CRDF Commercial Product Delivery Project Progress Report

Quarter Ending June 30, 2014

Project Title: 1. Antimicrobial Strategies

Subproject Title: 1a. Antimicrobial Strategies: Conventional Antibiotics

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

1. Forge a partnership with companies that are capable of driving the product development, regulatory and overall commercialization process for streptomycin and oxytetracycline against HLB, and provide financial and other resources to facilitate and accelerate that process.
2. Broaden the reach of currently approved antibiotics for use on grapefruit to include oranges.
3. Track RMC and CPDC research projects relevant to the identification, screening, formulation and delivery of conventional antibiotics against HLB and integrate findings into project planning.

Narrative of Progress against Goals:

1. CPDC and CRDF Board approval was granted in May to provide funding to AgroSource to accelerate field work on commercial antibiotics (oxy-tetracycline and streptomycin) for use on Florida citrus. During the month of June CRDF staff drafted contracts and, with support of the CRDF Board President, negotiated agreements for Excom/Board ratification.
2. At the April CPDC meeting, AgroSource presented proposals for CRDF support to accelerate implementation of its plans for combatting canker and HLB based on its Firewall (streptomycin) and Fireline® (oxytetracycline) based products. At the meeting, the Committee requested that CRDF staff review detailed plans, roles and responsibilities, costs, milestones, and potential IR-4 support, and define the benefits to CRDF from its support.
3. In May, on the recommendation of the CPDC, the CRDF Board of Directors approved four proposals submitted by AgroSource. The first proposal pertains to the Section 18 SLN renewal of Firewall® for grapefruit. It provides for resistance monitoring and data gathering on HLB impacts when the foliar application is made for canker suppression. The second proposal is for expanding the Section 18 SLN label for Firewall® to target canker on round oranges. It calls for funding to compile the economic and related data required to apply for the SLN registration. The third proposal is to develop information on the efficacy of Firewall® and Fireline® which contain streptomycin and oxytetracycline respectively against HLB. This would involve large scale field trials. The fourth proposal is to conduct studies specific to a regulatory support package that would include residue in treated plant

materials, eco-toxiciology, soil profiling, resistance monitoring and other data requirements that are met under GLP protocols.

4. Subsequent to Board approval, CRDF staff was tasked to develop and negotiate the contractual agreements. Unresolved issues were discussed at the June 12 Board meeting. With support from the CRDF Board president, negotiations continued throughout June.

Significant Meetings or Conferences:

The most significant meetings related to the negotiations between CRDF staff with support of CRDF Board President) and AgroSource. UF legal also provided important support to the effort.

Obstacles Encountered and Breakthroughs:

The most challenging obstacles related to negotiating of acceptable terms in the agreement that protected CRDF and grower interests. The breakthroughs came with concessions by AgroSource to make the document acceptable to the CRDF Board.

Subproject Title: 1b. Antimicrobial Strategies: Agricultural Antibiotics

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

1. The project goal for agricultural antibiotics is to evaluate the performance of Zhongshengmycin (ZS) and Validoxylamine A (VA) against HLB in a CRDF-supported field trial conducted by UF (Powell) started January'14. Citrus effects of the treatments will be investigated, including reduction in fruit drop, increased canopy density, reduced HLB symptoms, increased root uptake, residues and phyto-toxicity.

Narrative of Progress against Goals:

1. 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, Zhongshengmy;cin (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 include reduction of C Las titers, phytotoxicity, tree health, et.al. First applications were made in January '14.
2. The four month assay, 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 phytoxicity 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.

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

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

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

1. Screen library of Company C biopesticides (products and proprietary active ingredients) using *L. crescens* assay at University of Florida (UF) and offer to transfer the know-how for this assay to Company C.
2. CRDF-Company C coordination for a new field trial based on new plantings that are uninfected with HLB, using Company C biopesticide and host immune modifier products against HLB, including a dose escalation study above currently permitted levels. The purpose of the trial is to examine whether the products can prevent infection.
3. CRDF-Company C coordination of on-going field trials: measure HLB levels on ongoing Company C canker trials, measure canker levels on ongoing CRDF HLB trials.
4. Track RMC and CPDC research projects relevant to the identification, screening, formulation and delivery of biopesticides and host immune modifiers against HLB and integrate findings into project planning.

