# CRDF Commercial Product Delivery Project Progress Report FY 2014-15

# Quarter Ending June 30, 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 treatments for the first year and data collection and analysis is being completed. Year two sites for project one, two and three have been selected and some applications have been completed. All but one sample has been analyzed in the residue study and a report is expected early next quarter.
An oxy-tetracycline residue study with NuFarm has been funded by CRDF, this study covers the citrus group, sites have been selected and several Florida trials have commenced.

**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.

## 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 are no longer being evaluated in CPD projects because of the lack of strong activity and because these are antibiotics not distributed in the US.

2. Track research projects relevant to non-antibiotic tetracycline derivatives against HLB
Project number 584 was recently completed, this study found Zhongshengmycin to be less effective than Validoxylamine A. Nanoemulsions of VA was found to be effective against HLB.

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

**Narrative of Progress for Project Goals:**

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

1. Screen library of Company C biopesticides using *L. crescens* assay.
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. Top candidates will be tested in the greenhouse assay next quarter, meetings have occurred to discuss possible candidates, application methods and doses.

2. A new field trial based on new plantings that are uninfected with HLB.
   This trial completes year one in the next quarter, most of the evaluations will be made at that time. 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 evaluations have been completed for the company C HLB trial, no canker was found in the evaluation trees.

**Significant Meetings or Conferences:**
During the quarter, there was regular communication with Company C and Company I 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 therefor 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
   One of the tetracycline derivatives in combination with thermal therapy has been added to the Powell #910 field trial. Treatments were initiated in this quarter.

## 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
      No progress has been made with these materials in the past quarter. Some of the materials tested are candidate for greenhouse assay testing that will commence in the next quarter.

   b. Bacterial protein targets LdtR and SecA

CRDF-funded project #773 examined SecA and lipid A inhibitors as possible antimicrobial compounds. Two compounds are being tested in the field trial to determine the effective dose and application frequency.

## 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.
   The field trial to evaluate biopesticides/minimum risk pesticides has been set-up and materials will be applied early next quarter. 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.

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 and #617 using foliar and bark applications and gravity bag infusion. These materials continue to be studied in project #910; combination therapies in the field have begun for this project.

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

   Ongoing treatments (quarterly or monthly) were began in May/June 2014 and are being applied with and without an organic mulch at the 3 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 submitted on time.

   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: Valencia/Swingle trees are 6 years old. As of 19 Mar 15, the Ecofriendly treatment

(n=28, ± mulch) has a higher visible disease index (worse DI) than the untreated control treatment (UTC) but the Bio Flourish has a lower DI than the UTC. Other treatment DIs were intermediate and did not differ. Average fruit yield per tree varied from 110 to 115 lbs per tree and there was no effect of treatment on yield. Ecofriendly trees had the largest trunk cross sectional area (CSA; 60 $cm^2$) whereas Quantum and Bio Flourish trees were the smallest (51 $cm^2$). Bio Flourish treated trees had the largest canopy volume (6.9$m^3$) whereas Aliette treated trees were the smallest (5.5 $m^3$). With respect to juice quality, there was no overall effect of treatment on brix or pound solids per box but the mulch treatment had a lower Brix/acid ratio (15) than the ratio in no mulch treatment (15.5).

SW FL site: Valencia/Swingle trees are 11 years old. As of Dec 2014, there were no treatment effects on visible DI. The Bio Flourish treatment had the greatest yield per tree (405 lbs) followed by the Aliette treatment (378 lbs/tree) which were both greater than the UTC (327 lbs/tree). There were no treatment effects on trunk CSA (range 103 to 272 $cm^2$) but the Bio Flourish and Aliette treated trees had the largest canopy volumes (29 and 28 $m^3$, respectively). Canopy volumes in Quantum and Ecofriendly treated trees were intermediate whereas and Serenade trees did not differ from the smallest UTC trees at 17 $m^3$. With respect to juice quality, all treatments had lower pounds of juice per box than the UTC (=57 lbs) and Aliette treated trees were significantly lower at 55 lbs of juice / box. There were no treatment effects on Brix/acid ratio (range 15-16) or pound solid /box (range 6.5-6.7). The mulch treatment significantly increased brix to 11.9 from 11.7 in the no mulch treatment.

