

CRDF Commercial Product Delivery Project Progress Report

Quarter Ending September 2014

Project Title: 1. Antimicrobial Strategies

Subproject Title: 1a. Antimicrobial Strategies: Conventional Antibiotics

Narrative of Progress by Project Goals:

1. Forge a partnership with a company for commercialization for streptomycin and oxytetracycline.

In July the CRDF Board approved agreements between CRDF and AgroSource under which CRDF would provide funding to accelerate field work on commercial antibiotics (oxy-tetracycline and streptomycin) for use on Florida citrus against HLB. AgroSource has advised that field trials are underway.

2. Broaden the reach of currently approved antibiotics for use on grapefruit to include oranges.

One of the agreements calls for expanding the Section 18 SLN label for Firewall® use against citrus canker on grapefruit to include round oranges. It specifically calls for funding to compile the economic and related data required to apply for the SLN registration.

3. Track RMC and CPDC research projects relevant to conventional antibiotics against HLB.

CPDC staff has been in communication with both growers and a sponsoring company conducting on-going field trials on Florida citrus involving its streptomycin and oxy-tetracycline products. CRDF's intent is to monitor progress and understand sponsoring company plans involving these products for use on Florida citrus.

CRDF has also been funding research under a three year project with Dr. Jim Graham of UF CREC to monitor streptomycin resistance in canker-infected citrus in support of the FireWall® registration for Canker. This is to comply with EPA monitoring requirements, and uses a protocol developed for the Section 18 use of kasugamycin against the Fire blight pathogen in Michigan.

Significant Meetings or Conferences:

On August 28 there was a telephonic meeting relating to regulatory issues surrounding antimicrobial tools for Citrus HLB. Participants included representatives from CRDF, Florida Fruit and Vegetable Association (FFVA), Florida Department of Agriculture and Consumer Services (FDACS), U.S. Environmental Protection Agency (EPA), Food and Drug Administration (FDA) and Centers for Disease Control and Prevention (CDC). The purpose of the meeting was to continue discussion of regulatory issues related to antimicrobial candidates being pursued for HLB therapy. All relevant government agencies with purview over use of antibiotics and other chemicals that have potential impact on human health and food supply safety were present.

This meeting set the stage for further discussions at the IR-4 regional Food Use Workshop, Antibacterial Mini-Summit, and post-summit meeting during the period September 9-11 in Atlanta. At the Antibacterial Mini-Summit, Dr. Jim Dukowitz presented the CRDF antimicrobial strategy against HLB, while other presenters described issues and experiences in treating a number of specialty crop diseases using antimicrobial compounds, with special attention given to the only three antibiotics approved for use in plant agriculture: streptomycin, oxytetracycline, and kasugamycin. Representatives of EPA, CDC and FDA were all present and provided their perspectives on the regulatory hurdles associated with use of conventional antibiotics.

The afternoon discussion following the mini-summit was hosted by CRDF and included representatives of CRDF, FFVA, IR4, FDACS, EPA, CDCP, and FDA Discussion specifically focused on the use of antimicrobial compounds against HLB.

The bottom line presented by the regulatory professionals was that oxy-tetracycline and streptomycin should not pose significant problems for label expansion to include HLB across Florida citrus, and that, once the residue profile is developed, it would be reasonably straightforward to develop a Section 18 package. Government officials cited the significant regulatory challenges posed by penicillin due to lack of EPA approved data sets for use in plant agriculture; its high potential for creating resistance that carries across all of the other related materials being used for treatment today; allergenicity issues; and its impact on mid-gut micro-flora in animals and humans. Also discussed were legal risks, public relations issues, and market acceptance concerns created by use of penicillin on Florida citrus. These meetings were important to set the stage for an ongoing dialogue between the Florida citrus industry and federal regulatory agencies.

Obstacles Encountered and Breakthroughs:

The various meetings described involving all relevant stakeholders helped to create greater clarity on how the regulatory agencies view conventional antibiotics for use by the Florida citrus industry against HLB, and the issues, challenges and roadmaps associated with each candidate that must be pursued.

Other Information:

The link to the proceedings of the meeting, which includes the presentations and the notes taken by IR-4 staff, can be found on the IR-4 home page: <http://ir4.rutgers.edu/>. It is referenced with a link in the upper right hand side of the screen, which is copied below:

<http://ir4.rutgers.edu/Biopesticides/workshoppresentations/Proceedings%20Understanding%20the%20ABCs1.pdf>

Subproject Title: 1b. Antimicrobial Strategies: Agricultural Antibiotics

Narrative of Progress by Project Goals:

1. Evaluate Zhongshengmycin (ZS) and Validoxylamine A (VA) against HLB

CRDF-supported studies by Powell (#617-enhanced) that ended on June 30, 2014 examined various parameters with specific antimicrobial candidates. It tested in the field a limited number of high

potential antimicrobial compounds that were found to be effective against C Las in the graft-based assay. This included two Chinese agricultural antibiotics, Zhongshengmycin (ZS) and Validoxylamine A (VA). Two samples per tree were taken at two month intervals for qPCR evaluation. Application methods included foliar-spray, basal bark application and gravity bag infusion. Measurements includes reduction of C Las titers, phytotoxicity, tree health, et.al. First applications were made in January '14.

