Citrus Research and Development Foundation, Inc.

CRDF-Funded Projects Summary Progress Report FY 2019-2020
Quarter Ending March 31, 2019

RESEARCH TOPICS COVERED IN THIS REPORT

1. *CLAS* PATHOGEN INTERVENTION
a. Bactericides/antimicrobials

K. Pelz-Stelinski **18-018** "Disrupting transmission of Candidatus Liberibacter asiaticus with antimicrobial therapy" –Feeding solutions containing oxytetracycline (Fireline) or streptomycin (Firewall) were orally administered to D. citri. D. citri mortality was significantly higher when insects fed on artificial diet solutions containing 1mg ml-1 oxytetracycline, 5mg ml-1 oxytetracycline, or imidacloprid. Approximate 40% and 100% mortality occurred among D. citri that fed on 5 mg ml-1 of oxytetracycline after 3-d and 10-d, respectively. Greater mortality occurred in response to the high oxytetracycline concentration than the low concentration on day 3 (25%) and day 10 (63%). D. citri mortality in response to untreated diet was approximately 20% and 30% on days 3 and 10, respectively. Neither 5 mg ml-1 nor 1 mg ml-1 streptomycin was associated with significant D. citri mortality as compared with untreated diet. After 30d, 40% of D. citri that fed on untreated or streptomycin diets survived. Subsequent assays will evaluate the effect of foliar applied antimicrobial treatments on D. citri survival and CLas transmission.

S. Santra **18-020** "Novel multi-metal systemic bactericide for HLB control" - Different formulations of Mg-hydroxide or Zn-hydroxide loaded with Cu-chelates were successfully synthesized using sol-gel chemistry. The total metal content is 40,000 ppm (wt/V) including up to 50% of Cu-chelates (equivalent to 20000 ppm of metallic Cu; the A.I.): (1) Cu: Mg (10%: 90%, MM10C90M), (2) Cu: Mg (25%: 75%, MM25C75M), (3) Cu: Mg (50%:50%, MM50C50M), (4) Cu: Zn (5%:95%, MM5C95Z), (5) Cu: Zn (10%:80%, MM10C90Z), (6) Cu: Zn (25%:75%, MM25C75Z). The stability of each formulation was observed for 48 h post-synthesis. Most formulations (except for MM50C50M and MM25C75Z) formed a stable colloid as no phase separation was observed. Hydrodynamic diameter of the colloidal solution was estimated using Dynamic Light Scattering (DLS) technique. The hydrodynamic size for MM50C50M, MM25C75Z and MM25C75M was estimated to be around 880 nm, 580 nm and 450 nm, respectively, suggesting aggregation of primary particles. MM25C75M particle size was comparable to MM10C90C size. DLS particle size of MM5C95Z and MM10C90Z could not be reliably estimated due to low scattering intensity (less than 10kcps, below the detection limit). This suggests that MM5C95Z

and MM10C90Z formulations might contain highly-dispersible ultra-small size particles. Further study using electron microscopy is needed to confirm primary particle size. Phytotoxicity was tested in a plant growth chamber set for summer conditions (T > 80°F, RH 60-80%). Ornamental vinca plant was used as a model system due to their high susceptibility for metals. Three different foliar application rates (300, 500 and 900 ppm wt/V of total metal content, similar to field application rate) were tested. Phytotoxicity symptoms were evaluated after 3 days of incubation based on visual observation as described in our previous paper (Plant Disease 2016, 100(12), 2442-2447). All multi-metal treatments showed reduced plant leaf damage at all tested concentrations when compared to copper alone. Future reports will include phytotoxicity and antimicrobial efficacy results.

Z. He **18-040C** "Evaluation of the spatiotemporal dynamics of bactericides within the citrus tree via different application methods" - The purpose of this project is to reveal mechanisms of bactericide uptake and transport in citrus and establish a theoretical basis for developing technologies to improve the efficacy of bactericides. Objective (1) to compare the uptake and dynamic movement/distribution of bactericides applied either as a foliar spray, truck injection or by root administration. (2) to clarify the systemic movement and transportation mechanisms of bactericides within the phloem of tree; and (3) to investigate the effects of citrus variety and age on the delivery efficacy of bactericides. Samples of citrus leaf, stem, and fruit were collected from field trials and treated with antibiotics to be used for testing and validating existing methods for detecting trace amounts of antibiotics in plant tissues and fruit. The work planned for the next quarter will develop an improved robust analytical method for detecting and quantifying antibiotic concentration in citrus samples. Laboratory experiments and analyses to test existing methods for detecting antibiotics in citrus plant tissues and fruit samples. We will continue to collect plant tissue and fruit samples from citrus fields where oxytetracycline and/or streptomycin were frequently applied. Laboratory methods (LC-MS/MS) to improve antibiotic extraction and cleanup procedure will be developed based on the results from preliminary experiments to establish reliable methods to characterize antibiotics in citrus samples. Citrus plants will be cultured according to the work plan for the greenhouse experiments.

b. Diagnostics

Irey **17-002C** "Continued Support for the Southern Gardens Diagnostic Laboratory" - This project is a continuation of funding that has been provided to Southern Gardens Citrus (SGC) to provide growers and researchers with a facility to do testing to detect Candidatus Liberibacter asiaticus. This report covers the third quarter of year two funding. For the period of January 1, 2019 to March 31, 2019, a total of 6,951 samples were processed and tested by qPCR. Of these 95% were plant samples and 5% were psyllid samples. Virtually all of the plant samples were from grower, private entity, or grower research trials. To date, for the two-year project, a total of 42,466 samples have been processed and tested. Based on the current trends, it is expected that the total number of samples that will be processed during the grant period will be approximately 50,000 (budgeted amount was 60,000). If the lab sample load does not reach the budgeted amount, the final bill will be adjusted as necessary to reflect the total number of samples actually run. One trend that is changing is that more customers (50%) are requesting copy number information instead of just a positive/negative determination. In addition,

requests are coming in to provide testing using different primer sets and to return the DNA extracts back to the customers for additional in-house or custom testing. When possible, the SGC lab has tried to accommodate these requests.

2. ASIAN CITRUS PSYLLID VECTOR INTERVENTION

a. Asian Citrus Psyllid management

C. Vincent **16-020C** "Dyed kaolin to repel Asian citrus psyllid in field conditions" - The long-term field trial continues with weekly psyllid counts and quarterly CLas infection testing. Treatments continue to have similar effects on ACP counts, however, the differences between red and white kaolin in CLas infection are no longer persist. The two kaolin treatments, however, now have approximately 50% infection, while the control and the foliar insecticide treatment, have nearly 100% infection. Plants in both of the kaolin treatments continue to show higher growth rates than the other two treatments. The red treatment has the highest growth rate, trunk cross-sectional area, and canopy volume. Kaolin treated trees that are infected grow more than untreated-infected trees, but less than treated uninfected trees. The Master's student funded by this project completed his thesis and defended it successfully. We now have substantial evidence that both kaolin treatments improve growth, while the red improves water use efficiency. In potted studies whole-plant water loss was dramatically reduced through application of the red particle film. Despite this, we know that growth is increased in the field. We submitted an additional IP disclosure to include the possibility of manipulating whole plant water use through the color of the particle film. We are still exploring options for commercialization. The current experimental repetitions use the earlier designs, but they use adjusted rates so that all the particle films allow the same quantity of light, so that only light quality is different.

