Citrus Research and Development Foundation, Inc.

CRDF-Funded Projects Summary Progress Report FY 2021-2022
Quarter Ending December 31, 2022

RESEARCH TOPICS COVERED IN THIS REPORT

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CLAS PATHOGEN INTERVENTION

a. Bactericides/antimicrobials

K. Pelz-Stelinski 21-021 "CLas Inhibition with Antisense Oligonucleotides for Management of Citrus Greening Disease" – 55% Completion of Objectives (November 2022 report)

1. FANA antisense oligonucleotides targeting specific CLas genes were tested by trunk injection in field trees at CREC and compared to oxytetracycline (OTC), insecticide, and untreated controls. The FANA oligos reduced CLas titers through 90 days similar to OTC treatment.
2. Evaluate injection of FANA oligos on psyllid transmission of CLas. Transmission to healthy plants was reduced by the FANA treatments, but less than that for OTC treatment that completely prevented transmission.
3. Progress on the objectives and use of the budget are as they should be at the stage.

b. Diagnostics

M. Irey 21-002C "Continued Support for the Southern Gardens Diagnostic Laboratory" – 98% Completion of Objectives, based on volume of testing (December 2022 report)

This report is for the second quarter of year 2 of Project 21-002C - All samples were plant samples, mostly from large scale research trials. To date 40,112 samples have been run, which is under the budgeted amount of 43,126 for 6 quarters of work.

Lab samples run appear to be on track or possibly slightly under what was budgeted. Whether or not it stays at this level most likely will depend on how many samples growers and researchers submit related to trials with OTC.

To date, the project is essentially on budget. A total of 40,112 samples were run versus a budget of 43,126. As has been done in previous years, the number of samples run at the end of year 2 will be reconciled against the budgeted amount and the last payment will be adjusted up or down as needed.

Whether or not usage stays at this level most likely will depend on how many samples growers and researcher submit related to trials with OTC.

ASIAN CITRUS PSYLLID VECTOR INTERVENTION

a. Asian Citrus Psyllid management

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

S. Santra 22-002 "Management of tree health and huanglongbing disease pressure using advanced Zn formulations" – 30% Completion of Objectives (November 2022 quarterly)

In Central Florida trial (Avon Park grove), all data regarding tree height and canopy as well as canker and HLB incidence were collected. Also, samples for time 0 nutrient analysis and auxin quantification were collected. Unfortunately, Hurricane Ian destroyed many trees and produced a great amount of fruit drop, so Hamlin plots were harvested before data collection was completed. In the Southwest Florida trial (Duda grove) all trees were assessed for tree height and canopy volumes and assessment of HLB and canker incidence has begun. For HLB, most of the trees are category 3 = 26-50% of the canopy with foliar disease symptoms, and category 4 = 51-75% of the canopy with foliar disease symptoms. Canker incidence is so far negligible. Samples were collected for leaf nutrient analysis and auxin quantification.

Treatments with the three advanced liquid Zn products (Zinkicide TMN111, FertiZink, and NuZinc) were initiated. Treatment trees continue to show greener canopies than controls. Control trees had some off-blooms in September and October. These off-blooms were not observed in the treatment trees. Fruit drop is being recorded in Hamlin weekly. Unfortunately, Hurricane Ian increased fruit drop but since data is collected weekly, the fruit drop background noise can be minimized to detect whether treatments reduced fruit drop even under hurricane conditions. Some trees were lost due to the high wind. Interestingly, all trees lost were in a control plot. Other treatments in the same grove with commercial Zn sulfate are showing the same effect on tree loss. There is no clear explanation for this, but fortunately, treatments were not compromised and other trees in untreated plots are being used as new control replacements.

To evaluate the oxidative properties of the FertiZink and NuZinc product formulations, Amplex Red based reactive oxygen species (ROS) assay was performed to measure the production of ROS. Neither of the formulations significantly produced ROS even at 28,000 ppm Zn. These results suggest that the formulations would not cause oxidative stress to plants upon foliar application. Dynamic Light Scattering (DLS) studies were conducted on two batches of FertiZink and NuZinc that were fresh and 1 year old at the time of evaluation. Measurements were conducted at 400 ppm and 800 pm Zn to replicate field spray application rate. The DLS studies of the products showed the presence of micron and sub-micron size particulates, confirming that the products are not in the nano-regime. sedimentation of products was noticed during the DLS measurements, which resulted inconsistent readings. This inconsistency was apparent in both the 1-year-old and the fresh batch. Assessment of the particle size will be further examined through scanning electron microscopy.

Work anticipated for next quarter: Hamlin fruit will be harvestable in the SW Florida trial. Harvest has not been done yet as brix is still around 8.2 (December 13, 2022), but slowly increasing. Zn treatments will continue in the Valencia trees.

Outreach: Alferez, F. Understanding and Managing Fruit Drop in HLB-Affected Citrus. Invited Seminar at American Society of Horticultural Science. January 12, 2023.

Budget status: Underspend as the UF-subcontract has not been invoiced.

CITRUS HOST INTERVENTION

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

U. Albrecht 19-030C "Use of compost and interaction with low- and high-vigor rootstocks to accelerate young sweet orange tree establishment and enhance productivity" – 100% Completion of Objectives (October 2022 Final report)

This three-year project was designed to study the effects of regular applications of compost on Valencia orange tree growth and productivity during the first three years of establishment. Trees were planted on 22 acres of land in a commercial citrus grove in southwest Florida near Felda. The trial was established in 2019 on eight raised beds at a planting density of 15 x 22 feet. The experimental design was a split-plot design with eight replications and a total of 3200 trees (145/acre). The main plot was treatment at two levels: compost or no-compost. For the compost treatment compost was applied at a rate of 5 tons per acre twice annually (May and November). To study the interaction with rootstock, four different rootstocks were included as the sub-plot: US-802, US-897, US-812, and X-639. Trees were arranged in two rows per bed, each row containing 100 trees with each rootstock sub-plot containing 50 trees. As root health is a major concern in the HLB era, this project complemented standard horticultural evaluations with evaluations of root health indicators, including anatomical and metabolic root health traits as well as the rhizosphere microbial community composition. We tested the hypothesis that increased nutrient retention through application of compost during the early phase of tree growth will result in better tree establishment and health and therefore higher productivity once trees become mature. It was found that the biannual applications of compost increased several soil physicochemical properties such as CEC, pH, organic matter, and water content. Despite some variations across sampling times, soil potassium, magnesium, calcium, and boron content were generally increased after compost applications, while soil copper content was decreased. Increases in soil nutrient content translated into increases in leaf nutrient content in some instances such as for potassium. In addition to changes in soil properties and leaf nutrient status, compost amendments resulted in an increase in the fibrous root respiration rate, indicating a higher metabolic activity, but fibrous root length was not affected. Despite improvements in soil physicochemical properties and nutrient status, increases in growth or productivity were not measured after three years of field study. However, the total soluble solids content of fruits was decreased by the compost amendments. There were also differences associated with the rootstock, with US-897 producing the most and best-quality fruits. US-897 also had the longest and finest roots, which may be one reason for the higher fruit quality commonly associated with this rootstock. Changes in the rhizosphere microbiome were also measured in response to the compost amendments, but the impact was only correlated with specific changes in root nutrients for US-812 and US-897. One concern regarding the compost amendments was that they provided a favorable environment for weed growth, evidenced by a higher weed biomass measured in compost-treated vs. non-treated plots in some instances. The competition with weeds may have contributed to the lower soluble solids in fruit measured for trees from compost-amended plots. Taken together, biannual compost applications at the rate and with the methodology used in this study did not result in any measurable effect on tree growth and productivity during the first three years after tree establishment despite some measurable improvements in soil and root health parameters.

