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Journal of Citrus Pathology

2024 IRCHLB VII Abstracts

Abstracts presented at the 7th International Research Conference on Huanglongbing (IRCHLB), in Riverside, California, USA, March 26-29, 2024 are now available on line at the **Journal of Citrus Pathology (JCP)**, International Organization of Citrus Virologists (IOCV) (http://journalofcitruspathology.com/).

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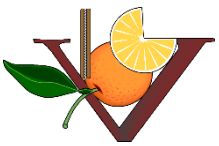
Recommended format for citing abstracts is presented below, using as an example the first abstract O1-1, in page 1:

Mandadi, K., Ramasamy, M., Irigoyen, S., Padilla, C., Yang, C., Ibanez, F., Ancona, V., Albrecht, U., El-Mohtar, C., Irey, M. (2024). Identification and evaluation of new chemicals and antimicrobial peptides for HLB management. *Journal of Citrus Pathology*, 11(1):1. http://dx.doi.org/10.5070/C411163652 Retrieved from https://escholarship.org/uc/item/33r648r0



MARCH 26-29, 2024
RIVERSIDE CONVENTION CENTER
RIVERSIDE, CALIFORNIA, USA

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Identification and evaluation of new chemicals and antimicrobial peptides for HLB management

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Abstract: '*Candidatus* Liberibacter asiaticus' (CLas) associated with citrus greening (aka huanglongbing [HLB]) poses an immense threat to the citrus industry and causes billions of dollars of economic losses. A few bactericides, such as oxytetracycline and streptomycin, were recently approved for emergency use in citrus and HLB management in Florida. Identifying alternative (or complementary) therapies effective against CLas is critical for sustainable HLB management. Unfortunately, screening antimicrobials effective against CLas is a bottleneck due to the unculturable nature of CLas. Here, using an innovative citrus hairy root-based efficacy testing system, we identified multiple novel chemicals and antimicrobial peptides that showed activity against CLas. Ongoing field evaluation with selected candidates in HLB-affected trees in Texas and Florida showed promising improvements in fruit yield and tree health. One or more of these antimicrobials could be further commercialized as products for HLB management.

Non-technical summary: Recently, oxytetracycline and streptomycin-based products were approved for use in huanglongbing (HLB) management in Florida. However, over-reliance on only a few active ingredients could lead to antibiotic resistance. In this study, we have implemented a pipeline for discovering and evaluating new HLB therapies. The new therapies could be deployed as alternative or complementary therapies in the future for sustainable HLB management.

Identification of antimicrobial compounds from the citrus microbiome with potential to manage HLB disease

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Abstract: Huanglongbing (HLB) is a devastating citrus disease, associated with the Gram-negative, phloem-limited and unculturable bacterium, '*Candidatus* Liberibacter asiaticus' (CLas), and transmitted by the psyllid, *Diaphorina citri*. Secondary root pathogens, such as *Phytophthora* and *Fusarium* spp., are linked to accelerated HLB tree decline. In previous work, we investigated the citrus-associated microbiome profile of HLB-impacted trees, which generated a citrus microbe culture collection. *Bacillus* isolates from our culture collection that showed *in vitro* inhibitory activity against *Liberibacter crescens* (Lc), have been screened against two species of *Phytophthora* and *Fusarium* spp. in *in vitro* assays. Isolates that showed inhibition of Lc and *Phytophthora* and *Fusarium* spp. have been selected for metabolite profiling using LC-MS. Mass spectrometry analysis revealed the presence of amicoumacins in inhibitory fractions to Lc; for example, the known antibiotic amicoumacin A was observed in an isolate identified as *Bacillus safensis*. MS/MS-based molecular networking analysis of the crude extract revealed a network of amicoumacins and related compounds, as well as a pumilacidin/surfactin network. In addition, samples containing amicoumacin A showed significant inhibitory activity to CLas in an in *vivo* assay. Purification of amicoumacin A is still ongoing and bioassays with purified amicoumacin A compound will reveal its potential use in HLB-affected plants. We identified several *Bacillus* spp. inhibitory to citrus root pathogens *Phytophthora* spp. and *Fusarium* spp. in dual-microbial assays. Further investigation of the crude extract of these isolates and comparison with the predicted BGCs will aid in the identification of target compounds to be obtained either via purification or heterologous expression. We are building upon our previous findings and discovering the chemistry and functional diversity of the citrus microbiome, identifying potential natural products and bioinoculants that can be used to manage HLB as a disease complex.

Non-technical summary: We are mining the citrus associated microbiome to find antimicrobial compounds with potential to suppress '*Candidatus* Liberibacter asiaticus' (CLas), the huanglongbing (HLB) associated pathogen, and secondary citrus root pathogens that are known to aggravate root decline in HLB trees. We found one antimicrobial compound, amicoumacin A, produced by a bacterial isolate that showed inhibitory activity to *Liberibacter crescens* and CLas and we identified several *Bacillus* spp. inhibitory to citrus root pathogens *Phytophthora* spp. and *Fusarium* spp. We are building upon our previous findings and discovering potential natural products and bioinoculants that can be used to manage HLB as a disease complex.

Soil Health: Foundation of Sustainable Huanglongbing Disease Management in Citrus Groves

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Abstract: Soil is a fundamental component of agriculture and soil-water-root dynamics are critical for grove management. An optimum soil microclimate can sustainably improve tree health and productivity of huanglongbing (HLB) affected citrus groves. In Texas, irrigation water scarcity and poor quality including high salinity, and *Phytophthora* intensify HLB-induced root losses that affect tree productivity and fruit quality. Therefore, various grove floor management strategies have been evaluated to alleviate these abiotic and biotic stresses and improve tree health. The effects of the application of biochar and compost as soil amendments and the deployment of woven black plastic mesh as ground cover (GC) on soil microclimate, root growth and pre-harvest fruit drop of HLB affected 'Rio Red' grapefruit trees were studied in Texas. Individually, the application of biochar or compost and the deployment of groundcover improved soil moisture retention relative to the bare ground control, while stacking any soil amendment with ground cover had additive effects. In addition, soil amendments and the deployment of ground cover significantly lowered soil sodium (39-57%) and chloride (75-86%) contents in the root zone. Grove floor management treatments affected root growth during fall and spring. Application of the two biosolids either alone or covered with the black plastic mesh significantly increased root growth index, but substantially reduced fruit drop relative the untreated bare ground. This study highlights the importance of grove floor management practices in mitigating stresses and maintaining the productivity of HLB affected grapefruit trees.

Non-technical Summary: Soil health is a key for production sustainability in the times of climate change. Soil amendments and soil matting play a vital role in developing healthy rhizosphere that relieves huanglongbing (HLB)-induced stress in the citrus trees and improves fruit quality as well. Therefore, grove floor management is becoming an inevitable part of HLB management in the Texas citrus groves.

