. Beginning in 2018, the CRDF Board meetings began being held in different citrus-growing regions of the state and were co-hosted with the state's regional citrus producer associations (Gulf Citrus Growers Association, Indian River Citrus League, Peace River Valley Citrus Growers Association, and Highlands County Citrus Growers). This resulted in six meetings in Lake Alfred and five at selected venues around the state (Grower Associations + Florida Citrus Industry Annual Conference). Regional growers will be encouraged to attend, with greater membership and participation on CRDF committees sought.

5. **Educational Seminars:** In addition to moving CRDF Board Meetings throughout the state, the meeting's agenda was expanded. Typical Foundation business was conducted in the morning, followed by one to two 1 to 2 educational seminars featuring scientists involved in citrus-related research. These educational seminars were offered by live streaming video to offsite viewers. In addition, the seminars were recorded and made available on the CRDF website.

6. **Engaging UF/IFAS Cooperative Extension:** UF/IFAS is fortunate to employ highly competent Regional Specialized Extension Agents (RSA) focused on Citrus. The CRDF helped fund Agent travel to CRDF meetings so the works of CRDF and the research it funds could be better communicated to our clientele. An expectation of this effort will be a summary compilation of CRDF news and information to be shared among the Agent group and included in their monthly newsletters. In addition, CRDF will engage IFAS Citrus Agents in a broader discussion on how further investment into their *Annual Plan of Work* may help contribute to effectiveness of CRDF communication.

7. **News You Can Use:** Working with our website host manager and UF/IFAS Communications, CRDF will design an electronic 'blast' using E-mail and social media (Facebook and Twitter)

titled *News You Can Use* from CRDF. This one-page document will lead the user directly to three or four informative links that will highlight topics important to our CRDF clientele. This effort will actively feed our clientele timely information while directly linking them to the source.

8. **CRDF Brochure:** Working with the UF/IFAS Communications Department, CRDF designed and published an attractive and informative, printed document (bi-fold) that illustrates the three W's: *Who we are, What we do, and Why it's important*. The document will be updated annually, distributed at multiple venues throughout the state, and provided to members of the Florida Legislature.

CRDF Final Report on 5-Year USDA-SCRI-NIFA nuPsyllid Project

The Final Report on the 5-year, $9M, nuPsyllid project, which ended August 31, 2017, can be found at: https://citrusrdf.org/wp-content/uploads/2018/01/BoD_NuPsyllid-Project-Annual-REEport-Aug-2017-Final.pdf The Public Outreach effort connected with this project became a hub of communication about citrus technologies for both grower and the general public, critical to building trust that technologies, especially those supported with public funding. It also helped diminish the perception held by some that such projects are developed in secret. Dispelling this perception furthers the establishment of trust between scientists and the general public that technology solutions under investigation and development are communicated as they are developed. The outreach team compiled a fact sheet titled *What makes lemons, oranges and limes look and taste different,* defining crop and insect genetic engineering in the context of citrus, including the nuPsyllid project as an example This sheet is available on the web site and is often used as a handout at grower meetings.

Postbloom Fruit Drop, Citrus Canker, and Citrus Black Spot

Dr. Megan Dewdney, Associate Professor of Plant Pathology and Extension Specialist, UF CREC, gave a presentation on Postbloom Fruit Drop, Citrus Canker, and Citrus Black Spot on February 20, 2018. Her presentation can be found at the following link: https://citrusrdf.org/wp-content/uploads/2018/03/2018-02_Dewdney-Fruit-School-Feb-20-L.pdf

Rootstock Field Day (March 7, 2018)

CRDF hosted a Field Day at Peace River Packing Company in Babson Park, FL to show the CRDF rootstock trial being conducted cooperatively with Peace River Packers. Presentations were given by Dr. Jude Grosser, UF, CREC, and Dr. Kim Bowman, USDA-ARS. It was attended by 118 persons.

PRESENTATION AT CITRUS GROWERS' INSTITUTE (April 3, 2018)

Dr. Catherine Hatcher, CRDF Project Manager, gave a presentation at the Citrus Growers' Institute on April 3, 2018, outlining CRDF Research Goals, Research topical areas, an overview of 40 Bactericide Projects, 87 ACP Vector Intervention Projects, 73 Citrus Plant Improvement & Resistance Projects, and 76 Horticultural Projects. The power point presentation can be found at: https://citrusrdf.org/wp-content/uploads/2018/05/CRDF-Citrus-Grower-Institute-Update-2018_CH.pdf

Grower Education Session – Presenters Dr. Vincent and Dr. Johnson

The first Grower Education Session was held on May 22, 2018, following the CRDF Board of Directors' meeting by the following researchers:
Dr. Christopher Vincent – "Effects of heat treatments on antimicrobial uptake and translocation in citrus trees," and "Dyed kaolin to repel Asian citrus psyllid in field conditions."
Dr. Evan Johnson – "Progress on Zinkicide efficacy trials."
Their presentations, as well as subsequent GES presentations, can be found at: https://citrusrdf.org/crdf-grower-education-session

Bayer and CRDF Partnership to Combat Citrus Greening

Bayer and CRDF entered into a research collaboration agreement to find solutions to citrus greening disease, which currently threatens the global citrus production and juice industry. Link to October 12, 2017 presentation: https://citrusrdf.org/wp-content/uploads/2012/09/CPD-D1aIII_Bayer-CRDF-Partnership-Project-Overview.pdf The June 13, 2018 update given to the CRDF Board can be found at the following link: https://citrusrdf.org/wp-content/uploads/2018/08/2018-06-13_CRDF-Board-Meeting-Bonita-Springs_Bayer-Crop-Science.pdf Quarterly updates have followed and will be posted when cleared by the parties.

CRDF RESEARCH PORTFOLIO

The balance of this report provides an overview of the key topics of focus for CRDF efforts and includes reference to specific projects within the portfolio. While most references are to activities during the recent fiscal year, the report necessarily alludes to prior progress leading to current project activities and progress. Projects are identified in this report by PI and CRDF project number. More details can be obtained on individual projects by searching the CRDF website at citrusrdf.org. The body of the report is organized according to interventions targeting the HLB pathogen Candidatus Liberibacter asiaticus; the insect vector, Asian citrus psyllid, Diaphorina citri; and the array of citrus host plants affected by HLB.