U. Albrecht 21-005 "Comparison of field performance of citrus trees on rootstocks propagated by seed, cuttings, and tissue culture" – 25% Completion of Objectives (December 2022 quarterly)

This is a continuation of project 18-028C.

Objective 1: Assess rootstock propagation method and rootstock cultivar effects on growth, health, and productivity of grafted Valencia trees during the early production years (years 4-6 after planting) in two commercial citrus production environments.

Objective 2: Assess rootstock propagation method effect on tree performance, root architectures and uprooting resistance of Valencia trees after 5 years of growth.

Regular observations on root growth using minirhizotrons continued. Tree health ratings and tree measurements were conducted.

Work anticipated for next quarter:

Rhizotron measurements and data analysis will continue and tree ratings will be conducted. Depending on grower collaborator's schedule, harvest the fruit and analyze fruit quality will procced.

Budget status is as expected.

K. Bowman 21-008 "Development of Next-Generation SuperSour rootstocks with tolerance to HLB" – 15% Completion of Objectives (October 2022 quarterly)

This project has two major objectives related to the development of next generation SuperSour rootstocks with tolerance to HLB.

Objective 1. Collect field performance data from Stage 1 and 2 replicated rootstock field trials and release new rootstock cultivars as justified by superior performance in multiyear field trials. Collect field performance data. Since the beginning of the project, three of the 31 replicated trials listed on the original proposal were removed, and three new replicated trials have been established during previous reporting quarters. Therefore, the current list of active replicated rootstock trials for the project remains at 31. During this quarter, canopy health data was collected from all trees in the trials. For two trials with an early mandarin scion, fruit yield and fruit quality data were collected during this quarter. Preparations began for the collection of yield and fruit quality data from trials with Hamlin and lemon scions, which will be completed during the next quarter.

Supplemental testing of promising selections. When rootstock selections appear promising in field trials and are being considered for commercial release, additional information about stress tolerance and other traits is highly desired. This will help inform appropriate sites and management for commercial use. During this quarter, rootstock plants were prepared for a study to evaluate salinity tolerance among selected promising new rootstocks. Seedlings of the most promising advanced rootstock selections were grown for assessment of trueness-to-type or uniformity from seed and will be evaluated by morphology and molecular markers.

Preparation for release. The most promising USDA rootstock selections are provided to the FDACS-DPI program for shoot tip grafting and disease testing. Those that have completed the process are held as trees at FDACS and USDA, Ft. Pierce, for use as seed trees and sources of clean budwood. The introduction of new plant material to this FDACS program was temporarily suspended in 2022 but is expected to begin again soon. The two new USDA rootstocks tentatively planned for release in 2023 have already gone through the FDACS program and are available as clean budwood.

Release of new rootstocks. New rootstocks will be officially released by USDA for commercial use when justified by multi-year performance in the early stage replicated field trials. Based on outstanding performance in field trials, the proposed release documentation is being prepared for two new USDA rootstocks, coded as US-1688 and US-1709. It is anticipated that these two rootstocks will be released during 2023. New USDA clones are assigned official names at release, and the new names for these have not yet been approved.

Objective 2. Create hybrid rootstocks that combine germplasm from parental material with good rootstock traits and HLB tolerance, propagate the most promising of these hybrids, and establish replicated field trials with commercial scions. Current focus of USDA rootstock work is primarily on testing of hybrids previously created. Selected new crosses are planned for spring 2023, with parental combinations based on new information and breeding values of particular clones assembled over the past year.

Propagate hybrids. New hybrids to be used in trials will be propagated by uniform nucellar seed or stem cuttings. Trees were budded in the nursery with two new superior scion cultivars on 20 rootstocks, in preparation for field planting in 2023. Plant material was prepared in the nursery for one new Stage 2 trial with Valencia scion including the important commercially available rootstocks and the most promising of the new rootstocks. It is anticipated that these trees will be ready for field planting in spring 2024.

No new field trials were planted during this reporting quarter.

Tree care in trials. Periodic care was applied in the 31 field trials to maintain tree health and productivity and manage weeds as needed.

R. Shatters 21-025 "Transgenic capable field site to assess HLB-resistant and other improved citrus" – __% Completion of Objectives

Project start date was postponed to October 1, 2022; the initial progress report has not been submitted.

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

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" – (December 2022) Executive Board Summary for CRDF on Bayer/USDA project.

The validated screening process for identifying field candidates to test on HLB has been continuously running to look at leads from Bayer and external companies which has included:

Analysis of >20,0000 microbial strains, >200,000 synthetic compounds and a number of external candidates from the start of the project.

In 2022:

- 460 Hairy Root assays were carried out at Texas A&M AgriLife Research resulting in 12 leads that reduced HLB levels in infected citrus roots
- 130 compounds were tested in greenhouse assays for HLB at UF, SWFREC in Immokalee resulting in one strain that cured infected trees and four synthetic compounds that significantly delayed disease development (preventative)
- Four new field trials were established to test lead synthetic and microbial candidates in 2022. Two trials initiated in 2020 were completed and two trials started in 2021 are ongoing including analysis by qPCR and metabolomics. One trial was set up to test the active microbial strain on infected trees.
- Metabolomics analyses of both field and greenhouse for disease included 13,000 samples. A new metabolomics screen to look at plant vigor in presence of HLB was developed and samples are being analyzed.
- Currently no candidates have shown efficacy in the field. The most advanced candidates that were applied in 2022 can be assessed in 2023 when disease begins to develop in the newly planted trees.
- A regulatory strategy for each approach (microbial and synthetic) is being developed to outline predicted toxicology testing, costs, and timelines to be completed in Q2 2023

F. Gmitter 18-010 "Upgrading Citrus Genome Sequence Resources: Providing the Most Complete Tools Necessary for Genome Editing Strategies to Create HLB Resistant Cultivars" – 75% Completion of Objectives (July 2022 Final)

This is the final quarterly report for the project. The aim is to provide the highest possible quality genome assemblies to a chromosome scale and with the greatest accuracy and contiguity technically possible. Sufficient PacBio long read coverage is lacking for Ruby Red grapefruit and Shekwasha to complete the series of seven commercially important citrus. New HMW DNA preparations were made from the last two selections, and PacBio sequencing has begun. To provide the best possible annotation of the genomes, broader collections of tissue types were required to maximize the number of expressed genes for annotation of the assemblies. samples of tender flush, mature leaves, flowers, young and nearly mature fruit, bark, were collected and for some accessions leaf tissue with and without symptoms of CLas infection and citrus canker was collected. RNA samples were prepared and for two of the seven genomes, pooled sample RNAseq using the PacBio Seq IIe platform was completed to have full-length transcript reads, and tissue-specific barcoded samples Illumina short reads, to enable tissue specific gene expression studies.

Five of the seven genomes have been assembled using both the PacBio and Hi-C sequencing and assembly using Hi-Rise; preliminary assemblies of the last two listed above will be completed once the new PacBio reads are available. The focus has been on finalizing two of the seven genomes that are most near to full completion, while continuing to generate genome and transcriptome data for the remaining five. These two have their chromosomes properly phased, multiple previous unanchored sequence contigs have now been integrated, centromeric regions have been defined, telomeric regions have been polished, and nearly all highly repetitive sequences in these regions have been resolved. These two are the most accurate citrus genome assemblies possible with current sequencing technologies and assembly tools. The RNAseq data for these two are being used currently to complete the annotation of the assembly and it is anticipated these two assemblies will be made public soon. The same steps as described above will be implemented for taking the remaining five genomes to the same level of completeness.

