CRDF Commercial Product Delivery Project Progress Report Quarter Ending December 31, 2014

Subproject Title: 1a. Antimicrobial Strategies: Conventional Antibiotics

Narrative of Progress by Project Goals:

1. Forge a partnership with companies for commercialization of streptomycin and oxy-tetracycline for HLB.

Field trials for the CRDF-funded project with AgroSource accelerating fieldwork on commercial antibiotics (oxy-tetracycline and streptomycin) for use on Florida citrus against HLB were initiated in the first quarter and are progressing on schedule according to the work plans.

FireWall® 50WP (streptomycin sulfate) by AgroSource has been granted a second year of EPA Section 18 registration for control of Citrus canker in Florida grapefruit. As a condition of Firewall® registration, EPA requires monitoring of *Xanthomonas citri* subsp. *citri* (Xcc) for streptomycin resistance. Dr. Jim Graham conducted the survey for 2014 under CRDF-funded project 730 and a report was submitted to EPA by end of 2014. The AgroSource project also is charged with resistance monitoring in accordance with terms of the Section 18 label.

CPDC staff has been in communication with both growers and NuFarm, a sponsoring company conducting ongoing field trials on Florida citrus involving its streptomycin and oxy-tetracycline products. This company has expressed an interest in submitting a proposal for project funding and CRDF has been tracking progress. A site visit and discussion was held with NuFarm officials and consultants conducting 2014-15 field trials with NuFarm antibiotics.

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

Data have been processed on Hamlins towards the proposed expanded Section 18 labeling for Firewall® use against citrus canker in round oranges. Valencia data will be collected in the next quarter.

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

Significant Meetings or Conferences:

On December 12 a meeting took place between ASI, FDACS, FFVA, CRDF and researchers to discuss the data needed for the expanded Section 18 labeling for Firewall® use against citrus canker in round oranges. ASI is in the process of gathering the supporting data and information needed for this application with a goal to submit the application in early spring.

Subproject Title: 1b. Antimicrobial Strategies: Agricultural Antibiotics

Narrative of Progress by Project Goals:

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

The CRDF-supported study #617 is testing 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 including Zhongshengmycin and Validoxylamine A. Results are being collected and including reduction of C Las titers, phytotoxicity and tree health. Final results from this project will be available in the next quarter.

Significant Meetings or Conferences:

The project manager has met with the PI of this project to discuss progress and results.

Obstacles Encountered and Breakthroughs:

The key obstacles are uncertainty 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:

- Screen library of Company C biopesticides using L. crescens assay.
 A compound library from company C (12 products) and company I (300 samples from culture library) were screened in the L crescens assay.
- 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. This project has been initiated and is on schedule.
- 3. Company C measure HLB levels on Company C canker trials and canker on CRDF HLB trials CRDF reached agreement with Company C to expand two ongoing citrus canker field trials that Company C is undertaking to include C Las PCR sample collection and analysis facilitated by CRDF. This initiative is underway and on schedule, and allows CRDF to get "curative" information from the two on-going trials.
- 4. Track research projects relevant to biopesticides and host immune modifiers against HLB.

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. In addition, a presentation was made to the Florida Fertilizer and Agrichemical Association, highlighting the range of bactericidal strategies being pursued and the need for commercial partners to assist in commercialization and marketing of emerging products.

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.

CPDC project (775C) for the investigation of non-antibiotic t etracycline analogs and formulations against HLB was completed this quarter. The study examined two specific compounds in trans-bark and phloem transport experiments with intent to develop the optimal formulations.

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

Additional derivatives are still being synthesized and tested in the L crescens and Gonzalez flush-based assay, and pro-drug forms will be developed with intent to enhance plant uptake and bioavailability. Three new samples were sent to the assay lab for testing this quarter and one sample is being tested in the flush-based assay. Results are expected in January 2015.

CPDC staff also identified a company (Company K) with a library of novel tetracycline derivatives that were developed for animal and human use. A subset of these has been identified for evaluation using L crescens, with likely follow-up on promising compounds using the Gonzalez flush-based assay. One of these non-antibiotic tetracycline derivatives was withdrawn at a late stage of development and, according to the CEO, has significant food safety and toxicity studies completed.

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, a company-driven development plan is in place with identified CRDF roles and support responsibilities.

