CRDF Commercial Product Delivery Project Progress Report FY 2014-15

Quarter Ending March 31, 2015

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 projects with AgroSource accelerating fieldwork on commercial antibiotics (oxy-tetracycline and streptomycin) for use on Florida citrus against HLB completed the third quarter objectives, work continues to complete year one of the projects.

An oxy-tetracycline residue study with NuFarm has been funded by CRDF, this study covers the citrus group, field applications will begin early in the next quarter.

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

Data has been collected on all crops towards the expanded Section 18 labeling for Firewall® use against citrus canker in round oranges.

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

CRDF-funded project #617 ended this quarter. This project evaluated twenty-four antimicrobials at three temperatures and by root or trunk application. Six treatments were applied by trunk infusion. Seven materials from this project were moved forward into CRDF project #910 including three antibiotics. Chemical applications by trunk infusion have occurred over the past ten-month period, thermotherapy will be coupled with chemical therapy in the next quarter. Nutrient therapy as a treatment will be added in the future.

CRDF-funded project #584 was completed this quarter. This project examined methods of introducing materials to the plant phloem. Single materials and combinations of materials were examined in greenhouse studies. In the field, trunk infusion and trunk painting of materials was examined. Trunk infusion was the most effective method of material delivery.

Significant Meetings or Conferences:
CRDF attended a meeting that occurred between AgroSource and EPA to discuss the Section 3 and Section 18 applications. This was an informational meeting with the expectation of follow-up conversations towards registration goals.

Subproject Title: 1b. Antimicrobial Strategies: Agricultural Antibiotics

Narrative of Progress by Project Goals:

1. Evaluate Zhongshengmycin (ZS) and Validoxylamine A (VA) against HLB
   Zhongshengmycin was less effective than other antibiotics tested in the *L. crescens* assay. Validoxylamine A was ineffective at the rates tested. These materials continue to be evaluated in Powell #910 in combination with thermotherapy and nutrient therapy and using trunk infusion or foliar application.

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

Narrative of Progress for Project Goals:

1. Screen library of Company C biopesticides using *L. crescens* assay.
   Company C and Company I biopesticide libraries continue to be screened to find candidates for movement through the assay system. These are fermentation or plant secondary metabolite materials that these companies are dedicated to making available to growers if shown to be effective.

2. A new field trial based on new plantings that are uninfected with HLB.
   The trial approved in May to test Company C biopesticides and host immune modifiers is on schedule, objectives for the quarter have been met. The purpose of this study is to test the preventative effect of the materials. The study measures tree health, foliar nutrition, disease rating, HLB status, root density, yield and fruit quality.

3. Company C - measure HLB levels on Company C canker trials and canker on CRDF HLB trials
   Canker testing will occur at the appropriate time in mid-summer.

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

Significant Meetings or Conferences:

During the quarter, there was regular communication with Company C researchers to work to advance materials through the assay system. Meetings will continue through the next quarter.

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.

Two tetracycline derivatives have been chosen to continue investigating. These materials will continue to be investigated in the Wang RSA #935. These materials may be next-generation solutions for HLB and therefore are not a priority for CRDF field trials aimed at gathering data on near-term materials, but showing efficacy in CRDF funded assays Company E may find industry partners to bring a next-generation therapy to citrus growers.

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

Company K tetracycline derivatives continue to be tested in the L. crescens assay. One of these non-antibioitic 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

   During the quarter, 16 compounds from Company D were tested using the Gonzalez flush-based assay and results forwarded back to Company D and funding partner Company H.

   Also during the quarter Company H was engaged in an outreach program to seek company partnerships to move forward with commercialization activities on the technologies.

   b. Bacterial protein targets LdtR and SecA

   CRDF-funded project #773 examined SecA and lipid A inhibitors as possible antimicrobial compounds. Several of these have been identified and small-scale field trials are in place. Both trunk injections and foliar applications are being tested. The Lipid A work has been expanded due to the high level of antimicrobial activity.