The genomes of the five major cultivars identified in the proposal were assembled and an additional four important cultivars were included as the cost of sequencing was reduced, and these also were assembled. Two assemblies have been highly polished and represent what will be the highest quality citrus genomes to date. At grant termination, we were assessing the current inventory of sequence resources available and identified additional needs to produce genome sequence assemblies to a chromosome scale. We were still lacking sufficient PacBio long read coverage for two accessions, so new HMW DNA preparations were made. We planned to expand our collection of RNA transcripts, both with Illumina short reads and PacBio long reads, from a broader collections of tissue types to maximize the number of expressed genes for annotation of the assemblies. Samples of tender flush, mature leaves, flowers, young and nearly mature fruit, bark, and for some accessions leaf tissue with and without symptoms of CLas infection and citrus canker were collected for RNA extraction and sequencing. Seven of the nine genomes were assembled from both the PacBio and Hi-C sequencing and assembly via Hi-Rise. Quality control and assessment was underway for 7 of the 9 assemblies to properly phase chromosomes, to identify and anchor unanchored sequence contigs, to find haplotype swaps, to define centromeres, and to polish telomeres and resolve highly repetitive sequences in these regions. Work will proceed to full completion despite project termination.

All 9 completed high-quality assemblies will be made publicly available upon completion and publication, through Citrus Genome Database at WSU (https://www.citrusgenomedb.org/).

E. Rogers 18-019 "Phloem specific responses to CLas for the identification of novel HLB Resistance Genes" – 80% Completion of Objectives (August 2022 Final)

METHODOLOGY: The 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). A recently developed methodology for woody crops is being used that allows gene expression profiling of phloem tissues. The method leverages a translating ribosome affinity purification strategy (called TRAP) to isolate and characterize translating mRNAs from phloem specific tissues. This approach is unlike other gene expression profiling methods in that it only samples gene transcripts that are actively being transcribed into proteins and is thus a better representation of active cellular processes than total cellular mRNA. Sweet orange, and HLB-resistant Poncirus and Carrizo (sweet orange x Poncirus) have been transformed to express the tagged ribosomal proteins under the control of characterized phloem-specific promoters; transformants expressing the tagged ribosomal proteins under control of the nearly ubiquitous CaMV 35S promoter were also constructed as a control. Transgenic plants were exposed to CLas+ or CLas- ACP and leaves sampled 30, 60, 90, and 120 days later. Ribosome-associated mRNA is being sequenced and analyzed to identify differentially regulated genes at each time point and between each citrus cultivar. Comparisons of susceptible and resistant phloem cell responses to CLas will identify those genes that are differentially regulated during these host responses. Identified genes will represent unique phloem specific targets for CRISPR knockout or overexpression, permitting the generation of HLB-resistant variants of major citrus cultivars.

PROGRESS: During the three year and nine-month grant period, our group has made at least four high-expressing lines for each of the nine promoter/genotype combinations and shipped them from the Stover lab to the Rogers lab. More than 150 rooted cuttings from these lines were exposed to CLas+ or CLas- ACP in no-choice psyllid inoculation experiments. Each rooted cutting was sampled four times, yielding more than 600 leaf samples for ribosome affinity purification and mRNA isolation. Approximately one-third of the samples have been processed and almost 60 have been sequenced.

In a parallel approach, the tagged ribosomal proteins were cloned into a citrus tristeza viral expression vector. This vector and a control empty vector were moved into both Nicotiana benthamiana and Citrus macrophylla. Proof of concept experiments testing whether CTV can yield mRNA samples enriched for phloem genes appear promising.

There were several issues that hindered progress on project milestones. First, laboratory closures and occupancy caps due to the COVID-19 pandemic drastically limited personnel time in the lab and greenhouse. The Poncirus genotype is very slow-growing and the last Poncirus transgenic lines were not available until the end of year 3. The post-doc moved on to a permanent job after about 2 1/2 years on the project. In spite of extensive advertising, no suitable replacement post-doc candidate was identified. Additionally, mRNA yields from both of the citrus specific promoters were low and an ultracentrifugation step to concentrate ribosomes will be added prior to affinity purification.

In summary, significant progress was made towards our goal of identifying phloem gene expression changes in response to CLas infection even though the final studies are not complete.

M. Leslie 20-015C "Vismax™: A novel peptide-based therapeutic for mitigation of citrus diseases, including HLB" – 55% Completion of Objectives (November 2022 quarterly)

1. The objective of the 2-year project is to determine whether Vismax treatment promotes resistance to other major citrus diseases other than HLB.
2. In year 1 of the project, Vismax treated orange trees were significantly more resistant to citrus canker when the formulated peptide was applied as a dilute foliar spray or soil drench applied 7 days prior to leaf inoculation.
3. In year 2 of the project, greenhouse testing conducted by Dr. Megan Dewdney indicated that Vismax foliar applications gave statistically significant reductions in canker severity both in

the presence and absence of surfactant, and that commercially available surfactants were compatible with Vismax. However, the canker protection does not persist, and the treatment must be reapplied to effectively provide canker protection.

4. Currently, a phytophthora root rot trial is underway, evaluating rates of drench-applied Vismax in combination with and comparison to Foliar-applied Vismax for their ability to promote resistance to phytophthora root rot in susceptible orange seedlings by comparing dry root mass and scoring roots and leaves for symptoms of phytophthora rot.

5. The change in researchers from Johnson to Dewdney along with some problems in obtaining trees put the project behind schedule. A no-cost extension is being requested. Progress appears to be continuing at this time if extended.

H. Jin 21-003 "Test of SAMP efficacy in the field and greenhouse on important citrus varieties in Florida" – 100% Completion of Objectives (November 2022 commercialization report)

Objective 1: A field trial to test foliar spray treatment of SAMP on 4-year-old fruit-bearing 'Hamlin' sweet orange trees at FL to test if SAMP can remediate an HLB-affected young citrus grove. – test ongoing

This trial aims to test the SAMP effect on fruit yield. This trial includes 25 non-treated and 25 treated trees. This experiment is conducted in the Ridge region of Florida on the deep sandy soils. The trial started at the end of 2021. SAMP solution was sprayed every two months. The treatment is ongoing. This trial is expected to run for three seasons. The data in these trials will be analyzed for statistically significant differences using either mixed model methods such as PROC GLIMMIX in SAS or with non-parametric methods such as described in Shah and Madden (2004) and Mondal et al. (2012). The underlying distributions will be changed to suit the data, and the incidence data will be analyzed with a binary distribution.

Objective 2: Field trial of SAMP efficacy test using foliar spray on newly planted young 'Hamlin' sweet orange. – The treatment 2, which with nursery spray followed by foliar spray every two months has the lowest declining rate of trees.

A field trial to determine if newly planted 'Hamlin' sweet orange trees could be protected from HLB by SAMP (collaboration between Dr. Dewdney and Invaio). The trees were planted on October 15, 2020, in Lake Alfred, FL. The experiment was laid out in a completely randomized design with three treatments and sixty trees per treatment. The treatments included untreated control, treatment or no treatment in the nursery prior to planting, and field applications by foliar spray every two months or injection every six months. Some trees die early after planting in the field which may not be due to HLB. The number of living trees is shown in Table 1. The tested area has high pressure of ACP. According to the recording result on September 16, 2022, which was 23 months after tree planting in the field, the trees are showing decline symptoms caused by HLB. In four treatments. treatment 2, which with nursery spray followed by foliar spray every two months has the lowest decline rate of trees.