FY 2017-18 CRDF-Funded Project List

Project No#	Principal Investigator	Project Title	Original Contract Total	Start Date	End Date
15-002	Bowman, Kimberly	Development of Supersour and Other Promising Rootstocks for Florida.	$959,860.00	7/1/2015	6/30/2018
15-003	Bowman, Kimberly	Metabolomic profiling to accelerate development of HLB tolerant rootstocks	$539,104.00	7/1/2015	6/30/2018
15-005	Dewdney, Megan	Asexual inoculum production of Guignardia citricarpa, the causal agent of citrus black spot	$255,227.00	7/1/2015	12/31/2018
15-009	Gabriel, Dean	Exploiting the Las phage for potential control of HLB	$419,500.00	8/1/2015	7/31/2018
15-010	Gmitter, Fred	Development and Commercialization of Improved New Disease Resistant Scions and Rootstocks - the Key For a Sustainable and Profitable Florida Citrus Industry	$1,797,148.00	11/1/2015	10/31/2018
15-013	Grosser, Jude	Understanding and Manipulating the Interaction of Rootstocks and Constant Nutrition to Enhance the Establishment, Longevity and Profitability of Citrus Plantings in HLB-Endemic Areas.	$340,778.00	10/1/2015	3/31/2019
15-016C	Hall, David	High-Throughput Inoculation of Transgenic Citrus for HLB Resistance	$427,083.00	8/1/2015	12/31/2018
15-017	Killiny, Nabil	Disrupt LuxR solo quorum sensing that mediates plant virulence and insect transmission of Candidatus Liberibacter asiaticus to control the disease	$157,144.00	8/1/2015	1/30/2018
15-020	Mou, Zhonglin	Create citrus varieties resistant to Huanglongbing (HLB) through transgenic and nontransgenic approaches.	$358,922.00	7/1/2015	12/31/2018
15-021	Pelz-Stelinski, Kirsten	Regulation of Las transmission and microbial colonization by the Asian citrus psyllid immune system	$185,732.00	9/1/2015	2/28/2018

Project No#	Principal Investigator	Project Title	Original Contract Total	Start Date	End Date
15-022	Reuber, T. Lynne	Engineering citrus for canker resistance	$367,598.00	7/1/2015	6/30/2018
15-023	Schumann, Arnold	Citrus nutrition studies for improved survival of HLB-affected trees	$281,804.00	7/1/2015	12/31/2018
15-024	Stelinski, Lukasz	Predicting When, Why, and Where Asian citrus psyllids move to increase effectiveness of insecticide sprays.	$161,116.00	8/1/2015	7/31/2017
15-025	Stover, Ed	HLB Resistance and Tolerance in Citrus Scion Breeding	$441,935.00	10/1/2015	9/30/2018
15-026	Stover, Ed	Implementing Transgenic Tools to Produce Commercial Scion Cultivars Resistant to HLB and Canker	$540,758.00	8/15/2015	8/14/2018
15-028	Wang, Nian	Control citrus Huanglongbing (HLB) by counteracting the SA hydroxylase of Candidatus Liberibacter asiaticus	$430,697.00	7/1/2015	9/30/2018
15-030C	Rogers, Michael	Continuing Field Trial Support for CRDF CPDC	$322,129.00	7/1/2015	9/30/2017
15-033C	Orbovic, Vladimir	Support role of the Citrus Core Transformation Facility remains crucial for research leading to production of Citrus plants that may be tolerant or resistant to diseases.	$306,438.00	7/1/2015	12/31/2018
15-034C	Batuman, Ozgur	Continuation of diagnostic service for growers for detection of Huanglongbing in citrus and psyllids to aid in management decisions	$287,688.00	7/1/2015	12/30/2017
15-035C	Rogers, Michael	Continuing support of Citrus Health Management Areas (CHMA's)	$155,017.00	7/1/2015	9/30/2017
15-036C	Rogers, Michael	Correlating pesticide residue analysis with psyllid feeding to improve protection of young trees.	$451,603.00	7/1/2015	6/30/2018
15-037C	Santra, Swadeshmukul	T-SOL™ antimicrobial for the management of citrus canker and HLB	$240,224.00	7/1/2015	12/31/2017

Project No#	Principal Investigator	Project Title	Original Contract Total	Start Date	End Date
15-039C	Stover, Ed	Secure site for testing transgenic and conventional citrus for HLB and psyllid resistance	$260,980.00	1/1/2016	12/31/2018
15-042	Wang, Nian	Control citrus Huanglongbing using endophytic microbes from survivor trees	$467,000.00	7/1/2015	9/30/2018
15-045C	Zale, Janice	Continued Funding for the Mature Citrus Facility to Produce Disease Tolerant, Transgenic Citrus.	$1,092,752.00	7/1/2015	12/31/2018
15-049C	Booker, Brad	Evaluation of minimal-risk and biopesticide products as a protectant and therapy for HLB	$53,354.00	11/1/2015	6/30/2018
16-001	Li, Yi	Enhancing Genetic Transformation Efficiency of Mature Citrus.	$316,168.00	7/1/2016	6/30/2019
16-005	Wang, Nian	GFP labeling of Candidatus Liberibacter asiaticus in vivo and its applications.	$472,753.00	10/1/2016	9/30/2019
16-007	Duan, Yongping	Field evaluation of the selected variants of Ruby Red grapefruit volunteer seedlings for greater HLB resistance/tolerance.	$296,945.00	4/1/2017	3/31/2020
16-009C	Triplett, Eric	Developing second generation antimicrobial treatments for citrus greening disease.	$248,744.00	7/1/2016	12/31/2018
16-010C	Dewdney, Megan	Enhancement of Postbloom fruit drop control measures.	$498,580.00	3/1/2016	2/28/2019
16-011C	Adair, Robert C.	Increasing the yield and decreasing the bearing age of citrus trees in new plantings by using metalized reflective mulch while determining ACP populations.	$138,873.30	7/1/2016	1/31/2018
16-012C	Triplett, Eric	RSA - Antimicrobial assay for inhibition of Liberibacter crescens, the closest cultured relative of the citrus greening pathogen, Ca. L. asiaticus.	$5,425.00	8/1/2016	7/31/2017