Ridge Site : Valencia/Swingle trees are 17 years old. There were no treatment effects on visible DI. The mulched trees tended to be consistently larger (though not significantly so) but within the smaller UTC trees, mulched trees had a significantly larger trunk CSA but not CV. Bio Flourish trees had the largest trunk cross sectional area (229 $cm^2$), the others were intermediate and dis not differ, whereas the UTC trees had the smallest CSA (146 $cm^2$) . There was no treatment effect on canopy volume however, ranging from 25 $m^3$ in the UTC to 31 $m^3$ in the Bio Flourish treated trees. Juice from the Bio Flourish treatment had similarly high acid as the UTC (0.84), Ecofriendly, Quantum and Aliette were intermediate and Serenade had significantly lower acid (0.74) than the UTC. Since there was no treatment effect on Brix, Serenade also has the highest ratio (15.5) and the UTC had and lowest ratio (12.8). There was no treatment effect on pound solids per box and no mulch effect on any juice quality characteristics.

3. Provide communication on project goals, progress and results to CPDC, CRDF and growers
   CRDF Project reviews at Citrus Expo, August 19, 2015, Ft Myers, FL.

**Significant Meetings or Conferences**:
CRDF Project reviews at Citrus Expo, August 19, 2015, Ft Myers, FL

**Obstacles Encountered and Breakthroughs**:
None

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

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:

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>.
There have been 7 new thermotherapy (TT) field trials established in which the CRDF staff is participating in tree evaluations. Trials are located in private groves in St. Lucie and Hardee County (bedded) and Polk and Lake County (Ridge), and we have been invited by the grove owners to evaluate the effects of the thermotherapy. Trees include Valencia, Rio Red Grapefruit, and Ray Ruby Grapefruit on a variety of rootstocks (sour, Swingle, Carrizo). Most trees being evaluated are in varying stages of the decline due to HLB; most are heavily managed for psyllid control, nutrient

applications, root health, etc. One grove in Lake county, however, is under organic production practices and there is one grove using conventional production practices but is under managed for comparison of TT results.

There are 3 different thermotherapy machines being used in the trials—all are making visible improvements in tree appearance. The first steam machine was constructed by a private grower, Larry Davis, from Wauchula and operates in a similar fashion to Dr. Ehsani's steam machine using 120˚F for 20 seconds based on successful visible results of treatments in his grove. The second hot water/steam machine is built on a 'goat' frame by Premier Energy. The water used is purified prior to being heated 122˚ – 128˚ F for 20 seconds. There are 3 levels of 100 psi nozzles inside an octagon-shaped over-the-tree canopy. Most of the time there is foliage dropped at the top of the tree following treatment. So far no fruit drop directly due the treatment has been observed. The 3rd machine is owned by the Scott Family on the east coast who specialize in grapefruit. Their machine is similar the goat-based, hot water/steam Premier Energy machine but the treatment water is not pre-purified prior to heating. The round over-tree canopy has a single row of four 1000 psi nozzles that are 14" from the ground that spray at a 45-degree angle that create a "vortex" inside the canopy. The current TT treatment is 130˚ F for 15 seconds. Although this machine does not cause leaf drop, visible temporary leaf bleaching is common. Nonetheless, this method of thermotherapy may be visually the most promising but quantitative tree evaluations are yet to be analyzed.

The quantitative tree evaluations, comparing treated and non-treated trees where possible, will include pretreatment photographs and leaf qPCR and post TT treatment PMA-qPCR to separate short-term living vs dead CLas DNA. Follow up leaf qPCR samples will be re-evaluated in the winter months (December). Current and winter-time tree height, canopy diameters, trunk circumference, fruit drop counts, % leaf drop, % leaf bleach and visible Disease Index will be measured. Summer time (July-August) leaf nutrient analysis will be evaluated along with fruit yield and juice quality testing where appropriate. Data analysis will focus on current and winter time tree comparisons to be followed annually to evaluate recovery and/or relapse.