Table 1. Field trial of newly planted 'Hamlin' sweet orange trees treated with SAMP (collaborate with Dr. Dewdney and Invaio). The trees were planted at the end of 2020.

	Treatment method	Living Tree number	Declining trees number/rate 09/16/2022	Nursery treatment	Frequency
1	Untreated control	53	11/21%	N/A	N/A
2	Foliar spray	49	8/16%	1 week prior to planting	every 2 months
3	Injection	57	12/21%	1 week prior to planting	every 6 months (less than ideal)
4	Foliar spray	54	10/18%	N/A	every 2 months

Please note that the recommended injection frequency is every 3 months, but due to some technical and personnel issues at Invaio, the application ended up being every 6 months, which is not enough to fight against such a high pressure of reinoculation from dense ACP at that plot. The goal is to improve the situation for next year.

Objective 3: Greenhouse trial of SAMP efficacy test using foliar spray on 'Valencia' sweet orange

Dr. McCollum retired at the end of last year. As a results it was not possible to test 'Valencia' sweet orange trees in his greenhouse at USDA FL. Alternatively, 'Navel' sweet orange and 'Tango' Mandarin were tested at UC Davis. One foliar spray treatment was applied as a protectant for 'Navel' sweet orange (Table 2). Trees pre-sprayed with the SAMP had a 23% infection rate compared to 100% infected in buffer treated set (mock). For the post-infection treatment, tree were tested using trunk injection and foliar spray on the early infected tree (4 weeks after ACP exposure) (Table 3). The SAMP-treated 'Navel' sweet orange and 'Tango' Mandarin both have a lower infection rate compared to the buffer-treated set (mock).

Table 2. Test of using SAMP foliar spray to protect trees from *C*Las infection. The month is marked as 'M'. The *C*Las DNA is detected by qPCR after 8 months of ACP exposure in a greenhouse test.

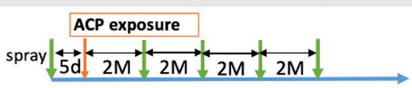
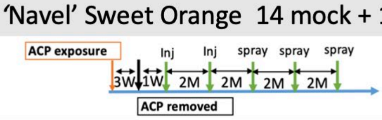
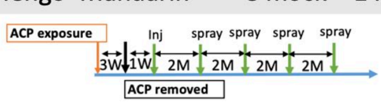
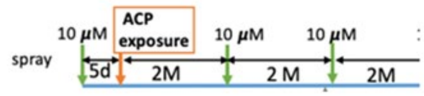
Varieties	Treatment	Result	Mock	SAMP
'Navel' Sweet Orange	8 mock, 8 SAMP		Mock	SAMP
		Infected trees	8/8	2/8
		Infection rate	100%	23.0%

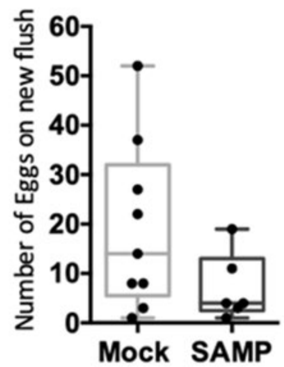
Table 3. Test of SAMP treatment using trunk injection (inj) and foliar spray on the early infected trees (4 weeks after ACP exposure). Week and month are marked as 'W' and 'M', respectively. The CLas DNA is detected by qPCR after 8 months of ACP exposure in a greenhouse test.

Varieties	Treatment	Result	Mock	SAMP
1 'Navel' Sweet Orange	14 mock + 13 SAMP		Mock	SAMP
		Infected trees	11/14	3/13
		Infection rate	78.5%	23.0%
2 'Tengo' Mandarin	8 mock + 14 SAMP		Mock	SAMP
		Infected trees	6/8	2/14
		Infection rate	75%	14.2%

Unexpectedly, the SAMP was observed to have a suppression effect on ACP, and the treated plants have much less eggs and no/little hatched nymphs as compared with mock treated control plants after expose to the same number of hot psyllids (Figure 1 below).



	Mock	SAMP
New Flush	9	8
New Flush w/ Eggs	9	6
Eggs	172	42
Nymphs	13	0



Availability of citrus products or application of growers' practices found through funded research: Through a partnership with our Industry licensee, a Biotech company, Invaio Sciences, a cost-effective manufacturing method has been developed to produce large-scale SAMP for application. From this preliminary result, the following are some preliminary conclusions:

1. The pre-treatment in nursey may provide a lower decline rate by comparing treatments 2 and 4 in objective 2. The pre-treatment may make trees more tolerant or resistant to the HLB.

2. Injecting once per six months may not be sufficient. The greenhouse result suggests one injection per two months.

Though this early result indicates that treatment 2 provides a better outcome on tree decline rate, which proves foliar spray of SAMP provided a suppression effect on HLB, the treatment program can be improved by coordinating the application method and frequency. In the greenhouse test, the injection method indeed has a better effect on the suppression of *C*Las in trees compared to foliar spray. However, frequent injections may cause more damage to the trees, therefore, it is suggested to combine the injection and foliar spray SAMP solution to make an optimal HLB treatment program for the next set of field trial.

The status of the commercialization process of such products or practices:
In the field trial with 4-year-old trees in objective 1, the expectation is to complete the test in late 2023 and run the trial for three seasons. Invaio Sciences is working on using their injection device to test different tree sizes on multiple field trials. The field trials may take 2-3 years to have conclusions.

A new on-going experiment was not originally proposed :
The most sustainable and cost-effective approach for HLB control is to generate SAMP cisgenic rootstocks and scions to provide growers HLB resistant/tolerant citrus trees. A cisgenic DNA cassette has been generated that contains the SAMP gene (originated from Australian finger lime, recently was classified as a citrus variety) and all citrus-derived DNA expression promoters and

terminators, for transformation into citrus rootstocks US942 and Kuharske, and scion Hamlin by the citrus transformation facility in University of Florida. Additional funding is needed to support confirmation and analylsis of these plants.

N. Wang 21-028 "Generation of non-transgenic HLB-resistant sweet orange varieties using CRISPR-Cas technology" – 8% Completion of Objectives (November 2022 quarterly)

Objective 1. Generate non-transgenic HLB resistant/tolerant Valencia and Hamlin sweet orange plants by mutation of HLB susceptibility genes.

In total, six putative S genes will be edited. Constructs needed for CRISPR genome editing are being made. Multiple edited lines were generated for ACD2 gene. However, further confirmation demonstrated none of them was biallelic/homozygous mutant.

Objective 2. Generate cisgenic genome modified Valencia and Hamlin sweet orange plants by knock-in the gene encoding MaSAMP from Microcitrus.

A knock-in method is being optimized for using the CRISPR technology.

Progress is being made as expected for this period.

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

C. El-Mohtar 21-014 "CTV-T36 vectors as a tool to induce efficient flowering in citrus seedlings" – 40% Completion of Objectives (December 2022 Quarterly)

1.Objectives. Year-1 Generate CTV infectious clones that express different FT3s or downregulate negative regulators of flowering to inoculate into Citrus macrophylla. Prepare different citrus genotypes for inoculation with the generated CTV vectors.