L. Stelinski **17-001C** "Insecticide resistance management in Florida citrus production" - This study was designed to investigate the capacity of field collected Asian citrus psyllid (ACP) to develop resistance to the pyrethroid insecticide, fenpropathrin and to determine the biochemical and genetic mechanisms of resistance to this popularly used chemistry for ACP management. We established an insecticide resistant strain of ACP in the greenhouse. The selected adult ACP population was originally collected from commercial citrus groves from Wachula, FL on July 15, 2018. They were treated with the LC50 concentration of fenpropathrin for nine generations of continuous rearing and then for a subsequent seven generations at a higher insecticide concentration. Bioassays were conducted using the bottle bioassay to assess the resistance of adults during each successive generation. Insecticides were dissolved to make 5-7 concentrations in acetone that gave 0 to 100% mortality. Control ACP were treated with acetone. Selection was performed by exposing adults to treated glass vials at the LC50 concentration. The ACP that survived were released in rearing cages to serve as parents of the next generation. The value of LC50 was increased from 0.12 to 3.71 ng/µL after nine generations. The resistance ratio was 30.91-fold. Biochemical assays were performed with detoxifying enzymes, namely esterase (EST), glutathione S-transferase (GST) and cytochrome P450 monooxygenase (P450). These were quantified every two generations and compared with the laboratory susceptible population. The activity ratio of EST enzymes was 1.530-fold higher for the selected population compared with the laboratory population at eight generations and

there were significant differences between the two populations ($p < 0.001$). The activity of GST enzyme was 1.486-fold higher, and for P450 the activity ratio was 1.10-fold higher for the selected population compared to the laboratory population. There were significant differences between the two populations for GST activity ($p = 0.038$) and for P450 activity ($p = 0.045$). Future experiments are planned to better understand the genetic basis of resistance by determining the expression levels of genes involved in the detoxification of pyrethroid insecticides. The results of this study provide insight into the development of insecticide resistance and designing appropriate resistance management strategies for ACP.

L. Stelinski **18-056C** "Functional IPM for Asian citrus psyllid under circumstances of chronic HLB" - The objective of this study is to determine how different rotation schedules of commonly used insecticides with different modes of action may impact the level of insecticide resistance Asian citrus psyllid (ACP) populations. An associated goal is to determine if ACP populations can be managed to reduce resistance in those populations where it already exists to a particular insecticide under rotations. Finally, the rotations must be effective in managing existing ACP populations to acceptable grower standards. We have selected two locations where resistance to neonicotinoid insecticides has been demonstrated and is known to exist. At each location, three rotational schemes of insecticides for ACP management will be established in 5-acre plots in Lake Alfred and 4.2-acre plots in Wauchula. The trees are 1-2 years old `Hamlin' trees with a variety of rootstocks. We are collecting adult ACP currently from these locations to determine their baseline insecticide resistance levels compared with a susceptible laboratory population of ACP using a leaf dip assay. Field populations have been collected from the Wauchula site and bioassays are underway currently. We will be collecting psyllids from the Lake Alfred site shortly. We will use commercial formulations of dimethoate, fenpropathrin, imidacloprid and cyantraniliprole to determine baseline resistance levels for these populations. Five to six concentrations of each insecticide will be tested and replicated 5 times. We will begin insecticide applications to fully evaluate our rotation treatments in early April 2019. We will collect samples chosen at random from the central rows for both the Lake Alfred and Wauchula sites. The plots will be sampled weekly beginning in late March 2019. The tap sample method will be used to sample adults. Ten samples will be taken per plot. For eggs and nymphs, 10 randomly selected flush samples will be taken per plots and number of eggs and nymph per flush samples will be counted. When counts of adults, eggs or nymphs in any plot reaches a predetermined threshold, a spray will be applied with the next insecticide in the rotation. Also, we will collect adults from the rotation sites to determine the relative expression of ten CYP4 and six GST genes that are implicated in insecticide resistance in ACP compared with the laboratory susceptible population. Finally, our goal is the development of a more refined method of an effective insecticide resistance management strategy. Our newly developed methods will have positive impact on suppression of ACP populations by stabilizing or reducing resistance and will be economically viable.

b. New technologies, e.g., RNAi, attract and kill traps, reflective mulch

T. Eyrich **16-016C** "Use of RNAi delivered by the Citrus Tristeza Virus Viral Vector to control the Asian Citrus Psyllid" - CTV RNAi Trials 1. In February, sentinel trees were tested using ELISA to

detect the presence of CTV and gel electrophoresis and rtPCR to detect the presence and stability of CTVvv-RNAi.
2. A repetition the experiment began in early March and will continue for a period of five weeks. The protocol established is as follows: Selected trees were scouted for flush or pruned to induce flushing. Areas of flush were bagged and inoculated with 20 parent ACP. Parent ACP were removed from bagged flush after two weeks. First generation ACP were counted two weeks after removal of parent ACP. The presence of native ACP life stages also documented.
3. Aphid scouting continues on a biweekly basis. The presence of brown aphid has not been detected.

3. CITRUS HOST INTERVENTION

a. Deployment of disease resistant or tolerant citrus rootstocks and scions

F. Gmitter **15-010** "Development and Commercialization of Improved New Disease Resistant Scions and Rootstocks - the Key For a Sustainable and Profitable Florida Citrus Industry" – Final reporting due 2/28/19 not received.

K. Bowman **18-004** "Development of SuperSour and other outstanding rootstocks with tolerance to HLB" - 1) During Oct-Dec 2018, six new replicated rootstock trials were field planted, including three on the East coast region, two in the Central ridge region, and one in the Southwest region. Replicated randomized experimental designs were used and included several commercial standard rootstocks for comparison.2) Seventeen rootstock trials planted prior to summer 2018 (as described in the Proposal Appendix ii) were monitored and used for data collection on field performance, as appropriate during this quarter for the scion involved. Yield and fruit quality data were collected from four Hamlin and one pummelo field trial. Three new USDA rootstocks were released on 2 November 2018, identified as US SuperSour 1 (SS1), US SuperSour 2 (SS2), and US SuperSour 3 (SS3), and are freely available without restriction. An informational sheet with performance data on the three rootstocks was prepared and distributed widely to industry, and provided to CRDF. All three new rootstocks demonstrated good fruit quality with sweet orange and superior fruit yield in trials, compared with Sour orange, Swingle, and Cleopatra rootstocks. Seed source trees have been planted with all three new rootstocks, although initially propagation will be limited to cuttings and micropropagation from FDACS clean source material.

T. McNellis **18-016** "Testing grapefruit trees expressing an anti-NodT antibody for resistance to HLB" - The research agreement contract was finalized in January 2019, and funds became available in February 2019. Dr. McNellis initiated a search for a graduate student to perform the work through the Department of Plant Pathology and Environmental Microbiology graduate program and the Plant Biology graduate program at Penn State. A suitable candidate was interviewed in February 2019, and an offer of admission was made in March 2019. A rotation student in the Intercollege Program in Plant Biology at Penn State also expressed interest in the project and began a rotation in Dr. McNellis' lab on March 5, 2019, which will continue for at least the remainder of the spring semester. It is likely that one or both these students may become available to work on the project. The grapefruit trees expressing the FT-scFv anti-HLB

antibody protein were continuously maintained at Penn State and at the USHRL in Ft. Pierce, FL, and continue to grow normally and are ready for analysis.