Field Evaluation of Small Molecules for Citrus Huanglongbing Management in Texas

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Abstract: Huanglongbing (HLB), caused by '*Candidatus* Liberibacter asiaticus' (CLas), is an important disease of citrus that has caused catastrophic damage to citrus trees worldwide. The disease causes reduced fruit quality and yield and eventual tree death. Several studies have shown that ampicillin and tetracycline can reduce CLas titers and temporarily improve citrus fruit quality and yield. However, these bactericides pose a threat to the emergence of antibiotic-resistant bacteria. In this work, our team has screened and discovered several small molecules that have efficacy against CLas in citrus hairy root bioassays to develop new HLB management strategies. The effects of three small molecules (CL3, CL6, and CL8) were further evaluated on HLB-affected grapefruit trees in the field by trunk injections alongside oxytetracycline (OTC), water, and solvent controls. Quantitative PCR results indicated that CLas titers were significantly reduced by all three small molecules 15 days after injection, similar to OTC, while water and solvent controls had no significant effect on CLas titers. In addition, fruit yield was increased in all three molecules and OTC compared to water and solvent controls. The highest yield was recorded in the CL8 treatment, followed by CL3, while CL6 and OTC had similar yields. Juice analysis showed that only CL8 significantly increased Brix in HLB-affected citrus juice. CL8 also significantly enhanced canopy color and density in HLB-affected citrus after injection on par with OTC. In conclusion, field evaluation of the three small molecules shows promising results for CLas titer suppression, increased yield, and improved HLB-affected citrus tree health.

Non-technical summary: Huanglongbing (HLB) causes loss of citrus production worldwide. HLB therapies that prolong citrus productivity are needed as a short-term solution against this disease. Although antibiotics can reduce '*Candidatus* Liberibacter asiaticus' (CLas) titers and improve the field performance of HLB-affected citrus, they also result in safety concerns for the environment and human health. This work evaluated three new small molecules that suppressed CLas titers and enhanced the yield and performance of HLB-affected citrus trees. These molecules are promising alternative therapies for HLB management in the near future.

Flipping the HLB therapeutic screening paradigm: That's right we went straight to the field

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Abstract: There is an immediate need to provide the citrus industry with relief from huanglongbing and a return to profitable citrus production. Years of research and more than a billion dollars have been spent looking for a solution. Standard screening methods for therapeutic molecules involve various laboratory-based assays to quickly select molecules with the desired properties. Typically, these will be either antimicrobial or insecticidal properties depending on the target of the research. Some drawbacks of this standardized framework are 1) the correlation between the lab assays and tree rejuvenation are unknown; 2) antimicrobial activity is only one of many potential mechanisms to restore tree health; 3) systemic movement cannot be determined though it is one of the most important properties of a therapeutic molecule; and 4) laboratory testing delays getting molecules into the field to determine if it works. A design-of-experiment framework base was developed to rapidly screen molecules in field citrus to select molecules that improve tree health over the course of a season. Utilizing the injection system developed for the delivery of oxytetracycline into citrus for '*Candidatus* Liberibacter asiaticus' control, we injected 88 molecules into 8-year-old Valencia citrus over the course of six weeks. The framework and the results from these injections will be presented.

Non-technical summary: The citrus industry is in desperate need of a huanglongbing (HLB) solution. To meet this need, a novel framework designed to quickly screen treatments for HLB control was created. To date 88 molecules have been injected into Valencia citrus trees. These will be monitored for health changes over the course of a year.

Symbiont™ Technology: development and optimization of a novel delivery approach for therapeutics to control '*Candidatus* Liberibacter asiaticus'

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Abstract: Citrus huanglongbing (HLB) presents a significant challenge in disease management due to the systemic, vector-transmitted, and phloem-limited lifestyle of the causative agent '*Candidatus* Liberibacter asiaticus' (CLas). Effective therapies for HLB will require scalable manufacturing and reliable, affordable, low-maintenance delivery into the phloem of trees in the field. We describe the development of Symbiont technology that coopts Agrobacterium tumefaciens ability to insert DNA into plant genomes (the transferred DNA is called T-DNA). By modifying the T-DNA to express only plant growth regulator (PGR) genes and a gene of interest, we created a cluster of dividing plant cells that produce therapeutic biomolecules. We call the structure a Symbiont because of its benefit to the plant. The Symbiont has vascular connections to the plant and continually produces therapeutic peptides. No opine genes are encoded in Symbiont plasmids, and thus, disarmed Agrobacteria do not persist in Symbionts. Optimization of light exposure, temperature and humidity greatly enhanced growth and homogeneity of marker gene expression for Symbionts grown on citrus trees in controlled conditions. Certain plasmids led to more rapid development of Symbionts with increased longevity. Ongoing work is focused on using Symbionts to screen antimicrobial peptides that reduce CLas titer and improve tree health and to enhance peptide export from the Symbiont. Symbiont technology is also a promising platform for plant cell based in vitro production of biomolecules, which can be purified and injected into diseased plants. Seven binary vectors were constructed that contain, within the T-DNA, various iterations of the PGR genes, a cloning site for candidate antimicrobials and/or other therapeutics, a fluorescent/visual reporter, and an antibiotic resistance gene for transformed cell selection. Each vector has a unique phenotype in Symbionts and in Symbiont cells in in vitro culture. While early-stage technology, Symbionts may one day provide a biological approach to deploying plant therapeutics.

Non-technical summary: We developed a plant cell-based system called Symbionts (patent pending) that administers a continuous supply of therapeutic molecules to a plant to combat diseases. This system combines genes that help plants grow more cells on their stems with genes that produce special molecules to fight diseases. A preliminary screen of antimicrobial peptides in potted citrus trees found four molecules that reduced the amount of harmful bacteria causing huanglongbing and made the disease symptoms less severe.

N-acetylcysteine as tool for Huanglongbing management: results in the Brazilian orchards

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Abstract: The control of huanglongbing (HLB) disease in orchards is based on using insecticides against the vector and eradicating symptomatic plants. However, the number of diseased plants in the field has increased recently, signaling a gap in disease control. HLB-diseased plants struggle with the high levels of oxidative stress that impacts plant physiology and development. To deal with this problem, we used N-acetylcysteine (NAC), a cysteine analog widely used as an antioxidant in medicine and with positive effects in citrus plants affected by citrus variegated chlorosis (CVC) and citrus canker. The first trials regarding HLB were performed in a greenhouse. HLB-positive plants were sprayed monthly with NAC. As a result, NAC promoted a significant reduction of the levels of reactive oxygen species and increased the activities of antioxidant enzymes in those plants. Currently, a long-term field trial is ongoing on two orchards of sweet orange varieties ('Rubi' and 'Valencia') in a region where HLB incidence increased from 20% in 2020 to 40% in 2023. The plants were evaluated for callose deposition, disease incidence and severity, fruit drop, and fruit production on treated and untreated plants. During the first three years of evaluation, it was verified that NAC-treated Ruby plants showed a 53% reduction in HLB incidence, and the severity was 50% lower than in untreated plants. NAC also enhanced productivity while reducing fruit drop by 30% compared to untreated plants. Similar results were observed in Valencia, with a reduction of 55% in fruit drop, while production increased by 6 tons/ha in plants treated with NAC. Taken together, the results from the greenhouse and field study demonstrate that NAC could be incorporated into integrated disease management to reduce the damage caused by HLB. Additionally, it is a sustainable and environmentally friendly molecule easily applied in agriculture.

Non-technical summary: The N-acetylcysteine (NAC) molecule has proven useful to control many citrus bacterial diseases, including huanglongbing (HLB). This molecule is sustainable and environment- and human-friendly. Since 2020, a NAC-based strategy has been field-tested for HLB management. Experiments carried out in the State of São Paulo, Brazil, showed that orange trees, when treated with NAC, exhibit greater productivity, less fruit drop, and reduction in the incidence and severity of HLB.