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

During the quarter, twelve compounds from Company D were tested using the L crescens assay and seven compounds using the Gonzalez flush-based assay. Sixteen new materials were sent to the Gonzalez lab for testing and results are expected in January 2015.

b. Bacterial protein targets LdtR and SecA

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

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

Significant Meetings or Conferences:

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

CRDF and UF continue to collaborate with a large user of citrus (Company H) as they work with a supplier of new bactericidal materials (new molecular entities) (Company D) to evaluate potential commercialization partners in the agrochemical industry. One of the agrochemical companies has agreed to conduct proof-of-concept testing and, if acceptable results are achieved, enter into an extended R&D collaboration to develop HLB control agents. The proof-of-concept testing includes efficacy and bioavailability screens on about a dozen compounds. This is a long-term endeavor requiring significant time and money (about 10 years and about \$150MM) and potentially new and creative funding models. The companies are currently negotiating material transfer terms and expect to have proof-of-concept results by second quarter 2015.

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 four cationic selective antimicrobials (ceragenins). All had performed well in the L crescens assay. These were provided by companies other than Company D. The ceragenins showed promise and talks have been initiated with these companies to discuss manufacturing, cost, regulatory and IP concerns.

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, p-cymene, carvacrol and thyme oil emulsions from the UF Particle Engineering Research Center (PERC) were tested and minimum inhibitory concentrations established using the L crescens assay. Thyme Guard (AgroReaearch) has also been tested in this assay. Further evaluation is necessary to determine if these materials will perform well in field trials.

In the next quarter the novel therapeutic zinc particles will be tested in the L crescens assay and the flush assay.

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

Nanoemulsions of two plant essential oils, p-cymene and carvacrol, have been tested in greenhouse and field trials in study #584 using foliar and bark applications and gravity bag infusion. Data from qPCR measurements of CLas titer, plant health and fruit quality data is being collected and analyzed. P-cymene and carvacrol nanoemulsions continue to be formulated and tested by researchers at PERC.

CRDF has 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.

Significant Meetings or Conferences:

A meeting was held to discuss the strategy for EPA approval of the novel theraputic zinc compound for use on canker. The project researchers, consultants from TPR (Dan Botts and associate) and CRDF project managers (Tom Turpen and Stephanie Slinski) attended this meeting. A White Paper is being written by this group for EPA and meetings with EPA will be held in early 2015 to discuss registration for citrus canker.

Obstacles Encountered and Breakthroughs:

The zinc materials appear to be a significant near term substitute for copper use against canker. Efficacy against HLB is in progress.

Other Information:

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

Narrative of Progress by Project Goals:

- Track ongoing research on soil microbes and their role in HLB and tree health
 Nothing to Report
- Conduct field trials to test commercially available naturally occurring microbes
 Experimental protocols were developed to provide a sound scientific assessment of effects
 of five commercially available microbial soil amendment products plus a water treated
 control on HLB.
 - -- All treatments (quarterly or monthly) were began in May/June and are being applied with and without an organic mulch at the 3 mature tree Valencia/Swingle trial sites Ridge, East Coast, South FI-- using a professional crop consultant company at each site.
 - -- An additional newly planted young tree trial was initiated to test 4 soil drench treatments of Alliette, Serenade, a numbered compound plus a water treated control on a Ridge site in Lake Wales. Trees were Valencia on X639 rootstock planted in late Fall 2013.
 - -- All required field work at all 4 sites is on schedule and all the data has been be submitted on time
 - -- Leaf samples for PCR analyses have been collected in Nov/Dec 2014 for individual trees at each of the 3 sites and are awaiting analyses in the US Sugar Lab.

IR site: Results from visible Disease Index (DI) tree ratings for HLB status generally show no treatment effects either with or without organic matter. When pooled within quarterly dates, DIs are positively correlated with each other: r = 0.65 to 0.70.

-- Results from July/Aug leaf samples for nutritional analyses show that trees generally show optimum nutritional status with few treatment effects.

Ridge site: Results from visible Disease Index (DI) tree ratings for HLB status generally show no treatment effects either with or without organic matter. When pooled within quarterly dates, DIs are positively correlated with each other: r = 0.74 to 0.85.

--Results from July/Aug leaf samples for nutritional analyses show that trees are generally low in leaf N (168 tree avg = 1.9 % N) but generally adequate nutritional status for other elements with few treatment effects.

SW FL site: Results from visible Disease Index (DI) tree ratings for HLB status generally show no treatment effects either with or without organic matter. When pooled across within 2 quarterly dates, DIs are positively correlated with each other: r = 0.72.

-- Results from July/Aug leaf samples for nutritional analyses show that trees generally show optimum nutritional status with few treatment effects.

Lake Wales young tree ridge site. Initial PCR leaves sampled 8-15-14 revealed that 5 of the 120 total 1-yr-old trees were positive for HLB.

-- Results from Aug leaf samples for nutritional ana3. Iyses show that the young trees generally show optimum nutritional status with few treatment effects. Trunk caliper in August 2014 averaged 1/2" and tree height averaged 40" with no effect of treatment.