Z. Mou **18-017** "Establish early-stage field trials for new HLB-tolerant canker-resistant transgenic scions" – Objective 1) Remove the flowering-promoting CTV and the HLB bacterial pathogen in the transgenic plants HLB-tolerant transgenic plants were treated under alternating temperature conditions (25°C for 4 hours and 42°C for 4 hours). New shoots that emerged from the previously treated trees were tested by ELISA for CTV and by qPCR for CLas. Both CTV and CLas were not detected in the new shoots. The new shoots were grafted onto rootstocks to generate CTV and CLas free plants, but the number is small. More shoots to make additional grafted plants are being produced.
Objective 2) Graft CTV- and HLB-free buds onto rootstocks. About 50 transgenic progeny plants that are HLB tolerant were confirmed to be CTV negative by ELISA and CLas positive by qPCR. The majority of these plants have no HLB symptoms and three plants show mild HLB symptoms. These trees will be planted in May at the Picos Farm with assistance from Dr. Ed Stover, Ft Pierce USDA ARS.

E. Stover **18-022** "Delivery of Verified HLB-Resistant Transgenic Citrus Cultivars" - 1) Mthionin Constructs: Assessment of the Mthionin transgenic lines is continuing apace. Detached leaf assays, with CLas+ ACP feeding, have been conducted and lines with the most promising results have begun greenhouse studies. These studies (With 9 Carrizo lines and 4 Hamlin lines, 98 total plants with controls) include graft inoculation of Carrizo rooted cuttings with CLas+ rough lemon, no-choice caged ACP inoculation of Carrizo rooted cuttings, and no-choice caged ACP inoculation of grafted Hamlin on Carrizo with all combinations of WT and transgenic.
The first field plantings with Mthionin transgenic Carrizo (45 plants) have been made with leaves of non-transgenic rough lemon on transgenics showing higher average CLas CT, significantly decreased leaf mottle and significantly increased health values after 6 months. Plants for follow up field plantings of transgenic Hamlin on WT Carrizo (112 plants), WT Hamlin on transgenic Carrizo (84 plants), WT Ray Ruby on transgenic Carrizo (118 plants) and WT Valencia on transgenic Carrizo (118 plants) with WT controls are being propagated.
Seeds for additional scion variety transformations have been collected and germinated. Shoots will be developed enough to yield epicotyl tissue for Mthionin construct transformations in 2 (Hamlin), 4 (Ray Ruby) and 6 (Valencia) weeks.
2) Citrus Chimera Constructs: Detached leaf assays, with CLas+ ACP feeding, were conducted on lines representing chimera constructs TPK, PKT, CT-CII, TBL, LBP/'74', `73', and `188'. Multiple lines from several constructs were moved forward into greenhouse studies based on these results as noted below. Definitive results for TPK, PKT, CII, and TBL were hindered by low inoculation rates. Assays for these constructs are being repeated to identify which lines of each are best suited for greenhouse studies. Detached leaf feeding assay protocols have also been adjusted to improve sensitivity (See section 4). No-choice caged ACP inoculation has been conducted on 8 lines of citrus Thionin-lipid binding protein chimeras (`73', and '74'). Three-month data has been collected, while many plants are yet to show CLas DNA amplification, there is a statistically significant reduction (13x) in CLas titer for transgenics vs WT in the CLas+ plants. An additional 475 rooted cuttings have been propagated from chimera constructs (6 lines of `188', 7 lines of `74' and 12 lines of `73') for the next round of ACP inoculation trials.

3) ScFv Constructs: Greenhouse studies on the 5 scFv lines in the 1st round of ACP-inoculation has been completed with the best performing lines showing significantly reduced CLas titer over the 12-month period (up to 250x reduction) and a much higher incidence of no CLas rDNA amplification in all tissue types at the conclusion of the study. The best lines have been used as rootstock for WT Ray Ruby scions and will be moved to the field once the graft union is strong enough. An additional 129 rooted cuttings are propagated for additional grafts and field plantings. ACP inoculations were conducted on 150 more plants from 12 scFv lines. Data from 3 months post inoculation have been collected, but too few plants are testing positive at this time for a conclusive analysis. An additional 370 rooted cuttings have been propagated from the remaining scFv constructs/lines to be tested and will soon be mature enough for ACP inoculation.
4) Screening Development and Validation: Details of the high throughput ACP homogenate assay, and its use for selecting lytic peptides for activity against CLas, has been submitted for publication and remains in use for early screening of therapeutics in the lab. The detached leaf ACP-feeding assay has undergone several small revisions to improve sensitivity and maintain consistent inoculation; increasing from 10 to 20 ACP per leaf, decreasing the feeding period (7 days to 3) and adding a 4-day incubation period between feeding and tissue collection.
An array of phloem specific citrus genes has been selected for investigation as potential reference genes to improve detached tissue and plant sampling techniques. The use of a phloem specific endogene would allow for samples to be normalized to phloem cells instead of total citrus cells, more accurately evaluating bacterial titer and potential therapeutic effects with the phloem limited CLas.
5) Transgene Characterization: Transgenic Carrizo lines expressing His6 tagged variants of chimeric proteins TBL (15 lines), BLT (15 lines), TPK (17 lines), and PKT (20 lines) have been generated and confirmed for transgene expression by RT-qPCR. These plants will be used for generating data on the movement and distribution of transgene products in parallel to antibody-based approaches.

U. Albrecht **18-028C** "Comparison of field performance of citrus trees on rootstocks propagated by seedlings, cuttings, and tissue culture" – One field trial was established at the UF/IFAS SWFREC farm in November 2017. Two commercial trials were established in Collier County and in Polk County in April of last year. Prior to planting, a subset of trees was destructively sampled to assess root system architectures and other plant growth parameters in detail. Data from these measurements are being prepared for publication. Since trial establishment, measurements of tree height, canopy size, scion and rootstock trunk circumferences, and canopy health. Bases of rootstocks were assessed for potential abnormalities arising from propagation. Root growth has been monitored monthly in the SWFREC trial with minirhizotrons (long plastic tubes) and an associated camera and software system. Additional rhizotrons were prepared for installation to the root zone in a subset of trees in March at the Polk County and Collier County locations. Root growth measurements will commence in April. Propagation of new plant material in the DPI-approved USHRL greenhouses commenced for 4 rootstock cultivars were used. Rootstock liners were generated from seed, from tissue culture, and from cuttings, and were grafted with `Valencia' scion. Trees are anticipated to be ready for field planting in September of this year.