Project No#	Principal Investigator	Project Title	Original Contract Total	Start Date	End Date
16-016C	Eyrich, Tim	Use of RNAi delivered by the Citrus Tristeza Virus Viral Vector to control the Asian Citrus Psyllid	$554,486.00	9/1/2016	8/31/2019
16-017C	Tetard, Laurene	Quantitative Detection and Mapping of Bactericides in Citrus.	$52,642.00	7/1/2016	9/30/2017
16-019C	Pelz-Stelinski, Kirsten	RSA - Small plant assay for testing the efficacy of antimicrobial materials against HLB.	$121,004.00	8/1/2016	12/30/2017
16-020C	Vincent, Christopher	Dyed kaolin to repel Asian citrus psyllid in field conditions.	$273,908.00	12/1/2016	11/30/2019
16-022C	Richardson, Taw	Large Scale Lab/Greenhouse/Field Trial Evaluation - HLB.	$884,509.00	10/1/2016	9/30/2017
16-023C	Etxeberria, Ed	Determining the Efficacy of a New Class of Adjuvants in Increasing Penetration of Antimicrobials into Citrus Leaves.	$23,603.00	2/1/2017	7/31/2017
16-024C	Ables, Camilla	A Review of the Citrus Greening Research and Development Efforts Supported by the Citrus Research and Development Foundation.	$492,096.00	2/1/2017	6/30/2018
424	McNellis, Timothy	Functional disruption of the NodT outer membrane protein of Candidatus Liberibacter asiaticus for rootstock-mediated resistance to citrus greening using a phloem-directed, single-chain antibody	$55,000.00	7/1/2011	6/30/2018
784-801	Multiple Sub-awardees	Rear and Release Psyllids as Biological Control Agents - An Economical and Feasible Mid-Term Solution for Huanglongbing (HLB) disease.	$9,000,000.00	9/1/2012	8/31/2017

Project No#	Principal Investigator	Project Title	Original Contract Total	Start Date	End Date
940C	Beeson, Richard C.	Propagation of Rootstock Tree Production in Greenhouses by Seed, Stem Cuttings and Tissue Culture to Accelerate Budded Tree Production for Outplanting.	$333,774.00	10/1/2014	9/30/2017
943C	Rogers, Michael	Support for scale-up of Thermal Therapy Treatment: Evaluation before and after thermotherapy heat treatments to combat HLB	$164,912.00	3/1/2015	11/30/2017
99900	Botts, Dan	Work with CRDF Board and the Boards research partners to achieve regulatory approvals	$92,500.00	7/1/2014	9/30/2018
99901	Botts, Daniel	Neonicotinoid and Other Insecticide Label Modifications to Manage Asian Citrus Psyllid (ACP) to Prevent Transmission of Huanglongbing (HLB) in Newly Planted Citrus Trees	$229,500.00	7/1/2014	9/30/2018
99904	Browning, Harold	CRDF billings to Sponsors for project oversight. Etc.	$0.00	7/1/2014	10/31/2017
17-006	Triplett, Eric	Monitoring of citrus groves for non-target antibiotic resistance prior to and after application of streptomycin and oxytetracycline.	$195,034.00	4/1/2017	3/31/2018
17-005C	Vincent, Christopher	Effects of heat treatments on antimicrobial uptake and translocation in citrus trees.	$64,372.00	4/1/2017	3/31/2018
16-025.2C	Booker, Brad	Comparison of chemical uptake with laser ablation and conventional foliar application - Phase 1 Crop Consultant.	$17,170.00	4/15/2017	8/15/2017
16-025.1C	Drouillard, Greg	Comparison of chemical uptake with laser ablation and conventional foliar application – Phase One.	$41,231.00	4/15/2017	8/15/2017
16-025.3C	Wang, Nian	Quantification of oxytetracycline in plant samples.	$54,186.00	4/15/2017	4/14/2018

Project No#	Principal Investigator	Project Title	Original Contract Total	Start Date	End Date
16-026C	Meissner	Establishment and application of tools to allow a systematic approach to identify and characterize hits with confirmed in planta HLB activity.	$12,610,000.00	5/1/2017	6/30/2020
17-001C	Stelinski, Lukasz	Insecticide resistance management in Florida citrus production.	$226,247.00	7/1/2017	6/30/2019
17-002C	Irey, Mike	Continued Support for the Southern Gardens Diagnostic Laboratory	$451,953.00	7/1/2017	6/30/2019

CRDF Commercial Product Delivery Progress Report

Quarter Ending 30 June 2018

Project Title: 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 (Explanations of projects largely taken from PI project submissions):

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 relatively 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. A no-cost extension was requested 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 analyzed within the neural network software. Based on leaf size and leaf nutrient values from a 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 the future to guide growers on how much fertilizer to apply to HLB-affected groves. In a greenhouse study, three nutrient solutions were used 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 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 the SWFREC. Detailed results of years 1 and 2 of this experiment were presented at Citrus Expo on Aug 15, 2018. Results showing a therapeutic affect from overdoses of manganese against HLB and interactions with B were 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 run by Drs Orbovic and Zale developed transgenic plants for researchers. Various events have putative resistance or tolerance towards HLB and citrus canker. The laboratories confirm the 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.

NOTE: ALL Quarterly Project Managers' Progress Reports can be found at citrusrdf.org

2018 RFP

The CRDF project management staff met with the various citrus industry organizations, UF Center Director, Dr. Michael Rogers, and USDA Center Director, Dr. Brian Scully, to poll each group for their input on specific objectives for the 2018 Request for Proposals.

After compiling these extensive lists, the Research Management and Commercial Product Delivery Committees each developed their individual Request for Project Funding and announced a general call for proposals in April 2018. Following is the general timeline:

2018 RESEARCH AND PRODUCT DELIVERY RFP TIMELINE

2018	RMC-18 and CPDC-18 Pre-Proposal & Proposal Selection Process
March	CRDF Project Management Team meets with Center Directors M Rogers (UF) & B Scully (USDA), and Citrus Industry Groups to discuss research priorities
3/23/2018	Commercial Product Delivery Committee Meeting to discuss RFP priorities
3/27/2018	Research Management Committee Meeting to discuss RFP priorities
4/1/2018	National Academy of Science draft report received
4/16/2018	RMC Meeting to review NAS report, propose RFP wording
4/17/2018	CPDC Meeting to review NAS report, propose RFP wording
4/24/2018	CRDF Board of Directors Meeting (off site- Gulf Citrus Growers, Immokalee)-approve RMC-18 and CPDC-18 RFP's
4/25/2018	Announce RFP to Research Community
5/21/2018	Deadline for submission of RMC-18 and CPDC-18 Pre-proposals (46 RMC and 67 CPDC received)
6/4/2018	Scientific Advisory Board review of RMC-18 Pre-proposals (telephone meeting)
6/5/2018	RMC selection of Pre-proposals recommended to submit full proposals
6/6/2018	CPD-Scientific Advisory Board review of CPD-18 Pre-proposals (telephone meeting)
6/8/2018	CPDC selection of Pre-proposals recommended to submit full proposals
6/13/2018 (Wed.)	BoD approval of Pre-proposals invited for full proposals (26 RMC and 36 CPDC invited)
6/14/2018	Announce / post list of Pre-proposals invited to submit full proposals
7/20/2018 (Fri.)	Deadline for submission of RMC-18 and CPDC-18 Full Proposals
8/22-23/2018	C-SAB Panel meeting to review & make recommendations of CPDC-2018 Proposals for funding to CPDC ; review current portfolio progress reports
8/29-30/2018	SAB Panel meeting to review & make recommendations of RMC-2018 Proposals for funding to RMC; review current portfolio progress reports
9/4/2018	RMC recommendations meeting - receive SAB recommendations and prepare list of projects recommended for funding for BoD. Include contingencies for funding
9/5/2018	CPDC recommendations meeting - receive SAB recommendations and prepare list of projects recommended for funding for BoD. Include contingencies for funding
9/21/2018	RMC meeting to review and approve projects with contingencies for funding
9/24/2018	CPDC meeting to review and approve projects with contingencies for funding, and 4 Service & Support Proposals
9/25/2018	BoD approval of Proposals: RMC (14 approved, 1 deferred) and CPDC (18 approved; 4 deferred))
10/12/2018	RMC meeting for further discussion and review of deferred proposal (18-011 Gmitter)
11/15/2018	CPDC meeting for review and discussion of 4 Service & Support Proposals
12/11/2018	BoD approval of 4 Service & Support Proposals