The data collected after four months, while still preliminary, showed that both ZS and VA treatments were more effective in reducing Las titers than the negative control (water), but not as good as the positive control (ampicillin). All treatments except water decreased the Las titer in new leaves at 4 months after initial treatment. Temporal phytotoxicity was observed in some gravity bag infusion treatments, but all treated trees recovered after 4 months. After four months, trees treated with ZS and VA still had high titers of HLB bacteria in the older leaves, but less titer in the young leaves.

Significant Meetings of Conferences:

None

Obstacles Encountered and Breakthroughs:

The key obstacles are related to Intellectual Property and access to product of consistent quality and quantity.

Subproject Title: 1c. Antimicrobial Strategies: Biopesticides and Host Immune Modifiers

Narrative of Progress for Project Goals:

Make the following progress with partner Company C during FY 2014-2015:

1. Screen library of Company C biopesticides using *L. crescens* assay.

In September, CRDF Program Management finalized a first list of products from the Company C library of antimicrobial compounds for evaluation using the *L. crescens* assay, and cleared all company legal and administrative hurdles to enable shipment of its first 12 compounds to the University of Florida (Triplett lab).

During the quarter we worked with Company I with a significant library of natural products and bio-pesticides, and finalized agreements between company, CRDF and University of Florida. As of the end of the quarter, Company I had shipped approximately 300 products from its library for testing, with more to come.

2. A new field trial based on new plantings that are uninfected with HLB.

In May, CPDC and the CRDF Board approved a new trial funded by CRDF that will be based on new plantings that are uninfected with HLB using Company C biopesticides and host immune modifier products (both commercially available and in development). The purpose of the study is to examine whether the products can prevent infection. The study will measure tree health, foliar nutrition, disease

rating, HLB status, root density, yield and fruit quality.

3. Comp C - measure HLB levels on Company C canker trials and canker on CRDF HLB trials

CRDF also reached agreement with Company C to expand two in-place field trials by Company C to include C Las this season.

4. Track research projects relevant to biopesticides and host immune modifiers against HLB.

Ongoing

Significant Meetings of Conferences:

During the quarter, there was regular communication with Company C and Company F relationship managers, as well as legal, scientific and product development teams to move things forward.

Obstacles Encountered and Breakthroughs:

None

Other Information:

None

Subproject Title: 1d. Antimicrobial Strategies: Non-antibiotic Tetracycline Derivatives

Narrative of Progress by Project Goals:

1. Working with partner Company E, optimize three non-antibiotic tetracycline derivative compounds.

CRDF has funded a CPDC project (775C) for the investigation of non-antibiotic tetracycline analogs and formulations against HLB. The study, which will conclude in October 2014, is examining two specific compounds in trans-bark and phloem transport experiments with intent to develop the optimal formulations. Additional derivatives will be synthesized based on the findings of the Triplett laboratory, and pro-drug forms will be developed with intent to enhance plant uptake and bioavailability.

2. Track research projects relevant to non-antibiotic tetracycline derivatives against HLB

During the quarter Company E provided 2 different samples of non-antibiotic tetracycline derivatives, which were evaluated at a range of different concentrations using the *L. crescens* assay, and 3 samples were evaluated using the Gonzalez flush-based assay.

CPDC staff also identified a company (Company G) with a library of novel tetracycline derivatives that were developed for animal and human use. A subset have been identified for evaluation using *L. crescens*, with likely follow-up on promising compounds using the Gonzalez flush-based assay.

Significant Meetings of Conferences:

None

Obstacles Encountered and Breakthroughs:

None

Other Information:

None

Subproject Title: 1e. Antimicrobial Strategies: New Actives in Development

Narrative of Progress by Project Goals:

1. Identify CRDF roles and support responsibilities defined for the following technologies:

a. Polycation polymers

Working with partner Company D, have a company-driven development plan in place with identified CRDF roles and support responsibilities.

CRDF Program Management conducted regular conference calls during the quarter with technical teams associated with polycation polymers.

During the quarter Company D provided six promising compounds for evaluation using the L crescens assay, and also provided four compounds for evaluation using the Gonzalez flush-based assay.

b. Bacterial protein targets LdtR and SecA

Working with partner Company D, have a company-driven development plan in place with identified CRDF roles and support responsibilities.