U. Albrecht **18-029C** “Evaluation of citrus rootstock response to HLB in large-scale existing field trials using conventional and automated procedures” - Due to the urgency of data collection from the Lykes large-scale field trials, preparation for harvesting `Hamlin' fruit in the months began prior to start of funding. Existing field maps were modified, and new worksheets were created containing unique tree identification numbers. Multiple field visits were conducted to label individual trees with plastic tags containing the unique identification numbers. In December 2018, yield data was collected from the `Hamlin' Fort Basinger location (Highlands County) and in January 2019, yield data were collected from the `Hamlin' Lake Wales location (Polk County). Data were sent to CREC for growers' access on the https://citrusresearch.ifas.ufl.edu/trial-overview/ website. Horticultural assessments include tree height, canopy volume, and canopy health ratings commenced. Due to the large scale of the trials, these measurements are taking considerable time. Considerable effort is also being dedicated to accurately document and organize data for statistical analyses. In March 2019, fruit were collected to conduct fruit quality analyses from `Valencia' trees at both Fort Basinger and Lake Wales. Due to the high costs of fruit quality analysis and for statistical purposes, only trees of replicated rootstock cultivars were sampled and six replications per rootstock were included. Harvest of Valencia trees is scheduled for April 2019.

Y. Ampatzidis **18-033C** “Automated root mapping to enhance field trial evaluation of citrus rootstocks in the HLB era” - The influence of several factors on the capability of ground penetrating radar (GPR) to accurately detect citrus roots were evaluated. Single-factor experiments were conducted for: (i) GPR frequency (900 and 1,600 MHz); (ii) root diameter; (iii) root moisture level; (iv) root depth; (v) root spacing; (vi) survey angle; and (vii) soil moisture level. Two multi-factor field experiments were conducted to evaluate the performance of the GPR for development of 3D morphology root maps.
Initial experiments showed that the 1,600 MHz GPR was more accurate in detecting citrus roots and their location than the 900 MHz GPR. Upon target (root) detection, the GPR generated a hyperbola in the radar profile. The width of the hyperbola was used to determine the diameter of roots larger than 0.5 cm. GPR was able to distinguish live from dead roots.

K. Pelz-Stelinski **18-051C** “Improving bactericide therapy for young tree protection and inoculum reduction” - During the past quarter, we have identified field sites, procured materials needed for the upcoming field trials, and finalized the experimental design for the trials that will take place over the next two years. These experiments will be conducted in three separate groves, two commercial sites (young trees) and one research grove (mature trees) at the CREC. Trees in the study will receive insecticide treatments (6 application per year) consisting of a combination of soil-applied insecticides and foliar sprays. As a negative control, young trees will be enclosed in exclusion netting (‘Tree Defender’) to prevent ACP feeding and Las inoculation. These trees will be compared to unenclosed trees treated with foliar applications of bactericides. Special permitting is not required to test any of the products used in this project. Treatments will be applied to trees as follows: 1) Fireline (oxytetracycline HCL), monthly application alternating with Firewall (streptomycin sulfate), semi-annual application of each product (4 total applications/yr); 2) Fireline (oxytetracycline HCL), monthly application alternating with Firewall (streptomycin sulfate), bi-monthly application of each product (6 total

applications/yr); 3) negative control (insecticide + exclusion netting); and 4) positive control (insecticide only). All antimicrobial treatments will be applied at label rate with a recommended adjuvant. The initial treatment will be applied after bloom, during the spring flush in late March/early April.

E. Stover **18-058C** "Fort Pierce Field Test Site for Validating HLB and/or ACP Resistance" - A number of trials are underway at the Picos Test Site funded through the CRDF. In the last quarter:
1) A replicated planting of 32 transgenic trees and controls produced by Dr. Jeff Jones at UF was established. These trees include two very different constructs, each quite specific in preventing infection be the citrus canker pathogen.
2) An experiment on pollen flow from transgenic trees was initiated. Data on flowering overlap was collected for transgenic US-802 and non-transgenic FF-5-51-2. The FF-5-51-2 trees, located slightly more than 1000 ft from the US-802, are self-incompatible and mono-embryonic. When seed mature in the FF-5-51-2, thousands will be collected and tested for nptII, the transgene in the US-802.
3) Controlled crosses were also made of US-802 pollen onto 36 flowers of FF-5-51-2. This will demonstrate cross-compatibility and provide seed to validate testing protocols.
4) If pollen from transgenic trees is not detected from open-pollination, it should reduce isolation distances required by BRS.
5) Annual BRS inspection of the planting site was conducted.

N. Wang **18-064C** "Evaluation of the control effect of bactericides against citrus Huanglongbing via trunk injection" – The purpose is to evaluate the control effect of bactericides via trunk injection. Objective 1. To determine whether application of bactericides via trunk injection could efficiently manage citrus HLB and how bactericides via trunk injection affects Las and HLB diseased trees. Three field trials to investigate how the application of bactericides via trunk injection affects citrus growth, production, HLB symptom development, and Las population in different aged trees at different levels of HLB disease severity. We evaluated the inhibitory activity of OTC against Las in greenhouse and field experiments. Citrus trees were trunk-injected with OTC, and leaves were inspected for Las populations and OTC residues using qPCR and HPLC assays respectively, at various times after OTC treatment. We have acquired data about the MBC of OTC in planta. We will repeat this experiment. Objective 2. To examine the dynamics and residues of bactericide injected into citrus and systemic movement within the vascular system of trees and characterize the degradation metabolites of bactericides in citrus. A field trial has begun to determine the concentrations of bactericides in leaf, stem, root, flower, and fruit using HPLC at the following time points: 2, 7, 14, 28 days, 2, 4, 6, 8, 10, and 12 months after injection at different doses. Objective 3. To determine whether trunk injection of bactericides could decrease Las acquisition by Asian citrus psyllids (ACP). We will determine whether trunk injection of bactericides at three different doses could decrease Las acquisition by ACP in greenhouse and in the field. We are conducting the experiment right now. Objective 4. To monitor resistance development in Las against bactericides and evaluate potential side effects of trunk injection of bactericides. Las-specific primers were designed to target the putative binding sites of OTC in 16SrRNA gene of Las. Plant genomic DNA was extracted from citrus trees received OTC injection for three years. PCR were performed with the primers and

DNA samples, and the products were purified and subjected to DNA sequencing. No mutation was identified yet. We will continue to monitor the resistance development against OTC and Streptomycin.

E. Stover **18-065C** "High -Throughput Inoculation of Transgenic Citrus for HLB Resistance" - CRDF funds support high-throughput inoculations with CLas+ ACP to evaluate HLB resistance in citrus germplasm developed by Drs. Ed Stover and Kim Bowman.
1) As of December 2018, a total of 14,111 plants had passed through the inoculation process. A total of 361,255 psyllids from colonies of CLas-infected ACP were used in inoculations. After inoculations, plants were returned to the breeders and subsequently subjected to further evaluation in the field.
2) In addition to inoculating germplasm, infected psyllids were supplied to other researchers for other purposes. This side of the project grew over time, but detailed records were not maintained on how many were given out until 2018. In 2018, more than 10,000 infected psyllids were supplied to the research community for an array of experiments. Recipients included researchers with USDA in Fort Pierce, Ithaca and Beltsville, UF in Gainesville, Cornell in Ithaca, University of California, and University of Nevada.
3) In most recent quarter, the 35-day federal government shutdown, and the threat of a possible shutdown on Feb 15, directly disrupted initiation and conduct experiments using the CLas+ ACP colonies. In addition, considerable rehabilitation of colonies and supporting plants was necessary due to the minimal care that could be provided during the shutdown. During this quarter, 2400 CLas+ ACP were used for detached leaf assessments of plants expressing three different transgenic constructs. Return to normal supply and demand in the current quarter is anticipated.

b. Gene technology, e.g., deploying resistance genes, antimicrobial peptides

Y. Li **16-001** "Enhancing Genetic Transformation Efficiency of Mature Citrus" – Progress Report due 4/15/19 not received.