Root density analyses: (3 mature tree sites) Eight soil cores from the wetted zone under each measurement trees were collected as described in the proposal protocol. Roots were extracted from 500 cc aliquant of each soil sample over a 2 mm mesh sieve by hand and the dry weight of roots was measured. The overall average root density was 1.14 g/500 cc soil.

The root density did not show any significant difference between treatments, either within each site nor when pulled together

3. <u>Provide communication on project goals, progress and results to CPDC, CRDF, and growers.</u>
Nothing to report.

Obstacles Encountered and Breakthroughs:

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 were repeated in Dec 2014 and await laboratory analyses at US Sugar Corporation.

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.
 - Dr. Kirsten Pelz-Stelinski of UF, IFAS, CREC, has significant experience in characterizing aspects of CLas transmission by Asian citrus psyllid. In response to discussion, Dr. Stelinski submitted a plan to examine the question: "can ACP acquire CLas from trees treated with thermal therapy?". This plan will evaluate levels of transmission prior to and over a time series following thermal treatment. These experiments will be begin in spring, 2015 and will continue through the 2015 growing season. Steam treatment will be applied to test trees and the experiment is being coordinated with Dr. Reza Ehsani. The plan was presented and approved by CRDF at the October CPDC and Board meetings, and has been contracted. This project also is being forwarded to the MAC group for inclusion in the portfolio of project supported by MAC in the area of thermal therapy.
- 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. Progress in evaluating time/temperature characteristics important to effective use of thermal therapy is being made by the programs at the core of this area. Dr. Ping Duan and his group continue to develop time/temperature relationships in their greenhouse, growth chamber and in field settings where solar thermal energy is captured in singe-tree enclosures. Simultaneously, Dr.

Reza Ehsani is continuing efforts to fine tune application of short-duration steam and other supplemental heat to accomplish CLas suppression. Results of these studies are reported in progress reports.

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 received numerous proposals for scale-up of thermal therapy and are finalizing plans for at least three separate projects that span solar enclosures to large-tree treatment with supplemental heat. The specifics of approved projects have not yet been announced but will be made public in ensuing weeks. The results of this funding program could move additional efforts forward into field trials as early as spring, 2015.

Numerous growers and companies within Florida have begun independent trials of thermal therapy application, with some focused on applying steam growing from Dr. Ehsani's work. Increasing numbers of trees are being treated with the various methods with varying levels of evaluation. In an effort to coordinate learning from the use of thermal therapy in Florida groves, CRDF has developed a plan and methods to evaluate thermal therapy trials as a part of overall field trial administration.

At its November, 2014 meeting, CPDC reviewed and recommended forward a proposal to provide personnel and facilitation to evaluate thermal therapy trials as they are installed in groves. The evaluation measures are consistent with those used in evaluation of field trials targeting other HLB management tactics. As such, results of all such trials have a common basis for comparison. With Board approval the CRDF Field Trial Coordinator and Field Trial Administrator are gearing up for thermal therapy evaluation in 2015. Initial field scouting and validation of the methods has been accomplished to line up logistics and finalize the evaluation methodology.

Field sites will be chosen by growers and TT applicators. Standardized CRDF field evaluations will include: **Pre-treatment grove evaluations** will include tree age, scion, rootstock, soil type, soil pH, general moisture status, and cultural practices including irrigation scheduling, fertility programs, previous leaf nutrition, pest/psyllid control, yield records and fruit quality. Good horticultural care should continue. **Tree evaluations:** Photographs, Disease Index, Leaves for PMA-qPCR (living vs. dead DNA), Trunk diameter, Fruit drop, TT Treatment details.

Post-treatment tree evaluations: Photographs (immediately after TT treatment, 3 months after, annually thereafter), Leaves for PMA-qPCR (immediately after, 3 months, annually thereafter), Disease Index (annually), Trunk diameter (annually), leaves for nutrient analysis (July/August), Fruit drop (bi-monthly), fruit yield and quality (annually).

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. The topic of thermal therapy has been included in presentations and discussions of near-term strategies, and will be a major topic of the January 2015 Citrus Show in the Indian River area.

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. The efforts of the MAC group to invite proposals from outside groups to scale-up thermal therapy has resulted in several new partnerships.

Project Title: 4. Plant Growth Regulator Interactions with HLB

Narrative of Progress by Project Goals:

1. <u>Track RMC research projects evaluating the effects of PGR application on plant physiological</u> processes and on pre-harvest fruit drop

Percentage fruit drop data from mature Valencia trees in the 4 Central FL 2,4-D (Retain) field trials (Minter and Yonce) from Dec 13 through March 14 varied from 20-70%. Overall treatment effects were not remarkable but one site did show a positive reduction in fruit drop. The CPDC recommended and approved funding for repeating all 4 trials at the same locations but using different trees. Applications went out in Dec 2014 and fruit drop counts will begin in Jan 2015. More detailed tree and site evaluations should enable a better interpretation of effects the 2,4-D treatments in 2015.

