United States Department of Agriculture

2019 Project Summaries

Huanglongbing Multi-Agency Coordination Group

December 2019

UAV and Ground-based High Throughput Phenotyping In Citrus Utilizing Artificial Intelligence

Company
Principal Investigator: Dr. Yiannis Ampatzidis
Agreement Number: USDA-APHIS-10025-PPQS&T00-19-0154
Amount Obligated: $182,331
Agreement Period: 8/1/2019-7/31/2021

Summary
The main goal of this project is to provide low-cost and high-throughput phenotyping tools to citrus growers for sustainable management of HLB-infected citrus trees. These tools will allow growers to better evaluate citrus varieties, rootstocks, and management practices.

Project Status

15%

Complete

Project Update

Objectives	Percentage Complete
1. Automated UAV-based high throughput phenotyping and plant assessment technique.	0-24%
2. Automated ground-based high throughput phenotyping technique.	0-24%
3. Field Evaluations and development of an outreach program.	0-24%
4. User-friendly and cloud-based wed application to visualize the collected data.	0-24%

Figure. Cloud and AI-based software to visualize data collected from aerial (e.g., UAVs) and ground sensing systems. a) field statistics (e.g., total number of trees and tree gaps) of every grove. b) interactive software to categorize trees based on their height, canopy size, and health status (zoom-in in grove "3b"); the user can select and display only specific categories (e.g., trees less than 10 ft height).

Achievements

- A cloud and AI (Artificial Intelligence) based application (Agroview) to analyze and visualize data collected from UAVs (Unmanned Aerial Vehicles), satellites, and other platforms (e.g. small airplanes) was development.
- This interactive and user-friendly platform can: (i) detect, count and geo-locate trees and tree gaps (locations with dead or no trees); (ii) measure tree height and canopy size (tree inventory); (iii) develop individual tree health (or stress) status maps (health index maps).
- Field data were collected from a commercial orchard (around 2,000 acres) and maps were developed.
- The first prototype of the ground-based sensing and high-throughput phenotyping system is under development.
- Outreach events: A Citrus Seminar was organized on September 25 with around 40 participants in SWFREC to demonstrate the Agroview technology.

Next Steps

- More field data will be collected next quarter with both the UAV-based and the ground-based system from commercial citrus groves.
- A yield prediction algorithm based on the collected data (e.g., plant height, canopy size, and health) will be developed.
- An AI-based algorithm to analyze the collected data and a cloud-based application (software) to visualize the data will be further developed.
- Manual data (e.g., tree height and canopy size, fruit counts per tree, etc.) will be collected from around 200 trees and compared with the data collected from the remote sensing platforms (UAV and ground).

Asian Citrus Psyllid Detection Canines For California

Canine Detection Services
Principal Investigator: Lisa Finke
Agreement Number: AP20PPQS&T00C001
Amount Obligated: $247,095
Agreement Period: 07/18/2019-07/17/2020

Summary
Purpose: To determine if detection dogs are able to locate ACP populations in regions of California where the psyllid populations are very low in order to assist with psyllid eradication efforts. This is a proof of concept project.
Goal: Reducing the spread of HLB by improving insect vector detection and local eradication where densities of psyllids are extremely low. In addition, ACP finds will be flagged and reported to CDFA for follow up, including sample collection and analysis.

Project Status

30%

Complete

Project Update

Objectives	Percentage Complete
1. Procure, train and test 3 dogs to detect ACP nymphs and adults in an indoor training facility.	90%
2. Evaluate three dogs through scientific experiments in commercial orchards and residential settings.	0%
3. Analyze data and distribute findings.	0%
4. Develop pathways for commercialization of the technology.	45%
5. Develop third-party certification protocols for ACP canine/handler teams.	45%
6. ACP detected by the canine/handler team in low incident rate areas will be flagged and then collected by regulatory authorities who will test them for CLas, the causal agent of HLB.	0%