N. Wang **16-005** "GFP labeling of Candidatus Liberibacter asiaticus in vivo and its applications" - We are testing new methods for culturing Las using co-culturing of Las with citrus tissue culture and psyllid tissue culture. Currently, we are in the process of establishing a pure psyllid cell culture. We have used two approaches to label L. crescens. Preliminary data showed one approach works for Las in vitro. We are testing whether we can label Las in vivo and observe its movement. 2) We have conducted Las movement and multiplication in planta based on qPCR method. We have tested approaches to prevent Las movement in planta. In addition, based on the movement of Las in planta, we have developed a method for targeted early detection of Las before symptom expression. 3) We have been testing the effect of different control approaches including application with bactericides. One manuscript entitled: "Control of Citrus Huanglongbing via Trunk Injection of Plant Defense Activators and Antibiotics" has been published by Phytopathology. In addition, based on the movement of Las in planta, we have

developed a method for targeted early detection of Las before symptom expression. A manuscript entitled "Targeted Early Detection of Citrus Huanglongbing Causal Agent 'Candidatus Liberibacter asiaticus' Before Symptom Expression" has been published by Phytopathology. We also determined the in planta minimum inhibitory concentration of oxytetracycline against Candidatus Liberibacter asiaticus effective for control of citrus Huanglongbing which is being submitted to Phytopathology. This will be important for oxytet residue work.

Y. Duan **16-007** "Field evaluation of the selected variants of Ruby Red grapefruit volunteer seedlings for greater HLB resistance/tolerance" - Progress Report due 4/15/19 not received.

E. Triplett **16-009** "Developing second generation antimicrobial treatments for citrus greening disease" – Progress Reports due 1/15/19 and 4/15/19 not received.

BAYER Executive Summary: D. Manker **16-026C** "Establishment and application of tools to allow a systematic approach to identify and characterize hits with confirmed in planta HLB activity" – During the first quarter of 2019, the screening cascade has become operational. Screening of Anti-bacterial microbes is on track with 2053 strains screened from 260 distinct species. From this set, 116 have gone through confirmation and 81 strains have been scaled up. Identification of active chemistry was identified from advanced strains and work is ongoing with lead strains from the screening process. On the PDM team, twenty compounds from the lead hit class have been synthesized and are being profiled to see if improvements have been achieved on plant defense response as well as other characteristics such as phytotoxicity and stability. For the in planta assays, improvements were made to reduce variability in the tomato Lsol assay in a collaborative effort between Bayer and UC Davis. Lead strains have been tested for phytotoxicity at UF in citrus and the first test substance has been applied to HLB infected trees in the greenhouse assay. Three field sites have been identified to begin field trial set up which includes three geographic regions with testing on Valencia, Vernia and Hamlin. Two trial sites have been planted and third is set up to be planted by May. First applications of PDM treatments were applied at two sites at the end of April.

M. Dutt **18-007** "Investigating the role of transgenic rootstock-mediated protection of non-transgenic scion" - 1) Expression of AtNPR1 gene in transgenic Swingle and Carrizo using juvenile and mature tissue transformation was confirmed by qPCR and trans-protein production using western blot analysis.
2) Cuttings of several lines have been propagated and budded with non-transgenic Valencia sweet orange for outplanting at an USDA-APHIS approved field site.
3) Seed source trees are being prepared by budding onto US802 rootstock.
4) Transgenic rootstocks expressing other promising transgenes are being produced using the mature tissue transformation.

E. Rogers **18-019** "Phloem specific responses to CLas for the identification of novel HLB Resistance Genes" - Our project is examining phloem gene expression changes in response to CLas infection in HLB-susceptible sweet orange and HLB-resistant Poncirus and Carrizo (a sweet

orange - Poncirus cross). Identified genes will represent unique phloem specific targets for CRISPR knockout or overexpression, permitting the generation of HLB-resistant variants of major citrus cultivars. Our grant started December 1, 2018. In the last three months, we have processed all the paperwork needed to establish the grant and begin spending funds at ARS. We have identified a qualified and interested post-doctoral researcher, Dr. Tamara D. Collum, who we will be hiring with grant funding. However, all ARS hiring actions, even those using soft funds, are currently on hold at the Department of Agriculture level. Now that the department has a full-year budget, we hope this hold will be lifted shortly so we can bring Tami on board in the next couple weeks. Objective 1 (development of transgenic constructs) is close to completion and work has begun in the Stover lab on Objective 2 (production of transgenic citrus lines). For objective 6 (Additional Approach: Phloem limited citrus tristeza virus vectors will be used to express the His-FLAG-tagged ribosomal protein in healthy and CLas infected citrus) inserts have been assembled and sent to Dr. Dawson's lab for inclusion in CTV vectors and subsequent introduction into citrus.

N. Wang **18-025** "Optimization of the CRISPR technology for citrus genome editing" – 1) As a proof of concept, CsLOB1 was targeted through transient expression of Cpf1-crRNA via Xcc-facilitated Agrobacterium infiltration. CRISPR-LbCpf1 was demonstrated to generate transgenic Duncan grapefruit line #D35s4 that blocked XccΔpthA4:dCsLOB1.4 infection.
2) The promotor driving expression of Cas9 and Cpf1 was optimized for CRISPR gene editing
3) Transient expression of Cas9/sgRNA plasmid and Cas9 protein/sgRNA ribonucleoprotein complex was conducted in citrus protoplast. The plasmid-transformed protoplast has 1.7% editing efficiency, and the RNP-transformed samples have approximately 3.4% editing efficiency. The genome modified protoplast cells are undergoing regeneration. The goal is to increase the efficacy to over 20% and eventually generate non-transgenic genome modified citrus.

c. Gene delivery, e.g. plant transformation technologies, CTV vector

N. Wang **18-026** "Control citrus Huanglongbing by exploiting the interactions between Candidatus Liberibacter asiaticus and citrus" - 1) 21 outer membrane proteins have been cloned and the putative targets in citrus are being identified using Yeast 2 hybrid (Y2H) system. Potential PAMPs from Las (either homologous to known PAMPs or pilin genes) LasFlaA (flagellin), LasEF-Tu, LasCSP (cold shock protein), LasSSBP (single strand binding protein) and pilin assembly genes (named LasPil85, LasPil95, LasPil105 and LasPil115) were cloned under 35S promoter and the Arabidopsis phloem specific promoter SUC2 and introduced into Agrobacterium. Receptors will be tested in transgenic tobacco and citrus.
2) Selected PAMP receptors are being cloned and overexpressed in citrus.
3) Y2Hs are used to identify effector targets in Valencia sweet orange. Targets are being confirmed using other approaches including BiFC and co-IP assays.

V. Orbovic **18-066C** "Support role of the Citrus Core Transformation Facility remains crucial for research" - 1) In the first quarter of 2019, CTF received 5 orders for transgenic Duncan grapefruit plants and 4 orders for transgenic Valencia plants.