- -- AlbrigoTrials. 2014-15 harvest season trials 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. Since at least one test of 2, 4-D applied 2 years ago in December gave a significant reduction in preharvest fruit drop in Valencia, four PGR tests were started in December 2014 including a 2, 4-D treatment on preharvest drop of Valencia. One test was applied near Frostproof, another test was applied in Auburndale and a third test was made in Lake Alfred. Two of these tests included Headline. A fourth test, in Auburndale, was grower applied to 5 acre plots and included 2, 4-D and Headline.
- -- In the fall of 2014, six PGR tests using 6 to 10 tree replicates were applied to Hamlin blocks and two tests were applied by growers using single 5 or 4 acre sprayed plots without replication. In

these grower tests, matching sprayed plot trees and adjacent control trees (24 trees/plot in four 6 tree groups) were compared for % fruit drop.

- 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 Nothing to report.
- 3. Communicate to CPDC, Board and growers on the project progress, results and interpretation

Significant Meetings of Conferences:

Fruit drop results from the Albrigo trials will be reported at the IR FL Citrus Show 29 January 2015.

Obstacles Encountered and Breakthroughs:

Tree to tree variation with HLB status is a recurring 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.

Project Title: 5. Strategic Inoculum Removal to Manage HLB in Florida Narrative of Progress by Project Goals:

- 1. <u>Coordinate with Florida Department of Agriculture and with CHMAs to encourage emergence of Inoculum Removal Strategies</u>
 - It is generally accepted that eliminating Asian citrus psyllids (ACP) in citrus groves over large acreages is an important tool in sustaining the productivity of the citrus industry. The establishment of Citrus Health Management Areas (CHMA) has proven to be successful in reducing HLB inoculum when growers within a CHMA fully cooperate in coordinated application of pesticides for control of ACP as well as rotating modes of action of pesticides. The purpose of this project is to reduce a significant source of inoculum by destroying abandoned groves where ACP is not managed that are in proximity to well managed groves within a given CHMA.
 - With funding support committed from the USDA, APHIS MAC group, the Florida Department of Agriculture and Consumer Services (FDACS) has moved forward to develop plans to strategically remove high risk unmanaged citrus blocks in the vicinity of managed citrus groves. Using the current CHMA system and real-time Asian citrus psyllid survey data, FDACS has identified a series of properties across numerous CHMAs that fit criteria for selection to be removed. Property owners have been contacted for consent agreements, and bids are being developed for tree removal. The target for removal is a total of 2,500 acres across many CHMAs. The tree removal will allow evaluation via the ACP population numbers and with subsequent field monitoring. Young groves in the areas around grove removal will be followed to track changes in incidence of HLB.

Finally, FDACS is working with various facilitators of alternative energy development in Florida with the goal to utilize citrus tree biomass upon removal. This would provide disposal of the tree material as well as to subsidize the cost of grove removal.

This project will demonstrate the benefit of inoculum removal and will investigate various methods through which this could be accomplished. CRDF will continue to monitor this project and assist when possible.

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

The plans and details of this program will be communicated to industry through association and other industry group meetings in January, 2015. This includes presentation at the January CRDF CPDC meeting by Ms. Callie Walker, FDACS CHMA coordinator.

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.

No further progress has been made in this area due to changes in personnel in all of the leadership positions within UF and USDA who were poised to evaluate the new procedures. With new leadership in place, the project will be reinitiated.

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

No Progress to report

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

No Progress to report

4. 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.

A Research Management Committee site visit was made to two areas where the field experiments of project #447 are being conducted (Fort Pierce and Immokalee). The PIs presented current status of the project and in particular, how the trials could be expanded with additional treatments. Following those tours, this project has been revised and action will be taken at January RMC and Board meetings.

The USDA APHIS MAC group project supporting increased production of Tamarixia radiata for field release in states affected by HLB, including Florida is now in place. 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.

Review of projects related to alternative suppression strategies for ACP is underway within CRDF, and will be part of recommendations presented at the January Committee and Board meetings.

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

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 expired 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. The petition to renew the 24(c) label has been submitted through FDACS to EPA and approval has been communicated from both EPA and FDACS.

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. This action is still pending and the PRIA date for section 3 consideration of Belay has been delayed.