4. <u>Continue outreach efforts to inform growers of the availability of thermal treatment and to refine conditions that will lead to effective thermal treatment.</u>
The CRDF-initiated Thermal Therapy field days are being planned for Winter 2015 to inform growers of this emerging tool, and also attract entrepreneurs, innovators, 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**:
CRDF Project reviews at Citrus Expo, August 19, 2015, Ft Myers, FL.
**Obstacles Encountered and Breakthroughs**:
None

## 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.
   Two years of contracted field trials on effects of a single application of 2,4-D on mitigating preharvest fruit drop in Valencia oranges have been completed. This research was approved and supported by CRDF/CPDC. A manuscript has been submitted for publication in the Proceedings of the FL State Hort. Society in June 2015. Title: A Single Application of 2,4-D Can Decrease Preharvest Fruit Drop in HLB-Affected 'Valencia' Orange Trees, by J.P. Syvertsen, Tom Minter, Henry Yonce, and W. Bandaranayake

   The goal of these field trials was to determine if a single spray of 2,4-D in late December could mitigate 'Valencia' preharvest fruit drop from HLB affected trees. An on-label application of 2,4-D (Citrus Fix) was applied to mature 'Valencia' trees at four different sites in the central Florida ridge area in Dec 2013 and repeated using different trees at the same sites in Dec 2014. All trees had visible HLB symptoms. At each site, there were four reps of 6 trees treated along with 4 reps of 6 untreated control (UTC) trees. Fruit drop counts in 2014 and 2015 came from the 4 middle trees in each rep so there were 16 trees in the treated and UTC at each site. The grove floor under each tree was raked prior to treatment and approximately every two weeks thereafter for fruit drop counts until harvest in March or April. The percentage of fruit dropped was calculated after counting the remaining on-tree fruit at harvest and dividing the number of fruit dropped on each sampling day by the total number of fruit dropped + fruit harvested. At three sites in 2014, average total fruit drop ranged from 24 % to 76 % and there was no significant effect of the 2,4-D treatment on fruit drop. At a forth site, fruit drop was significantly decreased by 28% from 47 % drop in the UTC to 19 % drop the 2,4-D treatment. In 2015, at 2 sites where disease symptoms were visible in 37-38 % of each tree canopy, fruit drop was significantly decreased by 5% and 18%, respectively, in the 2,4-D treated trees compared to the UTC. There was no significant effect of the 2,4-D

treatment on fruit drop at a third site where symptoms were visible in 55% of the canopy. Combining the % fruit drop results from the 7 trials at 4 sites over the 2 years, % fruit drop was significantly reduced from 5-28% by the single 2,4-D treatment in 3 of the 7 trials. The site with the highest visible disease index, however, did not have the highest total % fruit dropped so disease index was not a good predictor of % fruit drop**.**

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, GA3 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 or Conferences**:

CRDF Project reviews at Citrus Expo, August 19, 2015, Ft Myers, FL.

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. See also the summary in:

Albrigo, L.G. and J.P Syvertsen, 2015. Status of citrus fruit drop in relationship to HLB. Citrus Industry (July): 14-17.

**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. A single application of 2,4-D can decrease preharvest fruit drop in HLB-affected 'Valencia' orange trees—but not always.

## 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 & Citrus Health Management Areas (CHMAs)

**Narrative of Progress by Project Goals:**

1. Pursue actions that will support expanded tools for ACP management
   Efforts continue in an effort to expand the rate available for application of Syngenta's Thiomethoxam neonicotinoid insecticide. Registrants and CRDF regulatory consultants are communicating this need and status of external issues that affect this moving forward.

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.
   Discussions are underway with Syngenta regarding expansion of the Thiomethoxam label for young citrus.

3. Continue participation in pesticide stewardship activities
   Work (Rogers 590) continued on our previously reported efforts to determine the level of imidacloprid in leaf tissue required to prevent ACP phloem feeding behaviors. In particular we have focused efforts at measuring the levels of neonicotinoids in different parts of the leaf tissue and how that changes with time. While this work is still underway, progress to date does show uneven distribution of neonics within a leaf that changes over time. There also appears to be some difference in movement patterns between the different neonics which may be due in part to their differences in water solubility. Further work is ongoing to better characterize these movement

patterns and use that information to then determine when retreatment of plants is needed in order to ensure psyllids are not able to feed successfully on treated plants.