CRDF Program Management conducted regular conference calls during the quarter with technical teams associated with bacterial protein targets LdtR and SecA.

Significant Meetings of Conferences:

A major source of communication has been CRDF project management participation in regularly scheduled conference calls with the technical teams for both projects.

Company D is conducting research through funding support from the parent company of a major citrus juice processor (Company H), which has initiated contact with several significant “large Ag” companies regarding interest in becoming commercialization partners for the most promising compounds against HLB. CRDF staff has participated in several of these calls over the quarter.

Obstacles Encountered and Breakthroughs:

One of the key issues during the quarter was to clarify the “go forward” commitment to this projects by Companies D and H.

Another issue is finding the right partners to take these technologies through the commercialization process. As a new material, it will likely require 5+ years and considerable expense to obtain regulatory approval.

Other Information:

CRDF also supported testing in the Gonzalez flush-based assay for two cationic selective antimicrobials (ceragenins) and one silver-based antimicrobial compound. All had performed well in the L crescens assay. These were provided by companies other than Company D.

Subproject Title: 1f. Antimicrobial Strategies: GRAS-like Compounds

Narrative of Progress by Project Goals:

1. Have at least one GRAS-like compound commercially available by June 2015.

During the quarter, 19 micro-emulsion oils of natural products provided by the UF Particle Engineering Research Center (PERC) and 2 plant derived organic acids provided by a grower were tested in L crescens. In addition, samples of the commercially available product Thyme Guard, produced by AgroResearch, was forwarded to PERC for evaluation. Based on that evaluation, a roadmap will be developed for continued testing of the product or formulation variants of the product.

The CRDF Antimicrobials Project Management team is highly interested in a novel therapeutic zinc particulate-based formulation for its potential against HLB, and is working with researchers and the technology transfer office at the University of Central Florida and University of Florida to facilitate completion of material transfer agreements and confidentiality agreements to evaluate this product on L crescens and potentially other CRDF-funded assays..

2. Track projects with GRAS-like compounds against HLB and integrate findings into project planning.

Two plant essential oils, p-cymene and carvacrol, were included in a limited field trial that evaluated a number of high potential antimicrobial compounds found to be effective against C Las in the graft-based assay. These studies were #584/#617 enhancements. Application methods included foliar spray, basal bark applications and gravity bag infusion. Data from qPCR measurements of C Las titers, along with measurements of phytotoxicity, and overall tree health should be available in the December-January time frame.

CRDF has also funded a one-year project with UF CREC to evaluate a novel therapeutic zinc particulate based formulation for preventing citrus canker and HLB. Because of the natural disease cycle of citrus canker, field trials were initiated before the official start of the proposal, and trials are well underway in a grapefruit block that is well established for canker management trials. The HLB project was initiated in June, and a grove of 3 year old infected Valencia was identified, scouted for disease rating and sampled. Sampling and measurement of the initial titer response in these trees is currently underway.

Significant Meetings or Conferences: None

Obstacles Encountered and Breakthroughs:

Through the various assays we are identifying promising plant essential oils that demonstrate potency against HLB or L crescens. We continue to learn more about formulation and delivery methods through the work at PERC as well as ongoing studies by UF and USDA.

Other Information: None

Project Title: 2. Naturally-Occurring Microbial Product Interactions with HLB

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

1. Track ongoing research on soil microbes and their role in HLB and tree health
2. Conduct field trials to test commercially available naturally occurring microbes
3. Provide communication on project goals, progress and results to CPDC, CRDF and growers

Narrative of Progress against Goals:

1. Experimental protocols were developed to provide a sound scientific assessment of effects of 5 commercially available microbial soil amendment products plus a water treated control on HLB.
 - All treatments (quarterly or monthly) are being applied with and without an organic mulch at the 3 Valencia/Swingle trial sites – Ridge, East Coast, and South FL-- using a professional crop consultant company at each site.
 - Treatment product samples from the company reps have been given to N. Wang for microbial characterization.
 - Results from initial visible tree ratings for HLB status and results from leaf samples for nutritional analyses are currently being statistically analyzed.
 - Thus, all required field work at all 3 sites is on schedule and all the data has been submitted on time.

Significant Meetings of Conferences:

Obstacles Encountered and Breakthroughs:

1. Leaf samples for PCR analysis were gathered at all 3 sites in June and sent to US Sugar but an instrument problem rendered the data useless. Samples will be repeated this winter.

Other Information:

Project Title: 3. Thermal Therapy to Reduce CLas Titer in Infected Trees

Narrative of Progress by Project Goals:

1. Determine impact of thermal treatment on CLas acquisition by ACP.

The ultimate value of thermal therapy will be determined by level of CLas reduction and tree response, as well as the duration of impact once treatment is applied. When applied to new trees as an alternative to tree removal and replacement, the additional question that can be posed is whether the CLas reduction is adequate to prevent psyllids from acquiring the pathogen from treated plants. Since secondary spread is an important aspect of HLB infection of new plantings, this questions may inform how useful thermal therapy will be on spread as well as the initial tree recovery.