Other Pesticides for use against ACP:

Bayer CropScience has been developing information and support for a new insecticide that is being petitioned for use in US agriculture, including citrus. It has activity against ACP and could provide another tool in the rotation of materials for psyllid management, particularly during bloom periods. Some details of the new material, with the trade name Sivanto are:

- SIVANTO is a new insecticide and the first in the new chemical class of chemistry, the Butenolides. It will control a spectrum of sucking pests including aphids, leafhoppers, psyllids, whiteflies and others.
- SIVANTO was designated as a "reduced risk" candidate by the US EPA. This designation was based on a favorable mammalian toxicity risk profile and a low risk to honeybees compared to current alternatives as well as its fit in integrated pest management (IPM) programs.
- SIVANTO will be registered on a broad range of horticulture crops and most broad acre crops which allows for inclusive and flexible crop rotation programs.

3. Continue participation in pesticide stewardship activities

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. The FFVA organized a meeting entitled "Florida Agroecosystems and Honey Bee Hive Health" in late September, 2014, bringing together representatives from citrus, tomato and melon industries, beekeepers, academics, registrants, and government regulatory agencies. The agenda included updates on the status of crop dependence on honeybee pollination, movement of bees into and out of Florida, and addressing the incidence of losses to bee hives before, during and after tenure in Florida. Experts provided information on movement numbers, risks associated with bee exposure in different environments, and the role that Florida crops and natural vegetation played in overwintering and permanent bee hive health.

One of the purposes of the meeting was to identify data gaps and other information needs, and to achieve consensus on how the Florida situation with regards to horticulture/beekeeper interactions can be improved. State apiary researchers provided overview of ongoing research that will shed light on some of the questions.

CRDF-sponsored research to address honeybee issues continues and results are provided to registrants of pertinent materials for use in addressing questions from regulatory agencies.

Project Title: 8. Candidate HLB Tolerant Rootstock Plantings

Narrative of Progress for Project Goals:

1. <u>Facilitate identification of best performing candidate rootstocks that appear to have some HLB</u> tolerance from Florida (and other) breeding programs

New research project. In September 2014, 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 Out planting". 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.

- 2. Encourage early release of new commercial rootstocks and other strategies to make these rootstocks available to growers
 - Nothing to Report
- 3. <u>Implement Phase I and II grower field trials of most promising candidate HLB tolerant rootstocks using standard varieties as scions.</u>

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 budded with '1-14-19 Valencia' for scion uniformity 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 FI, in Spring 2015. At least 2 sites, Ridge and SW FL, were selected in Dec 2014.

Grove site evaluation will include soil type, soil and water pH, and cultural practices including irrigation scheduling, fertility programs and pest/psyllid control. Best management practices will be determined by the individual cooperator and will be uniformly applied to all trees at each site. Biannual access to tree evaluators must be granted with reasonable (1-2 weeks) notice. Cultural practices will include:

- Aggressive psyllid management according to current CHMA recommendations or equivalent for young trees and early mature trees. Active participation in a CHMA or cooperative treatment area is encouraged as relevant
- Irrigation, nutrition and grove floor management consistent with best management current practices to promote root health and growth in the presence of HLB
- Freeze protection should be a component of the planting plan.

Record-keeping on the field trial planting should include dates, materials, rates and application methods for all practices. Grower cooperators and CRDF will coordinate on data collection on these field trials and will share information gained from the trials. CRDF will summarize and share publically general features of the trial, but details of disclosure of specific information from the sites will be discussed and

agreed upon with the cooperator before being made public. Once the field trial planting is established, CRDF would coordinate with the grower host for a periodic (no more than annual) field day to demonstrate the status of the trials and progress to date in evaluating HLB disease and overall tree performance. This would be organized to minimize disruption to the cooperator operations and to be respectful of business operations. Details on the level of public access to the trial should be established and documented in the CRDF/Grower agreement covering provision of trees for the trials.

CRDF protocols for tree evaluation: Each tree should be assigned a unique treatment and replicate number. Tree evaluations will include:

Digital Photographs of the **diagonal (8 trees)** in each of 5 replicates (40 trees on each of the 7 rootstocks = **280 measurement trees** at each site) will be taken annually. Photographs will be taken a standard distance, direction and size, and saved to a flash drive.

Disease Index (DI= 0 to 40, Gottwald et al., 1989, see below). DI will be evaluated annually on the 280 trees at each site, using 8 canopy sectors in trees greater than 3 years old and on 2 sides of trees 1-2 yrold.

Leaves for qPCR from the 280 trees at each site will sampled soon after planting and in December of year 1 each year thereafter. 6-8 mature leaves (with petioles attached) will be sampled from around the canopy. If visible blotchy mottle symptoms are present, sampled leaves should be mature symptomatic leaves and placed into a sealable (e.g. zip lock) plastic bag, labeled with the tree with the unique ID code (as above) for each measurement tree and GPS coordinates if available. Sealed sample bags should kept cool and out of the sunlight (eg., in ice chests). Samples should be immediately delivered the Southern Gardens Diagnostic Laboratory (SGDL) accompanied by the site information and sample form at http://www.flcitrusmutual.com/content/docs/issues/canker/sg_samplingform.pdf

Trunk diameter. Two perpendicular trunk diameters will be measured with calipers on each of the 280 trees soon after planting and in December of year 1 each year thereafter. Diameters will be measured at exactly 20 cm above the bud union.