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

Narrative of Progress by Project Goals:

2. Have at least one GRAS-like compound commercially available by June 2015.
Many essential oils have been tested in the L. crescens assay including carvacrol, p-cymene and thyme oil. These materials have been shown to be bactericidal at low concentrations and may be effective if introduced into the plant phloem. A field trial has been initiated to test the efficacy of essential oil (EO) formulations against HLB both as a preventative and curative treatment. The field trial will be set up in early May and material applications will occur in mid to late May. Five materials will be evaluated including Thyme Guard, Ecotrol, two EO materials in development by company J and one from Company N. All materials will be available to growers in a short period of time if shown to be effective against HLB. Thyme Guard and Ecotrol are available presently and labeled for citrus.

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

Nano-emulsions of two plant essential oils, p-cymene and carvacrol, have been tested in greenhouse and field trials in study #584 and #617 using foliar and bark applications and gravity bag infusion. These materials continue to be studied in project #910, which tests combinations of therapies including chemical, nutritional and thermotherapy.

Project #909 ends early next quarter. This project goals were to develop oil-in-water emulsions for delivery of essential oil into the plant phloem. This project was expanded to test a combination of thermotherapy and essential oil treatment to enhance the individual treatment effects.

Significant Meetings of Conferences:
A meeting was held with EPA to discuss considerations for registration of Zinkicide. Data requirements are being discussed and CRDF will continue to communicate with the EPA to move this process forward.

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

Narrative of Progress by Project Goals:

1. Track ongoing research on soil microbes and their role in HLB and tree health
   No activity
2. Conduct field trials to test commercially available naturally occurring microbes
   Experimental protocols were developed to provide a sound scientific assessment of HLB effects of 5 commercially available microbial soil amendment products (BioFlourish,

Ecofriendly, Serenade, Quantum and Aliette) plus a water treated control, in multiple applications per year as recommended. A subset of trees within each treatment was mulched with mature cow manure.

Treatments (quarterly or monthly) were began in May/June 2014 and are being applied with and without an organic mulch at the 3 mature tree Valencia/Swingle trial sites, Ridge, East Coast, South Florida, using a professional crop consultant company at each site. All required field work at all 3 sites is on schedule and all the data has been be submitted on time.

Nutritional analysis of the 5 products revealed that no treatment was applying more than one lb per acre per year of any fertilizer nutrient except Aliette applications, which totaled 7 lbs of P per acre per year.

Leaf samples for PCR analyses have been collected in November/December 2014 for individual trees at each of the 3 sites and have been analyzed. Overall, at this early stage, there are few significant treatment effects on PCR status unless noted below.

The Field Trial Project Manager, the Field Trial Administrator and Staff are monitoring the project activities. CRDF established data repositories for each project site so that all photos, data and treatment data are provided to CRDF as they are collected.

Indian River 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. Although there were 24 mulched trees and 144 un-mulched, the mulched treatment lowered the DI (so the mulched trees had visibly reduced symptoms) in May, October and December 2014.

Results from July/August 2014 leaf samples for nutritional analyses show that trees generally show optimum or above nutritional status with few consistent treatment effects. The Ecofriendly treatment increased leaf P. The mulch treatment significantly increased leaf K and B.

Ridge site: Results from visible Disease Index (DI) tree ratings for HLB status generally showed that the Aliette treatment had a low DI, indicating this treatment visibly appeared better than the other treatments. When pooled within quarterly dates, DIs were positively correlated with each other: r = 0.74 to 0.85.

Results from July/August 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 consistent treatment effects. By November 2014, Alliette trees also had the highest CT values from PCR and untreated control trees had the lowest CT (more CLas bacteria). The mulch treatment did not affect CT values. Untreated control trees had the smallest trunk cross sectional area and tended to have smaller canopy volumes.

SW FL site: Results from visible Disease Index (DI) tree ratings for HLB status generally show no product 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. The organic much treatment consistently lowered the DI rating in May, Oct and Dec 2014.

Results from July/Aug leaf samples for nutritional analyses show that trees generally show optimum nutritional status with few treatment effects. Aliette trees had high Mn and Cu but low P. The untreated control trees had the highest CT values (less CLas bacteria).

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 across the 3 sites.

3. Provide communication on project goals, progress and results to CPDC, CRDF and growers
   No activity

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.
   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. A project plan was developed by Kirsten Pelz-Stelinski of UF, IFAS, CREC and has been approved by CRDF, and subsequently approved for funding through the USDA MAC HLB program. The one-year research project is underway and has the following update.