To that end, discussions with scientists who have worked with ACP transmission have been requested to present a plan to answer this question. Since thermal therapy reduces *CLas* titer, measuring ACP acquisition (adults as well as nymphs) in a time series following treatment and comparing to untreated trees in the same block will characterize the level of protection from secondary spread provided by thermal therapy. This proposal will be presented for consideration by CPDC in October and, if approved the work would commence in spring 2015.

2. Refine requirements and environmental conditions for most effective thermal treatment.

Progress on all fronts is fine-tuning the conditions required for effective thermal therapy and its scale-up. From broader use of enclosures placed over trees to accumulate solar heat to ongoing testing of steam and other supplemental heat sources, the time/temperature relationships are being evaluated. They will be quite different depending upon the thermal therapy tool. Size of tree affected, general health of the tree, and perhaps even time of year.

Extensive grower testing has occurred since the April, 2014 field day and subsequent extension activities to more broadly spread the information. Thousands of trees, mostly of smaller size classes, have been exposed to steam treatment in conjunction with Dr. Reza Ehsani's project at UF. Placement and duration of steam application has been the main focus of this effort. In addition, Dr. Ehsani is evaluating the conditions under which trees experience partial to considerable defoliation from steam treatment, and establishing the balance between foliage (and fruit) loss and *CLas* reduction.

Repeated field trials will calibrate this against field conditions and variability in condition of trees being treated.

A field tour of this project was conducted in September and evidence for progress was observed.

However, longer-term objective evaluation requires metrics that include PCR measure of *CLas* titer, tree growth response and productivity. Where possible, evaluation of fruit for retention, volume and quality will be conducted in the field trials.

Work also continues in evaluating the combinations of factors that will lead to successful treatment of trees by trapping solar heat, as well as evaluating the hydro-solar treatment through USDA, ARS. Similar measures of effect will be applied to these studies as well.

CRDF, with its emphasis on field evaluation of solutions through CPDC, Dr. Jim Syvertsen has developed a series of protocols to measure the impact of various treatments against *CLas* and HLB in field trials, and these metrics are likely to have value in evaluating thermal therapy as well. They combine observational with data collection methods to provide an overall measure between treatments, and experiments conducted using these protocols can be compared against each other via common metrics. The CRDF Field trial Administrator, Brandon Page is working with thermal therapy research teams to become familiar with their needs and to establish baseline measures for later comparisons.

3. Encourage scale-up of individual tree, over-the row and root supplemental thermal therapy

Since the first evidence of thermal therapy has emerged from field trials in the East Coast of Florida, individuals and companies have shown interest in participating in scale-up, or anxiously supporting the commercialization of this treatment for broader application on young and older trees. Logistics of applying thermal therapy to mature hedgerow plantings requires different approaches, and there are a number of innovators who are attempting scale-up, primarily with their own resources at present.

Among these are growers adapting solar "tents" and commercial efforts to mass produce and market reusable and stackable tents. On the equipment front, companies within Florida are experimenting with

steam delivery, building on the efforts of Dr. Ehsani. Further afield, implement manufacturers are considering over-the-row application of steam for HLB treatment, building upon existing platforms. These are not yet ready for field evaluation.

The USDA, HLB Multi-Agency Coordinating Group is completing evaluation of ideas for “shovel-ready” solutions to HLB and has received a number of proposals to move this effort forward. The results of this funding program could move additional efforts forward into field trials as early as spring, 2015.

4. Continue outreach efforts to inform growers of the availability of thermal therapy

CRDF continues to publicize the thermal therapy field evaluation on its website, magazine articles, and presentations. In addition, considerable information was provided during the August Citrus Expo in Fort Myers, and through various Extension outlets.

As mentioned earlier, at least two multi-county agent-led educational activities followed the April 2014 field day with programs in July and August.

Obstacles Encountered and Breakthroughs:

An obstacle to rapidly moving this technology to commercialization is the identification of private parties with resources and willingness to develop and test these practices on a larger scale. This is occurring and new field tests are emerging. In addition, CRDF is proposing to provide the evaluation support to make sure that these field trials are adequately measured and comparisons can be made between methods and over time.

Project Title: 4. Plant Growth Regulator Interactions with HLB

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

1. Track RMC research projects evaluating the effects of PGR application on plant physiological processes and on pre-harvest fruit drop
2. Track and report on the portfolio of CPD field trials that have been completed, are underway during 2014-15 and those that will be initiated during this period
3. Communicate to CPDC, Board and growers on the project progress, results and interpretation

Narrative of Progress against Goals:

1. Percentage fruit drop data from the 2 Central FL 2,4-D (Retain) field trials from Dec 13 through March 14 were overall, not remarkable. Overall, % fruit drop varied from 20-70%.