RMC-18 Request for Project Funding

Revision 1 (5/1/2018)

Application Schedule:

April 25, 2018 Call for pre-proposals announced
May 21, 2018 Completed pre-proposals must be received by 5:00 pm EDT
June 14, 2018 Invited list for full proposal submission posted to CRDF webpage
July 20, 2018 Complete invited full proposals must be received by 5:00 pm EDT
Sept. 26, 2018 Approved funded projects list posted to website

Purpose:

The Mission of the Citrus Research and Development Foundation is to "Advance disease and production research and product development activities to insure the survival and competitiveness of Florida's citrus growers through innovation". The purpose of this CRDF request for proposals is to support the Foundation's mission by addressing key industry needs. Questions and research priorities were discussed during citrus grower input sessions, researcher brainstorming sessions, and from the recently completed National Academy of Sciences (NAS) study. These were developed into priorities for the two separate 2018 CRDF RFPs (RMC-18 & CPDC-18). The priorities identified are expected to lead proposals for hypothesis-driven, knowledge-building research projects, the results of which will lead to downstream solutions to HLB. These research priorities were selected and approved by the Research Management Committee (RMC) and the CRDF Board of Directors, which are composed of citrus industry members.

Pre-proposals and invited full proposals will be reviewed by the RMC, the Board of Directors, and a scientific advisory board. External, ad hoc expert reviewers will review invited full proposals. Decisions on pre-proposal and proposal approval will be made by the Board of Directors based on the recommendations of the RMC.

RMC-18 Research Priorities:

The CRDF Research Management Committee 2018 (RMC-18) call for funding applications focuses on priorities for hypothesis-driven, knowledge-building research projects, the results of which will lead to solutions to HLB downstream. Successful applicants to this program will have developed research projects addressing one or several of the research priorities in this section. These priorities should not be combined with priorities from the CPDC-18 RFP. Proposals addressing priorities from the CPDC-18 RFP must be submitted as independent pre-proposals.

1. **Effective bactericide use in Florida citrus groves**
   A. Examine the effect of bactericides on tree health including root health, yield and fruit quality, in different aged trees and different disease severity levels.
   B. Test or develop tools to accurately measure and track live bacterial titers in citrus trees.
   C. Examine the dynamics of bactericide introduction into the tree and systemic movement within the vascular system of trees.
   D. Develop novel, commercially viable application technologies to improve the uptake of bactericides.
2. **Asian citrus psyllid (*Diaphorina citri*)**

A. Improve the understanding of ACP population pressure and repeated inoculation of mature citrus on disease development and decline.
B. Examine the impacts of bactericides or other molecules on ACP survival and fitness and determine if bactericide or other molecule use impacts transmission of *C*Las.

3. Horticultural practices for disease management

A. Investigate how root systems are impacted by HLB and how they can be treated to restore or prevent further tree decline and interactions with other root pathogens?
B. Investigate the most efficient use of micro and macro nutrients on HLB diseased trees and possible correlations with *C*Las titer.
C. Investigate chemicals or horticultural practices that can mitigate or exacerbate the symptoms of HLB including phloem collapse.

4. Plant Improvement

A. Identify the genetic basis, of citrus host responses to HLB to identify targets for conventional or biotechnological approaches for the development of HLB resistant or tolerant citrus varieties.
    i. Seek new resistance R-genes in citrus or other species that counteract *C*Las effectors.
    ii. Describe the varietal target and experimental approach targeting HLB resistance. Indicate the percent effort, time and resources dedicated to each variety.
    iii. Evaluate germplasm developed specifically for HLB resistance through conventional or biotechnological techniques. Describe phenotypic and molecular characterization protocols for laboratory, greenhouse and field experiments; reference the appendix for phenotyping protocols.
B. Develop tools for reliable, high-throughput characterization of citrus germplasm for HLB resistance or tolerance using current knowledge of HLB symptomology and the molecular characterization of citrus.

5. Citrus black spot (*Phyllosticta citricarpa*) management

A. Develop strategies for citrus black spot management in Florida citrus groves, including strobilurin and non-strobilurin fungicide use and cultural practices.
B. Develop management strategies to control the spread of citrus black spot throughout Florida, e.g. tarping and CHMAs.

CPDC-18 Request for Project Funding

Revision 2 (5/2/2018)

Application Schedule:

April 25, 2018 Call for pre-proposals announced
May 21, 2018 Completed pre-proposals must be received by 5:00 pm EDT
June 14, 2018 Invited list for full proposal submission posted to CRDF webpage
July 20, 2018 Complete invited full proposals must be received by 5:00 pm EDT
Sept. 26, 2018 Approved funded projects list posted to website

Purpose:

The Mission of the Citrus Research and Development Foundation is to "Advance disease and production research and product development activities to insure the survival and competitiveness of Florida's citrus growers through innovation". The purpose of this CRDF request for proposals is to support the Foundation's mission by addressing key industry needs. Questions and research priorities were discussed during citrus grower input sessions, researcher brainstorming sessions, and from the recently completed National Academy of Sciences (NAS) study. These were developed into priorities for two separate CRDF RFPs for 2018 funding (CPDC-18 & RMC-18). These research priorities were selected and approved by the Commercial Product Delivery Committee (CPDC) and the CRDF Board of Directors, which are composed of citrus industry members.

The CPDC and the Board of Directors will review pre-proposals and invited full proposals. Full proposals will also be reviewed by external ad hoc expert reviewers. Decisions on pre-proposal and proposal approval will be made by the Board of Directors based on the recommendations of the CPDC.

CPDC-18 Research Priorities:

The CRDF Commercial Product Delivery Committee 2018 (CPDC-18) call for funding applications focuses on priorities that will lead directly to a near-term product, tool or service. Successful applicants to this program will have developed research projects addressing one or several of the research priorities in this section. These priorities should not be combined with priorities from the RMC-18 RFP. Proposals addressing priorities from the RMC-18 RFP must be submitted as independent pre-proposals.