The objective of this project is to evaluate the effect of thermal therapy treatment on *Candidatus* Liberibacter asiaticus (Las) transmission by the Asian citrus psyllid (ACP). Since the initiation of this project, a citrus grove, located at the Citrus Research and Education Center, has been identified for conducting bioassays. Trees in this grove are 4-year-old Hamlin oranges. Of the 203 trees tested using quantitative PCR (qPCR), 50 trees have been identified with cycle threshold (Ct) values below 36, indicating the presence of Las.

The experiments to characterize the ability of psyllids to acquire bacteria from thermally treated trees I underway and the preliminary (pre-treatment) acquisition exposures are being staged, with timing set for both availability of psyllids in the colony and the field readiness for application of the thermal treatment on the experimental plots. This first phase will be conducted in the next 30-45 days.

Initial experiments have commenced to quantify Las acquisition by adult psyllids prior to thermal treatments. Thirty teneral adult psyllids were caged on flushing branches of trees with a fine mesh sleeve for one-week acquisition feeding periods (AFPs). Thereafter, insects will be housed on uninfected citrus for one-week latent periods prior to testing with qPCR to determine their Las infection status. Eggs laid on branches will be monitored until adult emergence. Adults will be collected for qPCR analysis to demine initial nymph acquisition efficiency from trees pre-thermal treatment.

Dr. Reza Ehsani's lab has agreed to treat trees using a steam treatment device, when it becomes available in approximately 2-3 weeks. Acquisition of Las from Las-infected or uninfected trees receiving thermal therapy treatments will be compared to untreated Las-infected or uninfected trees in subsequent assays. Ten trees will be evaluated per treatment.

2. <u>Refine requirements and environmental conditions for most effective thermal treatment.</u>
   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. Dr. Ehsani of UF reported that application of steam heat of 58 C (136 F) for 30 sec resulted minimum plant injury, reduced PCR titer and recovery of tree health for at least 18 months. Such guidelines are currently being used by 3 commercial steam companies and several growers who are establishing large scale and small thermal therapy trials. This will have to be followed and perhaps modified seasonally as tree conditions change throughout the year.

3. <u>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.</u>

   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. Two Mac projects were approved to facilitate scale-up and both were in place at the end of this quarter.

Evaluation of thermal therapy conducted by those involved in scale-up is being initiated by the CRDF evaluation team. Six enterprises are either field testing machines in Florida or will have machines ready for testing or will have them field-ready within the next couple of months. Those with capability are operating at multiple locations in Florida, and the evaluation team is in the field conducting the evaluations.

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 before and after protocol was developed to document tree and environmental 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 publicized on the CRDF web page so growers can do some self-assessments of their own thermal therapy trials and been implemented on a small scale with grower and research trials. The protocol will become standard in the MAC funded CRDF project to evaluate thermal therapy scale-up described above. An overview of current field activity that the CRDF evaluation team is engaged in follows:

Visited sites where 4 of the 6 enterprises are operating equipment to deliver thermal therapy to HLB-infected citrus trees. This represents 8 or more sites in the past two months.

The Evaluation team has been trained on the parameters to be measured and have worked with the equipment people to determine timing of pre-treatment baseline information gathering, as well as the timeline for follow-up evaluation. The Protocols presented in the work plan have been field tested and data forms and logistics have been developed.

Two first level evaluations have been established with different machinery operations, allowing for the pre-treatment collection of PCR samples, tree health evaluations and other baseline measures. Post-treatment evaluations are scheduled and underway for this short version of the evaluation protocol

Establishment of one site for full evaluation of pre and post-treatment impacts of thermal therapy has occurred, with all pre-treatment assessments accomplished and the post-treatment protocols in place.

A report format has been established to collect general information relating to the field assessment of thermal therapy by passive (tents) and active (supplemental heat) methods. This report form will be used to gather data from thermal therapy providers as well as growers interested in implementing thermal therapy and will focus on measures of adoption and other features, including:

Number of machines (or tents) in use
Number of sites receiving thermal therapy
Number of trees treated by each operation
Number of acres treated (by tree size)
Perspectives from growers on effectiveness
Perspectives from operators

These data will inform the assessment of adoption and general performance of the use of thermal therapy, and will be supplemented from more in-depth data collection from the specific site evaluations.