Canopy height of the 280 trees can be measured in Dec in year 1 and canopy height, and 2 diameters can be measured in Dec of each year thereafter.

Leaf nutrition. In July/August of year 1 and each year thereafter, 2 leaves from each of the 8 diagonal trees can be sampled and pooled for the 5 replicates of each of the 7 rootstock (35 nutritional samples) at each site.

Fruit Drop. All fruit on the ground and in tree canopies will be annually in March.

Yield: After year 1, yield can be evaluated on the 5-64 tree blocks on each of the 7 rootstocks (35 total) at each site.

- 4. Evaluate ongoing grower plantings of candidate rootstocks at 3 different sites: Central Ridge, East Coast Indian River and Southwest Florida.
 - Nothing to report
- 5. <u>Communicate progress and results of evaluation of rootstocks to industry</u>
 Nothing to report

Project Title: 9. RNAi Molecules/Psyllid Shield

Narrative of Progress by Project Goals:

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

Based on results from experiments with caged psyllids and young citrus trees inoculated with RNAi, 5 dsRNA sequences have been selected for further development. These sequences were derived from experiments in the laboratories of Dr. Bryce Falk (UC Davis), and Bob Shatters (USDA) and from the InnoCentive™ discovery promotion. Each of these sequences have been tested multiple times in caged experiments and show substantial reduction in the ability of psyllids to complete their life cycle on citrus flush. In addition, any surviving adults have are found to be free of CLas bacteria (samples are negative by PCR testing).

2. Incorporate the RNAi Molecules project and its research results into the Psyllid Shield project.

These results have been incorporated into the experimental design of candidate field trials.

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

Dr. Jed Keesling (UF) has led the modeling effort to predict how the Psyllid Shield effect would be predicted to protect solid block new citrus plantings from dissemination of HLB.

4. Refine the model with vector entomologists and epidemiologists.

Dr. Keesling's team has developed and validated a epidemiological model using data collected from an infestation of lime trees in Mexico.

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

Each of the sequences selected have been tested multiple times in caged experiments and show substantial reduction in the ability of psyllids to complete their life cycle on citrus flush. In addition, any surviving adults have are found to be free of CLas bacteria (samples are negative by PCR testing).

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

There is a pipeline of new RNAi candidates for evaluation that is coming from the nuPsyllid and other sponsored research. However, it is not clear whether any improvement in efficacy would be necessary for practical evaluation of field trials. Therefore, it is a priority to advance the current candidates.

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

Additional modeling validation and parameter refinements are in progress.

8. <u>Decision to initiate field trials and regulatory approval process.</u>

All experimental data and theoretical modeling to date support the evaluation of this concept in field trials.

Significant Meetings of Conferences:

The full team of researchers and regulatory consultants met in person on November 24th in Lake Alfred to discuss and refine technical and regulatory strategy to further field trials. This team included Dan Botts and Lois Rossi (TPR), Tom Turpen and Stephanie Slinski (TIG), Rick Kress and Mike Irey (Southern Gardens), Bill Dawson, Jed Keesling, JoAnn Lee and Ross Ptacek (UF).

Obstacles Encountered and Breakthroughs:

Next steps will include identifying a lead sponsor and/or consortium to finance larger scale field trials, product registration and commercialization.

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

Narrative of Progress by Project Goals:

Assemble work group to discuss tactics and tools available for new citrus plantings
 Discussions on the need to facilitate new citrus plantings continue, with several measures of progress emerging during the current quarter. Among these are:

Tree Assistance Program: CRDF joined with Florida Citrus Mutual and other Grower Organizations, FDACS to approach the USDA, Farm Services Administration (FSA) to discuss how citrus tree loss could be considered within the existing USDA, Tree Assistance Program (TAP). Meetings in Florida with FSA officials, and in November, with leadership of USDA and FSA in Washington, DC, focused on how growers who could demonstrate the loss of citrus trees to HLB might qualify for assistance through the TAP program. Mr. Rick Dantzler, lead for the FSA, TAP in Florida, was a critical partner in these discussions. As a result, Florida citrus growers now qualify for cost-share on tree replanting. The program and its leaders are to be acknowledged for their willingness to work with citrus interests to modify the details of the program to make it relevant to the loss of economic viability that growers have experienced in the era of HLB.