1. **Determine the best use of bactericides in citrus**
   - A. Develop effective and best use of bactericides in citrus
     - i. Investigate the effect of registered or novel bactericide treatments on bacterial titer and tree health, including root health and regeneration, at various levels of tree decline.
       - a. Identify the optimal seasonal use pattern of bactericides, including material rotation.
       - b. Determine if effective bactericide treatments are feasible based on established fruit residue tolerance levels.
   - B. Investigate the effect of bactericides in new citrus plantings as preventative or therapeutic treatments.
   - C. Investigate the dynamics of bactericides within the tree
     - i. Investigate the systemic movement of bactericides within the phloem
     - ii. Improve methods for tracking and quantifying oxytetracycline and streptomycin in the tree.

D. Optimize delivery of bactericides
   i. Determine which adjuvants (penetrants, surfactants and water conditioning agents) most efficiently facilitate uptake of oxytetracycline and streptomycin.
   ii. Determine if tank mix components (including copper) affect the efficacy of oxytetracycline and streptomycin.
   iii. Can non-conventional application technologies improve uptake of bactericides?
      a. Determine if effective methods are feasible based on established fruit residue tolerance levels.

2. Improve Asian citrus psyllid (*Diaphorina citri*) management tools and strategies

A. Investigate barriers to prevent psyllid feeding and how to improve their use (e.g. rainfastness of kaolin clay products, netting, reflective mulch, etc.).
B. Investigate biopesticide products (including botanical oil products) as complements to conventional insecticides.
C. Revise ACP control strategies (IPM, CHMAs) to improve ACP management, including in young trees.
D. Implement effective pheromone tools and acoustic technology in citrus groves for ACP control.
E. Investigate the role of repeated feeding and inoculation by ACP on disease development and tree decline.

3. Plant Improvement

A. Field Trial Data Collection

i. Collect standardized data on existing field trials to evaluate citrus scion and rootstock response to HLB, other pests and diseases, horticultural performance, fruit and juice quality including flavor profile. The PI should describe existing field trials as described in the appendix of the RFP guidelines.

ii. Evaluate horticultural performance in existing commercial plantings and field trials of Vernia, Parson-Brown, Sugar-Belle, OLL-4, OLL-8 and mandarins and other varieties that appear to maintain productivity with HLB infection, as described in the appendix of the RFP guidelines.

B. Establish field trials of new HLB resistant or tolerant varieties for both scions and rootstocks as described in the standardized methods in in the appendix of the RFP guidelines.

C. Evaluate rootstock performance derived from tissue culture compared with conventional propagation materials.

4. Horticultural practices to rehabilitate declining trees

A. Develop economical seasonal recommendations for optimum micronutrient and macronutrient levels in leaves and/or roots in HLB diseased trees?
   i. Examine if nutrient therapies effect *C*Las titers, ameliorate root dieback, and improve tree health and yield in HLB diseased trees.
   ii. Investigate how water quality and soil-applied, carbon-based materials may impact nutrient uptake, root/tree health and yield in HLB diseased trees.
   iii. Investigate how plant growth regulators may stimulate root growth, reduce fruit drop and/or affect plant defenses.

5. Other Citrus Disease Management

A. Investigate chemicals, tools or cultural practices, and incorporate all available post-bloom fruit drop management experience and knowledge from other citrus growing regions, to economically improve management of PFD in Florida.
B. Investigate non-copper treatments for citrus canker including streptomycin and oxytetracycline, and investigate copper use in rotation with non-copper treatments.
C. Investigate the susceptibility of released rootstocks to nematodes and other root pathogens.

Appendix

Plant improvement germplasm evaluation guidelines

Section 1. Guidelines for proposals seeking funding to collect data on existing germplasm evaluation trials

CRDF recognizes that there are existing preliminary greenhouse and field trials as well as advanced stage field trials that may require funding for data collection. Although, these trials may not fit the criteria outlined for new trials, they may be considered for funding for data collection. Programs seeking funding should follow these guidelines when preparing a proposal to seek funding to evaluate existing trials.

Preliminary greenhouse and field trials

These trials should be embedded in core breeding proposal objectives as they occur earlier in the breeding cycle. Principal investigators seeking funding for data collection for these projects should describe the objectives, population development, targeted traits of interest, evaluation materials and methods, statistical design and analysis of ongoing experiments. PI's should submit available summary preliminary data for each cycle of evaluation on the trials.

New greenhouse experiments and preliminary (early stage) field trials should adhere to the minimum guidelines below which were adopted from a document developed by the team of plant improvement researchers from Arizona, California, Florida and Texas, nominated at the National Citrus Breeding Collaboration meeting on Feb 27th in Dever, Colorado.

Greenhouse experiments

Experimental design:

- Experiments must use control (non-inoculated) plants of the same variety and age as the inoculated or infected plants.
- Experiments must maintain controls and infected plants under the same conditions. Plants should be randomized.
- It is suggested to use at least 10-12 plants per treatment and variety to conduct disease/health evaluations. For additional genomics, metabolomics, or other analyses, a small but representative subsample of plants can be used.
- Plants to be used in the experiments should be as homogeneous as possible
- Use a minimum of 10 psyllids and 2 tissue pieces per plant for psyllid transmission and graft transmission of *C*Las, respectively. Number of psyllids and tissue pieces may be adjusted based on the size of experimental plants. Bud stick grafting is also a suitable method for inoculation. Provide information on percentage of infection of psyllid colony and titer levels of plant variety used for inoculation. Use standardized PCR methods and provide Ct-values and DNA concentration of plants from which inoculation material was obtained.
- Experiments must define the source of *C*Las used and reference to any previous publications or work with the same source.
- Researchers must do one of the following:
    - Use a *C*Las strain that is maintained in the exotic pathogens collection of citrus at USDA-ARS, Beltsville, MD.

- Use a strain that has been sequenced or extensively genotyped.
- Maintain the strain that they use.
- Maintain the strain that they use as -80 frozen tissue for future sequencing or other analysis.