The focus in the 3rd and 4th quarter of this project was to monitor flowering in the Citrus macrophylla genotypes infected with the different CTV-FT3 (Arabidopsis and Hamlin FT3 (Hamlin FT3 had a consensus sequence and two variants as revealed by sequencing) constructs and CTV-RNAi (supposedly suppress negative regulators of flowering). Hamlin CTV-FT3 consensus sequence constructs did not induce flowering in C. macrophylla. On the contrary, the two Hamlin FT3 variants induced early flowering in Citrus macrophylla. All CTV-RNAi constructs targeting downregulating the expression of negative regulators of flowering failed to induce early flowering. CTV constructs expressing Arabidopsis FT3 failed to induce flowering in infected Citrus macrophylla. This prompted testing of the stability of Arabidopsis FT3 in the CTV vector via Revere transcription polymerase chain reaction with primers upstream and downstream of the insertion site. The size of the RT-PCR product was slightly smaller than the plasmid PCR product. This suggested minor recombination that was confirmed by sequencing that revealed a 76 nts deletion in the sequence.

The transgenic FT3 Carrizo lines rooted have not yet flowered under growth chamber conditions and are not yet big enough to bud them with other citrus genotype.

2. Work is anticipated for next quarter: In the 5th quarter, citrus will be reinfected with the CTV-FT3 vectors that failed to flower to make sure of the result.

3. Please state budget status (underspend or overspend, and why): Budget is on target.

d. Horticultural Practices

D. Kadyampakeni (previously E. Johnson) 20-004 " Organic acids compared to conventional acidification for improved nutrient uptake and root physiology" – 70% Completion of Objectives (December 2022 quarterly)

1. Please state project objectives and what work was done this quarter to address them:

Objective 1: Determine effects of lowered soil pH on CLas populations and root physiology including internal root apoplast and vascular tissue pH. Due to a collapse of the inoculum trees, trees for these experiments are being re-inoculated with CLas. A good number of trees are now positive for CLas and will be subjected to varying pH levels in rhizotrons.

Objective 2: Field test multiple acidification materials including organic acids for tree response CLas suppression, nutrient uptake, and root and vascular pH changes. In this quarter, soil and leaf tissue samples and samples show sufficiency in all treatments. Root density, and PCR of selected trees are now being evaluated. Treatments with organic acids and elemental S are being applied in the appropriate treatments and are monitoring canopy changes and soil trends as described in the project deliverables,

2. Please state what work is anticipated for next quarter: Fruit harvests and juice quality evaluations will be completed in the next quarter.

3. Please state budget status (underspend or overspend, and why): The budget is on track and meeting the project milestones.

T. Vashisth 20-011 "Right Leaf Sampling-The first and most critical step to good nutrition program" – 65% Completion of Objectives (September 2022 quarterly)

In this quarter the summer flush was tagged on all the trees of both varieties and locations. In summer, the trees were fertilized based on spring and summer flush nutrient analysis. Another set of nutrient analysis were performed as well. In addition, leaf samples have been collected for comparison of fruiting and non-fruiting branches.

The preliminary analysis shows that the mildly affected HLB trees are responding to the fertilizer treatments based on summer flush nutrient analysis. Mild HLB trees receiving additional fertilizer did not lose as much canopy density as the control trees. The response to fertilizer is not the same for mild and severely affected trees. This observation explains the variability in grove response to nutritional treatments.

Work anticipated for next quarter:

1. Data analysis and interpretation
2. Collecting samples for nutrient analysis

3. Applying fertilizer treatments based on leaf nutrient analysis results

The budget is being spent as per the plan wherein major funds have been used for nutrient analysis.

C. Pederson, Agromillora 21-001 Trees for Phase 3 Rootstock Trials – Contract signed; 50% deposit, balance on delivery of rootstock trees, expected late summer/fall 2022.

This was a project of experimental rootstocks with very limited information and even more limited propagation material. So limited and such a tedious experiment that this is the only nursery in the State with the ability to attempt it. There are 17 different rootstocks, with 3 different scions, for a total of 51 different combinations. The growers hope to be ready to plant this fall.

Two of the experimental USDA varieties have repeatedly shown some sort of physiological problem that makes it seem that propagation in a nursery will severely limit its usefulness. Bud live was great each time and the trees pushed a nice growth, yet the stems become brittle and snap. Better to learn that in the nursery than for growers to plant that variety and have it fail.

T. Wood, CRAFT 21-004C "Large Scale Field Trials Cycle III" – 90%

As of December, 2022, seven Cycle III contracts remain, all of which have been approved by the Board and are being finalized and signed in January 2023.

U. Albrecht 21-032 "Rootstock field trials - CRDF - Phase 3"

This contract provides for assistance from UF with the planning and establishment of rootstock field trials in different commercial groves, with evaluations to include tree size measurements, tree health/canopy assessments, and fruit quality and yield determination. As well, it will provide assistance with leaf sample collection for nutrient and Clas analysis and data interpretation.

T. Wood, CRAFT 22-010 Cycle IV – December, 2022.

The deadline for online applications for CRAFT Cycle 4 was November 30, 2022.
CRAFT Cycle 4 will continue the practices established in Cycles 1–3 of partnering with Florida citrus growers to evaluate the effectiveness and economic feasibility of various HLB mitigation strategies in large-scale, real-world commercial settings. Approved projects will be eligible for payments of no less than $3,500 per acre for solid set plantings and $1,080 per acre for reset plantings.
CRAFT has established a list of factors of interest that includes areas of focus that are considered of higher interest for Cycle 4. The factors are rootstock and scion combinations, nutrition, pest management, biostimulants and resets. These factors of interest are not all-inclusive, and

growers are encouraged to submit all project applications that they believe maybe beneficial in the fight against HLB.
The CRAFT Technical Working Group is currently meeting with growers in outlining their individual details of the Cycle IV projects.

A. Schumann 21-024 "Determine optimal timing for application of fertilizer to improve fruit quality and reduce preharvest drop" – 24% Completion of Objectives (September 2022 Quarterly)

The overall goal of the project is to develop fertilization strategies to best match nutrient supply and demand and develop recommendations for optimal nutrient application timing as compared to a simple constant supply, which will improve fruit yield, quality, and reduce fruit drop.

Objective 1: Test if a reduced N-P-K nutrient supply in the fall is safe for sustaining HLB-affected citrus, and whether it can improve fruit quality to facilitate earlier maturity / harvesting and reduce fruit drop: In order to compare the effects of early versus late / sustained fertilization on health and fruit production of Hamlin and Valencia trees, the following completed treatments were evaluated in the third quarter of 2022: Treatments: Early fertilization as % completed of the recommended fertilization (RF; 160 lb N/acre) before June

T1: 25% of RF (T25%)
T2: 50% of RF (T50%)
T3: 75% of RF (T75%)
T4: 100% of RF (T100%)

The remaining mid- to late-season fertilizer treatments were applied during the third quarter and the results will be reported in the following quarter.

Leaf samples were collected on monthly intervals and analyzed to determine the leaf nutrient concentrations. The preliminary results showed that if 50% of total nutrient requirement was applied before May, there was improvement in tree health (NDVE) and vigor (NDVI) indices, and canopy volume.

The same treatment resulted in higher leaf nutrient concentrations but only nitrogen was statistically significant.

The fruit growth (diameter) was not significantly different for early fertilization treatment whether applying 75% or 100% of total fertilizer.

Early fertilization with higher rates increased fruit size but growth increments diminished over time in all treatments, as expected when fruit development enters growth stage 2 in summer.