Formulated Zinc particles significantly improve HLB-affected tree health

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Abstract: Application of Zn on huanglongbing (HLB)-affected citrus trees holds promise in maintaining health and improving productivity. Field performance of several industry-grade Zinc particulate (nanometer- and micron- sized) based formulations were evaluated on HLB affected citrus trees in Florida, USA. Multi-year field trial (2016-2017, 2017-2018) of nano-sized ZnO (~4.0 nm average size; Zinkicide®) conducted on grapefruit and Valencia sweet orange showed significant dose-dependent (0.25 lb – 2.0 lb/acre Zn) positive effect on yield (~ up to 30% improvement in comparison to untreated control, UTC), fruit size (grapefruit) and juice quality (Valencia brix-acid ratio: 10.5 -12 with Zn treatment and ~ 10.0 for UTC) at harvest. Six year-old grapefruit trees responded to nano-ZnO treatment better than 20+ year-old Valencia trees, which were heavily damaged by HLB and hurricanes. Grapefruit trial results showed that foliar application was effective but further improvement in yield was achieved when combined with soil drench. Both application methods were important to observe significant yield and juice quality response for Valencia. This suggested limited Zn uptake efficiency in old trees as compared to young trees. Zinkicide® demonstrated systemic activity and reduced hurricane damage in young trees. Recently, field performance (2022-2023 season) of two micron/sub-micron-sized fertilizer-grade Zn formulations, Zn hydroxide (Ferti-Zink) and Zn sulfide (NuZinc) on Valencia fruit yield and quality was evaluated. Irrespective of the dosage used, trees that received three applications of both compounds increased yield by 53% as compared to UTC (215 Kg of fruit per 3 trees replicate as compared to 140 kg per three-tree replicate), and by 10% as compared to trees treated with Zn sulfate (grower standard control). Brix was not significantly affected, but ratio was increased irrespective of the number of applications (around 11 as compared 9.7). Overall, the above findings suggest that formulated Zn products have significant positive effect on HLB affected trees.

Non-technical summary: Formulated Zn particulate products, irrespective of type and particle size, improved huanglongbing effected tree health and productivity. Treatment effect on yield was significantly higher than untreated or grower standard control while maintaining marketable fruit and juice quality.

Combining individual protective covers and brassinosteroids prolongs young citrus tree health under endemic HLB conditions

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Abstract: Preserving the health of newly planted citrus trees in an huanglongbing (HLB)-endemic environment is paramount. The risk of infection is exceptionally high for young trees, which flush frequently, attracting the Asian citrus psyllid (ACP). With increasing psyllid resistance to insecticides, individual protective covers (IPCs), a psyllid exclusion tool, are being adopted in Florida to protect newly planted trees. However, IPCs must be removed after 2-3 years, leaving trees exposed to psyllids and eventually get ACP infestation and disease infection. Brassinosteroids (Brs) are a class of plant growth regulators approved for commercial use in Florida citrus. Brassinosteroids induce immunity in plants including citrus, and may delay HLB progression. This study investigates the combined use of IPCs and Brs for protection against HLB and other diseases. We hypothesize that Brs use will prolong the health of young trees after IPC removal. We are studying several parameters in the response of young citrus trees to this combined treatment strategy, including (i) effects of IPCs, Brs, and their combination on tree physiology and immunity; (ii) effects on fruit production, including yield and quality; and (iii) effects on HLB incidence and progression. We started a monthly Brs spray regime when IPCs were removed from trees after 36 months. The infection rate of Brs-treated trees was significantly delayed, and by four months, 80% of trees remained non-infected compared to 40% for untreated trees. Genes related to the salicylic acid pathway, including isochorismate synthase, isochorismate mutase, and phenylalanine ammonia-lyase, were significantly upregulated for up to 6 weeks after each Br treatment, and fewer ACPs were observed in new flushes. Fruit yield was increased due to both more fruitset and fewer drop. Taken together, the use of IPCs followed by Brs provides an effective management strategy to prolong the health of young citrus trees growing under heavy HLB pressure.

Non-technical summary: Monthly brasinosteroid treatment of young citrus trees after removing individual protective covers provided protection against '*Candidatus* Liberibacter asiaticus' infection, reduced Asian citrus psyllid populations, and increased fruit yield.

Proteomic, transcriptomic, and yeast di-hybrid analysis of the '*Ca.*' Liberibacter-psyllid vector pathosystem to dissect pre- and/or acquisition stages of circulative, propagative transmission

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Abstract:'*Candidatus ('Ca.')* Liberibacter solanacearum' (*C*Lso) is the causal agent of zebra chip and vein-greening diseases of potato and tomato, respectively, and is transmitted by the potato psyllid (PoP) *Bactericera cockerelli* (Sulc.) in a circulative, propagative manner. Some similar and distinct biological characteristics are shared by the sister patho-system, citrus greening disease, which is caused by '*Ca.*' Liberibacter asiaticus (CLas) transmitted by the Asian citrus psyllid (ACP). A recent study of CLas-ACP pathogenesis involving gut and circulative invasion implicated perturbation of physiological processes in late-nymphal and teneral adult stages, the life stages biologically-associated with pre- and post-ACP acquisition of CLas. In contrast, PoP transmits CLso when ingested by immature and adult instars. To elucidate organ-specific mechanisms involved in Liberibacter spp. invasion of the psyllid host gut and salivary glands-acquisition, comparative analyses of both pathosystems, as was possible, based on transcriptomic, proteomic, yeast di-hybrid, and/or TEM analysis data, were carried out to dissect pathogenesis-related genes and predicted mechanisms involved in gut invasion and salivary glands-acquisition. Results indicated that '*Ca.*'-Liberibacter utilizes psyllid-host/vector endo-exocytotic pathways and enlist cytoskeletal remodeling to invade, colonize, and exit the psyllid gut. Salivary glands profiles further support late-nymphal/adult activity in response to '*Ca.*'-Liberibacter-infection. Intracellular stationary and motile lifestyles appear to be essential for '*Ca.*'-Liberibacter navigation psyllid host organs and tissues. These observations are consistent with biological evidence that infection of late nymphal stages is requisite to efficient ACP adult acquisition and transmission.

Non-technical summary: New knowledge about molecular and cellular interactions involved in Liberibacter-psyllid vector circulative invasion and acquisition processes that culminate in transmission will further the understanding of fundamental aspects, processes, and mechanisms governing transmission of fastidious bacteria by homopteran insect vectors. Further, identification of psyllid genes required for Liberibacter-gut and salivary glands invasion can guide the identification of gene targets to facilitate RNA-interference biopesticide technology development.

Silencing of the most abundant gut surface proteins of Asian citrus psyllid

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Abstract: The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is the major vector of '*Candidatus* Liberibacter asiaticus' (CLas), the presumed causative agent of citrus greening disease or huanglongbing. This disease has significantly impacted citrus production in most citrus-producing regions of the world. CLas is acquired by ACP on feeding and the bacterium interacts with the surface of the gut epithelium prior to infection of ACP. Disruption of CLas interaction with proteins on the gut surface could disrupt ACP infection and subsequent transmission of CLas to citrus trees. In this study, double-stranded RNA (dsRNA) was used to knockdown the expression of the nine most abundant gut surface proteins of ACP (Tavares et al, 2022), based on the hypothesis that CLas binds the most abundant proteins on the surface of the ACP gut. Gene silencing or control dsRNAs were fed to ACP in artificial diet in membrane feeding assays. No significant mortality over controls was observed by day five. The knockdown efficacy of transcripts encoding nine ACP gut surface proteins as measured by RT-qPCR ranged from 20 to 51% at 72 h, and 41-63% at 120 h. The impacts of these dsRNAs on the acquisition of CLas by ACP for the development of RNAi-based disease management tools will be assessed. Reduced production of key ACP proteins on the gut surface that impedes CLas acquisition and load in the psyllid could alleviate the burden of this disease to the citrus industry.

