3. CITRUS HOST INTERVENTION

3B. Horticultural Practices and Impact on HLB

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

1. Track ongoing research on horticultural aspects of HLB management and tree health.
2. Provide communication on project goals, progress and results to CPDC, CRDF and growers

Citrus Host Intervention projects focus on horticultural management practices to manage HLB. There are ongoing projects on controlling HLB using soil/tree microbes and tree nutrition as well as a project on evaluating HLB tolerance/resistance in grapefruit. There also is a report on a continuing service project.

Microbes.

E. Triplett's **16-009** project is on "Developing second generation antimicrobial treatments for citrus greening disease". No Dec 2018 report yet.

From Sept 2018: We are continuing to improve the defined medium (Cruz-Munoz et al. 2018) for the culture of Liberibacter crescens, the closest cultured relative of the citrus greening pathogen. Laboratory tests have shown that low levels of glyphosate (5 mM; Roundup) inhibit L. crescens. Both the L. crescens and CLas genomes possess ESPS synthase, the target of glyphosate. The predicted proteins in both genomes are expected to be sensitive to glyphosate based on amino acid sequence of the codon involved in glyphosate sensitivity. So, in collaboration with Michael Rogers at the CREC, we are now interested in testing whether glyphosate can control citrus greening disease. Even if glyphosate adversely affects citrus, we can engineer it in a non-transgenic way to make it resistant. A provisional patent on the idea has been filed by UF. Our next step is to get field efficacy data.

N. Wang's **16-005** project is on "GFP labeling of Candidatus Liberibacter asiaticus in vivo and its applications." The goal of this project is to generate green fluorescence protein (GFP) labeled Ca. Liberibacter asiaticus (Las), test its application in study of Las movement and its distribution in trees planta, and investigate the control effect of different measurements including heat treatment and antimicrobial treatment. We have elucidated plant-Las interaction through real-time monitoring of Las movement and multiplication in planta using GFP labeled Las. We are investigating the effect of different control approaches on the dynamic population of Las in planta using GFP labeled Las. We are testing co-culturing 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. We have conducted Las movement and multiplication in planta based on qPCR method. We have tested approaches to prevent Las movement in planta. We have developed a method for targeted early detection of Las before symptom expression. We tested different control approaches including application with bactericides: "Control of Citrus Huanglongbing via Trunk Injection of Plant Defense Activators and Antibiotics" has been published by Phytopathology.

Nutrition. J. Grosser's **15-013** project is on "Interactions of Rootstocks and Constant Nutrition to Enhance Profitability of Citrus Plantings in HLB-Endemic Areas". Objective 1. (Greenhouse

experiment): qPCR analysis was completed on all trees to determine CLas titers, and results were received from the Southern Gardens Diagnostic Laboratory. Fourty-four trees that were previously infected, tested negative for CLas, mostly from WGFT+50-7, UFR-3, X639 and Swingle, especially with treatments #5 (Harrell's 12-3-9- St. Helena mix) &6. This suggests that over time, slow release of strong micro-nutrient packages can have a therapeutic effect. Trees have been trimmed and made ready for field planting in the spring, in a possible collaboration with AllTech via Ed Dickinson (and will require a DPI permit). Objective 3: To evaluate the effect of complete, balanced and constant nutrition on HLB-affected mature trees (composition, delivery and economics). In this time period, we did final the final round of fertilization (3rd application). We also collected the final tree health data for 2018 including leaf nutrient analysis. The results with some treatments/locations showing yield and fruit quality improvements, were presented to CRDF board meeting in October 2018. Results were also presented at Nutrition day in December, which was followed by the field day at the Fort Meade trial location. Field day was very well received by growers and grower feedback was very good. Objective 5. (funded by Lee Groves, using donated fertilizer products): Alligator Vernia/Rough Lemon Enhanced Nutrition Experiment – Treatments: 6 tree plots (randomized), 2 plots per treatment – treatments 2 times per year. Positive results showing a therapeutic affect from overdoses of manganese against HLB were presented at the annual ASHS meeting in Washington DC, and a manuscript has now been accepted for publication in HortScience pending acceptable revisions.