Use of EPG techniques to determine the level of imidacloprid needed to prevent phloem feeding behaviors has continued. While still ongoing, we have narrowed down the LC50/LC90 for feeding disruption and should be able to be begin work soon for the other neonicotinoid compounds. This work will be continued in the coming year to help refine the use of soil-applied neonicotinoids for protecting young trees from HLB via disruption of psyllid phloem feeding behaviors.

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.

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. 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. Presentations on these topics occurred at the April Citrus Growers Institute held in Avon Park. Fourth Quarter activities also included 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.

   Over the past year of funding, grower interest and participation in the CHMA program has continued to remain high. At present there are 48 CHMAs functioning across the state with additional groups functioning as CHMAs but not necessarily formalized on the CHMA website. The CHMA program has continued to keep information up-to-date on its website that has been used to document areas where the program is working well and areas where improvement is psyllid control is needed through better coordination of activities. To further document the success and benefits of the CHMA program, information has been gathered from growers from around the state to compare production costs and fruit yields. The results show that participation in a CHMA can have a positive effect on fruit yields. Dr. Ariel Singerman presented this information recently at the Florida Citrus Mutual Annual conference in Bonita Springs. These data will help to further increase and motivate grower interest in the CHMA program in the coming year. Plans for the coming funding cycle include further analysis of this type of data to show the continued benefit of participation in a CHMA over time.

   During this reporting period, the CHMA assistant provided support to the CHMA program by attending various local CHMA meetings as well as statewide and multi state meetings. Specifically, Mr. Page attended 11 meetings including the following: Citrus grower's Institute in Avon Park (4/7/15), citrus extension planning retreat (4/8/15 - 4/9/15), CHMA meeting in Avon Park (4/15/15),

Hardee CHMA meeting (4/17/15), CHMA meeting in Immokalee (4/21/15), Volusia county CHMA meeting (4/24/15), CHMA meeting in Wauchula (4/24/15), Lake county grower round table (5/20/15), grower round table discussion in Wauchula (5/21/15), Florida Citrus Mutual Annual meeting (6/17/15), and the East coast CHMA planning meeting (6/24/15).

In addition to helping plan and CHMA meetings, Mr. Page continued to keep the CHMA website updated, sending out updates to the individual CHMA listserves, adding names to the listserves, and updating the ACP trend graphs and CHMA rankings. Mr. Page also continued to work with the new Citrus Economist at CREC to collect data from growers in different CHMAs and use that data to analyze citrus production in trends in areas with and without functioning CHMAs to determine the value of CHMAs in terms of increased production value.

## 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 were selected and propagated in large numbers: 5 experimental rootstocks from the UF and USDA breeding programs along with 4 standard rootstocks (812, Sour, Carrizo, Swingle) for comparison at individual sites.

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, in the he first ridge site (Peace River, Babson Park) at the end of April, and will be planted at the 2nd ridge site (BHG, Venus) in July 2015. Trees on two rootstocks, UFR 3 and UFR16, were not large to be planted in the 2 ridge plantings so gaps have been left to be planted this fall.

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

   Grove site evaluations 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 coordinate 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. CRDF will 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.

   Standardized 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 and fruit yield and quality when appropriate using a subset of measurement trees in each replicated block.

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

**Significant Meetings or Conferences**:

Dr. Grosser reported on statewide rootstock trials in the May CPDC meeting.

CRDF Project reviews at Citrus Expo, August 19, 2015, Ft Myers, FL

Planning for a rootstock field day in Oct/Nov 2015 has begun to occur at the initial SW FL planting (Duda) which also will include an 8-yr-old ongoing rootstock trial at the same site.

**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.
   **This goal has been accomplished**.

   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.
   **This goal has been accomplished**.

   Results from RNAi research are being incorporated into the experimental design planning for candidate field trials. Two separate CPDC projects were merged into one project.

3. Establish a mathematical model of RNAi effects on area wide protection of new plantings.
   **Basic model has been established.**

   Dr. Jed Keesling (UF) has led the modeling effort to predict how the Psyllid Shield effect will protect solid block new citrus plantings from dissemination of HLB. The model was developed and validated based on data collected from an infestation of lime trees in Mexico.