Preliminary results showed that there was a positive trend of crop health, fruit growth and leaf nutrient concentration with early higher fertilization but observed differences were not statistically significant.

Objective 2:

A) Develop an optimized, practical fertilizer timing management profile to boost fruit quality and reduce fruit drop for HLB-affected citrus based in part on the sigmoidal nutrient demand curve defined by four physiological growth phases (0=bloom/fruit set; 1=cell division; 2=cell enlargement; 3=maturation):

The growth and yield data associated with all different timed fertilizer events in year 1 is not complete yet and therefore there are no new results to report for this objective.

Investigations of use the intensity of leaf symptom expression throughout the season to help determine foliar nutrition in the field with smartphone apps is continuing.

B) New developments: The Sugarbelle trials focusing on solving the soft fruit quality issues are progressing well. three replicated foliar spray treatments have been applied to Sugarbelle trees at the city block and on healthy trees in the CUPS, consisting of KNO3 and KH2PO4. The purpose of the sprays is to increase peel thickness and strength to avoid soft fruit, as well as to improve fruit size.

C) Issues: The fruit drop and tree lodging/ branch breaking damage caused by hurricane Ian to the outdoor Hamlin, Valencia and Sugarbelle experiments is considerable. The Sugarbelle experiment in the CUPS sustained no damage. The expected reduced yield data will not be useful for calibrating the fertilizer timing models, but fruit quality data will be collected and use where possible.

Work anticipated for next quarter: The final late fertilizer applications will be made to designated treatment plots in November. Soil, lysimeter sampling, leaf sampling, processing and analysis will be ongoing, as will tree size and fruit measurement. Plan is to assess fruit yield and quality for the Hamlin and Sugarbelle experiments in December / January.

The third Aerobotics drone survey will now fly in late November due to delays from Hurricanes Ian and Nicole.

Budget status (underspend or overspend, and why): Spending rate is approximately on track.

U. Albrecht 22-001 "Directed research – Evaluation of different trunk injection devices and oxytetracycline formulations for efficacy against HLB, phytotoxicity, and feasibility" – 35% Completion of Objectives (December 2022 quarterly)

This project has 3 main objectives related to the injection of OTC into citrus tree trunks and are conducted at 5 trial locations. The objectives of the project are as follows:

1) Test the efficacy of different injection devices,

2) Determine the most effective formulation of OTC,

3) Determine the best month of injection and most appropriate OTC concentration based on tree size.

Project work conducted at each of the trial sites are as follows:

Trial 1: Located in SW Florida (Duda) - 8-year-old Valencia/Carrizo trees. – Assessment of tree health ratings, fruit size assessments, monthly fruit drop measurements, and tree size measurements were collected.

Trial 2: Located in SW Florida (Graves Bros) - 8-year-old Valencia/Kuharske trees. – Assessment of tree health ratings, fruit size assessments, monthly fruit drop measurements, and tree size measurements were collected.

Trial 3: Located on the east coast (Graves Bros) - 9-year-old Valencia/sour orange trees. – Assessment of tree health ratings, tree size measurements, monthly fruit drop measurements, and collected leaves for CLas detection were completed.

Trial 4: Located on the east coast (Graves Bros) - 4-year-old Valencia/x639 trees. – Assessment of tree health ratings, monthly fruit drop measurements and collected leaves for CLas detection were completed.

Trial 5: Located on the central ridge (King Ranch) - 4-year-old OLL-8/x639 trees. – Assessment of tree health ratings, monthly fruit drop measurements and collected leaves for CLas detection were completed.

Work anticipated during the next quarter will include fruit drop counting. The project team will prepare for fruit quality and yield assessment (pending the desired harvest time of the grower collaborator).

Budget status is as anticipated with most of the budget will be for fruit quality and residue analyses at the end of the first year).

F. Alferez 22-003 "Determining best timing for Brassinosteroid (Brs) application to achieve maximum beneficial effects on citrus tree health and fruit yield and quality" – 6% Completion of Objectives (October 2022 quarterly)

The objectives are: 1) To study the effect of Brassinosteroids (Brs) on priming immunity on young, newly planted trees. This will determine how long the immune response will last after Br application, so timing can be adjusted (number of applications); 2) To determine the best time of application (frequency) to achieve maximum protection against pests and disease in newly planted trees. 3) To determine the effect of Br application on advancing fruit maturation in both Valencia and Hamlin.

1) 40 Valencia trees on Carrizo and 40 trees on X639 trees were planted in a complete randomized block design. Applications of Br or water (as control) have been performed every 15 days. Psyllid infestation has been monitored and leaves collected for bacteria titer analysis. Tree growth is being monitored. It is still too early to have any statistically significant data, but psyllids are clearly less abundant in Br-treated plants. To better understand the effects, an experiment was started before August 1st under controlled conditions in the greenhouse with the same setup of trees, that were enclosed in individual cages and 4 pairs of psyllids per cage were introduced. Differences in oviposition have been observed and activation of SAR genes have been measured in response to treatment and the activation lasts for at least 6 weeks. This confirms the possibility of reducing the number of Br applications. Monitoring in the field trial continues.

2) This objective is related to the first one. In discussions with some growers on the SWFREC Citrus advisory committee, they raised the possibility of concentrating the Br application when psyllids are more abundant, during or at the beginning of flushes. This will be assayed as well.

3) To determine the effect of Br application on advancing fruit maturation in both Valencia and Hamlin. Br applications were started at Duda farm by the end of August in Hamlin. Since then, treatments have been performed twice per month under commercial conditions. While a statistically significant increase in Brix was not apparent in September, during October a slight increase Brix from 8.1 to 8.6 was measured. Interestingly, acidity has been consistently lower in fruit from Br-treated trees, making the ratio significantly higher. In view of this fact, collection of juice samples was initiated for further HPLC analysis of sugars and organic acids.

Fruit drop started for Hamlin and has been monitored weekly. Unfortunately, hurricane Ian accelerated fruit drop and some trees have been lost. However, close monitoring of fruit drop, that started before Ian will allow for assessment of the real impact of the hurricane on fruit drop.

Outreach:

-Invited talk. Brassinosteroids and fruit quality. Packinghouse day. August 27, 2022, Lake Alfred FL.

-Invited talk. Combining IPCs and Brs to prolong young tree health. Updates and future steps. Citrus Expo 2022, August 18, Fort Myers

Work anticipated for next quarter: For objective 3, harvest of Hamlin will begin when the commercial timing is right to assess yield and final quality. Treatments in Valencia will start in December. For Objectives 1 and 2, treatments and samplings will continue as in this quarter.

Budget status: Spending is on track. Student is on board, and more funding will be spent this quarter as in-depth molecular analysis begins.

e. Alteration of hormonal response to reduce fruit drop, standardize color break, eliminate minor blooms

F. Alferez 21-007 "Reducing fruit drop by altering hormonal responses within the tree through nutritional and hormonal therapies: a mechanistic affordable approach" – 18% Completion of Objectives (October 2022 quarterly)

The objectives are: 1) To determine the right timing for Zn and K treatments to minimize fruit drop; 2) To determine effects of GA3 and 2,4D applications on fruit retention when applied at different times during fruit development; 3) To develop a strong and proactive outreach program.