Data collection:

- One or more measures of plant size must be recorded at beginning and end of the experiment:
  - Stem diameter (preferred). For non-grafted trees measure at 10 cm above soil level. Height above graft union for measuring stem diameter of grafted trees is preferred at 5 cm but can vary depending on factors such as site of graft inoculation. Must be defined and consistent within an experiment.
  - Plant height or, if plants were pruned, the sum of the length of all branches after regrowth. Indicate times and frequency of pruning.
  - Biomass at the end of experiment. May be separated into shoot, leaf, and root portions.
- Greenhouse conditions and management practices should be recorded in as much detail as possible, including pot size, potting medium, nutritional program, temperature ranges, light levels, and any trimming or training of plants.
- Conduct foliar disease symptom ratings at different time intervals throughout the experiment using a scale from 1 to 5, with 1 = no foliar disease symptoms, 2 = foliar symptoms on less than 25% of leaves, 3 = 25-50% of leaves with symptoms, 4 = 50-75% of leaves with symptoms, 5 = more than 75% of leaves with symptoms.
- Document type of foliar symptoms (chlorosis, blotch mottle, reduced leaf size, vein corking, etc.).
- Collect at least 3 leaves (number may vary based on plant size) at different time intervals for *C*Las detection (may have this coincide with disease ratings). Choose mature leaves randomly from different areas throughout the canopy to account for variation. Pool tissue for analysis. Use petioles/midribs for *C*Las detection.
- If *C*Las testing of roots is to be part of the experiment, fibrous roots (≤ 2mm) should be used.
- Use the "Li primers" (Li et al., 2006) for real-time PCR detection of *C*Las. New guidelines on primers may follow.
- Report DNA concentration, Ct-values, and percent of infected plants. If the Ct values are used for classification of which trees are infected and which are not infected by *C*Las, the cutoff should be clearly indicated on reports.
- Duration of experiment and time intervals for disease ratings will vary depending on the age of plants used. Duration of experiments should be a minimum of 6 months following inoculation, but preferably 12 months. Time intervals for PCR detection may vary based on resources and main purpose of the experiment.

Early-stage field trials

Experimental design

- Experimental design should be completely randomized or randomized blocked. Design will depend on trial objectives, number of rows available, row length, and tree spacing. Should be balanced across treatments/genotypes as far as numbers of individuals and reps in the test.
- As much as possible, all trees to be compared in a trial should come from the same nursery and be planted at the same time.

- Variability of soil and irrigation conditions should be taken into account in the experimental design (blocking).
- A minimum of 6 replicates should be used. Depending on the trial objectives, replicates can be 3 trees, or more; a minimum of 3 trees is preferred. The relative balance of number of replications and number of trees per replication may vary according to the particular situation.
- Plant border trees at end of rows, and in adjacent rows on each side, when possible.

Data collection

Data to be collected from a field trial may vary by trial objectives, conditions, and resources available. The following metrics should be used:

- Tree size. Measure at one or more time intervals before the completion of the trial.
    - Trunk diameter for scion and rootstock. Measure at 5 cm above and below graft union. Be consistent and return to the same spot on the trunk every year. Measure in two perpendicular directions and use average. Alternatively, trunk circumference can be measured, and trunk diameter calculated using the formula [circumference/π]. Report trunk cross-sectional area (TCSA) using the formula [π x (diameter/2)^2].
    - Tree height to top of canopy (do not include height of vigorous shoots that extend significantly past the top of the canopy).
    - Canopy diameter (parallel and perpendicular to the row).
    - If hedging and/or topping are done to the block, this needs to be clearly noted, and may significantly change the value of subsequent canopy size measurements.
- Once tree height and diameter are measured, calculate canopy area and/or volume. Measure canopy diameter parallel and perpendicular to row.
- Calculate standard canopy volume according to the formula: [(diameter parallel to row x diameter perpendicular to row) x height]/4, modified from Wutscher and Hill (1995).
- Determine leaf macro and micronutrient concentrations annually during July-August from 12 mature, 4 to 6-month-old spring flush leaves from each or a subset of trees depending on experimental design.
- Report percentage of dead trees periodically or at the end of a trial period. Dead trees should be excluded from further ratings and analyses, or if included, this should be noted. Inferred or hypothesized cause of tree death may be noted. In many cases, trees that die in the first year are not the result of *C*Las effects and may be excluded from HLB-associated assessments.
- If a trial is located in an HLB-endemic environment, conduct foliar disease ratings using a scale from 1 to 5, with 1 = no foliar disease symptoms, 2 = foliar symptoms on less than 25% of leaves, 3 = 25-50% of leaves with symptoms, 4 = 50-75% of leaves with symptoms, 5 = more than 75% of leaves with symptoms. Calculate disease index as described below based on tree size and age:
    - For very small trees, rate the entire canopy as one unit. The maximum score per tree will be 5.
    - For medium trees, divide canopy into two sectors and apply ratings to each sector. The maximum score per tree will be 10.
    - For larger trees, divide canopy into 4 sectors and apply ratings to each sector. The maximum score per tree will be 20. If trees are very large, divide into 8 sectors for a maximum score of 40.

  - To standardize ratings across trees sizes, divide the total score by the number of sectors used, so that all tree ratings are expressed on a 1-5 scale.
- Conduct canopy thickness and color ratings using a scale from 1-5 as described below. Apply ratings to one, two, four, or eight sectors of the canopy depending on tree size, with a maximum score of 5 for smallest trees and 40 for large trees. To standardize ratings across trees sizes, divide the total score by the number of sectors used, so that all tree ratings are expressed on a 1-5 scale. Dead trees are not to be scored for canopy thickness or canopy color, and so will not affect average values in analyses.
  - *Canopy thickness*
    1 = very thin canopy, 2 = thin canopy, 3 = medium canopy, 4 = thick canopy, 5 = very thick canopy. It is recommended to illustrate differences between ratings photographically.
  - *Canopy color*
    1 = very yellow unhealthy canopy, 2 = yellow unhealthy canopy, 3 = moderately healthy canopy, 4 = healthy green canopy, 5 = very healthy dark green canopy. It is recommended to illustrate differences between ratings photographically.
- Document foliar diseases not associated with HLB if commercially relevant (e.g., canker) particularly when evaluating different scion varieties.
- Foliar disease and health ratings should be conducted at the same time of year. In Florida and Texas, fall is recommended for scoring disease symptoms, as that is the time they will usually be most pronounced (once temperatures are dropping). Additional ratings during spring and/or summer can provide important information and are recommended, particularly when evaluating new scion varieties.
- Tree appearance may be documented photographically using a measuring pole as reference.
- PCR evaluation of trees for *C*Las:
  - Collect mature leaves from most recent flush and use petiole/midribs for CLas detection. Depending on tree size, collect one or more leaves randomly from each of the four cardinal directions.
  - Collect fibrous roots (≤ 2mm) for *C*Las detection. Depending on tree size, collect fibrous roots from a minimum of two different cardinal directions, avoiding zones of overlap between adjacent trees.
  - Conduct leaf and root sample collections annually or at the end of the evaluation period (such as the end of four years of harvest). May coincide with disease and health ratings.
- Use the "Li primers" (Li et al., 2006) for real-time PCR detection of *C*Las. New guidelines may follow.
- Once trees reach maturity, collect fruit yield and fruit quality data each season. Conduct yield and fruit quality assessment at dates that are standard harvest times for that cultivar, or harvest times that are proposed for new cultivars. Report date of assessment.
  - Yield - assess directly by weighing fruits per replicate or indirectly by counting number of fruits per tree. Report as fruit weight per experimental unit. Alternatively, yield can be measured as boxes of fruit per tree.
  - Fruit weight – determine from random subsample of fruits from each tree, or group, depending on what is practical.
  - Fruit size - determine from subsample of fruits from each tree, or group, depending on what is practical for the situation. Measure the horizontal or vertical diameter (as appropriate) of the subsample of fruit collected for determination of fruit weight.