Non-technical summary: The bacterium that causes huanglongbing or citrus greening disease is delivered to citrus trees by the Asian citrus psyllid (ACP). The ACP ingests the bacterium from an infected tree when it feeds, and the bacterium associates with the surface of the gut before infecting the insect. We used RNA interference to reduce each of the nine most abundant proteins on the ACP gut surface with the goal of assessing the impact of reduced abundance of key proteins on infection of the psyllid. This approach could ultimately be used to reduce transmission of the disease agent, to the benefit of the citrus industry.

Citation: Tavares, C.S., Mishra, R., Ghobrial, P.N., Bonning, B.C. 2022. Composition and abundance of midgut surface proteins in the Asian citrus psyllid, *Diaphorina citri.* J. Proteomics 261:104580 doi: 10.1016/j.jprot.2022.104580

High-throughput Sequencing of Asian citrus psyllid (*Diaphorina citri*) in Distinct Populations and Developmental Stages from Florida

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Abstract: The Asian citrus psyllid (ACP, *Diaphorina citri*) vectors the '*Candidatus* Liberibacter asiaticus' bacterium and spreads the most destructive citrus disease, huanglongbing or citrus greening, throughout Florida groves. Researchers are currently investigating ACP-associated viruses as future biological and targeted control options of the ACP. These insect-specific viruses (ISVs) may have increased specificity and lethality toward the ACP compared to broad-spectrum and environmentally harmful insecticides. We previously surveyed ACP-associated viruses in Florida citrus grove populations and documented geographical distributions and abundances of these viruses. In this study, we used high-throughput sequencing (HTS) of laboratory-reared ACP adults and nymphs, and field-collected adults and nymphs to investigate viral associations between populations and developmental stages. We observed diverse characteristics of certain ACP-associated viruses and the taxonomic composition of these insects. Specifically, the RNA viruses Diaphorina citri flavi-like virus (DcFLV) and Diaphorina citri reovirus (DcRV) were seen at substantially high reads per kilobase million transcripts in the laboratory insects compared to zero detection in the field insect populations. DcFLV and DcRV were also consistently detected in these confined insects each month, suggesting a nonpathogenic, dual-infection association. Conversely, the RNA virus Diaphorina citri-associated C virus (DcACV) was seen in high abundance only in the field adults but with zero detection in the field nymphs or the laboratory populations. DcFLV, DcRV, and DcACV were seen in much higher abundance in the adult ACPs compared to their nymph counterparts. All populations had RNA sequences of Diaphorina citri densovirus-associated endogenous viral elements but no detection of the full-length virus itself. Finally, we identified a novel ACP-associated virus, tentatively called Diaphorina citri virga-like virus (DcVLV), exhibiting both plant virus and suspected ISV characteristics. Results of this investigation contribute to the characterization and evolving behavior of the Florida ACP virome, as well as ISV behavior in these distinct populations.

Non-technical summary: Viruses that infect the Asian citrus psyllid (ACP) are of high interest to manipulate for targeted control of the huanglongbing (citrus greening) insect vector. We found diverse viral associations and a putative novel virus within distinct populations of the ACP vector. This knowledge can help uncover the best virus candidates to use for future lethal infection and control of the ACP.

Generation of an optimally attractive scent for Asian Citrus Psyllid (ACP) biocontrol

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Abstract: A first generation of a scent lure for controlling the Asian Citrus Psyllid (ACP) is presented. ACP is an invasive insect vector spreading the bacterium causing huanglongbing (HLB) disease that is devastating the citrus industry. Intense insecticide application to control ACP populations provides only temporary relief as well as causes collateral damage to ecosystem, while continual replanting of diseased groves is unsustainable. An alternative strategy is suppressing ACP via efficient trapping using lures. Prior research has shown ACP is attracted to volatiles emitted by HLB-infected citrus more than healthy plants. A multi-component lure blend was demonstrated to be more attractive to ACP compared to controls in lab and field trials when combined with an insecticide. However, generating complex smells is challenging, as relatively small deviations in components' ratios could lead to diminished efficacy. Current wick-based release technology, most commonly used in volatiles release, does not allow to precisely control compounds ratios, and has unavoidable drift in released smell composition due to differing volatilities of released compounds. Recently, an ultra-low-cost graphene-based sorbent has been developed. This material allows both wicking and releasing volatiles via instantaneous heating. We use this technology for controlled multi-compound release to manufacture a portable, robust, low-cost lure device capable of emitting complex smell with the ability to precisely control compounds' ratios. This device allows fine-tuning volatiles release to optimize smell for maximum attractiveness to ACP. Consequently, in combination with a pesticide, this device can be used for an "Attract and Kill" (AK) strategy for ACP control. The device is fabricated using biodegradable 3D printing for simple manufacturing. The lure is currently undergoing lab testing. We aim to validate the lure technology for broad-use ACP control.

Non-technical summary: This project aims to control the Asian Citrus Psyllid, a destructive citrus pest using scent as a natural lure. Using novel material, we are developing a device that can generate complex smells by precisely controlling each component. An effective scent lure could provide a sustainable pest control solution for the citrus industry worldwide.

Low rate of processed kaolin to reduce *Diaphorina citri* population and huanglongbing incidence in a commercial citrus orchard

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Abstract: The application of processed kaolin at rates ≥ 2% has already been published as a promising tactic for managing *Diaphorina citri*. In the present study, the effect of low rate of processed kaolin (Surround WG) sprays on *D. citri* population and huanglongbing (HLB) incidence in a commercial citrus orchard was assessed. The experiment was performed on eight hectares of a *Citrus* × *sinensis* 'Natal' orchard located in the municipality of Pirajuí, SP, Brazil. The orchard was 27-month-old at the beginning of the experiment. The experiment was conducted in a randomized block design with four blocks (2 hectares/block) and two treatments: 1) fortnightly kaolin sprays 1,5% during Jul-Dec (psyllid population peak) and 1% during Jan-Jun; and 2) no kaolin sprays. Both treatments were insecticide sprayed every 10 days. Psyllid population was monitored fortnightly using yellow sticky cards, and HLB incidence was determined by quarterly visual inspections in 100% of the citrus trees. After 25 months, in the kaolin-sprayed plots, the number of psyllids/yellow stick card (mean of 0.8 psyllids) and HLB incidence (mean of 37.1%) were 20% and 52% lower than in plots without kaolin sprays, respectively. This study shows for the first time that fortnightly kaolin sprays at concentrations lower than 2% reduce the psyllid population and HLB incidence in a commercial citrus orchard.

Non-Technical Summary: The reason for low adherence to the use of processed kaolin by citrus growers is the high product cost per application. In the present study, it was demonstrated that fortnightly kaolin sprays at low rate (1 - 1,5%) reduce the psyllid population (20%) and huanglongbing incidence (52%) when compared with areas without kaolin sprays.