4. Refine the model with vector entomologists and epidemiologists.
   **This is ongoing**.

   Understanding the relationship between transmission of *CLas* and eventual development of HLB symptoms is crucial to deploying the model. Dr. Keesling has obtained survey data from Southern Gardens and has begun its analysis. Using the Southern Gardens survey data will require simulation of a much larger scale than is currently being used (approximately 100X more trees). This will require the University of Florida's High Performance Computing system, and they are in the process of optimizing code and porting it to C ++.

   Another key area for refining the model is the psyllid movement and migration portion. In an agent-based model, accurate simulation requires knowledge of how the vectors disperse. Contact has

been made with scientists at the USDA in Gainesville who are discovering how psyllid population density and citrus tree flush quality impact psyllid dispersal. The transmission parameter from infected flush shoots to psyllids was re-examined based on data from Dr. Dawson's experiments that evaluated plant characterization of dsRNA effects on all psyllid life stages.

5. Experimentally evaluate candidate protective effects of selected RNAi in CTV inoculated plants.
   **This is ongoing**.

   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 been 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.
   **This is ongoing**.

   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.
   **This is ongoing.**

   Additional modeling validation and parameter refinements are in progress.

8. Decision to initiate field trials and regulatory approval process.
   **This is in planning stage**.

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

**Significant Meetings or Conferences:**
None

**Obstacles Encountered and Breakthroughs**:

Next steps will include identifying a lead sponsor and/or consortium 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
   No significant progress is available to report in this quarter
2. Generate an overview of elements possible to incorporate into a new citrus planting

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

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

5. Communicate progress and results to CPDC, CRDF and growers
   Grower meetings in the April Annual Grower's Institute and the June Florida Citrus Mutual Annual Growers Conference highlighted the tools available for replanting. Individual talks from researchers focused on emerging information to refine planting details and post-planting management, while presentations and panels provided real-world examples of new plantings and how tools were being implemented into the design and management.

## 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.
   During this quarter, CRDF reviewed and approved several projects to continue core citrus breeding programs at UF, IFAS and at USDA, ARS for the next phase of conventional citrus breeding projects.

Projects have been approved and are underway as the new FY 2015-16 gets underway. Simultaneously, the USDA, APHIS MAC group also provided support for several breeding projects, which include field trial evaluation of HLB-tolerant or resistance scions

3. Develop and implement field trials as deemed necessary.
   As reported above, the emphasis of the projects approved for continuation of conventional breeding are focused on field evaluation of current scions that are ready to go to the field. A Knowledge Mapping analysis is planned for September, 2015 to further evaluation the need for and define the parameters for further field evaluations of HLB tolerant or resistant scions.

4. Provide communication regarding scion evaluation for HLB tolerance to CPDC, CRDF and growers.
   Presentations on projects that are focused on rootstock and scion resistance to HLB were made at all meetings held in this quarter, including the April Citrus Grower's institute, June FCM Annual Growers Meeting, and a presentation is scheduled for the August Citrus EXPO.

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

**Narrative of Progress by Project Goals:**
This is the final quarter of the project, so comments below will reflect progress over the life of the project.

1. Make measurable progress toward producing and introducing to Florida citrus growers new transgenic citrus lines based on mature tissue transformation of commercially available cultivars.
   These citrus lines will have disease resistance to citrus canker, and will flower and bear fruit in a short time period. For FY 2014-2015, measurable progress is defined as:
   a. Micro-propagate a number of plants to begin grafting the transformants in all combinations. Conduct at least one transformation of mature scion or rootstock every week.

      Dr. Zale continues to conduct the weekly Agrobacterium-mediated transformations and screen putatively transformed mature scion and rootstock shoots for clients. During the life of this project, 121 mature citrus transgenics were produced using genetic transformation with Agrobacterium. Sixty-six were transgenic for reporter genes and provided proof of concept that this protocol works in the hands of MCTF facility staff.

      In the last 21 months, 55 transgenics with disease tolerant genes, most without reporters, were produced and micro-grafted onto rootstock. Agrobacterium transformation efficiencies were relatively low, (3.47% positive shoots for constructs with reports in scion, and 3.0% positive shoots for constructs with no reporters in scion and rootstock. Only 0.78% shoots/total explants plated were transgenic. Many more transgenics were probably produced, but were lost in the micro-grafting process. Attempts are being made to increase micro-grafting efficiencies.