Encore


Tango


Eureka

Achievements
- Quality detection dog candidates were located and purchased July 28, 2019.
- Permit obtained from the Department of Food and Agriculture Plant Health and Pest Prevention Services to move live disease-free ACP from the UCR Quarantine facility to train canines at two locations.
- Initial training on target odor completed September 13, 2019.
- A nationwide, third-party certifying organization has agreed to review a new standard operating procedures (SOP) for the conduction of randomized, double blind certification testing of canine/handler teams locating ACP. The writing of this SOP is underway. External examiners would moderate the test once approved by the board of directors.
- The development of pathways for commercializing this technology is ongoing. It has been discussed with farming corporations, family farms, the Citrus Pest Detection Program Board of Directors and the Citrus Pest and Disease Prevention Committee Chairman.

Next Steps
- Begin training dogs outdoors to detect ACP using secure training aids within a scent training apparatus and on live potted citrus trees.
- Begin travel each week with dogs and ACP to Imperial County for field training in commercial groves.
- Locate areas for field training and testing in residential settings and obtain a permit.
- Schedule formal experiments indoors, in commercial orchard, and residential settings. One additional moderator will be present who is not affiliated with UC Riverside or Canine Detection Services.
- Analyze data and distribute findings.
- Flag incidental ACP nymphs located during training and report to CDFA for sample collection and analysis.

Evaluation of Citrus Tree Delivery of Neuropeptide Mimics To Control The Asian Citrus Psyllid

Boyce Thompson Institute, USDA ARS, AgroSource, Inc., University of Washington
Principal Investigator: Dr. Michelle Heck

Agreement Number: AP19PPQS&T00C079 (Neuropeptides)
Amount Obligated: $644,852
Agreement Period: 07/2019-06/2021

Summary

- New tools for controlling the psyllid are urgently needed.
- We will evaluate and commercialize a panel of modified, orally active insect neuropeptides and peptide hormones to control HLB.
- The orally active neuropeptides may act in several manners for HLB control, including blocking Clas transmission through psyllid feeding disruption or inducing insect mortality.

Project Status

5%

Complete

Project Update

Objectives	Percentage Complete
Peptide synthesis	50-74%
Small trials for peptide activity	25-49%
Scaled up synthesis	0-24%
Large-scale trials	0-24%
Develop project management plan	100%
Determine which candidates to advance	0-24%
Test effects of neuropeptides on psyllids	0-24%
Proteome analysis	0-24%
Data analysis	0-24%
Project products	0-24%

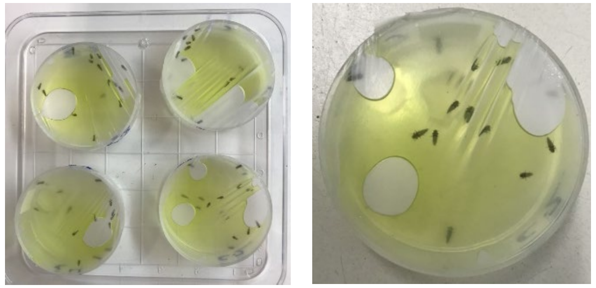


Fig. 1. Example artificial diet chambers that will be used for small-scale testing of the neuropeptides for activity against the Asian citrus psyllid.

Achievements

- Sub-contracts were executed by Sept. 2019. Thus, this Q1 report represents ~1 month of work.
- Scientists hired Heck and Shatters to work on the psyllid bioassays.
- Project management plan was developed and discussed among team members.
- Preparations to generate CLas+ trees for greenhouse studies.
- A manuscript describing the discovery and characterization of the Asian citrus psyllid. peptidome was submitted for peer-review.
- Synthesis of the following neuropeptides is underway (approximately 50% complete): kinin, pyrokinin, tachykinin-related, orcokinin, myosuppressin, and an analog of cap2b.
- Artificial diet delivery system was optimized to screen neuropeptides (Fig. 1).
- Solvent and volume concentrations were optimized (Fig.2).