Evaluating cover crops as habitat for the natural enemies of Asian citrus psyllid

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Abstract: Cover crops provide essential nutrients and improve soil health. They also provide habitat for the natural enemies (NE) of Asian citrus psyllid (ACP). NEs are facultative omnivores and opportunistically feed on nectar from extra floral nectar glands and flowers and on pollen. NEs forage primarily in shallow flowers with exposed nectar glands. NE primarily feed on alternate prey, such as aphids, that colonize cover crops and on their honeydew. From 2017 to present, test plants were grown in microplots at a botanical garden in south Florida and weekly evaluations were made of plant vigor and the occurrence of aphids and ACP predators. Plant species were selected based on their floral morphology, adaptation to local climate, and seed availability. All plants were annuals, and some could re-seed. Predator abundance followed aphid colonization of cover crop plants. Plants with the highest levels of aphids and predators in the cool season (February to April) included field mustards, coriander and cilantro, dill, and cowpea while warm season (May to October) plants included cowpea, okra, sorghum, and portulaca. Aphids did not colonize some plants, such as hairy vetch and Sunn hemp, while other species were eruptively attacked by herbivores. The most common NEs were lady beetles (coccinellids), hoverflies (syrphids), trash carriers (chrysopids), and the parasitic wasp, *Tamarixia radiata*. Video surveillance of orange jasmine flush infested with ACP showed that immature hoverflies decimated ACP colonies overnight and drove psyllid mortality. While sometimes abundant, lady beetles were not as important ACP predators as hoverflies. Exclusion tests showed that NEs could reduce or eliminate psyllid infestations on individual flush. The results suggest that certain cover crops can support NEs, which, in turn, effectively reduce ACP. Ongoing studies are showing that cover crops could be used as part of a push-pull strategy to direct insect movement within the grove.

Non-technical summary:
Since 2017, cover crop plant species have been grown and evaluated for their ability to attract and support natural enemies (NE) of Asian citrus psyllid (ACP) in south Florida. In our tests, immature hoverflies were the primary predator of immature ACP while lady beetles and trash carriers also contributed to psyllid mortality. Plants with the highest levels of NEs in the cool season (February to April) included field mustards, coriander and cilantro, dill, and cowpea while warm season (May to October) plants included cowpea, okra, sorghum, and portulaca. The results showed that cover crops can support psyllid NEs that, in turn, can reduce ACP populations.

High tree density as a cultural strategy for the HLB management at the edge of citrus orchards

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Abstract: Brazilian citrus growers increased tree density from 2004 to 2022 by almost 40%. High tree density (HTD) may intervene in huanglongbing (HLB) spread. We hypothesized that HTD at the edge of orchards may serve as a barrier to Asian citrus psyllids (ACP) carrying the HLB bacteria from external areas to the orchard´s center, and compensate the loss by HLB-affected trees. A sweet orange orchard was planted in May 2020 at 6.5 m between-rows without irrigation. Three in-rows spacings were evaluated at the edge (0-100 m from the orchard perimeter): 1.8 m (commercial standard), 1.2 m, and 0.9 m. Treatments were distributed in four randomized blocks with 16,500 m^2 per plot. From 100–200 m to the edge (central area), in-rows tree spacing was maintained at 1.8 m. Flush shoot (FS) dynamic, number of captured ACP per trap and HLB-symptomatic trees were assessed at the edge and central area from planting to August 2023. Tree size and FS frequency were similar among the evaluated treatments. However, trees were already hedged at 0.9 m spacing two years after planting. No differences were observed for ACP gradient and population until three years of planting. The progress of number of HLB-symptomatic trees was also similar among the spacings. Nevertheless, a dilution effect was observed, i.e., HTD reduced the cumulative HLB incidence (number of HLB-symptomatic trees per total number of trees in the plot), which was higher at 1.8 m spacing (16.2%) than 1.2 m (9.2%) and 0.9 m (6.7%) spacing. ACP population and HLB incidence were clearly higher at the edge than central area. There was a clear tendency towards a reduction in disease incidence in central areas as spacing at edge was reduced. Therefore, HTD should be considered in HLB management as a strategy to reduce HLB incidence and damage.

Non-technical summary: Citrus orchards with high planting density could be used as a cultural strategy for the huanglongbing (HLB) management, as it may compensate for the loss of HLB-affected trees. In this research, we investigated the effect of different tree density at the orchard edge on flush shoot dynamics, Asian citrus psyllid (ACP) capture e HLB incidence. Our results showed that after three years of planting the frequency of flush shoots, ACP abundance and number of HLB-infected trees are similar among different tree spacings in young sweet orange orchards under vector control. Nevertheless, high tree density may contribute to HLB management and damage mitigation, because the relative disease incidence is significantly lower at the orchard edge due to a dilution effect.

Canopy volume, productivity, and huanglongbing incidence on Natal sweet orange grafted onto semi-dwarfing and dwarfing rootstocks at ultra-high density

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Abstract: The use of ultra-high density (UHD) and low-vigor inducing rootstocks are cultural practices that may contribute to the huanglongbing (HLB) management. We evaluated the canopy volume, productivity, and HLB cumulative incidence on Natal sweet orange trees grafted onto two dwarfing rootstocks (tetraploid Swingle citrumelo/SW4x and Lindcove citrandarin/LC), two semi-dwarfing rootstocks (IAC 1711 and San Francisco/SF citrandarins) and the industry standard, Swingle citrumelo/SW. The orchard was planted in April 2016 in Matão, São Paulo State, at 5.5 x 1.5 m tree spacing (1,215 trees/ha). Trees were drip irrigated from 2018, and mechanically pruned in 2019 and 2020. Experimental design was a 5x5 Latin square design with five replications of 500 trees per rootstock in the plot (13 ha in total). The experimental area was surrounded by an edge of 15-25 m using trees grafted on the vigorous IAC 1710 citrandarin rootstock. Symptomatic trees were eradicated upon qPCR every three months, and the vector was controlled by strict insecticide applications. Tree size was measured in 2023 and the total canopy volume per area was estimated, giving about 23,000 m^3/ha for SW, whereas it decreased by 33% and 58% for the semi and dwarfing rootstocks, respectively. The cumulative productivity from 2019 to 2022 was the highest for trees grafted onto 1711 (4,764 90-lb boxes/ha), intermediate for SW and SF (mean of 4,285 boxes/ha) and the lowest for both dwarfing rootstocks (mean of 2,405 boxes/ha). Regarding to the HLB cumulative incidence from planting to July 2023, no difference among the evaluated treatments (mean of 2.22%). By way of comparison, cumulative HLB incidence was 10% for Natal trees grafted onto 1710 in neighboring blocks at same age but half tree density. Until the date, semi-dwarfing rootstocks at UHD conjugated high yield with less canopy volume, and much less HLB incidence under evaluated conditions.

Non-technical summary: Semi-dwarfing rootstocks could be readily used in ultra-high density orchards to maintain a competitive productivity while decreasing canopy volume due to a higher production efficiency. On the other hand, either closer tree spacing or adjusted nutrition and better-adapted varieties should be further investigated to improve the performance of dwarfing rootstocks. Moreover, such intensive production system based on dwarfed or semi-dwarfed trees may favor a more management of huanglongbing in addition to other benefits for the citrus industry in the next decades.