Complementing this effort, several industry sectors also are offering incentives for growers to replant. Processors and other groups recognize the value of continued replanting in the presence of HLB, and have communicated their commitment to the industry through these incentive programs.

UF, IFAS through the CREC and their local foundation, has developed a proposal to the USDA, APHIS MAC Group to install a demonstration of tools and techniques for new groves. In keeping with our goals, they propose to assemble a grower, researcher group to characterize how best to use existing and emerging tools in this field demonstration planting. The MAC group showed interest in this plan, and details are being worked out for MAC to provide funding to move this plan forward. More

information will be provided once the agreement has been approved, and CRDF will be interacting with this group to assist.

- Generate an overview of elements possible to incorporate into a new citrus planting
 This is a major effort of the IFAS project plan described above, and will ensue when approval is granted.
- 3. Encourage growers to consider use of these guidelines when planning for new plantings
 Nothing to report
- 4. <u>Establish one "model new grove" which incorporates the elements of integrated tactics and tools</u>
 This model grove will be an element of the approved UF, IFAS project approved by MAC. Details on this model grove will be forthcoming in the next quarter.
- 5. <u>Communicate progress and results to CPDC, CRDF and growers</u>
 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.

 Both USDA, ARS and UF, IFAS citrus breeding programs are cognizant of and pursuing evaluation of existing progeny of breeding efforts to evaluate scion tolerance to CLas and HLB. This is being accomplished in a number of ways and reported through several research project progress reports. The breeding programs have a range of scion progeny with Poncirus heritage that offer potential to evaluate scion tolerance. In addition, other potential sources of tolerance or resistance are being investigated by several projects, including Stover #605 "Development of Promising New Scions for Florida Citrus Exploiting HLB Resistance or Tolerance" and the core breeding program of UF, IFAS CREC.
- 2. Determine the need and scope of field trials to evaluate HLB tolerant scions. The breeding programs are conducting evaluation of trees for HLB health and fruit evaluation for quality and marketability from field trials that contain potentially tolerant scion genotypes. At this point, there has been no comprehensive effort to identify the need for further field trials of candidate tolerant or resistant scions.
- 3. <u>Develop and implement field trials as deemed necessary.</u> Nothing to report

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

Narrative of Progress by Project Goals:

- 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. <u>Micro-propagate a number of plants to begin grafting the transformants in all combinations.</u>

 <u>Conduct at least one transformation of mature scion or rootstock every week.</u>

During the quarter, Dr. Zale submitted a manuscript to *Crop Science Journal* for review that summarizes results from scion (Hamlin, Valencia, Pineapple and Ray Ruby) introduction into the growth facility by shoot-tip grafting (STG) from December 2011 to July 2013. Sixty-six mother plants out of 171 STGs introduced from FDACS were determined to be disease-free after micropropagation, budding and disease indexing. The biggest loss during this period was due to micro-grafting, as only 66 out of 157 (42%) GUS or GFP positive shoots survived.

There are currently approximately 3600 putative transgenics in the pipeline to be screened. For replicated disease screening, the number of transgenics will be increased at least threefold by budding, and expression in vegetative progeny can be determined. For one particular genetic construct, budding with transgenic immature rootstock can begin at any time to facilitate experiments to determine the contribution of each genotype in imparting tolerance. One mature Swingle rootstock tree, transgenic for a disease resistance gene, is over four feet tall and should flower soon for seed production.

- b. Conduct mature transformations with as many as 3 additional gene constructs.
 Mature transformation activities continue on scions and rootstocks using plasmids with disease resistant genes obtained from various scientists. Since most of these constructs have no GUS or GFP markers, all shoots are micro-grafted and screened with PCR, which is a more rigorous process than with reporters. Transgenics are double and triple checked with PCR and NPTII immunoscripts to ensure they are stable not chimeric, and expressing the NPTII protein.
- c. Improve laboratory and growth room productivity and mature transformation processes.

The lab continues to optimize biolistics in order to increase productivity. Results to date are promising and have recorded 200-300 transient GUS and GFP foci per shot in mature scion and rootstock shoots. If 0.01% of these foci develop into plants, 2 to 3 transgenics might be produced after each shot. During the optimization process, the lab has determined optimum stage height, gold particle size and helium pressure. The primary advantage of using biolistics is that it avoids all of the antibiotics used to suppress growth of Agrobacterium, which also suppresses shoot growth in scion and rootstock.

The growth facility is being certified as a nursery, which will hasten the pace of providing plants to scientists, growers, and industry. Routine disease testing in April will be conducted by FDACS.