4. Continue outreach efforts to inform growers of the availability of thermal treatment and to refine conditions that will lead to effective thermal treatment.
The CRDF-initiated Thermal Therapy field day in 2014 informed growers of this emerging tool, and also attracted entrepreneurs, innovators, individuals and at least 3 commercial 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:
Thermal Therapy was highlighted in presentations at January 29, 2015 Florida Citrus Show in Ft. Pierce and will be again at the Apr 7, 2015 FL Citrus Growers Institute in Avon Park.
There has been MAC planning meetings to include thermal therapy scale-up in shovel-ready projects

Obstacles Encountered and Breakthroughs:
At least 3 outside commercial companies have responded to the thermal therapy information and are developing steam 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

Narrative of Progress by Project Goals:

1. Track RMC research projects evaluating the effects of PGR application on plant physiological processes and on pre-harvest fruit drop
   No Activity

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.
   Percentage fruit drop data from mature Valencia trees in the 4 Central Florida 2,4-D field trials (Minter and Yonce) from December 2013 through March 2014 varied from 20-70%. Overall treatment effects were not remarkable but one site did show a reduction in fruit drop from 47% in untreated control trees to 19% in 2,4-D treated trees. The CPDC recommended and approved funding in December 2014 for repeating all 4 trials at the same locations but using different trees. Applications went out in December 2014 and fruit drop counts began in January 2015. Final percentage pre-harvest fruit drop will be calculated in May 2015 after harvest and final fruit counts. More detailed evaluations trees and site characteristics have been conducted and should enable a better interpretation of effects the 2,4-D treatments in 2015 than in 2014.

   PGR Field Trials Conducted by Dr. Gene Albrigo:
   In the 2014-15 harvest season, more Hamlin trials were included and larger plots used to include a greater number of replicate trees so that tree health (decline) status could 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 pre-harvest fruit drop in Valencia, four PGR tests were started in December 2014 including a 2, 4-D treatment on pre-harvest drop of Valencia. One test was applied near Frostproof, another test was applied in Auburndale and a third test was installed 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, a strobilurin fungicide. These tests will be harvested in April 2015 and data will be available shortly thereafter.

   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 percent fruit drop. Additionally, a white grapefruit test comparing GA, 2, 4-D and Headline was applied to double-row plots in the Indian River.
   In three of five Hamlin trials, GA_3 plus 2, 4-D showed some reduction in pre-harvest fruit drop compared to the controls. There were two of four tests that yielded reduction in fruit drop in Headline-treated plots. For both materials, the reduction sometimes was only in healthier or more declined trees and not both. In the grapefruit trial GA3 reduced fruit drop as did GA plus 2, 4-D, but the latter combination only reduced fruit drop in healthier trees. In Valencia trials, a combination of

three primary PGRs reduced drop slightly for healthier trees in one trial and in another Headline and 2, 4-D reduced drop in healthier trees only. Other Valencia trials are not yet completed.

3. Communicate to CPDC, Board and growers on the project progress, results and interpretation

   No activity

   Significant Meetings of Conferences:
   Fruit drop results from the Albrigo trials and the CPDC field trials will be presented at the annual Florida State Horticultural Society meetings in June 2015 and will be published as formal manuscripts in the subsequent Proceedings of FSHS.

   Obstacles Encountered and Breakthroughs:
   Tree to tree variation with HLB status is a reoccurring problem. It does appear, however, that trees with less severe HLB symptoms may respond better to PGR treatments by decreasing the percentage of pre-harvest fruit drop more than HLB declining trees.

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.
   No progress has been made in this project over the last quarter.

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.

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 refining our understanding of the seasonality of ACP population increase and movement, allowing for assessment of the best materials and time for ACP treatments. This is

important for CHMA coordinated sprays as well as choosing the timing and materials for the balance of the seasonal ACP suppression program.

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

   Preservation of the products that are available to control ACP populations is important and several CPD projects are working in this area. CRDF maintains communication with registrants relating to maintenance of or expansion of labels, and the most significant area at present is the neonicotinoids. A meeting with EPA, TPR and CRDF was held in February to discuss the issues surrounding neonicotinoids and honeybee health, providing an opportunity to emphasize the importance of this group of insecticides to Florida citrus. The reliance on soil insecticides for success of new plantings was communicated.