Moderate shading mitigates HLB severity and increases yield

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Abstract: Although it is generally recognized that huanglongbing (HLB) and adverse environmental conditions compound to impact tree health, no studies have addressed what above-ground conditions mitigate disease severity. Citrus shoots do not saturate photosynthesis under full sun conditions, but they cannot meet the water demand under full sun across the whole day. Because HLB disease impacts growth and photosynthesis in part by reducing root growth and source-sink dynamics, we hypothesized that shading trees could balance source-sink dysfunction and the demand for water imposed by high irradiance and HLB disease. In a multi-year study, we installed shade netting of various densities (30%, 50%, and 70% shading) and a non-shaded control above mature, infected trees. The shade environment alleviated symptoms and increased growth. Shade increased the total aboveground growth and fruit production, while reducing canopy density. Asian citrus psyllids per tree were reduced by reducing the intensity of flushing, despite increased biomass. Shaded trees were able to maintain higher photochemical performance, while investing less in stress protection. Sap flow and stomatal conductance of leaves suggest that moderate shading allows trees to transpire more than fully sunlit or heavily shaded trees. Hydraulics play a key role in HLB-affected ecophysiological dynamics and can be manipulated to enhance growth under endemic HLB.

Non-technical summary: Moderate shading of huanglongbing-affected citrus trees approximately doubled yield over three years. This was probably caused by better balance of water demand with water supply from the weakened root system. Vector populations were also reduced by shading.

Vulnerability of citrus to infection by '*Candidatus* Liberibacter asiaticus' is influenced by air temperature and the developmental stage of new shoots

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Abstract: Evidence that the developmental stage of new shoots (NS) and the environment impact the establishment of '*Candidatus* Liberibacter asiaticus' (CLas) infection in citrus plants was presented in previous works. A more detailed study was carried out. Batches of healthy 2-year-old potted plants of Valencia/Swingle were pruned at ca. 20 cm above the rootstock-scion junction, at one week interval. Excess of post-pruning NS were removed keeping a single NS on each plant. At sixth week, 6 lots of 25 plants each (corresponding NS v1 to v6) were selected and transferred to each of 4 environments (3 growth chambers and one acclimatized room) and exposed to continuous average air temperatures of 18, 22, 27 or 32°C (±2°C) and daily photoperiod of 12h:12h L:D. Then, five CLas-positive adults of *Diaphorina citri* were confined on each plant for 7 days. The plants remained on the same environments for 2 months and in a screened house favorable to huanglongbing (HLB) infection for additional 6 months, when they were evaluated for symptom expression and qPCR. Regardless of the environment, no plants at V6 stage (completely mature leaf tissues) became infected, and over 50% of successful infections happened on plants at V1 to V4 (tender actively growing tissues) exposed to 27ºC. Above or below 27°C, rate of successful infections reduced to avg. 0.33% at 18°C and 23.8% at 32°C for all NS stages. These results help to explain the regional variation in HLB incidences in Brazil, and can be used to improve disease management.

Non-technical summary: Flushing and mild temperatures favor infection of citrus trees by the huanglongbing bacterium, demanding from growers more rigorous actions aiming to protect the new shoots from te access of the insect vector.

Texas CRaFT: Mitigating the Effects of Citrus HLB in Texas Through Large-Scale Field Trials

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Abstract: Citrus huanglongbing (HLB) is widespread throughout the Texas citrus growing region. The disease was first detected in 2012 and since this date has become endemic. The citrus zone of South Texas is characterized as a semi-arid subtropical environment with sporadic flooding. These environmental conditions can lead to prolonged tree stress. As most mature commercial groves are planted on flat ground and flood irrigated, *Phytophthora* disease is also endemic. Citrus trees that are affected by abiotic and biotic stresses are more sensitive to the effects of HLB that can lead to significant loss of productivity and fruit quality. As a strategy for coping with the combined of effects of stress and HLB we have implemented the Texas Citrus Research and Field Trial (CRaFT) project funded by USDA-APHIS. This includes the commercial-scale trialing of grove floor management including the use of raised beds with ground cover equipped with pressurized irrigation as well as the use of individual protective covers (IPCs). The trees are being monitored for growth parameters, Asian citrus psyllid presence, and HLB incidence. Here we report the status of the Texas CRaFT as the project enters its third year of operation.

Non-technical summary: The Texas CRaFT aims to mitigate the effects of citrus huanglongbing (HLB) in Texas through the implementation of commercial-scale field trials. Treatments that are being tested include the use of raised beds, ground cover, and individual protective covers (IPCs) compared to untreated control plots. Parameters being measured include tree growth, Asian citrus psyllid presence, and HLB incidence.

HLB management at Cambuhy Agricola in Brazil: lower incidence in endemic region

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Abstract: The huanglongbing (HLB), transmitted by *Diaphorina citri* (Asian citrus psyllid), was identified in the central region of São Paulo State (SPS), Brazil, where the Cambuhy Agricola farm is located, in March 2004. Since its identification, the HLB management measures recommended by Fundecitrus based on preventive measures such as planting of healthy nursery trees, elimination of diseased trees, and vector control were applied and strictly followed. During the season 2009/2010, a quickly increase in the disease incidence (294% compared to the previous season) was detected in the farm, which caused the removal of 3.9% of HLB-affected trees. From that, supported by both research data and citrus grower experiences, the HLB control has been constantly improved. Regional disease management, higher frequency of insecticide spray on border blocks compared to inner blocks, row orientation, variety, and rootstock were carefully stated. Nowadays, (i) inoculum reduction by frequent removal (4 times/season) of symptomatic trees; (ii) insect population monitoring by yellow trap; (iii) targeted control of psyllid population by insecticide treatment, and; (iv) use of healthy trees for resets, are the main measures established in the Cambuhy. Moreover, external management in 5 km radius was implemented by (iv) removal of citrus seedlings in permanent preservation areas; (v) control of psyllid vector in backyard orchards by insecticide treatment or removal of symptomatic trees and; (vi) control of psyllid population in the commercial orchards by insecticide treatment. According to a survey published by Fundecitrus in 2019, the HLB incidence in the central region of SPS (municipality of Matão) was 17.2% while Cambuhy farm showed 1.2% of incidence. In the next surveys 2020, 2021 and 2022, the incidences were 14.4%, 9.7% and 8.9%, while in the Cambuhy farm were detected 0.7%, 1.0% and 2.2%, respectively. In the last season, all above mentioned measures represented 19.8% of the production costs which is more profitable compared to having a 100% diseased orchard. Therefore, to control the disease and obtain financial return, the Cambuhy Agricola perform all HLB management measures since 2004, included internal and external management.

Non-technical summary: The adoption of continuous and rigorous control of Asian citrus psyllid and removal of all huanglongbing (HLB)-symptomatic trees since the beginning of HLB epidemic, both inside of the citrus farm and in a 5 km radius neighboring commercial and non-commercial citrus areas, has kept the HLB at low and economically acceptable incidence even in a region where the disease is endemic in the center of São Paulo citrus belt, Brazil. This case can be considered a case of success in the HLB control and proves that the area-wide psyllid and inoculum control are effective to manage HLB.