-- Multiple field trials of various PGRs to decrease preharvest fruit drop in Valencias by Dr. L.G Albrigo. – Began Fall/winter 2013 and continued through harvest 2014. Fruit drop data are still being analyzed but overall, percentage fruit drop data were numerically reduced by some PGR treatments, particularly GA₃ plus 2, 4-D, but are statistically not different. Trials for upcoming 2014-15 harvest season will include more Hamlin trials and larger plots to include a greater number of replicate trees so that tree health (decline) status can be replicated more times in each plot.

-- Percentage fruit drop data from a field trial of 4 PGRs of Valencia on 2 rootstocks on CREC by Drs. Schumann and Brodersen in 2013-14, were not significantly affected by PGR treatment. There were differences between rootstocks, however, in that percentage fruit drop was 5-10% greater in trees on Carrizo than on Swingle. There was no difference in yield or fruit quality of retained fruit.

Significant Meetings of Conferences:

Fruit drop results from the 3 groups of trials above were reported at the ISHS meetings in August 2014.

Obstacles Encountered and Breakthroughs:

Tree to tree variation with HLB status is a reoccurring problem. It does appear, however, that trees with less severe HLB symptoms may respond better to PGR effects of decreasing percentage of pre-harvest fruit drop than HLB declining trees.

Other Information: None

Project Title: 6. Case Analysis of Grower Success in Responding to HLB**Narrative of Progress by Project Goals:****1. Envision a process to analyze individual citrus plantings in an HLB environment.**

The predecessor project that set the stage for this effort was UF project #614, directed by Drs. Young and Burns. This project ran from May 1, 2012 through June 30, 2014. The goals of project entitled “Enhanced nutritional application and productivity in endemic HLB grove situations in Florida – A statistical approach to determine efficacy was to learn from the numerous grower trials of various macro and micro-nutrient applications and to determine the common factors responsible for positive response. This project was funded at \$95,282 over the 2 year period and suffered from limited availability of grower-provided data. The final report indicates that without a broader participation, such efforts cannot provide meaningful analysis.

In this new project, a different approach is proposed, where domain experts use a “forensic approach” to determine the factors leading to identified “successes” in managing HLB in the field. The first step is a pilot study to be jointly organized by UF and UDSA, ARS. With leadership changes in both agencies, there has been progress to report on the pilot study.

3. Beta test analytic approaches on 1 or 2 cases to establish process.

No Progress to report

4. Commission appropriate groups to execute the process to evaluate successes.

No Progress to report

5. Communicate process and results to CRDF and citrus industry.

No Progress to report

Project Title: 7. Asian citrus Psyllid Management and Citrus Health Management Areas (CHMAs)**Narrative of Progress by Project Goals:****1. Pursue actions that will support expanded tools for ACP management**

Research continues on numerous alternative strategies for ACP management and its implementation at grower and CHMA levels. Among the recent activity is consideration of expansion of project #447

Stansly which includes inclusion of reflective mulches in new planting designs to reduce plant host-finding by ACP.

The USDA APHIS MAC group is approved funding for increased production of *Tamarixia radiata* for field release in states affected by HLB, including Florida. The FDACS rearing facilities at Davenport have been enhanced to double the annual production and release of ACP parasitoids, particularly in areas where ACP pesticidal applications are not being made.

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

Update on ACP control and Neonicotinoid Status provided by Daniel A. Botts, Third Party Registrations, Inc.

Neonicotinoid Insecticides:

Admire Pro® 24 (c) Label

The current conditional 24(c) label that expanded the use to allow a second application on larger trees expires at the end of the year. There were specific data required to continue the labelling that was being developed by the Registrant, Bayer CropScience. Efforts to coordinate the finalization of that data set and the subsequent meetings with FDACS are in progress and will need to be finalized prior to the end of the year.

Belay Insecticide Section 18

The Environmental Protection Agency granted a specific exemption to Florida Department of Agriculture and Consumer Services authorizing the use of Clothianidin on 3 – 5 year old bearing citrus trees to manage the transmission of Huanglongbing by the Asian citrus psyllid. This exemption expires on October 31, 2014, which corresponds with the last use date for the product on this size class trees for the 2014 calendar year. The Agency acknowledged that sufficient progress toward registration is being made. The Agency also indicated that they had made a preliminary determination that the use would be eligible for the re-certification program for Calendar year 2015. We are working with FDACS and the Registrant to ensure this application is made to prevent the loss of the applications during 2015. Approval letters for the Belay Insecticide 24 (c) from FDACS and the EPA Letter granting the Specific Exemption are available on request.