2) During this period, CTF produced 77 transgenic plants: 42 Duncan grapefruit, 19 Mexican lime, 11 pommelo, 4 kumquat, and one Pineapple sweet orange. These plants are the output from 15 different orders.

J. Zale **18-067C** "Continued Funding for the Mature Citrus Facility to Produce Disease Tolerant, Transgenic Citrus" - 1) During this quarter, 11 mature transgenics were produced with Agrobacterium and have been micrografted. These represent two genetic constructs with GFP transformed into Hamlin. An inordinate number of transgenics were produced which is unusal in mature citrus. This result is attributed to a cultivar x gene construct interaction.
2) Transgenics produced for Dr. Zhonglin Mou with a promising disease resistance gene did not rearrange in mature citrus. Using a RecA- Agrobacterium strain, the majority of transgenics retained the transgene in the proper sequence.
3) A manuscript accepted for In Vitro Cellular & Developmental Biology-Plant reports that phosphomannose isomerase (PMI) after biolistics in immature citrus increased transformation efficiency (~2 transgenics per paired shot). As a result of these findings PMI is being tested as a new selectable marker in mature citrus.
5) A protocol for precipitation of DNA onto gold particles that increases efficiency of biolistics in other plant species is being evaluated for citrus.

d. Horticultural Practices

A. Schumann **15-023** "Citrus nutrition studies for improved survival of HLB-affected trees" - Objectives: 1) Leaf nutrient thresholds for HLB trees. We implemented the full Diagnosis and Recommendation Integrated System (DRIS) method for leaf nutrient analysis that provides leaf nutrient analyses with protection from cross-correlation of variables and environmental effects. Reference nutrient data for DRIS was obtained from high-yielding 'Hamlin' trees growing in the Ft. Meade area prior to HLB (>700 boxes/acre average). Critical nutrient thresholds from HLB+ trees in this survey study were higher than published values for K, Cu, B; slightly higher for Mn, Zn {Note: these are not yet recommendations}. We obtained additional leaf data from collaborating grower's commercial grove databases in order to expand our new critical nutrient thresholds to include N, P, S, and Ca. The data that we collected during this project survey did not include enough values in the deficient range for those nutrients, which prevented us from establishing critical nutrient thresholds. The results of this research were presented at grower events: Citrus Nutrition Day, Bartow; Citrus School, Arcadia; Citrus Institute, Avon Park, and published in the April 2019 Citrus Industry magazine.
2) Determine soil conditions that favor root hair and VAM proliferation. Based on good results with healthy Carrizo rootstock trees grown hydroponically, we set up a final experiment where infected psyllids were allowed to inoculate some replications of the experiment with CLas. This treatment serves to monitor the impacts and interaction of HLB and nutrient solution treatment, as measured in root hair growth (until about May 2019). The final report will be completed in June. A paper on this research will be presented at the FSHS conference in June.

E. Triplett **18-024** "Foliar phosphate fertilization: a simple, inexpensive, and unregulated approach to control HLB" – Progress report due 3/15/19 not received.

F. Alferez **18-032C** "Preventing young trees from psyllids and infection with CLas through use of protective netting" - Objective 1. Assessing tree growth and absence of psyllids and HLB disease symptoms (including CLas bacteria titer) under individual protective covering (IPC). 2. Assessment of alternative netting approaches involved in 'targeted', 'alternated' or 'patterned' setup of IPC in groves for more cost-effective protection. 3. Monitoring the transition from vegetative to reproductive stage in the covered trees as compared to the uncovered. 4. Comparing IPC with CUPS-like systems. Progress Obj. 1: We assessed the trees (Valencia on Swingle) planted in our pilot study 14 months ago for HLB. 1/3 of the uncovered trees in the trial are already positive for HLB, all trees covered with IPC have tested negative. We are comparing leaf drop in both treatments. 2. We are now in the process of planting Tango', 'SugarBelle' and 'US Early Pride' trees with collaborators in Polk County. 3: The trial for this objective starts this month in Polk county and has started already in our grove at SWFREC. We have seen a significant advancement in flushing timing in IPC-covered trees. We have not seen any differences in blooming rate or intensity in IPC trees as compared to uncovered trees. We are rating the type of flushes on each tree. 4: We are currently planting 100 trees of Tango', 'SugarBelle' and 'US Early Pride' in our CUPS facility, after trellis installation. A Ms student, Susmita Gaire, has joined the Project and is performing part of this work for her Masters degree. Results from this Project have been already presented at the Citrus Show in Fort Pierce this past January, and an update was presented in Riverside at the IRCHLB meeting.

L. Duncan **18-036C** "Cover crops and nematicides: comprehensive nematode IPM across the grove landscape" – Three field trials were initiated in two orchards near Frostproof FL. Both had 15-month-old Valencia trees on Kuharske rootstock planted at 10x20 foot spacing. Trees exhibited variable growth and health and are infested by sting nematode. The first trial to determine the effect of perennial peanut in middles with or without oxamyl in rows was designed as a randomized complete block within four rows. Treatments comprise 4 adjacent trees replicated 4 times (complete factorial arrangement of untreated, peanut, oxamyl, peanut + oxamyl). We purchased the perennial peanut as sod that was installed on 5 March and is being watered by hand 3 times weekly until established. The second trial compares the efficacy against sting nematode of oxamyl, aldicarb, fluensulfone, fluopyram, fluazaindolizine, and an experimental compound (Syngenta) in a randomized complete block design with eight, four-tree replicates. All plots were plumbed across 16 rows to be treated by injecting the materials into a single, central manifold feeding the appropriate microjets. Two products per day are being applied with the irrigation cycle and the first treatments of all products will be completed this week. The third trial is in a nearby orchard in which Sunn hemp has been sown in tree middles in such a way that eight, four-tree replicates will be arranged in a randomized complete block design testing effects on sting nematode and tree health of the complete factorial treatments of untreated, oxamyl treated, sunn hemp treated, and oxamyl + sunn hemp. Sunn hemp was planted on 7 March. Trunk girth of all experimental trees in all trials was measured on 6-7 March.

E. Johnson **18-041C** "Characterizing HLB-pH interaction to improve management of root function and tree health" - Objectives: 1) Identify optimal pH range for root function and minimize root turnover on HLB-affected rootstocks and 2) determine how uneven pH levels in the root zone (e.g. irrigated vs. row middle portions of root system) affect the overall health of

the tree. This is being done in a split root system in the greenhouse where pH of different parts of the root system can be maintained.
We are in the final stages of rhizotron construction which was slightly delayed because of the late Valencia harvest this year for other projects combined with an unexpected loss of a staff member that will soon be replaced. The Masters student has assisted a member of Tripti Vashisth's lab with the 2nd repetition of the experiment that created the foundation of this project to become familiar with techniques that will be important for maintaining pH and collecting data. We expect to initiate treatments before the end of May.