   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 and expressing the NPTII protein.

Although 9 vectors carrying disease tolerant genes were used in sizeable transformation experiments, only 4 vectors yielded transgenics. (Most were NPR1.) The 55 mature citrus transgenics were produced with these 4 vectors to confer disease tolerance to HLB, canker, or both. These primary transgenics are being propagated into vegetative progeny to facilitate replicated field trials. Numbers were low for Ray Ruby grapefruit (3 transgenics) and efforts are being made to optimize the tissue culture and transformation protocol for grapefruit.

Experiments are underway to root mature scion because a larger scion could be easily micro-grafted onto rootstock with greater efficiencies. To increase chances of success, lab staff has been utilizing nurse cultures to supply additional nutrients to developing mature shoots.

Additional vectors are being acquired from scientists around the country and worldwide. Budding is now done entirely in-house.

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. PCR is expensive, time consuming, labor intensive and prone to error.

c. <u>Improve laboratory and growth room productivity and mature transformation processes.</u>

The lab continues to optimize biolistics in order to increase productivity. A gene gun was purchased in July, 2014 to develop a high –throughput biolistics transformation system for mature citrus. Transient expression levels are relatively high, and a few stable transgenics have been produced. Optimizations for mature citrus have been hindered by the limited supply of mature scion in the growth room, which was primarily used for Agrobacterium transformations. In the future, staff will purchase mature citrus from nurseries to continue optimizations.

During the biolistics 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.

The lab has also developed a high throughput screening system in which thousands of putative transgenics can be rapidly screened. A number of equipment expenditures were necessary to achieve the high level of efficiency, including a refrigerated centrifuge, a plate reader, a tissuelyser, a laminar flow bench and an incubator. A hybridization oven and dry baths were

purchased for molecular analysis. Maintenance expenses for lighting, AC repair, sensors and expansion cards for RCWebview, and the water softener in the growth room are ongoing.

**Obstacles Encountered and Breakthroughs**

As the Lab moves forward there are a number of issues to be addressed with support from the MCTF Steering Committee:

- Increasing the number of high quality gene constructs for screening and evaluation in the facility. This will require outreach strategies to identify, screen and evaluate high potential candidates.
- Increasing transformation and micro-propagation throughput of the facility for further field evaluation. This will require continued attention on improved efficiencies in both Agrobacterium and biolistic transformation protocols, micro-grafting efficiencies and benchmarking processes used by Dr. Pena (IVIA) in other locations.
- Ensuring a stable supply of healthy, viable rootstocks. Rootstock seeds of Swingle and Volkameriana had poor germination, and Macrophylia and Carrizo had disease/endophyte issues that negatively impacted budding of mature citrus and the tissue culture process.
- The Steering Committee will also work to help integrate the above efforts with ongoing breeding programs.

**Significant Meetings or Conferences:**
None

## Project Title: 13. Diaprepes Pheromone

**Narrative of Progress by Project Goals:**

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

Stelinski: The large-scale validation of citrus leafminer (CLM) disruption with the ISCA DCEPT CLM technology has continued. During this past quarter we have applied our 2015 application of the treatment. The deployment of the treatment was successful. Currently we are monitoring populations of CLM in treated and control plots, as well as, damage incidence. Male moth activity is being monitored with pheromone traps that were deployed after treatments were applied. Damage is being monitored by assessing CLM infestation of new leaves.

Lapointe: Recent efforts to disrupt mating of the leafminer Phyllocnistis citrella have focused on the use of SPLAT™, a flowable wax emulsion intended to serve as a slow-release matrix for pheromones. Early success with this approach was overshadowed by the expense and difficulty of application, and variation in wax component chemistry that contributed to reduced longevity of pheromone emission in the field. Solid elastomer dispensers (DCEPT CLM™, ISCA Technologies, Inc.) loaded with a 3:1 blend of (Z,Z,E)-7,11,13-hexadecatrienal and (Z,Z)-7,11-hexadecadienal, the major components of P. citrella sex pheromone, provided disruption of trap catch in commercial citrus orchards for periods exceeding 30 weeks. The triene component alone worked as well or better than the 3:1 blend. The height of dispensers placed by hand in the tree canopy had a significant effect on trap shutdown. Dispensers placed low (0.6 m) in the canopy resulted in a reduction of trap shutdown in the upper third (>3 m) of the canopy suggesting that the net movement of pheromone molecules was downward during the period of active moth flight. Although moth flight appeared equivalent among the heights tested, placement of dispensers higher in the canopy appears more effective given this downward movement of pheromone plumes. These studies suggest that season-long trap catch disruption can be attained in citrus with a single application of a hand-applied dispenser.