Specifically Targeted Antimicrobial Peptides (STAMPS) to Control *Candidatus* Liberibacter Species

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Abstract: Using a combination of computational, *in vitro*, and *in planta* genetic assays, we designed and evaluated the efficacy of a series of novel, specifically targeted antimicrobial peptides (STAMPs) for *Candidatus* (*C.*) Liberibacter spp. The STAMPs were engineered to target and kill *C.* Liberibacter spp. with much greater efficacy and specificity than non-targeted antimicrobial peptides (AMPs) alone. Efficacy testing was performed using *in vitro* assays against *C.* Liberibacter asiaticus (CLas)-surrogate bacteria and *in planta* citrus hairy root assays against CLas. The STAMPs exhibited ~2 to 12-fold greater efficacy than the non-targeted AMPs alone. We also designed and tested multiple versions of the STAMPs, varying in their compositions of the *C.* Liberibacter binders and linkers and in the orientation of the AMP (N- or C-terminus). We found particular combinations with enhanced antimicrobial activity. In addition, similar efficacies were obtained when the concentration of the STAMP was approximately ~2-fold lower than the non-targeted AMP. In conclusion, the *C.* Liberibacter spp., binders, and STAMPs developed in this study could be used to create highly effective prophylactic and curative HLB treatments.

Non-technical summary: Targeted delivery of antimicrobials into citrus trees can promote sustainable management of huanglongbing. From a practical standpoint, this could reduce the amount of antimicrobial needed to inhibit the pathogen as well as the associated costs, and lower any unintended effects on non-target beneficial microbes.

Understanding the citrus pathobiome to mitigate Huanglongbing as a disease complex

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Abstract: Using high throughput, DNA-based sequencing technologies, we derived an huanglongbing (HLB) disease ecology model that indicates as HLB severity increases, the root microbiome becomes enriched in soil-borne pathogens that include fungal taxa (*Fusarium*) and oomycete (*Phytophthora*) root pathogens. Fibrous root decline is a significant symptom of HLB that exacerbates tree decline. Our working hypothesis is that trees with HLB ultimately succumb to a complex of pathogens that include the primary pathogen (‘*Candidatus* Liberibacter asiaticus’ [CLas]), but also secondary soil-borne fungal/oomycete parasites that attack the root compartment of CLas-weakened trees. We are conducting functional microbiome studies to test this hypothesis. We have completed Koch’s postulates with a number of HLB-associated *Fusarium* spp. and determined they are pathogenic on several common citrus rootstocks. Moreover, using a combination of high throughput, automated culturomics and natural product chemistry, we are conducting experiments to empirically identify a consortia of biologicals (bioinoculants and antimicrobial natural products) derived from the native citrus microbiome that target the primary pathogen, CLas, and/or secondary pathogens, *Fusarium* and *Phytophthora.* The overall project goal is to develop HLB mitigation strategies that work with the citrus microbiome, rather than against it and support root health that feeds back into canopy health.

Non-technical summary: The overall project goal is to leverage the knowledge we have learned about how the citrus microbiome changes under increasing huanglongbing (HLB) severity. Using these data we are developing a consortia of microbes that will support root and canopy health by treating HLB as complex of pathogens.

High Throughput Culturomics Yields Potential New Tools in the Fight Against Huanglongbing

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Abstract: The community of microorganisms that live in and on plants, termed the microbiome, plays a pivotal but not yet fully defined role in plant health. Our ability to understand these roles is limited by our understanding of the microorganisms that make up these communities. Looking to move beyond DNA based inferences, the field of culturomics seeks to improve our ability to isolate microbiome members from complex environments and understand their contributions to their host. Previous studies have indicated that the citrus microbiome may have a distinct role in huanglongbing (HLB) development, and that certain microbiome members possess the capability to inhibit the growth of *Liberibacter*. To fully understand the disease suppressing capabilities of the citrus microbiome, we established a pipeline to isolate and screen citrus associated bacteria from field grown trees against *Liberibacter crescens,* the closest culturable relative to the associated causal agent of HLB. Utilizing automated high throughput microbial cultivation array technology, made possible by the Prospector® from Isolation Bio™, we were able to increase the diversity of our previously established citrus plant associated microbial culture collection. We captured several bacterial isolates that produce inhibitory compounds that could be promising microbiome informed management tools, in the form of bio-inoculants or natural products, to help ameliorate the existential threat Huanglongbing poses to citrus growing regions.

Non-technical summary: Utilizing new microbial isolation technology, we enriched our pre-existing citrus associated bacterial collection with an automated and high throughput workflow. We identified several bacterial isolates, native to the Citrus microbiome, that produce compounds inhibitory to *Liberibacter*, that could be developed into bio-inoculants or natural products for the treatment of huanglongbing.

Gene expression analysis reveals highly expressed defense response genes associated with HLB sensitivity

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Abstract: The regulation of 15 genes selected from a GWAS study conducted on a genetically diverse population of mandarin hybrids was observed to evaluate their role in *'Candidatus* Liberibacter asiaticus' (CLas) infection and huanglongbing (HLB) disease progression. According to the severity of HLB symptoms, three groups of plants, defined as tolerant, medium, and sensitive, were selected from the breeding population. RNA was extracted from leaves and the expression of the candidate genes was studied through qRT-PCR analysis. Six genes of the 15 under evaluation were differentially expressed among the three groups of plants. *Ciclev10033657m.g*, *Ciclev10030548m.g*, *Ciclev10004139m.g*, *Ciclev10025505m.g*, *Ciclev10026162m.g* and *Ciclev10026970m.g* genes were upregulated on plants belonging to the sensitive group in comparison with the ones belonging to the tolerant group, while the expression levels in the plants of the medium group displayed intermediate values. An unclear trend was identified in the expression of the genes involved in callose biosynthesis and deposition (*Ciclev10014015m.g, Ciclev10030560m.g, Ciclev10018456m.g*, *Ciclev10006304m.g).* The present study reveals that six genes involved in the plant immune response were highly upregulated on plants with more severe symptoms. This suggests that more sensitive genotypes had a greater immune response to the CLas infection.

Non-technical summary: Genetic studies reveal what makes mandarin hybrids more sensitive or tolerant to huanglongbing (HLB) disease. Results show that some genes can be associated with a higher sensitivity to HLB. These findings will help scientists to develop more efficient strategies to select more tolerant citrus varieties.

An effector of '*Candidatus* Liberibacter asiaticus' manipulates SNARE proteins to disrupt autophagosome-vacuole fusion

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Abstract: Autophagy functions in plant host immunity responses to pathogen infection. The molecular mechanisms and functions used by the citrus huanglongbing (HLB)-associated intracellular bacterium '*Candidatus* Liberibacter asiaticus' (CLas) to manipulate autophagy are largely unknown. Here, we identified a CLas effector, SDE4580, which contributes to HLB progression. Transgenic SDE4405 in Wanjincheng orange (*Citrus sinensis*) promotes CLas proliferation and symptom expression, and inhibits autophagy. SDE4580 interacts with the SNARE protein CsVTI12 and suppresses its expression. CsVTI12 contains a Qb-SNARE domain that forms a SNARE complex with CsSTX22 (Qa-SNARE), CsSTX61 (Qc-SNARE), and CsYKT61 (R-SNARE), which can activate autophagy in *Nicotiana benthamiana*. SDE4580 interferes with the interaction of CsVTI12 with CsSTX22 and CsSTX61 in a competitive manner, thereby inhibiting the formation of SNARE complex and then inhibiting autophagosome-vacuole fusion. Taken together, our findings reveal that SDE4580 subverts autophagy-mediated antibacterial defense by disrupting the SNARE complex formation to promote CLas infection.