Objective 1. A new set of treatments in Hamlin were applied by the end of September. As before, every set of treatments is performed in an independent block, so treatments are applied only once, to assess the best timing to achieve the best results. Trees under Zn and Zn+K treatments continue having greener canopies. Control trees had some off blooms in September and October with new fruit set clearly present. These off-blooms have not been observed in the treated trees. Fruit drop is being recorded in Hamlin. Unfortunately, hurricane Ian has increased fruit drop but since data are collected weekly, it will be possible to reduce the fruit drop background noise to see if treatments had a beneficial impact even under hurricane conditions in alleviating fruit drop. Some trees have been lost also due to the high wind. Interestingly, all the trees lost were in a control plot. There is no clear explanation for this, but fortunately, treatments were not compromised as other trees in untreated plots are being used as new control replacements.

Objective 2. GA3 and 2,4D treatments have continued as planned.

Objective 3. Outreach

Citrus Grower Forum. Citrus Fix. Guest speakers Vashisth and Alferez. Invited by AMVAC. SWFREC, August 30.

Citrus Grower Forum. Citrus Fix. Guest speakers Vashisth and Alferez. Invited by AMVAC. Stuart Conference Center, Bartow, August 31.

Work is anticipated for next quarter: For objective 1 December treatment in Valencia and for objective 2 treatments and assessment of fruit drop in Hamlin.

Budget status: underspending as the student joined late. As we start our analysis, more funds will be spent this quarter.

J. Curtis 22-006 "CRDF Study on Preharvest Fruit Drop Prevention Using Plant Growth Regulators (PGRs)" – This Project started July 1,2022.

This study will test the hypothesis that Citrus FixTM and ProGibb®, when used alone or in combination, may significantly reduce or prevent preharvest fruit drop, with the products used in conjunction with quality horticultural care. Both products are commercially available and have industry acceptance. It is hoped that after one season of the prescribed spray applications and data collection from multiple trial sites there will be enough evidence to draw tentative conclusions regarding the impacts of these treatments.

Nine foliar applied treatments are being evaluated plus an untreated control (10 plots). The 10 treatment plots are replicated five times with each plot containing seven trees. Evaluations will be focused on the center five trees of the seven-tree plot.

Timing of treatments, to begin after normal June drop:

Citrus FixTM - Split applications with an application in the months of July, September, November (treatment 3, 4 and 5, regardless of variety)

Citrus FixTM - two full-rate applications with an application in August and an application in October (treatment 6, 7 and 8, regardless of variety)

ProGibb® – a single nighttime application when the fruit transitions from dark green to light green (treatment 9)

ProGibb®+ Citrus FixTM – a single application at night (Hamlins – September, Valencia – November) (treatment 10)

Trial Site Locations:

1. East Coast – Scott's Family Grove; rootstock/scion: Sour orange/Valencia; 6 YO trees
2. East Coast – Williamson Ranch; rootstock/scion: Swingle/Hamlin; 7 ft. trees
3. Southwest – Pacific Tomato; rootstock/scion: Swingle/Hamlin; 8-10 ft. trees
4. Southwest – Pacific Tomato; rootstock/scion: Swingle/Hamlin; 8-10 ft. trees
5. Ridge – BHG; rootstock/scion: Swingle/Valencia; 8-10 ft. trees
6. Ridge – Wolfork Myers; rootstock/scion: Swingle/Hamlin; 7-10 ft. trees

OTHER CITRUS DISEASES

a. Post-Bloom Fruit Drop

b. Citrus Black Spot

M. Dewdney's 18-006 "Understanding the underlying biology of citrus black spot for improved disease management" terminated April 30, 2022; the final report was included on the June 2022 report.

c. Citrus Canker

Z. Mou's 18-017 "Establish early-stage field trials for new HLB-tolerant canker-resistant transgenic scions" terminated April 30, 2022; the final report was included on the June 2022 report.

d. Lebbeck Mealybug

L. Diepenbrock 20-002C "Developing near and long-term management strategies for Lebbeck mealybug (Nipaecoccus viridis) in Florida citrus" – 86% Completion of Objectives (December 2022 quarterly)

This project has a number of objectives focused on the control of Lebbeck mealybug in citrus groves. Not all objectives are being reported in each quarterly report due to which objectives are currently being focused upon.

1. Near term field management: (a) Develop methods to time management actions. In previous reports, discussion has been provided on efforts to understand the odors that appear to be attracting Lebbeck mealybug to help develop better scouting and potentially an odorant lure in the future to make sampling to determine management easier. To date, the primary components of the tree parts as well as damage have been identified and pure isolates of these odors have been obtained. Work is currently underway at testing attraction to combinations of

these odors and to individual odors to move closer to understanding what the mealybugs are attracted to. Because there is a strong recruitment of lebbeck mealybug to our traps around small wounds versus traps without wounds, it is anticipated that the odors associated with this damage will be the most attractive.

(c) Evaluate promising materials in open grove setting. In the fall of 2022, two field trials were completed to look at longevity of several promising foliar applied insecticides. The first trial compared those pesticides known to have contact activity against Lebbeck mealybug. Contact materials tested included: Agri-Flex (8.5 oz/a), Transform (2.75 oz/a), Voliam-Flexi (7 oz/a), Besiege (12.5 fl oz/a), AgriMek (4.25 fl oz/a), Actara (5.5 oz/a), Minecto Pro (12.5 fl oz/a), and Esteem (5 oz/a) (all with 0.25% NIS). Materials were applied to trees with flush in the grove and treated leaves that were soft but fully expanded were collected and brought back to the lab where they were challenged with mealybugs. In week 1, Agri-Flex had the greatest mortality with over 80% of juvenile mealybugs dying, closely followed by Transform (68%), and Voliam-Flexi (31.5%). All other tested chemistries were no different than the untreated control for mortality in the first week of application. Unfortunately, there was no residual efficacy for any materials in weeks 1, 2, and 3 after application.

In the second trial, studies focused on foliar-applied insecticides with systemic activity and included some variation in rate as well as adjuvant used. Treatments consisted of: Movento 16 oz/a + NIS, Senstar + NIS, Sivanto + NIS, Movento 10oz/a + 435 oil, Senstar + 435 oil, and control. In the initial week of application, Movento + NIS had the highest mortality at 38%, Senstar + NIS, Sivanto + NIS both had 28% mortality, and the rest fell below this. This isn't surprising as systemic materials need time to build up in leaves and most mortality was likely due to the adjuvant sticking to the insects and smothering them. One week after application, the Movento 10 oz + oil had increased mortality relative to the other treatments (37%) but no material showed the ability to kill 50% or greater of the nymphs at any point in the trial. This is not consistent with what has been observed in CUPS houses when these systemic materials were applied. A possible explanation for this is that the trees which were tested in field situations have had HLB for several years and materials are not likely moving through the vascular system as they would in a health tree. In healthy trees, systemic materials can translocate through the canopy, and it is likely that the vascular plugging exhibited by HLB affected trees reduces this.

II. Long-term management

c. Determine what insecticide chemistries inhibit feeding. While there has been some progress on this subobjective, it has been far slower than anticipated. Equipment used for testing has been sent for repair/ tune- up and are now reworking the initial feeding interaction data.

Working with the repaired equipment, we have been able to document the feeding waveforms, though with some noise, that we will need to move forward and complete documenting the feeding interactions. We can now see the E1/E2 ingestion pathway. This must be fully documented and reviewed to move into the next step, which will be determining if we can interrupt the feeding via insecticides and at what rate we need to interrupt feeding, halt offspring production, and lead to adult mortality.

d. Develop tools to minimize spread. Work to complete the solarization and freezing studies that were planned this past term were unable to be completed due to colony infestation by a predator impacting available insects to work with. However, progress has been made in understanding Lebbeck mealybug dispersal, which is important for determining broader methods for reducing spread.