Narrative of Progress against Goals:

1. During the quarter worked with Company C legal and product development team to put in place needed Material Transfer Agreements and CDAs to enable testing of products. Also conducted discussions with Company C about products to be tested. At end of quarter, these were all progressing, but not finalized.
2. 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. CRDF also reached agreement with Company C to expand two in-place field trials by Company C to include C Las this season.
4. During the quarter, we continued to evaluate a wide range of compounds and products, including biopesticides, in the L crescens assay. We also worked with other companies producing biopesticides to seek their involvement in the CRDF-funded assays.

Significant Meetings or Conferences:

During the quarter, there was regular communication with Company C's relationship manager, legal and scientific and product development teams to move things forward. This included a face to face meeting in Lake Alfred of Company C representatives with CRDF staff to develop plans for the cooperative field trials.

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

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

1. Working with partner Company E, have three non-antibiotic tetracycline derivative compounds optimized for activity, delivery and cost against HLB by June'15.
2. Track RMC and CPDC research projects relevant to the identification, screening, formulation and delivery of non-antibiotic tetracycline derivatives against HLB and integrate findings into project planning.

Narrative of Progress against Goals:

1. CRDF has funded as 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. In April, seven different samples of non-antibiotic tetracycline derivatives were evaluated at a range of different concentrations using the L. crescens assay. In June, 6 more samples were evaluated for a total of 13 during the quarter.
2. Company E representative played an important role in the Antimicrobial Advisory Team deliberations during the quarter about formulations, dosages, and delivery methods associated with moving the most promising antimicrobial compounds into field trials.

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

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

1. Working with partner Company D, have a company-driven development plan in place with identified CRDF roles and support responsibilities defined for the following technologies:
   a. Polycation polymers
   b. Small molecule ligands targeting bacterial proteins LdtR and SecA

Narrative of Progress against Goals:

1. CRDF Program Management conducted regular conference calls during the quarter with technical teams associated with polycation polymers (Project 1) as well as bacterial protein targets LdtR and SecA (Project 2).
2. During the quarter Company E provided 4 promising compounds for evaluation using the L crescens assay, bringing the total for the year to 11.

Significant Meetings or Conferences:

Major source of communication has been regularly scheduled conference calls with the technical team for both projects.

Obstacles Encountered and Breakthroughs:

1. Funding support for Company D comes from the parent company of a major citrus juice processor, so one of the key issues during the quarter was to clarify the "go forward" commitment to this project
2. Another issue is finding the right partner to take this technology through the commercialization process. As a new material, it will likely > 5 years and considerable expense to obtain regulatory approval.

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

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

1. The project goal for GRAS-like compounds is to identify, formulate and deliver antimicrobial compounds that are effective against HLB and that qualify for GRAS-like status within two years. The goal for FY 2014-2015 is to have at least one of these compounds commercially available by June 2015.
2. Track RMC and CPDC research projects relevant to the identification, screening, formulation and delivery of GRAS-like compounds against HLB and integrate findings into project planning.

Narrative of Progress against Goals:

1. ***PERC/L.crescens.***: Progress was made in the evaluation oil emulsions of a variety of plant essential oils, including carvacrol, p-cymene and thyme oil. This led to 122 different formulations that were evaluated using L crescens assay during the quarter. This work was conducted at the Particle Engineering Research Center (PERC) at UF under the leadership of Brij Moudgil and Parvesh Sharma. Results are being evaluated to determine most likely candidates for further evaluation using other assays as well as formulation and delivery studies in preparation for field trials.
2. ***Commercial Formulator:*** In May, CRDF Commercial Product Manager met with two key individuals from one of the leading formulators of natural products. The connection was made through another company partner. The key individual is based in Germany, and made last minute plans to visit USDA facilities in Ft Pierce to learn more about thermal therapy as well as efforts to treat HLB with natural products. Based on that meeting and several e-mail exchanges, as well as an executed confidentiality agreement, Company is developing some formulations involving natural products for our evaluation against HLB. Company has also provided valuable insights into the chemical properties required for effective phloem mobility, including molecular weight, acidity (pKA) and partitioning (log KOW).
3. ***CRDF-supported field trials*****:** Enhancements of studies by Powell (#584/#617) that ended on June 30, 2014 looked at various parameters with specific antimicrobial candidates that included two essential oils, p-cymene and carvacrol. They were included in a trial that 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. Application methods included foliar-spray, basal bark application and gravity bag infusion. Measurements include reduction of *CLas* titers, phytotoxicity, tree health, et.al. First applications were made in March'14, with a first look at preliminary results in four months from first application.