- Fruit quality – depending on the type of fruit and trial purpose, determine percent juice, brix, acid, brix/acid ratio, external color, and juice color from subsample of fruits according to standard laboratory methods.
- Sampling time will vary based on scion variety maturity and other factors. Select time that is most appropriate for the scion variety under evaluation.
- If appropriate, assess percentage of visually abnormal putatively greening-affected fruit per tree.
- If appropriate, assess fruit drop pre-harvest. Report as percent drop from fruit number data.

Existing Pre-commercial or large-scale field trials

PI's seeking funding to collect data on existing field trials should describe the field trials using the following guidelines.

1. What are the objectives of the trial?
   Describe the criteria used to select each nominated candidate rootstock or scion variety. Include a brief description of pedigree, population development, and experimental design and phenotyping methods used to characterize populations at each screening stage. Submit summary data from preliminary trials on each candidate rootstock or scion.
2. Provide information on ownership of the genotypes and technology associated with the candidates selected including but not limited to transfer agreements or constructs containing proprietary technology in the case of engineered genotypes.
3. Provide a timeline for the experiment describing establishment date. Provide all planting dates in cases where some genotypes were planted later. What was the source of plant material for the field trial?
4. Provide information on the site characteristics, field layout and a field map of the trial. The field map should provide sufficient detail to identify blocks, treatments (rootstock or scion) and site characteristics (ponds, ditches, slope, windbreaks, etc.).
5. Describe the field trial in detail including the number of candidate genotypes, commercial standards, number of sites, experimental unit (number of trees), observational unit (number of trees), replications and statistical design.
6. What are the horticultural practices used to maintain the trial? Please identify all treatments applied to the trial. If there are unbalanced, treatments explain how they are managed for statistical variation.
7. Describe in detail the traits of interest and data collection and analysis methods.
8. Submit summary data collected in the pre-commercial trial.
9. Identify candidates that have been submitted for disease indexing.

The scientific advisory board or ad-hoc reviewers, CRDF Committees and Board will review the information provided above to identify trials or portions of trials that merit funding for data collection will review the information provided above. As with new pre-commercial trials, CRDF will engage the citrus industry for comment on the relevance of the trials to industry needs to inform decisions on funding.

Section 2. Proposal guidelines for new Pre-commercial citrus field trials:

Proposals developed to solicit funding to conduct new large-scale pre-commercial variety field trials must describe the following.

Description of genotypes nominated for pre-commercial citrus field trials

1. Describe the criteria used to select each nominated candidate rootstock or scion variety including:
    a. A brief description of parental pedigree, population development, experimental design and phenotyping methods used to characterize populations at each screening stage.
    b. Provide information on ownership of the genotypes and technology associated with the candidates selected including but not limited to transfer agreements or constructs containing proprietary technology in the case of engineered genotypes.
    c. Submit summary data on each candidate rootstock or scion showing performance for all the traits of interest measured in preliminary trials. PI's should include a coded name for the candidates in a separate column. For example, US2018R. To protect intellectual property these codes will be used when presenting information in public meetings where pedigrees and other confidential information **will not be disclosed.**
    d. Indicate whether candidates have been submitted for disease indexing.
    e. What is the source of plant material for the field trial?
    f. Synchronized planting of the pre-commercial field trial is vital for direct comparisons. How much disease-free plant material is available for the field trial?
    g. Information provided in a – e above will be reviewed by a scientific panel and CRDF Board and Committees.

Final candidate list determination

The pre-commercial variety field trials serve as a powerful source of data for the citrus industry, and therefore it is important to engage the industry at large for input on the strength of the candidates and data summaries provided by the breeding programs. CRDF committees and Board will invite the principal investigators, collaborators, and industry stakeholders to evaluate the list of candidates and the associated summary data. Data presented at this public meeting will not provide confidential information such as pedigrees. Summary data of coded lines will be shared publicly along with ranking from the scientific panel, CRDF committees and Board, breeding program ranking and finally industry stakeholder ranking. At this meeting, the final list of candidates will be selected and approved for the pre-commercial field trial. Principal investigators MUST submit any/all revisions to the approved list of candidates for CRDF committees and Board consideration and approval before implementation.

General Pre-commercial field trial design requirements

The field trial design minimum requirements:

1. Number of sites: Dependent on the state geography and the targeted market. In Florida, at least 3 locations in the Central Ridge, Southeast, Southwest regions for most varieties.
2. Strategic site selection: To evaluate genetic, genetic x environmental and environmental effects on candidate variety performance.
3. At each site, the trial should be mapped to reduce variation and confounding factors.
    a. Account for topography, soil type changes, unbalanced windbreaks, drainage, ditches, ponds, etc.
    b. Multi-location trials should be set up given 3a so that genotypes can be compared for adaptation and regional performance.
4. Statistical design and analysis.

a. A randomized complete block design (RCBD) or Latin square design is preferred. If more factor levels are necessary, the statistical design must be clearly described for review and approval.
b. The trial should contain necessary standard (check) rootstocks or scions appropriate to the region for comparison to candidate varieties.
c. Number of replications: Minimum of 5 replications.
d. Experimental unit (plot) to which a treatment is applied: Minimum 8 trees. Treatments defined as the candidate varieties evaluated against industry standards.
e. The observational unit or sampling unit (e.g., trees, branches, leaves) from which data is collected must be clearly defined in trials where the experimental unit has more than the 8 minimum trees required in b, above.
f. The same observation trees will be measured in each data collection cycle. Due to the high variability of individual trees, a minimum of 8 trees is required for yield data collection. Death of observation trees should be noted in trial data summary reports each calendar year and if possible similar trees should be selected at random within the experimental unit (plot) for evaluation.
g. The trial should incorporate appropriate rows (2) of buffer around the trial and within the trial. Buffers between plots minimize the competition effect of removing genotypes due to poor performance.
h. The objective of the advanced field trial is evaluating candidate varieties for commercial relevance. Therefore total yield, fruit and juice quality traits must be measured every season after cropping. Early production is an important goal for new plantings due to higher production costs and shorter life expectancy for trees/groves. This situation has made it imperative that growers make an income as soon as possible so the field trials must be harvested each year until the trial is retired.
i. Trial tree spacing and grove design should be specified taking into account tree size data collected from preliminary field trials if it is available.
j. Horticultural practices: The trial should be managed using appropriate best management commercial practices for plant fertilization irrigation, pest management, pruning, hedging or topping. Grower cooperators will communicate horticultural practices annually. The incidence of wind, freezes, drought or flood or other phenomena that impact the trial should be recorded and reported. Pre-commercialization trials should not serve as a location for overlaying cultural practice treatments or to evaluate any other variable treatments which would interfere with the primary purpose of the trials.