A. Schumann's **15-023** project on "Citrus nutrition studies for improved survival of HLB-affected trees". End date extended to 12-31-18 (+ Requested No cost extension) to allow data analysis in hurricane damaged field sites. Purpose is to find the reasons for inconsistent responses of HLB-affected citrus to Enhanced Nutrient (EN) programs and to develop feasible and economical remedies that can consistently replicate successful HLB mitigation with ENs in all Florida groves. The Diagnosis and Recommendation Integrated System (DRIS) method has proved very valuable for indicating ranges of critical deficiency for K, Mn, Zn, Fe. Mg, B, and Cu (but not S and N). These results will be used to revise existing IFAS leaf nutrient thresholds for citrus. To determine soil conditions that favor root hair and VAM proliferation, we captured more SEM images to quantify root hair growth (Dec 2018) which was dramatically increased by tricalcium phosphate in equilibrium with the growing solution. We will quantify root hairs from the images in early 2019. When psyllid adults become available in February, we plan to inoculate the hydroponic citrus trees with CLas so that the impacts of infection and nutrient solution treatment can be measured on root hair growth (until about May 2019). The final report will be completed in May/June.

Y. Duan's **16-007** project is on "Field evaluation of the selected variants of Ruby Red grapefruit volunteer seedlings for greater HLB resistance/tolerance". Field evaluation of the selected variants of Ruby Red grapefruit volunteer seedlings for greater HLB resistance/tolerance. Objectives: to conduct a field trial using the selected Scott Grove grapefruit seedlings (from with greater HLB resistance/tolerance) to evaluate the quality of their fruit. This could produce a more resistant/tolerant variety that could be available without regulatory approval. Four lines of the seedlings were selected for further propagation on three rootstocks (commercial sour orange, newly selected USDA-sour orange and 942). The Brix, sucrose, glucose and fructose, soluble solids, pH, % TA and total ascorbic acid) of the four selected seedlings was not different from their maternal trees. These propagates were grown in our Picos farm under extremely aggressive HLB disease pressure. Here, the older the planting, the higher the disease index. The

new Picos farm HLB isolate also caused severe HLB disease on most of grapefruit seedlings and bud sport selections of in our greenhouse evaluations which were previously either resistant or tolerant to the greenhouse HLB isolates. A second group of the propagates on three different root stocks (Ca. 750 plants) have been budded and grown in our greenhouse and we are expecting planting in the Scott grove within 3 months.

Service Support Projects

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. For the period Oct 1, 18 through Dec 31, 18, a total of 2,120 samples were tested by qPCR. 91% were plant samples and 9% were psyllid samples. This is a marked decrease in the number of samples from the previous quarter and continues the trend of less sample submissions over the last few years. Almost all of the samples appear to come from research trials. For the two-year project, a total of 35,515 samples have been tested (budgeted amount was 45,000). SGC expects that the final number will be approximately 50,000 samples (budgeted amount was 60,000). The final bill will be adjusted as necessary to reflect the total amount of samples actually run.

3C. Deployment of Disease Resistant or Tolerant Citrus Rootstocks and Scions

Narrative of Progress against Goals:

Obj. 1- Track ongoing research projects evaluating emerging scion and rootstock genotypes for tolerance or resistance to HLB, citrus canker, and other diseases.

a) D. Gabriel **15-009** - Two Las repressors from were confirmed as functional transcriptional regulators of Las phage genes. One Wolbachia repressor was confirmed as a functional transcriptional regulator of Las phage genes. A likely Las virulence effector, a secreted peroxiredoxin enzyme, was identified that prevents citrus host phloem cells from killing Las and also blocks systemic host responses to Las. This secreted enzyme is a potential high value chemical target. A high throughput fluorimetric thermal denaturation screen was used to identify chemicals that bind to the Las peroxiredoxin target. Over 300 phytochemicals were screened, resulting in identification of 14 lead candidates for phytochemical control of HLB. One of these chemical candidates was confirmed to inhibit Liberibacter crescens but failed to provide a practical level of reduction of Las titer in heavily infected citrus. This compound will be evaluated further for efficacy in preventing new Las infections in commercial citrus replants.

b) J. Grosser **15-013** – Greenhouse trees on WGFT+50-7, UFR-3, X639 and Swingle rootstocks treated with constant feed fertilizer treatments tested negative for CLas by qPCR analysis. Constant feed fertilizer treatments of mature HLB-affected trees

improved yield and fruit quality in some treatments/locations. A therapeutic effect from high doses of manganese against HLB were observed in one location.