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 studies by UF and USDA. The Gonzalez leaf -based assay will validate antimicrobial activity against HLB, while the trunk injection- based assay (Wang) will provide a way to evaluate dosages in a field environment.

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:

Experimental protocols were developed to provide a sound scientific assessment of the effects of 5 commercially available microbial products on HLB. The 5 soil-applied products which were labeled for citrus were:

1. BioFlourish (Triangle Chemical). 3 applications per year
2. Ecofriendly Citrus Soil Amendment. 8 x per year
3. Serenade Soil (Bayer CropScience) 3 x per year
4. Aliette (Bayer CropScience) 3 x per year
5. Quantum product line 12 x per year

All treatments were applied with and without organic mulch. Experimental protocols and budgets were approved by the CPDC by April and bids were solicited from 6 vetted professional crop consultants for 3 trial sites – Ridge, East Coast, and south Fl. Three consultants, one at each location, were awarded contracts and the 3 year field trials began in May 2014. All trees at all 3 sites are bearing Valencia trees on Swingle rootstock.

All initial visible tree ratings for HLB status were done, leaves were sampled for PCR and tree photographs were taken in May. All soil materials were applied at all 3 sites by 1 June. Additional treatments and leaf nutritional samples are to follow in July/August.

Both the Field Trial Project Manager and the Field Trial Administrator are monitoring the project activities and the Field Trial Administrator received data and visited field sites as they were being established. CRDF established data repositories for each project site so that all photos, data and treatment data are provided to CRDF as they are collected.

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

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

1. Determine impact of thermal treatment on *CLas* acquisition by ACP.
2. Refine requirements and environmental conditions for most effective thermal treatment.
3. Encourage scale-up of individual tree, over-the row and root supplemental heat and evaluation of their performance in reducing disease and improving health of treated trees.
4. Continue outreach efforts to inform growers of the availability of thermal treatment and to refine conditions that will lead to effective thermal treatment.

Narrative of Progress against Goals:

1. Ongoing CRDF-funded research at UF and USDA does not identify how thermal treatment affects availability of CLas to be acquired by ACP feeding on treated trees. Discussions occurred on the need for this to be included in the MAC funding proposal addressing thermal therapy scale-up and research. Overlay of CLas acquisition testing on current field trials was suggested as a simple way to accomplish this goal.
2. Field research by UF and IFAS is obtaining further detailed information on the performance requirements needed in thermal therapy to reduce CLas titer. Tenting and steam application experiments have incorporated data recording devices to document thermal conditions for correlation with tree response. Presentation of data from both research programs at the April field day encouraged the scientists involved to summarize and share information that they have to date, and the meeting allowed discussion of what kind of additional information on time and duration of treatment is necessary. Dr. Ehsani of UF reported that early efforts with application of steam heat resulted in overtreatment and severe plant injury, informing his subsequent treatment protocols. Data from tenting of infected trees and grower experience has established guidelines that are currently being used by many growers who are establishing their own small-scale tests of solar thermal treatment of small trees.
3. Following the April, 2014 Thermal Therapy Field day at Ft. Pierce, significant interest in thermal therapy was generated and many discussions followed among researchers, growers and entrepreneurs. Demonstrations of both solar and thermal treatments allowed participants to envision their own designs and to consider scale-up prospects on their own. Within a week of the field day, a commercial supplier provided the design, specs and order information for single-tree solar covers made from rigid metal frames and nursery cloth covers. These “tents” are available for purchase and the details were posted to the CRDF webpage.

   CRDF CPDC moved forward with plans to coordinate evaluation efforts of thermal therapy. Building on the methods used to evaluate effects of other treatments (antimicrobials, soil amendments, etc.) on CLas and/or HLB and tree response, a protocol was developed to document the conditions surrounding thermal treatments and a data plan for follow-up so that individual trials will be evaluated similarly and treatments can be compared. This protocol has been implemented on a small scale with grower and research trials and will

become standard in the MAC funded CRDF project to evaluate thermal therapy scale-up described below.

The USDA, APHIS MAC group was charged to manage the federal funding to put HLB solutions in the hands of growers. This group quickly identified thermal therapy as a "shovel-ready" project area and encouraged development of project ideas and mechanisms to attract and encourage solvers to come forward with plans for scale-up, and to propose how this funding could facilitate rapid scale-up. USDA, APHIS responded with consideration of a mechanism that has been used by their agency previously in seeking solutions to challenges, and plans were established to solicit solvers for thermal therapy scale-up.