   Planning discussions for 2015-16 label needs are underway, with successful extension of the Bayer Imidacloprid Admire Pro product accomplished for this season. Discussions are underway with Syngenta regarding expansion of the Thiomethoxam label for young citrus.

3. Continue participation in pesticide stewardship activities

   Resistance monitoring of commonly used ACP suppression tools continues via a CRF project conducted By Dr. Lukas Stelinski. During this funding period, his lab investigated the levels of resistance in field populations of ACP in commercial groves across central and southern Florida to six classes of insecticides and compared those results to previous years. In years 2009 – 2012, a decrease in susceptibility to several major classes of insecticides was observed in field ACP. In contrast, surveys conducted in 2013 and 2014 showed the return of the LD50 response in field psyllids to be equal to that of the reference laboratory strain. Monitoring work and related research into resistance mechanisms continues.

   A CPD project with Dr. Michael Rogers is nearing its end that has focused on appropriate pesticide recommendation in relation to bee health. Seasonal product recommendations related to bloom period has been the focus, and this project is redirecting to better understand the movement and distribution of ACP pesticides in tissues upon which ACP feed. The recognition that not all plant tissues or locations have similar systemic materials present will allow incorporation into recommendations for timing and materials selection in ACP management.

4. Continue to support CHMA implementation of ACP and other HLB management tools

   The UF, IFAS project to provide recommendations and support for Citrus Health Management Areas (CHMAs) continues to engage individual CHMAs in assessment of success in implementing area-wide sprays, and the assessment of results coming from the CHRP Asian citrus Psyllid monitoring program. Brandon Page has analyzed population changes over time and contrasted that with the incidence of HLB emerging in new plantings within the various performing CHMAs. Informal surveys of the incidence of HLB infection in young (around 2-year old) plantings indicates that those new plantings in CHMAs where ACP populations are low experience a much lower infection rate than do new plantings that are located where ACP populations are less well managed. While this is not a

surprise, the information is important to tease out and share with growers so that they can see the impacts of regional ACP control.

Recent discussions indicate that the Indian River Region of the state is interested in forming CHMAs, bringing the majority of acreage that is not included in a CHMA into the discussion. FDACS and UF, IFAS CHMA coordinators are working with interested parties to move this forward with the goal of establishing additional CHMAs in which area-wide treatments can be applied. Presentations on these topics are scheduled for the April Citrus Growers Institute held in Avon Park.

5. Communicate progress and results of project to CPDC, CRDF and growers
   The CRDF-funded IFAS coordination efforts with CHMAs allows for significant educational outreach to growers. The January 2015 Indian River Citrus Show included displays and presentations on ACP management and the role of CHMAs. In addition, support for IFAS Citrus Extension Agents in the CHMA project allows for local and regional programs to deliver updated ACP management recommendations to growers in a regular programmatic context. Fourth Quarter activities include the Annual Citrus Growers' Institute in April, organized by citrus extension agents, and the Florida Citrus Mutual Annual Citrus Growers Conference in June.
   Finally the CRDF monthly column in Citrus Industry Magazine, the CRDF Newsletter, and the CRDF webpage provide ongoing communication of CPD activities on this topic.

Project Title: 8. Candidate HLB Tolerant Rootstock Plantings

Narrative of Progress for Project Goals:

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

2. Encourage early release of new commercial rootstocks and other strategies to make these rootstocks available to growers
   Progress in development of techniques for nursery management of new citrus rootstocks emerging from UF and USDA breeding programs is reported here from the project being conducted by Dr. Richard Beeson of the UF, IFAS MFREC, Apopka. CRDF approved funding to Dr. Beeson to investigate barriers to propagation of new rootstocks and supported the construction of an addition to existing facilities at MFREC. Construction of the facility is underway and plans are in place to evaluate seed germination, sanitation relating to seed contamination with citrus canker, and propagation methods for recalcitrant rootstocks.

3. Implement Phase I and II grower field trials of most promising candidate HLB tolerant rootstocks using standard varieties as scions.

   Nurseries have produced about 2/3 of the required 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 have been selected. Trees were planted in the Southwest Florida (Duda, LaBelle) site in March 2015 and will be planted at the first ridge site (Peace River, Babson Park) at the end of April. Additional tree will not be ready for the 2nd ridge site (BHG, Venus) in the fall 2015.