c) D. Hall **15-016** – This service project supported a high-throughput facility to evaluate citrus germplasm for HLB resistance. This screening program supports citrus breeding and transformation efforts by Drs. Stover and Bowman. Research indicated that the no-choice inoculation step averaged 77% effectiveness and speeds delivery of results from germplasm screening to the breeders.

d) V. Orbovic **15-033C** - The Citrus Transformation Facility (CTF) is a service lab for production of transgenic citrus plants for the research community involved in development of resistance of citrus to HLB and citrus canker. CTF produced 781 independent transgenic events in eight different cultivars: Duncan grapefruit, Carrizo citrange, Pineapple sweet orange, Mexican lime, Valencia sweet orange, Swingle citrumelo, Kumquat, and Pomelo. CTF processed 103 orders from 10 research programs including eight faculty members based at University of Florida, one faculty from University of California and one Foundation.

e) E. Stover **15-039C** - Several advancements in evaluation of resistant/tolerant genotypes have been documented at USDA Picos Farm:

1) The UF Grosser transgenic program: transgenic overexpression of an Arabidopsis defense gene was reported to enhance citrus HLB resistance (Dutt et al., 2015).
2) The ARS Stover transgenic program: Trees from many constructs exhibit modest differences while new material planted this spring that has shown promise based on greenhouse evaluation (Hao, Stover and Gupta, 2016).
3) A planting of more than 85 seedling populations from accessions of Citrus and citrus relatives (provided as seeds from the US National Clonal Germplasm Repository in Riverside, CA) demonstrates that P. trifoliata, Microcitrus, and Eremocitrus are among the few genotypes in the citrus gene pool that continue to show substantial resistance to HLB, P. trifoliata displayed reduced colonization by ACP, and measures of HLB-tolerance were associated with percentage citron in accession pedigrees. A UF Gmitter led mapping study is underway using the same planting, to identify loci/genes associated with HLB- and ACP-resistance.
5) More than 100 citranges, from a well-characterized mapping population, and other trifoliate hybrids (+ sweet orange standards) were planted in a replicated trial in collaboration with Gmitter and Roose. Plants were monitored for CLas titer development and HLB symptoms. Data from this trial should provide information on markers and perhaps genes associated with HLB resistance, for use in transgenic and conventional breeding. A manuscript reporting HLB resistance associated QTLs has been published.
6) Hall assessed ACP colonization on a subset of plants and further documented host morphological traits associated with ACP-colonization in Poncirus (Hall et al., 2017a&b).
7) Several USDA citrus hybrids/genotypes with Poncirus in the pedigree have fruits that approach commercial quality, were planted within the citrange site. As of April 2014 at the Picos Test Site, several of these USDA hybrids had grown to a height of seven ft (one

now released as US SunDragon), with dense canopies and good fruit set, while sweet oranges were stunted (3 ft) with very low vigor. These differences largely continue and the observations have encouraged aggressive use of this and other trifoliate hybrids as parents (Stover et al., unpublished).
8) A Fairchild x Fortune mapping population was planted at the Picos Test Site in an effort led by Roose from UCR to identify loci/genes associated with tolerance. This replicated planting also includes a number of related hybrids (including HLB-tolerant USDA 5-51-2) and released cultivars. HLB phenotyping and growth data have been collected and genotyping will be conducted under a new NIFA grant.
9) Valencia on UF Grosser tertazyg rootstocks hat the Picos Test Site for several years, were CLas-inoculated before planting, and several continue to show greater growth compared to controls (Grosser, personal comm.).

f) J. Zale **15-045C** - The Mature Citrus Facility (MCF) provided services including mature plant production using Agrobacterium harboring vectors with disease resistance genes & molecular analyses to show copy number of the transgenes & gene expression.