4. A field day on April 30, 2014 in the Indian River citrus growing area of Florida demonstrated ongoing work to evaluate the value of applied heat in treating trees affected by Huanglongbing (HLB). More than 175 growers and other interested parties participated in this day-long event organized by Parker Platts, Multi-County Extension Agent, Fruit Crops and sponsored by CRDF. The field day highlighted research that is evaluating use of tree covers to trap solar heat and several methods that are being developed to speed up the process of treating trees by application of supplemental heat. The event was hosted by USDA, ARS at their U.S. Horticultural Research Laboratory in Ft. Pierce, and included opening presentation overviews, a visit to the ARS research farm to see application methods and to view trees which had previously been treated with heat, and to a grower site where thermal therapy has been used for nearly three years. The challenge is to provide adequate temperature and duration conditions to reduce the titer of *CLas* bacteria, the causal agent for HLB, while not causing heat injury to the trees.

   Presentations by USDA, ARS and UF, IFAS research teams highlighted progress in understanding and delivering thermal therapy and were followed by observation of the technologies and methods in the field. These demonstrations signified that this HLB tool has moved from the lab to the field and is ready for adoption. Participants had many questions and discussed details of practicality, cost, tree injury, and when during the year such treatments can be expected to be effective. Other topics included impact on fruit quality and the likelihood that heat treatment would actually eliminate the bacteria from infected trees. Many of these questions are being evaluated further as more trees are treated and the methods continue to be refined.

   The field day continued with a visit to a grove site of Edsall Groves near Ft. Pierce, allowing participants to see evidence of treatment of young trees with solar thermal therapy and the results of trees which had been treated very recently and as far back as 2-3 years. Designs for the tree covers were discussed and an active discussion occurred between participants and Dave McKenzie, the production manager who has been an advocate and adopter of thermal therapy.

   CRDF initiated this field day to inform growers of this emerging tool, and also to attract entrepreneurs, innovators, individuals and companies, who are interested in helping to deliver thermal treatment to growers on a broader scale. This is an important step in moving from proof to use, and CRDF is strongly encouraging the partnerships that can make this happen. In addition, with recent federal funding being made available to bring HLB

solutions to the field, CRDF and the industry are pursuing the opportunity that exists to partner with the USDA Multiagency Coordinating group to direct some of the federal funding to encourage scale-up of thermal therapy.

More information and the presentation contents can be viewed by going to thermal therapy page at citrusrdf.org, which highlights the presentations made during the field days, photo galleries from the field visits, and additional information related to thermal therapy and its use in treating HLB-infected trees.

Significant Meetings of Conferences:

April 30 Thermal Therapy Field Day at Ft. Pierce as detailed above.

Thermal Therapy highlighted at June 11 Florida Citrus Mutual Annual Grower Meeting Seminar

MAC planning meetings to include thermal therapy scale-up in shovel-ready projects

Obstacles Encountered and Breakthroughs:

Several outside companies have responded to the thermal therapy field day with plans to develop machinery to delivery thermal therapy, seeking cooperation from the researchers involved in this area of HLB management.

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. Two field trials of 2,4-D (Retain) were established to decrease pre-harvest fruit drop in Valencias in central Florida by crop Consultant Tom Minter. The field trials began December 2013 and were completed March 2014. Percent fruit drop results were analyzed and the final report written and submitted in June 2014.
2. Two field trials of 2,4-D (Retain) were established to decrease pre-harvest fruit drop in Valencias in central Florida by Crop Consultant Henry Yonce. These field trails began December 2013 and were completed March/April 2014. Percent fruit drop results were analyzed and the final report written and submitted in June 2014.
3. Multiple field trials of various PGRs to decrease pre-harvest fruit drop in Valencias were established by Dr. L.G Albrigo. These trials began fall/winter of 2013 and continued through harvest 2014. Fruit drop data are being analyzed as the yields are completed.

4. A field trial of 4 PGRs of Valencia on 2 rootstocks was established at CREC by Drs. Schumann and Brodersen during the 2013-14 growing season. Data from the completed trials are being analyzed.

Significant Meetings of Conferences:

Fruit drop results from 1. and 2. above are to be reported at the Citrus Expo in August 2014. Results from 1. ,2., 3. and 4. above will be 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 than HLB-declining trees to PGR effects of decreasing percentage of pre-harvest fruit drop.