Thiamethoxam insecticide -- PRIA Petition

Work continues with Syngenta to monitor progress toward submission of the label expansion petition that has been held up due to internal company concerns and the on-going litigation and concerns over pollinator issues with this compound. The registrant has suggested a follow up discussion to ensure that the citrus industry still is interested in moving forward on this and other compounds they have in development.

3. Continue participation in pesticide stewardship activities

Update provided by Daniel A. Botts, Third Party Registrations, Inc.
Pollinator Stewardship.

The ongoing efforts being established through the leadership of the Florida department of Agriculture and Consumer Services will require additional effort as they move forward with refinements and changes supported by the impacted industries.

4. Continue to support CHMA implementation of ACP and other HLB management tools
5. Communicate progress and results of project to CPDC, CRDF and growers

An Asian citrus psyllid pest management update was provided by Dr. Michael Rogers of UF, IFAS, CREC at the August Citrus Expo Grower Seminar. Dr. Rogers summarized responses to most often asked questions.

Obstacles Encountered and Breakthroughs:

Other Information: None

Project Title: 8. Candidate HLB Tolerant Rootstock Plantings

Narrative of Progress for Project Goals:

1. Identification of best candidate rootstocks that have HLB tolerance from breeding programs.
2. Encourage early release of new commercial rootstocks to make rootstocks available to growers.

New research project. In Sept., the CPDC approved funding a new project entitled, "Propagation of Rootstock Tree Production in Greenhouses by Seed, Stem Cuttings and Tissue Culture to Accelerate Budded Tree Production for Outplanting". This research will be centered at MFREC and will address citrus rootstock availability issues at 3 levels of budded tree production; seed contamination, propagation of rootstocks by stem cutting, and acclimatizing tissue cultured rootstock to survive and thrive in commercial greenhouse production. There are 4 specific Objectives:

1. Develop guidelines for seed propagation that prevents contamination of seedling rootstocks with minimum loss of seed vitality.
2. Develop protocols for rooting of current citrus rootstocks from stem cuttings and optimize cultural practices to produce faster growth of rootstocks for budding.
3. Develop protocols for rooting of new citrus rootstocks from stem cuttings as they become available and optimize cultural practices to produce quicker rootstock growth for budding.
4. Develop protocols for large scale hardening of tissue culture produced rootstocks and guidelines for maximizing post-harden growth for budding.

Practical Application: Development of:

1. Guidelines for preventing canker contamination of seed.
 2. Protocols for rooting of existing rootstock species.
 3. Protocols for multiplying new rootstocks when they become available.
 4. Development of protocols for hardening off tissue culture plants.
3. Implement Phase I and II grower field trials of promising candidate HLB tolerant rootstocks

Nine candidate rootstocks have been selected: 5 experimental rootstocks from the UF and USDA breeding programs along with 4 standard rootstocks (812, Sour, Carrizo, Swingle) for comparison. Nurseries have been contracted to produce a number of trees of Valencia orange on each of the 9 rootstocks. Each genotype has 144 trees (64 measurement trees plus buffer trees) replicated 5 times. Trees have been propagated and are now growing in the nursery. Three cooperative growers with suitable sites between 28 – 35 acres, and resources will be selected in the fall. Trees will be planted at 3 sites, Ridge, East Coast, SW FL, in spring 2015.

Phase 1 propagation: Rucks Nursery reports that the test candidate HLB tolerant genotypes and standard rootstocks have all been propagated with a common scion in numbers adequate to meet the planting plans for the 3 regional commercial-scale plantings in Phase I. Some trees are far enough along to plant now, but all will be ready for spring 2015 planting.

Phase II propagations: The FDACS permit for micro-propagation of citrus rootstocks for the CRDF Phase II field trial were issued to Agromillora and transfer agreements with UF were executed. With this accomplished, Agromillora coordinated with FDACS (Dr. Peggy Sieberth, Mr. Michael Kessinger and Mr. Ben Rosson) to obtain materials representing UF releases selected for the Phase II trial. Having received the clean UF materials, Agromillora reports that they have 3 of the 4 UF selections now in culture. We will continue to try the missing one (UFR-DPIO- 435-006) until we have it in the culture. Agromillora also is working with micro-propagation of select USDA ARS candidate HLB tolerant rootstocks for which adequate seed are not available, working through a permit with a Florida Citrus Nursery.

4. Evaluate grower plantings of rootstocks at 3 sites: Central Ridge, E Coast Indian River and SW Fla.
Not in field yet, so no evaluation yet available.
5. Communicate progress and results of evaluation of rootstocks to industry
Presentations of updates and planning information were provided to industry through regular published communications and grower meetings during the quarter.