Efficacy of trap catch disruption was excellent in the 3 field trials and compares favorably with longevity of disruption previously reported for SPLAT CLM. The ability of the pheromone blend or triene-only formulation to disrupt trap catch of males in traps baited with the 'natural' 3:1 blend agrees closely with what we have observed and reported previously for SPLAT-based formulations. The longevity of disruption provided by DCEPT far exceeded results obtained with SPLAT and raises the possibility that a single application of emitters may provide year-long disruption of mating for P. citrella.

Dispensers loaded with the triene component performed equally well regardless of the presence of (Z,Z)-7,11-hexadecadienal. The two formulations tested contained the same amount of triene; the addition of the diene component did not increase disruption. We conclude that inclusion of the diene in mating disruption products for this species has no benefit that would justify the additional expense of its synthesis and formulation. Also, the fact that highly effective trap catch disruption was obtained with an off-ratio blend supports the conclusion that disruption in this species falls into the category of non-competitive mechanisms because the triene alone is not attractive to males. The height of traps did not affect the number of males caught suggesting that males were active throughout the canopy. But the location of pheromone dispensers in the tree canopy did have a significant effect on trap catch disruption. Dispensers placed low in the canopy (<0.6 m) resulted in decreased disruption in the center and tops of the canopy compared with disruption achieved low in the canopy. Similarly, dispensers located in the canopy center (2m) were more effective at

disruption of traps located low or in the center of the canopy; less disruption was achieved in the canopy top. These results suggest that net displacement of pheromone was downward perhaps due to the fact that the triene (molecular weight 234) is heavier than air and would tend to settle under calmer conditions.

2. Determine need to modify plans or details of project
   From ISCA:

   Indian River Exchange Packers ("VPI"): On April 10, 109,650 DCEPT CLM were shipped to VPI. The field application was completed before the end of the month. As of June 30th, performance has been excellent in all blocks across the farm with nearly no moth captures in the monitoring traps, verifying the sustained effect of the mating disruption. We will continue to pay special attention to blocks were we varied the application height of the DCEPT CLM and most importantly, the blocks that have received a reduced input of CLM specific insecticides.

   On June 1st, 15,500 DCEPT CLM were shipped to Packers. As of June 30th, we have not received further updates on the status of the trial.

   734 Citrus Holdings (previously "TRB", now "734"): On April 13, 185,549 DCEPT CLM were shipped to 734. The field application was completed before the end of the month. As of June 30th, performance has been excellent across the entire farm with nearly no moth captures in the monitoring traps, verifying the sustained effect of the mating disruption. Performance in this year's trials has certainly increased when compared to the 2014 trials. The current hypothesis is that it is due to the removal of application gaps; in 2015 every tree at 734 received a DCEPT CLM versus the row skipping we did in 2014.

3. Communicate progress and results of demonstration
   No Activity

4. Determine if CRDF has additional role in delivering this technology
   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. In addition, ISCA and the research team will measure DCEPT CLM performance and economic/market feasibility by placing a substantially larger focus on obtaining damage evaluation data. This will be the most important measure for the farmer and we believe the data will show a significant reduction in damage when DCEPT CLM is used. This will guide the need for further evaluation prior to widespread grower adoption.

## 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 or Conferences:**
None

**Obstacles Encountered and Breakthroughs**:
Nothing new to report in this quarter. 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**:
Any 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 completed 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. No further support funding is planned and the mechanism continues to exist for growers to communicate the presence of escapes as they encounter them.

2. Evaluate elements that can be implemented to advance project beyond its current scale.
   The formal program is winding down for lack of grower participation.

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

4. Communicate progress to CPDC, CRDF and growers