Project Title: 6. Case Analysis of Grower Success in Responding to HLB

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

1. Envision a process to analyze and interpret the variable successes of growers in managing individual citrus plantings in an HLB environment.
2. Beta test analytic approaches on 1 or 2 cases to establish process.
3. Commission appropriate groups to execute the process to evaluate successes.
4. Communicate process and results to CRDF and citrus industry.

Narrative of Progress against Goals:

1. Representatives of USDA, ARS and UF, IFAS met during this quarter to discuss the needs for evaluation of grower trials, and how to improve on the limited success derived from evaluation project funded by CRDF to evaluate field trials of nutritional treatments. Among the challenges identified was the limited participation by growers when production and treatment data were requested for incorporation into the analysis, and the lack of uniformity of how grower trials are set up and data collected.

   The outcome of these discussions is the consensus that UF and USDA will conduct a beta test of a "forensic approach" to site evaluation of successes, during which experts in various areas of citrus horticulture and pathology will evaluate the circumstances evident in a grove that is performing well under HLB pressure and to evaluate the factors involved in this "success". No concrete plans were provided by the team at the end of this quarter for the beta test. Results of this step will guide direction forward.

Project Title: 7. Asian citrus Psyllid Management and Citrus Health Management Areas (CHMAs)

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

1. Pursue actions that will support expanded tools for ACP management
2. Engage registrants and regulatory entities in need for label modifications

3. Continue participation in pesticide stewardship activities
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

Narrative of Progress against Goals:

1. Research focused on the season-long treatment of ACP in young as well as mature citrus groves continued this quarter. Data from evaluation of treatments applied around bloom period were analyzed for incorporation into registrant actions to expand labels and provide supporting data. Field experiments that are tracking effectiveness of systemic as well as foliar treatments provided additional information on expected response from individual treatment selections, reinforcing the need for consideration of a season-long approach to keeping ACP populations low.

   Tweaking of the use of CHMAs to encourage grower coordination of ACP management occurred during this quarter, with the new CHMAs emerging from the former regional CHMA in SW Florida. This re-organization into smaller CHMAs will facilitate closer local cooperation and increase participation by area growers in coordinated treatments.

   CHMA ACP population monitoring showed increased ACP populations in many areas around the state, reinforcing the need for aggressive ACP management and coordinated treatments. As more growers consider economics of production practices, ACP treatment are a primary economic consideration. Coupled with the persistence of untreated groves as a source of ACP populations, increased populations might be expected.
2. CRDF, through leadership by TPR and Dan Botts, communicated closely with registrants, citrus growers and FDACS and EPA regarding the portfolio of ACP products available to treat young and mature citrus. Of particular interest was the follow-up to April 8 issuance by Agriculture Commissioner Adam Putnam of a crisis declaration for the use of clothianidin (Belay® Insecticide from Valent) to control ACP. This expansion provides additional treatment options for young trees in the 3-5 year age range, the period when HLB begins to manifest itself in new blocks and in resets. All parties involved considered how best to incorporate this new tool with other available tools and information to revise the year-long ACP treatment recommendations.
3. Cooperative efforts were led by Dan Botts of FFVA/TPR to address the concerns over honey-bee exposure to pesticides during bloom period when the managed hives are located in citrus groves. Dan coordinated communication on behalf of CRDF and the citrus industry with FDACS and state beekeeper organizations, with several meetings focused on interpretation of pesticides labels and communication between beekeepers and citrus growers on locations of hives and scheduling of ACP treatments around the bloom period. According to FDACS, the 2014 citrus bloom period ended with no reported bee pesticide poisoning incidents.
4. CHMA support focused on continuing to stress the importance of following information available within the CHMA and coordination of spring/early summer ACP treatments. Information on availability of Clothianidin for 3-5 year old trees was incorporated into seasonal plans for young grove practices.
5. Information on the value of CHMAs and the timely control of ACP was distributed at the June Citrus Grower Meeting, as well as regularly through CRDF, UF-IFAS and FDACS communications.