Project Title: 9. RNAi Molecules/Psyllid Shield

In April, the CRDF Board approved funding for a two year project to improve and continue development of a mathematical model that characterizes the population dynamics of ACP and the spread of C Las. The model focuses on the flush shoots of the citrus trees that are the breeding sites for ACP. The objective is to use the improved model to bring the necessary precision to a field trial design by accurately modeling the performance of the psyllid shield concept over different spatial dimensions, neighboring psyllid and disease pressure, and RNAi performance.

Narrative of Progress by Project Goals:

1. Identify the specific dsRNA molecule(s) that can be advanced into practical psyllid control strategies.
Numerous RNAi sequences with strong lethal effects on adult psyllids have been identified in prior and on-going research.
2. Incorporate the RNAi Molecules project and its research results into the Psyllid Shield project.
At the September CPDC meeting, Dr. Bill Dawson (UF CREC) provided a summary of RNAi cage experiments that looked at the generation of psyllid progeny on RNAi-containing plants based on a

number of different CTV gene constructs identified for potential psyllid control. All 9 of the constructs tested to date in 2-9 replicated experiments showed 80-100% reduction in psyllid progeny. Furthermore, in 19 replicated control experiments the percentage of CLas positive progeny increased at least 2X from the input population while in RNAi protected plants the percentage of CLas infected progeny decreased at least 2X and in many cases all survivors had undetectable levels of CLas.

3. Establish a mathematical model of RNAi effects on area wide protection of new plantings.

At the September CPDC meeting, Dr James Keesling (UF Dept of Mathematics) provided an update on the Psyllid Shield Model. Using actual data on psyllid spread from a 2010-2011 study conducted in Colima, Mexico of 2909 four year old lime trees, grafted on macrofilia lemon, the team was able to refine and validate the model with actual field data.

4. Refine the model with vector entomologists and epidemiologists.

CPDC supported the continued efforts of researchers to model performance by varying the strength of the RNAi constructs, size of grove, and psyllid pressure (number of psyllids that invade the grove).

5. Experimentally evaluate candidate protective effects of selected RNAi in CTV inoculated plants.

See above.

6. Continue to evaluate new RNAi for improved activity with CTV vectors.

On-going.

7. Model performance of best RNAi for field trials and complete scale-up feasibility analysis.

The panel requested a comparison of 10Ac and 20Ac effects and a border row layout.

Significant Meetings of Conferences:

The key members of this team, Drs. Keesling, Lee, Dawson and Halbert have been meeting and discussing the crux of this problem of field trial design which is to obtain experimentally from small caged experiments and actual field data of psyllid spread estimates of the input parameters for the mathematical model so field trial feasibility can be assessed.

Obstacles Encountered and Breakthroughs:

One of the challenges in implementing the Psyllid Shield concept is how to bring the necessary precision to a field trial design to test the concept. This includes the size of the trial plot, estimates of adjacent psyllid pressure, and then measuring how well the RNAi works in the actual trial.

Other Information: None

Project Title: 10. Integrating HLB Management Tools into New Groves

Narrative of Progress by Project Goals:

1. Assemble work group to discuss tactics and tools available for new citrus plantings

Nothing to report

2. Generate an overview of elements possible to incorporate into a new citrus planting

Nothing to report

3. Encourage growers to consider use of these guidelines when planning for new plantings

Nothing to report

4. Establish one “model new grove” which incorporates the elements of integrated tactics and tools

Nothing to report

5. Communicate progress and results to CPDC, CRDF and growers

Nothing to report

Project Title: 11. Candidate HLB Tolerant Scion Evaluation in Field Trials

Narrative of Progress by Project Goals:

1. Encourage citrus Breeders to identify and prioritize candidates for scion tolerance to HLB.

Nothing to report

2. Determine the need and scope of field trials to evaluate HLB tolerant scions.

Nothing to report

3. Develop and implement field trials as deemed necessary.

Nothing to report

4. Provide communication regarding scion evaluation for HLB tolerance to CPDC, CRDF and growers.

Project Title: 12. Genetic technology (MCTF): Deploying Canker-Resistant Genes

Narrative of Progress by Project Goals:

1. Make measurable progress toward producing and introducing to Florida citrus growers new transgenic citrus lines based on mature tissue transformation of commercially available cultivars. These citrus lines will have disease resistance to citrus canker, and will flower and bear fruit in a short time period. For FY 2014-2015, measurable progress is defined as:

- a. Micro-propagate a number of plants to begin grafting the transformants in all combinations. Conduct at least one transformation of mature scion or rootstock every week.

Southern blots of citrus transgenic for market genes were conducted during the quarter using a non-radioactive DIG labeling kit. The results show that transgene integration and copy number are as expected for Agrobacterium transformation. Most citrus transgenics have one or two copies of the transgene. Additional blots are underway and a manuscript is in preparation.