Next Steps

- Synthesis of peptides to be completed for screening.
- Screening peptides for oral toxicity against the Asian citrus psyllid in artificial diets.
- Analyze the data and select candidates for greenhouse and field trials.
- Proteome experiments with best performing peptides to determine mode of action.

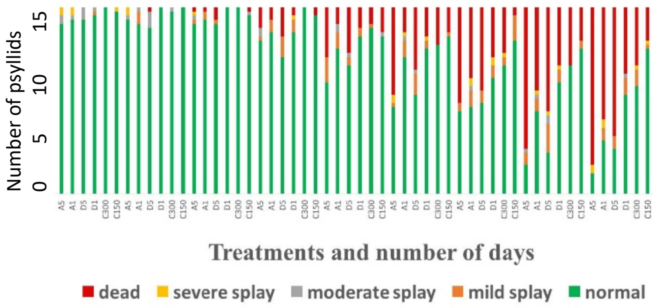


Fig.2. Effects of diets with up to 5% DMSO (D) or Armid FMPC (A) and reduced diet volume (150 vs 300µl) on psyllid mortality and wing splay over 9 days. DMSO is less toxic and volume of diet can be reduced to 150 µl with no increase in psyllid mortality.

Artificial Intelligence Apps for Smartphones: A Modern Diagnostic Extension Tool for Citrus Growers and Home Owners To Rapidly Identify Nutrient Deficiencies and HLB Symptoms in Florida Groves

University of Florida
Principal Investigator: Arnold Schumann
Agreement Number: AP19PPQS&T00C010
Amount Obligated: $27,000
Agreement Period: 3/1/2019-2/28/2020

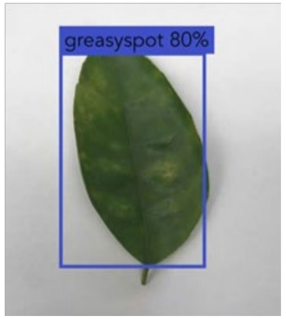
Summary
A smartphone app is being developed to help citrus growers diagnose common nutrient deficiencies, pests and diseases prevalent in HLB-affected Florida groves. The smartphone app uses artificial intelligence in deep-learning neural networks and machine vision to identify unique leaf symptom expression. The identification of common citrus diseases and pests from foliage symptoms was necessary to improve the accuracy of diagnosing nutrient deficiency symptoms which are critically important for mitigating HLB-affected trees.

Project Status

50%
Complete

Project Update

Objectives	Percentage Complete
1. Develop fast and accurate diagnostic artificial intelligence models for key nutrient deficiencies of citrus encountered when trees are impacted by HLB disease	100%
2. Transfer the best detection models developed to smartphone platforms (iOS and Android) and develop apps to use the models for a) identifying the above disorders in-field and b) to provide recommendations for their management with enhanced nutrition	50-74%
3. Demonstrate and distribute the prototype smartphone app to citrus extension agents and citrus growers, and provide initial support by creating extension and outreach materials on UF/IFAS EDIS libraries and by offering a workshop for using the new smartphone app.	25-49%



Achievements
- Using 1,800 digital images of leaves, we successfully trained an artificial neural network to identify visible symptoms of nutrient deficiencies, pests and diseases in images of citrus leaves
- Leaf symptoms that can be identified with an average 89% accuracy (63-100% range) include 1) nutrient deficiencies: iron, manganese, zinc, magnesium, 2) pests: two-spotted spider mites, 3) diseases: HLB, citrus canker, greasy spot
- The trained network was converted and transferred to the iOS platform for incorporation into our first draft smartphone app
- More details, including a video of leaf identification are on the project website