Obstacles Encountered and Breakthroughs:

Based on an analysis of the losses incurred during micro-grafting, it was determined that micro-grafting success is dependent on the transformation batch and shoot age, and must occur early after shoot development. Alternatively, rooting mature citrus must be established. Several of these transgenics flowered after trees the T5 fluorescent lights were replaced with LED lights, night temperatures decreased, drought stress was applied, or if the trees were moved to natural light. Because the facility has no natural lighting, a greenhouse with natural and supplemental lighting would help obtain early flowering and fruiting of desirable events.

Project Title: 13. Diaprepes Pheromone

Narrative of Progress by Project Goals:

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.

CRDF received notification in December 2014 that USDA has withdrawn CRDF's license application from consideration and will keep it on file. CRDF may reopen the license application at a later date and submit an updated license application provided the technology is available for licensing. The USDA notification came subsequent to a decision by CPDC at its October 2014 meeting to "park" the project for the time being.

The CRDF Project Manager has been in close communication with USDA Technology Licensing Office and understands this action was partly an end of year exercise to remove the pending status on applications that are not showing concrete progress towards a license. It also allows USDA to pursue other options, including bundling a group of USDA technologies and offering them to MBA programs as student commercialization exercises. USDA currently has no other licensees in mind, and wants to keep in touch with CRDF as the technology evolves and/or the opportunity landscape changes.

CRDF is currently funding a research project with Dr. Steven Lapointe of USDA related to Diaprepes pheromones that expires in June 2015. Plans for a longer field trial that would have been supported as part of the co-development and sub-licensing model proposed by CRDF to companies will not go forward.

Obstacles Encountered and Breakthroughs:

CRDF offered to assist USDA in commercializing the Diaprepes pheromone technology by helping them fund the field trial, and jointly developed the go forward model at their request. CRDF made it clear at the outset 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.

USDA made it clear that its decision to withdraw the license in no way impacts the strong relationship between CRDF and the USDA Technology Licensing Office. They indicated if something comes out of the MBA student evaluations they will plan to contact CRDF regarding commercialization assistance, assuming the technology is still available.

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. Reports for the December 2014 quarter have not yet been received. 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.

Funds are also provided to USDA, ARS and University of Florida, IFAS to support monitoring and analysis of the experiments at the three locations. Synopsis of their evaluations indicate the following as of September 2014. More recent reporting was not available at report time.

Disruption of trap catch is being monitored weekly throughout the growing season at the three sites. At the Emerald Grove location (NW St. Lucie County), adjacent blocks of grapefruit untreated with pheromone to the north, east and south of the treated blocks are being monitored as controls. At the SW St. Lucie County location, the entire grove has been treated. At the Charlotte county location, the effect of skip rows will be evaluated. All evaluations will be done at the end of the season as we are able to collect and collate grower practices along with estimates of mining damage in treated and untreated areas.

The large-scale validation of citrus leafminer (CLM) disruption with the ISCA DCEPT CLM technology has continued. The Stelinski UF, IFAS group has continued in assisting of collecting data to monitor the efficacy of the product during the project. They have been assisting in monitoring certain areas of the ca. 3,000 acres that were treated at three locations in southeast and southwest Florida.

- 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

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 focused on the expression of antimicrobial peptides delivered with CTV and targeting CLas.

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

Nothing new to report in this quarter. As reported in the 3Q2014 report, two companies have continued interest in 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 these assessments. CRDF Program Manager has offered his availability to provide perspective on the technology and it's potential.

Significant Meetings of Conferences:

See write-up under Project 9: RNAi Molecules/Psyllid Shield.

Other Information:

This project intersects with Project 9: RNAI Molecules/Psyllid Shield. Those aspects of the program related to Project 9 are being covered in that section.

Project Title: 16. HLB Escapes

Narrative of Progress by Project Goals:

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

The CRDF-funded project in far into its term, and the level of grower interaction in identifying potential escape sites has been relatively low. The UF, IFAS Extension Agents also report limited interaction with growers, despite the fact that they are the primary contact point for any escape reporting. Dr. Gmitter and Dr. Wang of UF, IFAS CREC provide the follow-up as relevant on the challenge of plant material with CLas (Gmitter) and evaluating the soil/root environment under potential escape trees.

Since the number of grower responses was low, there is little progress in identifying plant material or grove conditions that may resist HLB infection.

CRDF will be evaluating the progress over the life of the base funded project, and the Extension Agents will be asked to provide their perspective on how this project could be expanded to gain more grower interest.

- 2. <u>Evaluate elements that can be implemented to advance project beyond its current scale.</u>
 Nothing to Report
- 3. <u>Facilitate expansion and enable more Ext Agent involvement in observations of tree escapes.</u>
 Nothing to Report
- 4. communicate progress to CPDC, CRDF and growers

Progress Reports on the funded projects were submitted, and the final reports at project end will follow.