In the late summer of 2022, a wind tunnel was constructed in which we can control the velocity of wind. In this wind tunnel, plants can be introduced from below with varying infestation levels and at varying distances. While still preliminary, finding show Lebbeck mealybug are fairly easy to dislodge at low to medium wind velocities. 10 minutes at a high velocity of wind (exact speed will be reported later, the anemometer is broken) is sufficient to dislodge 50% of crawlers, 30% of immatures, and 15% of adult females. The ability to dislodge easily in wind events helps to explain the rapid spread of this pest and underscores the important of management actions shortly after major weather events.

II. Next quarter: Work is continuing to test promising materials in open groves and are in the process of planning a test of a subset of materials in a CUPS house as we have learned that chemistries don't all behave similarly in CUPS to the open field.

Studies will be conducted to document feeding interactions and plan to start insecticide trials in late spring to document influence of systemic chemistries on feeding, offspring production, and mortality.

Work will continue towards identifying appropriate odors to use towards future scouting tools.

Solarization and freezing tests for sanitation will be completed in spring 2023.

III. Budget status. Spending is behind budgeted levels after having a vacant postdoc position for several months, however we are on track in other categories.

e. Phytophthora

M. Dewdney (previously E. Johnson) 19-010 "Determining new cost-benefit guided Phytophthora propagule treatment thresholds for HLB-affected citrus" – 85% Completion of Objectives (December 2022 quarterly)

The goal of this project is to develop new soil propagule density management thresholds and recommendations for chemical management of Phytophthora root rot based on economic analysis of yield responses in different soil conditions.

Objective 1) Determine if labelled fungicides for Phytophthora management maintain efficacy in the field on HLB-affected trees for reducing fibrous root loss and improving yield.

In this quarter, root samples were collected from the Felda area grove. The root density was measured and the raw data appear to show that some treatments have greater density than the control for the Hamlin oranges. There is much more variation in the Valencia plots and the trends are unclear. Trends are also unclear from the populations of the two phytophthora species. analysis of these plots continues and the analysis of the data is being reworked using time as a factor. one of the hamlin sites has been harvested and the second site scheduled for harvest the first week of January. The fruit quality and size data have been gathered but not yet analyzed.

Objective 2) Determine benefit-cost thresholds for Phytophthora treatment on HLB-affected trees

The co-PI responsible for the economic analysis of this project, asked to have their funds returned. It is uncertain whether they will provide an analysis of this project.

Work is anticipated for next quarter: The second Hamlin harvest. The Valencia harvests and spring applications of the treatments. Data analysis will continue to confirm whether there any treatment differences in yield, fruit quality, root density, or inoculum levels of phytophthora.

Budget status (underspend or overspend, and why): The budget is underspent because when the project was taken over, staff had not been appointed to the project for several months. This caused funds not to be spent in a timely manner. Also, the plan was to ask for the no cost extension so that funds for harvest of the Valencia plots were available.

See also: M. Leslie 20-015C "Vismax™: A novel peptide-based therapeutic for mitigation of citrus diseases, including HLB" on page 9.

f. Nematodes

L. Duncan 21-013 "Integrated management of sting nematode in newly planted citrus trees" – 25% Completion of Objectives (November 2022 quarterly)

Trunk girth one inch above the bud union was 13% greater on covered than uncovered trees (NS). Nematicide treatment had no effect on the tree size. As noted previously, by summer the nematicides reduced the sting nematodes (P=0.02) by 87% on the uncovered trees and 13% on the covered trees. The apparent interaction for the nematicide effect was marginally significant (P=0.08). The final nematicide treatment of 2022 occurred in November and the effects on the winter population density will be measured in January.

The first 16 rootstocks from the UF breeding program were evaluated for sting nematode tolerance during the first week of November, approximately 18 weeks after planting in nematode infested tanks. Compared to unchallenged trees, the root damage was readily evident on the most susceptible lines and was documented photographically for each plant in the trial. The average fibrous root mass of the infested compared to the uninfested lines was highly correlated (0.80) with the subjective visual damage rating assigned each seedling and was used to rank the genotypes. The mean ratio of the average fibrous root mass infested/uninfested for the four most 'tolerant' lines was five-fold that of the four least tolerant lines. Three of the most tolerant lines were derived from crosses between the same tetrazygotic parents and the fourth had 3 of the 4 ancestral genotypes. Two additional tanks were established in July and a 2nd trial was initiated using USDA rootstocks and a number of conventional rootstocks. The second trial will be completed in February 2023.

g. Greasy Spot

M. Dewdney 21-012 "Evaluating the role of greasy spot and peel disorders in the greasy green defect on citrus fruit" – 25% Completion of Objectives (December 2022 quarterly)

The objectives are to 1) determine if the flush cycle and infection period for Z. citri-griseum have changed due to the influence of HLB on citrus physiology or changing environmental factors; 2) evaluate the potential promotion of "greasy-green" symptoms related to nutrition programs or to peel reactions like a chemical "burn" from different pesticide and combinations of pesticide tank mixes; and 3) evaluate if postharvest degreening treatments might be modified to adequately remove the green coloration while mitigating poor shelf life from anticipated longer degreening times.

Most of the progress to date has been on objective 1. Two sites were established in grapefruit groves in the Indian River region. These sites were identified to have significant problems with the greasy green disorder. In each block, twenty trees were selected for uniform canopy without excessive thinning or obvious sectoring from HLB and mapped, and ten flushes per tree were tagged to be observed for greasy spot symptoms later in the season. Every two weeks from the beginning of May, ten leaves and one fruit per tree have been collected. Since June fruit diameter has also been measured. The leaves were cleared and 5mm disk samples examined under the microscope to observe whether there is epiphytic growth. This method of sampling fruit was not giving satisfactory results so the protocol changed to applying clear nail polish to the fruit surface to remove the fungal growth for observation under the microscope. Much better success was obtained with the nail polish technique. Currently, the structures observed under the microscope are being molecularly confirmed as Zasmidium citri-griseum. Conventional PCR has been performed and the observed bands at the correct size are being prepared for sequencing. The microscopy samples are preserved, and during the winter season are being analyzed. Preliminary results were presented at the Southeastern Professional Fruit Workers Conference in Lake Alfred in November. From the first observations in May to October 2022, the majority of flush was fully mature with significant peaks of younger flush in June and September. The red and white grapefruit had similar patterns of flush but the red grapefruit had a longer flush period in the fall than the white grapefruit. Fruit growth increased in a typical exponential pattern until September when the growth rate was substantially reduced as expected. The average diameter in October was 8 cm.

Fruit are currently asymptomatic and spores and mycelial growth were observed from August. It is uncertain whether or not spores were detected earlier in the season because they were not there or because of an artifact in the way they were visualized. Hopefully the extent of epiphytic growth can be determined and when the epiphytic growth is the most prevalent used to compare with what is known from previous work. Ideally, this will allow adjustment of when fungicide applications occur to better control greasy spot as part of the greasy green disorder.

Work anticipated for next quarter: Samples will continue to be evaluated microscopically. The plan is to start evaluating flush in the early spring, probably mid-February or early March. Samples will continue to be collected. More fruit samples have been promised for the greasy-green physiology experiments and it is expected that several experiments following up on the ones already undertaken will be conducted.

Budget status (underspend or overspend, and why): No over or underspend on budget currently.

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