A genetic construct conferring tolerance to greening and canker has been transformed into mature scion of Valencia, Hamlin, Ray Ruby, Pineapple, and mature rootstock of Swingle and Carrizo. PCR positive plants have been double checked to ensure they are not false positives. Most transgenics have been transplanted to the soil and are acclimating in the growth room.

The transformation and micro-grafting pipeline has been changed so that all shoots are now micro-grafted onto immature rootstock, grown, and screened afterwards. The dedicated micro-grafting station is working well and about 160 shoots can be micro-grafted per day. At this time, however, only about half survive for screening.

The facility has 2672 micro-grafted shoots in the pipeline that must be screened.

b. Conduct mature transformations with as many as 3 additional gene constructs.

The service facility has obtained additional constructs from other scientists targeting canker susceptibility, which are being transformed into Hamlin, Valencia and Ray Ruby scion.

c. Improve laboratory and growth room productivity and mature transformation processes.

The gene gun is being tested on mature explants of scion with constructs carrying the GFP reporter and plants are being regenerated.

Significant Meetings of Conferences:

Copy number information and expression data were compiled for a poster presentation at the annual American Horticultural Society (AHS) meeting in Orlando at the end of July.

Obstacles Encountered and Breakthroughs: None

Other Information: None

Project Title: 13. Diaprepes Pheromone

Narrative of Progress by Project Goals:

1. Make a "go-no go" decision no later than July 2014 on a two year field trial.

Significant Meetings of Conferences:

1. During the quarter there was no further progress in identifying candidate companies interested in co-investing in field trials in exchange for an option to sublicense the technology for use in their own products. None of the four companies we have been working with have not made a commitment to work with CRDF and no new candidates have been identified.

Obstacles Encountered and Breakthroughs:

In our application to and subsequent discussions with USDA regarding this technology we made it clear that, while important to the Florida citrus growers, the overall market size is relatively small, and that any decision to move forward with a CRDF licensing agreement with USDA would depend on finding one or more commercial partners to co-invest in the further development of the technology.

Other Information: None

Project Title: 14. Citrus Leafminer Area-Wide Mating Disruption

Narrative of Progress by Project Goals:

1. Monitor progress in both commercial and research evaluation phases of demonstration project

At the September progress report and invoice period, more than 375,000 DCEPT CLM emitters have been purchased and installed in the field trials, along with 300 lure traps for monitoring CLM activity in the 3 commercial sites.

The latest progress report on this project indicated that the DCEPT has been deployed in the three grower locations as planned and evaluations up to 16 weeks post-application have been made. The sites vary in both leafminer control practices as well as age of trees and leafminer pressure. Generally, the treated areas showed lower or equivalent CLM levels in traps as well as when CLM damage was assessed. In some cases, cooperators continued to spray the non-DCEPT plots with biweekly insecticides and the CLM levels in the DCEPT (no chemical sprays) were comparable. IN the block where rows were skipped when DCEPT was applied, it appears that this contributed to higher CLM infestation and damage, providing some indication for the need for coverage in applying these pheromone test materials.

In the younger grove locations it appeared that a combination of higher CLM pressure on new growth and lack of canopy to shade the DCEPT led to possible early breakdown of the effectiveness.

Field trials areas continue and plans to follow evaluations are in place. It appears too early to fully evaluate the impacts of this area-wide treatment.

2. Determine need to modify plans or details of project

No significant changes in plans have occurred.

3. Communicate progress and results of demonstration

Whereas, ongoing results are being shared with grower cooperators, there has been no broader communication of the status of this evaluation

4. Determine if CRDF has additional role in delivering this technology

Nothing to report

Project Title: 15. CTV Vectors

Narrative of Progress by Project Goals:

1. Ensure delivery systems and candidate genes for HLB resistance are commercialized.

SGCN continues to pursue the goals and milestones as defined in the licensing agreement with UF.

2. Ensure the pipeline of CRDF-funded research on CTV vectors is mined for commercial potential

Two companies have expressed interest using the CTV vector to express RNAi in programs for insect control, and are evaluating the opportunity for use in citrus. Market size, penetration projections, and cost structures will be included in their assessments. CRDF program manager has offered his availability to provide perspective on the technology and it's potential.

Significant Meetings of Conferences:

None

Obstacles Encountered and Breakthroughs:

None

Other Information:

None

Project Title: 16. HLB Escapes

Narrative of Progress by Project Goals:

1. Assess progress in identifying citrus trees that escaped HLB disease.

Nothing to Report

2. Evaluate elements that can be implemented to advance project beyond its current scale.

Nothing to Report

3. Facilitate expansion and enable more Ext Agent involvement in observations of tree escapes.

Nothing to Report

4. communicate progress to CPDC, CRDF and growers

Nothing to Report