Introduced new, high yielding cultivars & tests into the transformation process. Increased micro-grafting efficiencies, bypassed it altogether, or used rooted mature scions. Tested different selectable markers & reporters. Developed a stable biolistic transformation protocol for immature and mature citrus and several transgenics were produced. Increased throughput of budded plants in the MCF.

g) Y. Li **16-001.** Genome editing is being applied to knock out the endogenous PAR1 gene in citrus cells to create paraquat resistance to reduce chimeras composed of both edited and non-edited cells. The goal is to produce transgene-free plants derived from mature citrus tissues. Kn1, AGO and NPRD genes, and NPA and IAA were tested for increasing transformation efficiency of mature tissue. Results have been inconsistent. An in-planta transformation method for mature citrus tissues is under development. This method uses Agrobacterium infection of damaged meristem tissues of lateral buds on mature shoots. So far the process has encountered problems depending on how much meristem tissues is surgically removed before Agrobacterium infection.

h) Y. Duan **16-007** - Conducted a field trial using the selected grapefruit seedlings with putative resistance/tolerance. By the end of this year, conclusions will be made from the planting in Picos Research Farm as to whether the selected seedling variants show resistance/ tolerance to HLB pathogens. A second group of propagates on three different root stocks has been budded and grown in our greenhouse, and are expected to be planted in Scott grove within 3 months. To confirm productivity of the selected trees, assays of two year graft-inoculation trees in greenhouse were conducted. Fruit quality (Brix, sucrose, glucose and fructose, soluble solids, pH, % TA and total ascorbic acid) of the four selected seedlings showed no significant difference from their maternal trees.

Obj. 2- Cooperate in in-depth evaluation and planning exercises related to Florida (and the US) citrus breeding to better focus on HLB solutions and rapid evaluation and deployment of

rootstocks and scions.

Obj. 3- Develop and implement plans for expanded management of tolerant and resistant citrus

Obj. 4- Facilitate identification of best performing candidate rootstocks that appear to have HLB tolerance or resistance from Florida (and other) breeding programs

Obj. 5- Implement and evaluate Phase I and II grower field trials of most promising candidate HLB tolerant rootstocks using standard varieties as scions.

4. Other Citrus Diseases (CBS, PFD)

Citrus Black Spot. M. Dewdney's **15-005** project is on "Asexual inoculum production of Guignardia citricarpa, the causal agent of citrus black spot". This project is 1) to determine the temperature and relative humidity optima for CBS infection and production pycnidiospores on citrus twigs, leaf litter, and fruit; 2) To determine whether CBS can survive and reproduce on citrus debris on grove equipment. We found that the more symptomatic trees had a greater amount of P. citricarpa DNA in their twigs than those with low severity. This establishes that there is a connection between the amount of P. citricarpa in the canopy with the severity of fruit infection. What remains to be shown is whether the twigs are the initial inoculum source for the fruit or a sign of the overall infection level. Relative humidity levels below 90% greatly reduced the number of pycnidia or conidia formed. Sporulation was more affected by temperature than relative humidity. This allows us to see when production of conidia is most likely to estimate the greatest inoculum potential. Results of the completed research are consistent with recommendations from FDACS in regards to efficacy of recommended disinfectants. The finding that efficacy diminishes when spores are associated with citrus debris offers an opportunity to update recommendations for hedging operations and other activities that may generate significant amounts of fine debris to ensure that debris is fully saturated with disinfectant solutions.

Post-bloom Fruit Drop. M. Dewdney's **16-010C** project is on "Enhancement of Postbloom fruit drop control measures" including fungicide treatments, Luna Sensation to protect early flowers and to determine if the period flowering of trees affected by HLB can be narrowed to eliminate the offseason bloom and PFD inoculum. There were few consistencies among treatments since in both trials, PFD disease was extremely low and it was difficult to determine if the number of fruit per tree side was solely due to the treatments or other inherent differences among trees. There were no fungicide treatment differences observed. The second year of bloom synchronization was undertaken in 2018. Off-season bloom was suppressed with gibberellic acid (GA) in both Navel and Valencia trees and the major blooms were compressed with fewer flowers after GA but there was no signficant reduction of yield compared to the untreated control. Trees treated with NAA (synthetic auxin) did not have a similar effect. Applications for the upcoming flowering period have been commenced and fruit number data will be collected by July. Field validation of the Citrus Advisory System (CAS) continued in 2018 at 2 sites, Polk City and Fort Meade. There

were no significant differences among the treatments for the number of fruit per tree. No applications were triggered by the CAS. This was the best forecast and significant cost savings could be had by using the CAS.

Obj. 2- Engage registrants and regulatory entities needed for label modifications

Obj. 3- Continue participation in pesticide stewardship activities

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

Obj. 5- Communicate progress and results of project to CPDC, CRDF and growers