D. Kadyampakeni **18-042C** "Development of Root Nutrient and Fertilization Guidelines for Huanglongbing (HLB)-Affected Orange and Grapefruit" - The purpose of the project is to develop new guidelines for restoring root health and improving overall tree nutrition in Florida oranges and grapefruit. Objectives: 1. Determine optimal nutrient concentrations in roots and leaves for grapefruit and orange varieties. 2. Compare fertigation, soil, and foliar fertilization to identify best application method for uptake of nutrients into both underground and aboveground components. 3. Investigate the relationship between root and leaf nutrient contents to tree health, yield, and fruit quality as well as bacteria titer. 4. Generate updated and new guidelines for optimal nutrient contents for roots and leaves for HLB-affected trees. Progress: At three sites, CREC, Southern Gardens Citrus near Clewiston, FL and Indian River Research and Education Center (IRREC), preliminary data on yield, canopy size, HLB and other disease ratings, soil characteristics and tree and root health and nutrition are underway and will be reported in the next quarter. Nutritional treatments will be applied starting in spring 2019. One graduate student (CREC), 5 agricultural assistants (IRREC and CREC) have been recruited to work on the project. A search for another graduate student (IRREC) has been completed and the person will start in Fall 2019. Co-PIs (Drs. Kadyampakeni, Rossi, Ferrarezi and Johnson) on the project presented some of their on-going work at the Citrus Show in Fort Pierce, FL and indicated that results from this project will be used to refine current citrus nutrition guidelines.

R. Niedz **18-050C** "The effect of the ionization state of iron and citric acid on the health of HLB-infected trees" – An experiment was designed and setup for Objective 3, Effect of Fe2+ and citric acid treatment on HLB titer of model HLB system determined. The experiment tests F11, a high Fe2+ product sold only in Japan and which was developed from Patent US 8,945,631, Liquid for treatment of citrus greening disease and treatment method using same. Greenhouse-grown citron plants propagated from HLB-infected citron were tested by RT-PCR. Twenty-two plants that tested positive and showed HLB symptoms were divided into two groups with eleven plants in each group. The plants are growing in Ruck's pots. One group is being treated with F11 + Siltrate, and one group is being treated with Siltrate only and serves as the control group. F11 is foliar and soil applied at 150 ppm Fe weekly. For soil application 40 mls is added to each pot. Siltrate Advanced (Meherrin Inc., Severn, NC), is a nonionic siloxane surfactant, and is used at 1.3 ml/L. The experiment was interrupted by the Federal Government shutdown and plants were not treated. The plants were cut back to 25 cm, cuttings weighed, and treatments resumed. Plants will be treated for 6 months. A similar experiment will be setup using Treatment solution B as described in Patent US 8,945,631. Treatment solution B is a high Fe2+ formulation that includes $FeSO_4 \cdot 7H_2O$ and citric acid.

The subcontractor is running the field experiments Trees were ordered and have been planted (12/19/18) for Objective 7, Determine the effect of Fe2+ + organic acid solutions on newly planted (<2 years old) field trees.

J. Qureshi **18-052C** "Sustainable Management of Asian citrus psyllid (ACP) and Citrus Production" – The goal of this project is to develop integrated, sustainable and economically viable psyllid IPM management programs for conventional and organic citrus producers and improved yields. Some organic ACP-HLB control programs such as Azera, Aza-direct, Entrust, Grandevo, Microthiol, Pyganic, Surround, Venerate and in combination with HMO FL 435-66, have shown a potential to provide significant reductions in psyllid populations and produce yields comparable to conventional program. Naturally occurring populations of the predators such as spiders, lacewings and ladybeetles and parasitoid Tamarixia radiata released on regular basis in these programs were additional factors which contributed to psyllid control and yields. The proposed IPM program for conventional growers will include synthetic and organic insecticides including biopesticide products to complement conventional products to reduce the risk of insecticide resistance. The IPM program for organic producers will include organic insecticides including biopesticides and oils. The conventional only and HMO only programs will serve as comparison against IPM programs with more tools available from both production systems to evaluate costs and benefits. These programs will also provide opportunities for beneficial insects to enhance biological control, which has been diminishing through the constant use of the hard chemistry insecticides. Naturally occurring populations of predators and parasitoids and additional introductions of commercial predators and mass reared Tamarixia will further strengthen these programs. Sampling of the experimental block designated for IPM programs in the Gulf region revealed psyllid populations at 0.3 adults per tap sample. Spiders and lacewings averaged at 0.14 and 0.05 per tap sample, respectively. Spray applications in different IPM programs will begin after the block is harvested in April. Positions for the postdoc and temporary assistant were announced and applicants are being evaluated.

J. Qureshi **18-055C** "Optimizing Benefits of UV Reflective Mulch in Solid Block Citrus Plantings" - Evaluation of young tree production on reflective mulches is being conducted in Gulf, Ridge and River regions of the state. Study locations and plants suitable for all three locations are being prepared. Evaluations include 1) assessment of effects of UV reflective mulch on ACP control, HLB incidence and severity, tree growth and ultimately fruit production, and 2) assessment of ACP control and resistance to insecticides in response to flush synchronization for ACP control using mulch/drip irrigation system on three different soils types. Economic analysis summarizing 3-year and projected costs and benefits of mulch system with and without flush control will be conducted. Positions for the postdoc and temporary assistant were announced and applicants being evaluated.

S. Strauss **18-059C** "Citrus row middle management to improve soil and root health" - Funding for this project was received in December 2018. The treatment blocks were flagged in both groves. Dataloggers and soil moisture probes were purchased and installed in both locations and are currently recording soil moisture every hour. In each grove, 30 minirhizotron tubes were installed 2 ft from the trunk and 1.5 ft into the soil. These will be used to monitor root growth in the cover crop treatments and assess the impact of the treatments on root health

and development. Soil and leaf samples were collected to determine pretreatment tree and soil nutrient concentrations and microbial community composition. Pretreatment assessments of total weed coverage and weed density by type and species were also made. Canopy size measurements were made to determine preliminary trees size characteristics. The first draft of a grove management survey has been prepared. Preparation is underway for the winter/spring cover crop mix to be planted in the next two weeks. Postdoctoral and graduate student candidates have been identified, and interviews are being conducted. Data collection will continue in the next quarter to capture fruit yield and other variables on the project.

T. Vashisth **18-061C** "Evaluating sustainability of yield and fruit quality of sweet oranges with use of controlled release fertilizer and micronutrients" – This project is a continuation of an existing CRDF funded project ending in March 2019) with some added treatments to be evaluated. The additional treatments are:

1. CRF + Tiger Micronutrients+ Mn 50%
2. CRF + Tiger Micronutrients+ Zn 50%
3. CRF + Tiger Micronutrients+ Fe 50%
4. CRF + Tiger Micronutrients+ B 50%
5. CRF + Tiger Micronutrients+ Mn +Zn 20%
6. CRF + Tiger Micronutrients+ Mn +Fe 20%
7. CRF + Tiger Micronutrients+ Zn +Fe 20%
8. CRF + Tiger Micronutrients+ Zn +B 20%
9. CRF + Tiger Micronutrients+ Fe + B 20%
10. CRF + Tiger Micronutrients+ Mn +Zn 50%
11. CRF + Tiger Micronutrients+ Mn +Fe 50%
12. CRF + Tiger Micronutrients+ Zn +Fe 50%
13. CRF + Tiger Micronutrients+ Zn +B 50%
14. CRF + Tiger Micronutrients+ Fe + B 50%

The treatment for objective 3:

1. CRF + Foliar Micronutrients + Tiger 90
2. CRF + Tiger Micronutrients

These treatments will be initiated once the block that was being used for hybrid fertilizer trial (comparison of different rates of controlled nitrogen) is harvested in spring 2019. In December-February 2019, we have surveyed the site, 'Valencia' on Swingle between 11-15 years old, set up an experimental layout for the newly added treatments, and collected pre-treatment data on tree growth, leaf and soil nutrient analysis. The fertilizer applications will be made in spring 2019 after harvest of the existing trial. We have also solicited fertilizer donation from Harrell's and Tiger Sul.