4. Evaluate ongoing grower plantings of candidate rootstocks at 3 different sites: 2 on Central Ridge and one in Southwest Florida.

   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. Periodic 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 plantings has begun and includes 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 has been assigned a unique treatment and replicate number. Tree evaluations initially include **tree height** and **trunk diameter; Digital Photographs, Disease Index** and **Leaves for** qPCR, on a subset of measurement trees will follow after 1 year. **Leaf nutrition** will be evaluated in July/August of year 1.

5. Communicate progress and results of evaluation of rootstocks to industry

Significant Meetings of Conferences:
Dr. Grosser has been scheduled to report on statewide rootstock trials in the May CPDC meeting.

Obstacles Encountered and Breakthroughs:
Availability of sufficient numbers of contracted trees to be ready to plant continues to be a limiting factor.

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.
   Five dsRNA sequences have been selected for further development based on results from experiments with caged psyllids and young citrus trees inoculated with RNAi. The experiments were conducted in the laboratories of Dr. Bryce Falk (UC Davis) and Dr. 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 been 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 an epidemiological model using data collected from an infestation of lime trees in Mexico**.** During the past quarter, the team received data for flushing patterns in Southern Gardens and is working to get an estimate for Florida flush patterns as an input for the model. The transmission parameter from infected flush shoots to psyllids was re-examined based on data from Bill Dawson's experiments.

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 psylids to complete their life cycle on citrus flush. In addition, any surviving adults have been found to be free of C Las 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**.** For example, during the past quarter Dr. Falk reported progress in using artificial micro RNAs (amiRNAs) to target psyllid mRNAs for better specificity and to lower the potential for off-target effects. He is currently evaluating the functionality and the efficiency of amiRNA targeting, and is also conducting feeding tests with different approaches, plant and artificial diets.

However, it is not clear whether any improvement in efficacy would be necessary for practical evaluation in 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. Decision to initiate field trials and regulatory approval process.
   All experimental data and theoretical modeling to date support the evaluation of this concept in field trials.

Significant Meetings of Conferences:

None

Obstacles Encountered and Breakthroughs:

Next steps will include trying to identify a lead sponsor and/or partnership to finance larger scale field trials, and product registration. Because the market for this early stage technology is limited to citrus it has proved very challenging to find additional commercialization partners.

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

Narrative of Progress by Project Goals:

1. Assemble work group to discuss tactics and tools available for new citrus plantings
   The UF, IFAS CREC proposal to USDA, MAC has been approved and plans are progressing to design and plant a model new grove to demonstration the integration of HLB prevention/management tools into new plantings. The land is being prepared and trees ordered for this field demonstration area.
2. Generate an overview of elements possible to incorporate into a new citrus planting
   As part of the project above, discussions are being held to describe the array of available tools, what role they might play in new plantings, and how they will be incorporated into the model grove project. While land preparation and tree propagation are underway, this group effort will assemble new planting options as well as “best practices” for how to use these tools.
3. Encourage growers to consider use of these guidelines when planning for new plantings

Once step 2 is well underway, IFAS and CRDF will communicate the outcome of discussions on new planting tools to growers for their consideration in design and installation of their new groves.

4. Establish one "model new grove" which incorporates the elements of integrated tactics and tools
   Discussed in #1 above.
5. Communicate progress and results to CPDC, CRDF and growers

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.
   A USDA, MAC project has been approved with Dr. Ed Stover, USDA, ARS, HRL as the lead to identify and test the scions that show promise for tolerance or resistance to HLB. This project will provide coordination to test scion candidates which have shown promise in reducing CLas infection and which contain Poncirus trifoliate in their pedigree. The MAC group also is promoting activity to identify and overcome constraints in moving citrus breeding material between states to facilitate testing of other scion candidates with potential tolerance or resistance from other US citrus breeding programs (Texas A&M, UC Riverside, etc.). A mechanism to identify and address challenges in getting field testing of scions underway is in place and breeders and regulatory officials in each state are working to address how the performance of evaluation can be accommodated across state boundaries.