5. Trial establishment : Tree survival should be monitored after planting, and a protocol defined if replanting or removing genotypes is necessary.
6. Location characteristics: Soil type, pH, organic matter content, irrigation water quality, incidents of drought, freezes, hurricanes or other phenomena which affect the field trial should be noted and evaluated. Trials that are affected negatively should be retired if the validity of the data collected is questionable.

Pre-commercial field trial evaluations

Environment

Evaluate candidate variety performance based on the following conditions if applicable: Data collected from grid soil sampling and testing. Soil samples to be collected within each block at the drip line to account for block and block × treatment interaction

1. Salinity
2. pH
3. Soil type
4. Organic matter content
5. Asian Citrus Psyllid infestation- relevant to HLB testing.

Horticultural Performance

Means of propagation: For each candidate variety, describe means and methods of propagation through seed, cuttings or tissue culture.
Tree height (m): Measured from the base of the tree to the top of uniform canopy ignoring errant vigorous branches. Pruning of vegetation as hedging, topping or skirting should be noted.
Tree skirt height (m): Measured from the base of the tree to the bottom of uniform canopy ignoring errant vigorous branches.
Canopy Diameter (cm): Two perpendicular diameters measured between trees along the row and perpendicular to the row.
Trunk diameter (cm): Two perpendicular trunk diameters measured with calipers on each of the observation trees. Diameters will be measured 10 cm above the bud union, a different criteria is used due to branching please describe the selected distance above the bud union.
Leaf nutrient content: Follow established sampling guidelines.
Bloom: Observations of bloom time and environmental conditions.
Yield and maturity date: Fruit maturity date and the total weight of fruit harvested per experimental unit.
Yield/acre: Calculation per experimental unit based on yield/experimental unit and planting density.
Juice quality: Juice weight, total soluble solids, brix/acid ratio, color rating and flavor profile measured on a random pre-determined sub-sample of fruit per experimental unit at harvest.
Fruit quality: Fruit size and color measured on a pre-determined random sub-sample of fruit per experimental unit at harvest.
Post-Harvest fruit evaluation: Where applicable in scion trials evaluate post-harvest handling traits, especially for the fresh-fruit market, such as diseases, bruising, degreening, etc.

Diseases and Pests

Huanglongbing (HLB): PCR testing annually for bacterial titer is required at least once a year during the highest stress period. Leaf tissue samples should be collected from each observational (sampling) unit.

HLB: Visual rating of disease incidence and severity (HLB Decline Index) adjusted for tree size.

Tree canopy decline index (DI) score: For each quadrant visually assess HLB symptoms on a scale of 1 to 5, with 1 = no foliar disease symptoms, 2 = foliar symptoms on less than 25% of leaves, 3 = 25-50% of leaves with symptoms, 4 = 50-75% of leaves with symptoms, 5 = more than 75% of leaves with symptoms.

1. Small trees (Year 1 and 2): Rate the entire canopy as one unit. The maximum score per tree will be 5.

2. Medium canopy trees (Canopy volume ≤ $3m^3$) divide canopy into bilaterally. Apply ratings to each sector. The maximum score per tree will be 10.
3. Mature trees, each canopy hemisphere is subdivided into four equal quadrants by two imaginary perpendicular planes (vertical and horizontal at mid-canopy height) passing through the axis of the tree trunk. The resulting eight sections are scored individually on a 0-5 scale indicative of the proportion of limbs expressing HLB disease symptoms within each section (0 **=** no limbs, 5 = all limbs). The summation of the eight scores for each tree will result in a severity rating of 0 - 40 for each tree on each survey date. Trees that were severely declined with initial DI scores greater than 32 (average DI 4 x 8 = 80% declined with symptoms) will not be chosen as measurement trees within each plot.

Evaluation of other pests and diseases based on incidence and severity.

1. Blight: Die-back and quick decline ratings in canopy sections developed for HLB DI ratings by tree age and size.
2. *Phytophthora nicotianae.*
3. *P. palmivora/Diaprepes* weevil complex and burrowing nematodes
4. Other citrus nematodes.
5. Citrus Tristeza Virus: Die-back and quick decline ratings the same as used for HLB disease rating
6. Post-bloom fruit drop: Count the number of buttons in canopy sections accounting for variability.
7. Citrus Canker: Percent lesions on leaves and fruit in canopy sections developed for HLB DI ratings by tree age and size.
8. Other: Incidence and severity of other pests and diseases should be recorded, and evaluation criteria developed and when necessary.

Guidelines specific to pre-commercial rootstock field trials.

Scion selection: For simplicity of trial design, data collection and interpretation, one scion clone is recommended for each replicated, multi-location rootstock trial.This leads to separate trials for each 'Valencia,' 'Hamlin', 'Mid-sweet', 'Grapefruit', 'Tangerine', 'Mandarin' etc. scion clone selected for each trial. Complex designs utilizing more than one clone for any scion type, for example, two Valencia clones, must be balanced and take into the effect of scion and scion/rootstock interaction on the validity of data collected.

Guidelines specific to pre-commercial scion field trials.

Rootstock selection: For simplicity of the trial design, data collection and interpretation, one rootstock variety and one scion variety-type (multiple scion genotypes) of similar maturity (sweet orange, grapefruit, etc.) is recommended for each replicated, multi-location trial. For example, Valencia candidate scions budded on Swingle only (Valencia1/Swingle, Valencia2/Swingle). This leads to separate trials for each 'Swingle, 'Sour orange, 'Carrizo'etc. scion/rootstock combination selected per trial. However, if the decision is made to test the candidate scions on more than one rootstock (e.g., valencia1/Swingle, Valencia2/Swingle, Valencia1/Carrizo, Valencia2/Carrizo) the following considerations should be made:

1. Each experimental unit (split-plot) should contain every scion/rootstock combination.
2. If known, from preliminary data, the effect of rootstocks on scion maturity should be taken into account.