Next Steps
- Additional leaf symptoms to be trained: potassium, nitrogen deficiencies, rust mites, thrips, Asian citrus psyllids, citrus scab, Phytophthora, citrus black spot
- Collect and use thousands of additional leaf images for model retraining to improve the detection accuracy of all symptoms and to add the new list of symptoms
- Develop the Android smartphone app for citrus leaf symptom diagnosis
- Test and demonstrate the first prototypes of Android and iPhone smartphone apps in field applications of nutrient, pest and disease diagnosis
- Report on the use of smartphone apps for citrus leaf diagnosis in extension meetings, trade journal articles and UF/IFAS EDIS libraries

Asian Citrus Psyllid and CLas Reductions from Psyllid Sprays Timed To Spring and Summer Flushes in Citrus

University of Florida
Principal Investigator: L. Gene Albrigo
Agreement Number: AP19PPQS&T00C165
Amount Obligated: $252,413.00
Agreement Period: 09/01/2019-08/31/2021

Summary
Although the Asian citrus psyllid vector (ACP) and Candidatus Liberibacter asiaticus (CLas), the bacterial cause of the citrus disease huanglongbing (HLB), are endemic in Florida, this project is primarily targeted to Vector Management (Goal 1, Minimizing HLB reinfection of citrus by timing ACP sprays to new flush development rather than calendar timing or to the presumed effective residue of each spray chemical. This method will minimize the ACP seasonal population build up and reduce the ACP feeding transfer of CLas to new flush, but this proposal is also related to Goal 3 (Remediation), by preventing CLas buildup in new flush.

Project Status

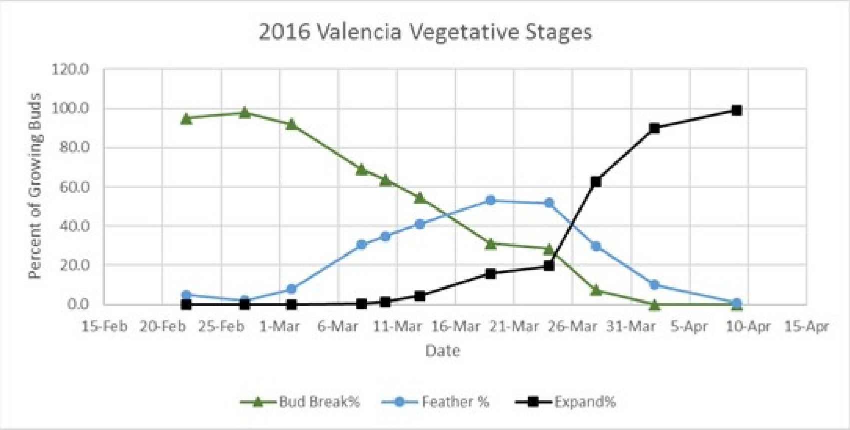
7%
Complete

Project Update

Objectives	Percentage Complete
1. Compare timing of psyllid sprays to grower spraying in 4 sites and 3 cultivars. Start counts and spray timings as appropriate from December to see which method saves sprays and which controls psyllids best • Grower control, flush timed sprays, 0.2 adults/tap spray and 0.7 adults/tap spray • Sample count adults, nymphs and eggs in three 10 tree sample areas/block	0-24%
2. Establish baseline photosynthesis in fall and follow through following two seasons to see if reduced psyllid feeding reduces loss of Pn function. • Test various ways to alter psyllid feeding. • Develop alternate method to assess blockage of sugar transport from affected leaves, Starch based.	0-24%

Achievements
- Test areas selected.
- Attempt to use iodine starch test to confirm that phloem transport is block and leaf starch has accumulated.
- Attempt on fresh tissue resulted in dark staining.
- Cryo-sectioned leaf tissue showed promise and will be examined further.

Next Steps
- The on-line flowering model will be followed to determine initiation of flower bud growth.
- First spray timing will be about two weeks later.
- Monitoring of psyllid stages will start first week of December.
- Each treatment spray after that will be determined by flush stage and/or psyllid counts depending on treatment.



The early bud break period, without feather flush, is an excellent time to control adult psyllids for the new season.