Project Title: 8. Candidate HLB Tolerant Rootstock Plantings

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

1. Facilitate identification of best performing candidate rootstocks that appear to have some HLB tolerance from Florida (and other) breeding programs
2. Encourage early release of new commercial rootstocks and other strategies to make these rootstocks available to growers
3. Implement Phase I and II grower field trials of most promising candidate HLB tolerant rootstocks using standard varieties as scions.
4. Evaluate ongoing grower plantings of candidate rootstocks at 3 different sites: Central Ridge, East Coast Indian River and Southwest Florida.
5. Communicate progress and results of evaluation of rootstocks to industry

Narrative of Progress against Goals:

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. The nursery was visited in June and the trees are growing out well. Challenges were encountered in nursery production with some of the new rootstocks, pointing to the need for fine-tuning the handling of each genotype 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, one each on the Ridge, East Coast, and southwest Florida, in spring 2015. A meeting of the grower/CRDF group to discuss considerations for the field trials and for selection of grower cooperators was held and discussion focused on the practices that should be considered for use in these trials. These considerations included ACP control, management of trees once infected, and other variables.

The plans for micro-propagation of candidate tolerant rootstocks for a phase II field trial in 2016 were advanced this quarter with the approval of the export/import permit for these materials. This is necessary for rootstock candidates for which adequate seed are not available for propagation. FDACS has issued a permit for importation of the resulting micro-propagation rootstock starts from the Agromillora laboratory in Oregon. This allowed the shipment of clean propagative material of UF candidates for planting in this trial. USDA materials were not available from a certified clean source, and these materials will be propagated in Florida.

Project Title: 9. RNAi Molecules/Psyllid Shield

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

Identify the specific dsRNA molecule(s) that can be advanced into practical psyllid control strategies.

1. Incorporate the RNAi Molecules project and its research results into the Psyllid Shield project.
2. Establish a mathematical model of RNAi effects on area wide protection of new plantings.
3. Refine the model with vector entomologists and epidemiologists.
4. Experimentally evaluate candidate protective effects of selected RNAi in CTV inoculated plants.
5. Continue to evaluate new RNAi for improved activity with CTV vectors.
6. Model performance of best RNAi for field trials and complete scale-up feasibility analysis.
7. Decision to initiate field trials and regulatory approval process.

Narrative of Progress against Goals:

1. 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.
2. At its April'14 meeting, CPDC recommended approval of 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. This funding was later approved by the CRDF Board at its April meeting.

Significant Meetings or Conferences:

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

Other Information:

The next scheduled meeting for the team will be in Sept. in Lake Alfred.

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

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

1. Assemble work group to discuss tactics and tools available for new citrus plantings
2. Generate an overview of elements possible to incorporate into a new citrus planting
3. Encourage growers to consider use of these guidelines when planning for new plantings
4. Establish at least one "model new grove" which incorporates the elements of the integrated tactics and tools
5. Communicate progress and results to CPDC, CRDF and growers

Narrative of Progress against Goals:

This project is under development through MAC Group funding and has been preliminarily approved for implementation as a parallel MAC project addressing inoculum removal is developed and implemented under leadership of FDACS. The objective of this project is to assemble the current "best practices" options of new plantings with regards to grove layout, tree selection and post-planting production and disease management.

Several growers are applying these general principles to new plantings, and presentations were given at the June, 2014 Citrus Grower Annual Meeting, and are scheduled for a Grower Panel at the August Expo.

An advisory group to consider how best to provide support to a grower-based "model grove" is being formed, composed of growers, nursery producers and researchers representing various aspects of citrus horticulture.

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

No activity this quarter. UF and USDA, ARS citrus breeders have been contacted regarding the need for additional field evaluation trials of HLB-tolerant or resistant citrus scions.

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

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

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. For mature transformations involving the NPR1 gene construct, micro-propagate a sufficient number of plants to begin grafting the transformants in all combinations. Conduct at least one transformation of mature scion or rootstock every week. Once accomplished, additional time will be needed to grow plants to a size suitable for greenhouse testing.
    b. Conduct mature transformations with as many as 3 additional gene constructs, once the criteria to commence work have been met.
    c. Improve laboratory and growth room productivity and mature transformation processes.

Narrative of Progress against Goals:

1. **Progress on NPR1 Gene construct.** : A genetic construct from Dr. Mou has been transformed into mature scion of Valencia, Hamlin, Ray Ruby, Pineapple and mature rootstock of Swingle and Carrizo. A number of PCR positive, putatively transgenic shoots

have been micro-grafted onto immature rootstock. There are numerous PCR positive shoots in all varieties, which will be verified by additional molecular methods once the plants are larger. PCR positive Pineapple sweet orange shoots have been secondarily micro-grafted in the growth room and are in soil. The lab has been able to promote rooting of immature tissues in one week with IBA but not NAA. After clonal propagation of desired transgenics, budding in additional combinations will commence.