4. OTHER CITRUS DISEASES

a. Post-Bloom Fruit Drop

M. Dewdney **16-010C** "Enhancement of Postbloom fruit drop control measures" - Objectives: 1.Determine the efficacy and economics of fungicide treatments; 2) Determine if Luna Sensation has enough systemic activity to protect flowers from before they fully develop and open; 3) Determine if the period flowering of trees affected by HLB huanglongbing can be

narrowed to eliminate the offseason bloom that contributes to the PFD inoculum increase in groves. In 2019, a field trial was set up and treatments were applied in a Valencia grove in Ft. Meade. Only one application was made based on the PFD-FAD or CAS model predictions. Button counts will be collected soon now that flowering has finished. Trees with flowers at different stages were treated in the field with Luna Sensation and branches were collected for inoculation in the lab but the petals fell off the open flowers rapidly so it will be difficult to determine how well the open flowers were protected. The third year of bloom synchronization was undertaken in 2019. All the foliar PGR applications were completed. The initial pre-flowering button counts were done from January to March. In January, the flower counts were started and are still underway. Harvest of the 'Valencia' trees was delayed until April. Navels were harvested and we are currently in the process of analyzing and interpreting the 'Navel' harvest data. Model outputs for predicting infection events were compared in 2 sites of 'Valencia' trees in Ft. Meade and Dundee, FL. In Ft. Mead, one application per the CAS model was applied, one application per the PFD-FAD model, and three weekly applications. In Dundee, there were no applications with the CAS, one application per the PFD-FAD model, and 2 weekly applications. No infected flowers were found in either grove on the major blooms. Button and fruit counts will be taken later in the year. A manuscript to be fully completed by the end of May is in preparation for the Citrus Advisory System (CAS).

M. Dewdney **18-034C** "Improved postbloom fruit drop management and exploring PFD spread in Florida" - Objectives: 1) Conduct field trials of new products and fungicide programs for PFD management and validation trials for the Citrus Advisory System (CAS); 2) Investigate the reasons for the movement of Postbloom fruit drop (PFD) to new areas and recent major outbreaks; 3) Evaluate methods for initial inoculum reduction on leaves so that early fungicide applications could be more effective and identify the constituents of the flower extracts using "omics" techniques.
Objective 1 was covered by project 16-010C and activities are reported there. Unfortunately, we are waiting for USDA-ARS permissions, delayed by the federal government shut down, so we have not started on the experiments with the wind tunnel. In the meantime, in Florida has been started. Five FAWN weather stations were selected for the work on modelling of leaf wetness to better predict PFD outbreaks work. The recorded data was compared to the output of four leaf wetness estimation models, singly and in combination. The most accurate models were considered for modifying the citrus advisory system (CAS). Further analysis to look at the number of recommended sprays and model accuracy are planned. In the initial experiment to assess the effect of available fungicides on the secondary conidiation of C. acutatum on citrus leaves, pyraclostrobin was used but no difference was seen in the secondary conidiation regardless if sugar or water were used for stimulation of conidia. In subsequent experiments, leaves were exposed to sterile water or 2.5% sucrose solutions to stimulate sporulation with or without ferbam. Leaves then were coated with nail polish and the conidia and appressoria stripped from the leaf surface and counted. Ferbam affected the viability of the spores but not the number produced. It also significantly reduced the number of appressoria. A similar technique will be used to evaluate the effect of flower extracts on conidiation to determine which extracts to investigate. Flowers were collected and extracted using water, methanol and ethyl acetate. The yields of all the extracts have been calculated as the future reference. All the

extracts have been dried for testing their antifungal effects. Once we receive the antifungal effects of the extracts, we will start the composition analyses.

b. Citrus Black Spot

M. Dewdney **18-006** "Understanding the underlying biology of citrus black spot for improved disease management" - In this first quarter, we found a field site with sufficient black spot to conduct the skirting and fungicide timing trial. We scouted 100 rows for presence/absence of black spot and chose the best 96. The plots have been laid out and calculations were done for the fungicides and organized with the grower to apply with an airblast sprayer. Two sites have been scouted for a fungicide spray trial. The details will be determined once the incidence and severity have been collected. We plan to be able to use at least 5 products and possibly more. The postdoctoral researcher in South Africa has been appointed from 1 March 2019, and preparations for genotyping-by-sequencing of a collection of Phyllosticta citricarpa isolates from USA has been initiated. There are also continuing contract negotiation between CRI and UF which we hope will be resolved shortly so funds can be received, and the postdoc can continue to work on the project. As we work to collect isolates for objective 3, Jeff Rollins has been working with the UF administration to make sure all the necessary permits are in place for him to be able to travel to Cuba and collect isolates to determine the mating type and species identifications. We are waiting to receive MAT 1-1 DNA from Australia and South Africa to use as positive controls for our experiments.

c. Citrus Canker

J. Jones **18-013** "Using a Multipronged Approach to Engineer Citrus for Canker Resistance" - 1) Transgenic Duncan grapefruit containing the Bs3-executor transgene were tested for resistance to an array of strains representing a worldwide collection. All of the strains elicited a hypersensitive reaction when infiltrated with a bacterial suspension of Xanthomonas citri into young leaves of the transgenic citrus plant but a susceptible reaction when infiltrated into non-transgenic susceptible Duncan grapefruit leaves. During the past three months the Bs3-executor transgene was inserted in a different vector that is acceptable for future regulatory purposes. The previous constructs contained an additional selectable marker that allowed for identifying putative transgenics with a higher success rate. Given that there was concern about the additional marker, the new construct contains only NPT as a selectable marker. The construct was recently sent to Vladimir Orbovic, who is in the process of transforming grapefruit and sweet orange. The Bs3-executor transgenic plant was grafted onto two rootstocks (812 and Sour Orange) for planting in the field at Fort Pierce within the next couple of weeks. We have also identified two other possible transgenics from plants received from Vladimir Orbovic that contain the Bs3-executor transgene. One of the putative transgenics has a growth defect and will be of no use in future experiments. The other putative transgenic tree that appears to contain the gene may be useful for future testing.

2) To determine if EFR-generated transgenic grapefruit plants are resistant to the citrus canker bacterium in field experiments in Fort Pierce. The two most promising EFR transgenic plants (based on ROS activity) were grafted onto two rootstocks (812 and Sour Orange) and are in the process of being planting in the field at Fort Pierce within the next couple of weeks. We have

additional transgenics from plants received from Vladimir Orbovic that will be tested for the presence of EFR.
3) To determine if bs5-generated transgenic Carrizo plants are resistant to X. citri and to generate transgenic grapefruit carrying bs5, trees are being grown and then will be grafted onto rootstocks for further testing.

NOTE: The full progress reports for these projects have been added to the Progress Report Search function of the citrusrdf.org web page.