2. Determine the need and scope of field trials to evaluate HLB tolerant scions.
   CRDF currently is requesting full proposals for the next phase of conventional citrus breeding projects being conducted by UF, IFAS and USDA, ARS. These proposals will address the overall programs of each institution, but will also address the specifics of tolerant or resistant scions and their field testing. Review and analysis of these project plans in April-May will lead to recommendations to the Board for June approval of the most promising candidates.

3. Develop and implement field trials as deemed necessary.
   In planning as per #2 above.

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

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

Narrative of Progress by Project Goals:

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:

1. Micro-propagate a number of plants to begin grafting the transformants in all combinations. Conduct at least one transformation of mature scion or rootstock every week.
   Dr. Zale continues to conduct the weekly Agrobacterium-mediated transformations and screen putatively transformed mature scion and rootstock shoots for clients. The total number of transgenics produced and detected thus far (excluding the 157 reported in the last quarterly report earlier) is approximately 100. About half survived primary and secondary grafting. Transformation efficiency was 2.8% for mature scion and rootstock, with mature rootstock giving a higher transformation efficiency than mature scion.

2. 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 for NPTII expression to ensure they are stable, not chimeric, and expressing the NPTII protein.

   As mentioned in previous reports, the weaknesses of this protocol are the high number of escapes, difficulties with micro-grafting, and for certain constructs with no reporter genes, the difficulties in screening by PCR.

3. Improve laboratory and growth room productivity and mature transformation processes.
   The lab continues to optimize biolistics in order to increase productivity. During the optimization process, the lab has determined the 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.

Significant Meetings of Conferences:
None

Obstacles Encountered and Breakthroughs:

GFP expressing transgenic shoots were regenerated from mature citrus after biolistics, but subsequently died after micro-grafting. The lab will consider purchasing mature scion from nurseries solely for

biolistics because they cannot be produced in sufficient quantities in the growth room to facilitate the weekly Agrobacterium transformations and optimize biolistics.

The lab has also had to find an alternate California seed source because certain varieties that are regularly used in tissue culture are contaminated by a fungus. Spraying fungicide does not eliminate this seed borne fungus.

Other Information:

A manuscript entitled "Genetic Transformation of Commercially Important Mature Citrus Scions" authored by Hao Wu, Yosvanis Acanda, Alka Shankar, Michael Peeples, Calvin Hubbard, Vladimir Orbovic and Janice Zale has been accepted for publication in ***Crop Science***.

Project Title: 13. Diaprepes Pheromone

Narrative of Progress by Project Goals:

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

As reported in last quarterly report, no further activities in this area since the notification in December 2014 that USDA had withdrawn CRDF's license application from consideration and will keep it on file.

Significant Meetings of Conferences:
None

Obstacles Encountered and Breakthroughs:
None

Other Information:
None

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

Narrative of Progress by Project Goals:

1. Monitor progress in both commercial and research evaluation phases of demonstration project (LaPointe) Disruption of trap catch was high at the three locations through 20 to 22 weeks after deployment of the DCEPT CLM devices. One device was placed in each tree within the treated blocks. Trap disruption appeared to decrease after 22 weeks suggesting that release of pheromone contained in the release devices was decreasing. 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 were monitored as controls. During the 22 week period when trap disruption was high, the number of leafminer mines were estimated by randomly collecting flush samples and recording the number of larvae and pupae contained in the leaves. The grapefruit blocks treated with the pheromone release devices had statistically equivalent numbers of larvae and pupae compared with adjacent controls that had received 11 insecticide applications during the period ending on June 23. A second survey was conducted in August and similar results were observed. However, significantly fewer 2nd and 4th instars and pupae were found in the pheromone-treated blocks compared with the control blocks (A blocks) to the east of the treated B blocks. By these measures, application of pheromone appears to as effective as multiple insecticide applications. However, the current insecticide application regimes for control of Asian citrus psyllid have greatly reduced or eliminated biological control agents of the leafminer including the introduced parasitoid Ageniaspis citricola. For this reason, leafminer populations are abnormally high in many groves. If IPM practices were implemented that succeeded in reducing the number of insecticide applications and allowing re-establishment of biological control (Ageniaspis), we hypothesize that mating disruption would be even more effective.
Applications for the second years of this study will begin in late March/early April, as detailed below.