Field Implementation of An Advanced Multimodal Attract-and-Kill Device (CAPUT Trap) for Sustainable Management of Asian Citrus Psyllids

University of Florida
Principal Investigator: Lukasz Stelinski
Agreement Number: AP19PPQS&T00C073
Amount Obligated: $721,270
Agreement period: 4/1/19-3/31/21

Summary
Improving design of our attract-&-kill device and performing multi-state field testing to further optimize trap components is the main focus of our current research. We have identified field locations for performing our field trials in young and mature plantings in Florida and are making progress in TX and CA. Also, we are working on improving and optimizing the trap components with focus on the attractants and insecticides. We have started testing updated prototype designs in our cage experiments in order to optimize them for larger field testing.

Project Status

10%
Complete

Project Update

Objectives	Percentage Complete
1. Field efficacy testing and optimization of CAPUT trap design.	0-24%
2. Quantification of trap residual longevity in mature and newly planted citrus groves, homeowner backyards, and horticultural nurseries.	0-24%
3. Trap evaluation with combined Kaolin clay application.	0-24%
4. Assessment of trap compatibility with natural enemy populations.	0-24%

Next steps: Evaluate CAPUT under field conditions in young and mature groves.

T1- AKD with insecticide and all ingredients & attractant
T2- AKD with blank SPLAT
T3- Yellow sticky card (control)

Achievements

- Mortality counts of psyllids following exposure to attract-&-kill device with different insecticides showed that pyrethroids are most effective in killing psyllids that visited the traps. Baythroid XL caused 78% mortality of psyllids compared to 5% mortality in the control traps after 4 h. Another pyrethroid, cyfluthrin, killed 62% of psyllids in 4 h compared to 2% in the control trap. All other insecticides (organophosphate, neem derivative, and cyazypyr) tested caused very low mortality of psyllids in laboratory bioassays. Based on these results, we are pursuing pyrethroids as toxicants for our attract-&-kill devices for field trials and further optimizing their dose.
- Three different citrus volatile blends were compared in a small field trial to measure their effectiveness in attracting psyllids. These blends were identified by researchers from Florida and Texas in their individual research programs. In Florida, all three blends tested performed equivalently in attracting ACP.
- Field locations in south and central Florida counties were scouted to identify young and mature citrus plantings with sufficient psyllid populations to complete larger-scale field experiments.

Next Steps

- Attractant lure blend comparison experiments will be performed in TX and CA to compare with the results obtained in FL.
- Experiments will be established in the locations we have identified to determine device deployment strategies including height, density and location of devices in citrus beds in young plantings and mature citrus groves.
- Preparations are under way to test efficacy of our attract-and-kill device in the background of a visually contrasting white kaolin product (Surround WP™) to maximize the visual attraction of our device. Device designs will be evaluated in kaolin-treated and untreated blocks in commercial groves.

Area-Wide IPM of the Asian Citrus Psyllid in Urban-Commercial Citrus Buffer Zones

University of California, Riverside
Principal Investigator: Richard Stouthamer
Agreement Number: AP19PPQS&T00C106
Amount Obligated: $541,362.00
Agreement Period: 5/2019-5/2021

Summary

- The purpose of this project is to combine and incorporate all available biocontrol tools into a single area-wide control effort as part of a demonstration project to show their effectiveness in an existing pest control district's control operations and to determine which tactics or combination of tactics can be most effective.
- The goal is to replace the chemical control now applied in urban buffer zones with a sustainable biological control.