2. **Screening Capacity Increased**> Capacity of PCR screening methods has been increased using a direct tissue PCR method, which does not involve performing DNA extractions. Putative PCR positives can also be determined by fluorescence of the PCR reaction in the tube on a UV light.
3. **Progress on Additional Constructs:** Additional constructs obtained from other UF scientist(s) have been transformed into the appropriate scion and/or rootstock. Shoots have been regenerated and micro-grafted onto immature rootstock. For some constructs, sequence and maps have still not been obtained. Work will commence on these once they are obtained.
4. **Copy Number and Expression Data Compilation:** Southern blots of plants transformed with marker genes are underway using a non-radioactive DIG labeling kit. Copy number information and expression data are being compiled for a poster presentation at the annual American Horticultural Society (AHS) meeting in Orlando at the end of July. Once copy number and gene integration has been demonstrated, it will be possible to determine the number of false positives.
5. **Additional Equipment:** A second-hand laminar flow bench was procured for the growth room that will serve as a dedicated micro-grafting station. The gene gun is operational and is being tested on mature and immature explants of both rootstock and scion.

Project Title: 13. Diaprepes Pheromone Commercialization

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

1. Make a “go-no go” decision no later than July 2014 on whether CRDF will license technology from USDA, enter into a CRADA with USDA, and pursue co-funding with partner companies of a two year field trial for continued technology validation.

Narrative of Progress against Goals:

1. During the quarter re-contacted three candidate companies to determine their interest in pursuing further discussions on a possible co-funding and partnership arrangement. Those companies did not express an interest in pursuing discussions and provided a variety of reasons, including: financially unable to make an up-front investment; technology not far enough along, so too risky; relatively small market; and did not place a high value on patenting.
2. Also made contact with a fourth company and, after discussion, provided information on the arrangement. As of the end of quarter, have not received a response from this company.

3. Also spoke with the inventor, Dr. Lapointe, to get his ideas on possible company contacts. He was generally familiar with the same companies that we have approached.

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 would depend on finding one or more commercial partners to co-invest in the further development of the technology.

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

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

1. Monitor progress in both commercial and research evaluation phases of demonstration project
2. Determine need to modify plans or details of project
3. Communicate progress and results of demonstration
4. Determine if CRDF has additional role in delivering this technology

Narrative of Progress against Goals:

This collaborative project between CRDF, ISCA, USDA-ARS and UF-IFAS was approved to demonstrate the large-scale benefit of leafminer disruption in reducing citrus canker incidence. The collaboration includes both cost-sharing on the purchase of adequate leafminer pheromone dispensers to be applied to 3 large test areas in Florida. The goal is to interfere with leafminer mating and thus reduce population pressure and leaf damage. The ultimate measure of success is lower canker incidence resulting from fewer pathways for infection and inoculum development that leaf mines provide.

During the current quarter, CRDF confirmed that the trials are in place and that the grower cost-share has been met, allowing for the payment of the CRDF component. Contracts with the USDA, ARS and UF-IFAS research groups were completed so that appropriate monitoring is being provided to assess the response to this broader scale treatment.

Project Title: 15. CTV Vectors

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

1. Help ensure that delivery systems and candidate genes for HLB resistance based on CTV vectors are commercialized to the best possible benefit of the Florida citrus industry.
2. Ensure the pipeline of CRDF-funded research on CTV vectors is mined for commercial potential

Narrative of Progress against Goals:

Representatives of University of Florida Office of Technology Licensing and Southern Gardens provided an update to the Board on the status of the licensing arrangements involving CTV vectors. OTL discussed plans for a continuation filing on the currently licensed patent with

expanded claims based on a new invention disclosure. OTL also noted that all required reports were being filed by Southern Gardens in a timely manner. Southern Gardens also provided an update on its activities related to this license.

Project Title: 16. HLB Escapes

Funded projects with UF in support of this objective are ongoing, but there is relatively little response from growers. The UF, IFAS Citrus Extension Agents are summarizing the reports from industry, but at report time, there have been only 6 contacts regarding potential escapes in the field. A broad communication of the goals of the program and progress to date will potentially generate additional response from the industry. Otherwise, this project will remain of little importance.