(Stelinski) We have been investigating the application of synthetic sex pheromones to disrupt mating of the citrus leafminer (CLM) as a possible effective and environmentally friendly alternative to pesticide applications. We have conducted large-scale field experiments combined with data modeling to optimize the use of this technology for the purpose of practical application. Optimizing mating disruption through examination of multiple interrelated variables was a method used for possible wider adoption of this technique in Florida citrus, especially since this particular pheromone active ingredient is quite expensive to produce. Simulations and field experiments were designed to produce response surfaces to investigate data. We varied the distribution and number of pheromone dispensers in plots. Moth catch data in traps were used to understand the efficacy of our treatments. We used commercially available dispensers for mating disruption of CLM obtained from ISCA Tech. For modeling, Monte Carlo simulations were used for a spatially explicit agent-based model. These resulted in nonlinear disruption profiles with increasing point source density. Field trials conducted in citrus infested by CLM varied the amount of pheromone applied at each point source and point source density using attractive and non-attractive disruption blends. Trap catch disruption in the field resulted in nonlinear disruption profiles similar to those observed with simulations. Response surfaces showed an interaction between the amount of pheromone applied and the number of point sources for the attractive blend, but not for the non-attractive blend. Disruption surfaces were combined with cost curves to optimize trap catch disruption under real world cost constraints.

Our bottom line results were as follows. Our data indicate that the more complex and more expensive 3:1 blend of two pheromone components was not more economically effective than the less expensive single component blend. Also, our data suggest that both formulations actually cause disruption by the same noncompetitive mechanisms. Therefore, we have definitively shown that the

less expensive single component dispenser is the most effective for practical adoption for use to manage CLM in Florida using a combination of field trials and data modeling.

2. Determine need to modify plans or details of project

   (ISCA): As of March 31, 2015, a signed invoice has been received to confirm participation in the 2nd year of trials. A total of 109,650 DCEPT CLM has been produced and will be sent to VPI in mid-April. This season, we will test new application methods in a select number of blocks. We will vary the height of DCEPT CLM placement to evaluate the effect of height on mating disruption performance. In addition, one block will receive reduced CLM specific insecticide input and another will have CLM specific insecticide input fully removed. TRB is now called 737 Citrus Holdings but it is the same farm that participated in DCEPT CLM trials last year. As of March 31, 2015, a confirmed invoice has been received to confirm participation in the 2nd year of trials. A total of 185,549 DCEPT CLM has been produced and will be sent to 737 in mid-April. For this year's trial, 737 have decided to proceed with a full application rate across the entire farm. Data from the 2014 trials showed evidence that reduced rates led to reduced performance. As of March 31, 2015, participation by the Packers of Indian River in the 2nd year of trials has been confirmed. A total of 15,500 DCEPT CLM will enter production in mid-April for application in early May. For 2015, Packers decided to reduce application to approximately 120 acres. We will continue to test the full application rate. The 2015 trials will be focused on testing an increased rate of pheromone. DCEPT CLM will have 2.4x more pheromone this year, which should lead to improved performance and field life.

3. Communicate progress and results of demonstration
   No Activity

4. Determine if CRDF has additional role in delivering this technology
   No activity

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

Significant Meetings of Conferences:

None

Obstacles Encountered and Breakthroughs:
As reported in previous reports, CRDF Program Management has offered their availability to provide perspective on the technology and its potential to companies who have an interest in using the CTV vector to express RNAi in programs for insect control, and are evaluating the opportunity for use in citrus.

Other Information:
Aspects of this project related to Project 9: RNAi Molecules/Psyllid Shield, are 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.
   This project is entering its last quarter of support from CRDF, and progress is limited to a few instances where Extension Agents or UF Escape Project scientists have been alerted to potential escapes in commercial citrus. In some cases (e.g. Lake County), the extension agent has worked with the grower to propagate from the candidate tree so that additional challenge can be made to the material. As the project nears completion, an assessment will be done of the reach that was accomplished to communicate with growers on the topic and what next steps might be necessary.

2. Evaluate elements that can be implemented to advance project beyond its current scale.
   No activity.

3. Facilitate expansion and enable more Ext Agent involvement in observations of tree escapes.
   No activity

4. Communicate progress to CPDC, CRDF and growers
   A popular article is being drafted to report the outcome of this effort to find and investigate escape trees in the field. Capability will continue to respond to reports of escapes and to provide the follow-up when necessary.