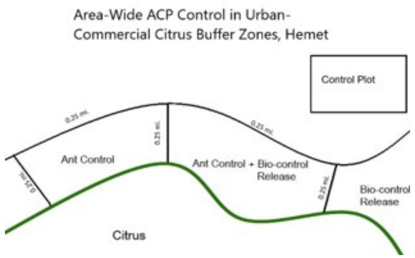
Project Status

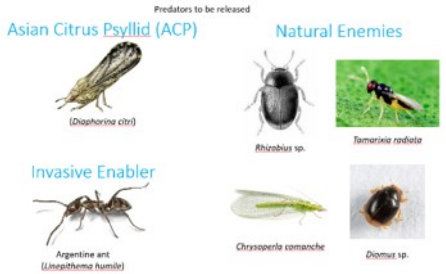
12%

Complete

Project Update

Objectives	Percentage Complete
1. Determine the viability of augmentative releases of natural enemies and ant control for Asian Citrus Psyllid (ACP) area-wide population control in urban citrus buffer zones next to commercial citrus.	0-24%
2. Determine if ant control alone in buffer zone areas where *Tamarixia* and other natural enemies are already present can achieve the same or better results than augmentative natural enemy releases.	0-24%
3. Determine if these same methods can be integrated into the current California Department of Agriculture HLB quarantine ACP control zones.	0-24%





Achievements

Program started in July 2019 and since this time we have :

- Hired personnel to do the field experiments
- Contracted with a commercial app development company to adapt an app for entering and uploading field data
- Contracted with insectaries for production of the natural enemies: *Rhyzobius lophanthae, Chysoperla comanche, Diomus pumilio* and *Tamarixia radiate*
- Developed methods to rear *Diomus pumilio*
- Assigned different zones surrounding commercial citrus to different treatments: all biocontrol agents, no treatment and ant control only. And each of these treatment zones in split into two, one with the standard pesticide treatments done by the California Department of Agriculture while the second zone does not receive this pesticide treatment
- Obtained permission from 174 home owners to sample ACP populations on their citrus trees in the different treatment areas.
- Set up a sticky trap sampling scheme in the different treatment areas
- Established experiment and started releasing natural enemies and deploying ant bait stations in September 2019
- Initiated sampling sticky traps and ACP populations on citrus trees through tap sampling September 2019

Next Steps

- Continue releases and sampling to determine impact of the different treatments
- Adapt natural enemy releases based on results to ultimately determine the minimal number of natural enemies required to obtain sufficient ACP control

Pre-symptomatic detection of HLB using commercially available, and economically scalable, remote sensors mounted to drones

Sentera, Inc.
Principal Investigator: Dimitris Zermas
Agreement Number: AP19PPQS&T00C239
Amount Obligated: $316,616.00
Agreement Period: 09/01/2019-08/01/2020

Summary
Conduct development and validation of a multispectral sensor mounted to a small unmanned aircraft system (sUAS) to remotely detect Huanglongbing (HLB) disease in citrus trees. The sensor uses narrow bands in the visual, near-infrared and long wave infrared spectra. The study attempts to replicate existing studies, aiming to validate and extend current results. The sUAS enables rapid, consistent, and extensive data collection.

Project Status

20%
Complete

Project Update

Objectives	Percentage Complete
1. Component selection and sensor design	100%
2. Development of hardware and software components of sensor	100%
3. Sensor prototype testing and integration to sUAS	50-74%
4. Data collection in Florida and California	0-24%
5. Data processing and detection algorithm development	0-24%
6. User interface design and commercialization	0-24%

Achievements
- Successfully created a 5 multispectral bands and 1 visual band sensor that can synchronously capture images from its 6 individual sensors
- Assembled the components (sensors and sUAS) needed for the collection of data
- Tested data collection for the custom-made 6 camera sensor
- Tested the integration of the 6-camera sensor on the sUAS

Next Steps
- Complete testing and integration of the data collection platform (sensors and sUAS)
- Perform the data collection flights in California, Texas, and Florida
- Gather groundtruth data on which trees have been verified to be HLB infected
- Preprocess data to bring into a usable format (All image spectra including thermal need to be aligned through image registration software)
- Perform analysis and testing to identify HLB deficient trees
- Develop software platform to expose the results of the HLB detection algorithms

Images

Sentera custom-designed 6 sensor camera without (left) and with (right) a stabilization gimbal

DJI M200 sUAV with a thermal camera and the custom Sentera camera