Non-technical summary: The anti- and pro-microbial roles of autophagy in host-pathogen (oomycetes, fungi and viruses) interactions have been studied but the molecular mechanism by which autophagy is modulated by bacteria is largely unclear. We identified a '*Candidatus* Liberibacter asiaticus' (CLas) effector, SDE4580, which interferes with the formation of SNARE complex and thereby inhibits autophagosome-vacuole fusion. These data illustrate the critical roles of CLas effector protein in manipulating autophagy and enrich the current understanding of the interaction between CLas and citrus.

Using Citrus tristeza virus (CTV) to identify citrus targets for CRISPR modification

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Abstract: The ultimate solution for the huanglongbing (HLB) epidemic is resistant/tolerant trees. CRISPR technology enables targeted gene editing like natural mutations and provides the ability to modify elite citrus lines directly. The obstacle is to determine which citrus gene/s to modify. For this purpose, we are using the benign CTV-T36 based RNA interference (RNAi) and overexpression vectors which systemically colonize phloem tissue of citrus without synergistically interacting with the Clas bacteria, to identify gene target/s that will induce tolerance to HLB disease. The first approach is to boost plant defense. We built 44 CTV RNAi vectors that downregulate the expression of negative regulators of citrus defense. As transgenic trees overexpressing NPR1 were tolerant to HLB disease upon infection with Clas, we used it as a molecular marker to identify targets of interest. It enabled us to identify 4 citrus genes whose knockdown upregulates the NPR1 protein. HLB screening of one of the two sets of gene targets confirmed the marker assisted selection result. The second approach is dependent on considering Clas bacteria as an auto immune disease inducer and working on modifying the plant response by targeting 20 citrus genes for downregulation by CTV-RNAi vectors independently, as well as 5 citrus genes for overexpression. Among the 15 sweet orange sets infected with CTV-RNAi targets screened, we identified one target gene that induced tolerance in sweet orange. We continue to screen other targets for their ability to induce tolerance for HLB disease in sweet orange seedlings. Further, as we expect almost all CRISPR modified plants to be juvenile, we aim to fast transition them to maturity by inducing early flowering using CTV vectors expressing FT3 and downregulating negative regulators of flowering.

Non-technical summary: CRISPR technology provides a tool to generate directed mutations like natural occurring ones. The problem is deciding which gene to modify in citrus to generate the desired huanglongbing (HLB) phenotype. As Citrus tristeza virus (CTV) vectors are relatively faster and easier to generate than transgenic or CRISPR ones, we are using CTV vectors to identify citrus genes that could, upon manipulation, produce the tolerant HLB phenotype in citrus. Those genes will be modified by CRISPR.

New HLB-Tolerant Citrus Rootstocks and the SuperSour Breeding Strategy

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Abstract: To overcome the challenges inherent to citrus rootstock breeding and accelerate progress in development of huanglongbing (HLB)-tolerant rootstocks, the USDA citrus breeding program has implemented a new multi-pronged SuperSour strategy for rootstock breeding. The new strategy expands the diversity of germplasm utilized in rootstock breeding, vastly increases the number of new hybrids evaluated concurrently, and reduces the time from cross to potential cultivar release. In addition, the new strategy integrates mapping of rootstock traits, and incorporates genetic/genomic selection into each breeding cycle. The target of this new SuperSour strategy is to obtain new rootstocks that possess the positive attributes of the traditional favorite rootstock sour orange (*Citrus aurantium*), along with increased tolerance to citrus tristeza virus and huanglongbing, higher yield, and higher fruit quality. Rootstock propagation that does not rely upon apomictic seeds is one key to the new strategy. By taking advantage of modern micropropagation technology, and eliminating the need for nucellar seeds, we can vastly expand the potential genepool in rootstock breeding to include additional parents and progeny with a series of outstanding traits and eliminate the 6- to 15-year delay in testing while waiting for the new hybrids to fruit. The use of genetic/genomic selection for important rootstock traits will eliminate a large portion of flawed new hybrids before field testing might begin, allowing focus of field testing on the most promising new candidate rootstock hybrids. A series of new replicated field trials with about 400 new hybrid rootstocks have been planted in Florida under the SuperSour strategy, and detailed performance information collected each season. Five new hybrid rootstocks have been released from the program between 2018 and 2023, based on superior performance in field trials. We will present additional detail about the SuperSour strategy, field trials, data collection, and the promising new rootstocks that have been released.

Non-technical summary: Citrus crops are almost always grown as two plant varieties grafted together, with one variety on top that forms the leaves and fruit, and a rootstock variety on the bottom that forms the lower trunk and roots. Using rootstock varieties with superior tolerance of stress and disease can greatly increase health and productivity of trees, but there are considerable challenges to the development of new citrus rootstocks that have caused rootstock breeding to be a very slow process. We will describe the new SuperSour strategy for breeding new superior rootstocks that has been implemented to overcome these challenges and accelerate the development of new rootstocks with greater tolerance to huanglongbing disease and high fruit productivity.

Citrus Pan-Genome: The Search for Genes of Huanglongbing Resistance in Australian Citrus

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Abstract: Citrus huanglongbing (HLB) is a disease in citrus trees that reduces fruit quality and results in tree death. It is currently devastating the commercial citrus industry internationally. While most commercial citrus cultivars are susceptible to HLB, wild Australian native Rutaceae species have demonstrated resistance to HLB. Commercial citrus species can be successfully hybridized with these resistant species, but the process is long and requires the maintenance of high fruit quality and production traits, presenting a barrier to breeding efforts. We present an Australian Rutaceae-focused citrus pan-genome to assist in these efforts. We selected a total of 12 citrus species with high-quality, chromosome-scale reference genomes and gene annotations, including 4 Australian Rutaceae species. Following the GENESPACE pipeline, our genomes were placed into synteny-constrained orthogroups, reflecting both their similarity to genes found in other species and their placement relative to genes in other species. The orthogroups unique to 4 Australian Rutaceae species were associated with DNA methylation, phosphorus metabolism, and peptidase activity. Selected F1 and advanced hybrids with Australian and cultivated citrus parentage will be added to the analysis to facilitate the identification of genes for resistance and fruit traits. The pan-genome contextualizes the differences between gene content in Australian Rutaceae compared to commercial citrus species and provides a platform for genetic mapping of traits, including HLB resistance and fruit quality, to accelerate breeding efforts.

Non-technical summary: Citrus huanglongbing (HLB) has been a devastating disease for the citrus industry. Our pan-genome identifies genes unique to Australian Rutaceae, which are associated with HLB resistance, and their location in the genome relative to other species. This will assist current efforts to breed production-ready commercial cultivars with both HLB resistance and high fruit quality.

Combatting HLB with Gene Editing

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Abstract: While traditional breeding approaches are one way of introducing disease tolerance into commercial citrus cultivars, these approaches are slow and often result in the loss of the carefully selected attributes of those commercial varieties. Thus, alternative approaches to rapidly generate new cultivars that are resistant to huanglongbing (HLB) and yet maintain their qualities would be extremely valuable. Maintaining varietal qualities can be achieved through specifically mutating susceptibility genes. To identify susceptibility gene mutations that can potentially confer HLB resistance, we are generating a library of multiplex CRISPR/Cas9 gene edited lines of Carrizo citrange. We identified 1,200 potential HLB susceptibility genes through analyses of 71 transcriptomic and proteomic datasets. To identify genomic sequences and successfully mutate these 1200 genes, we are generating a high-quality reference genome for Carrizo citrange. We are mutating up to four susceptibility genes in each Carrizo citrange line and utilizing low-cost genomic sequencing to quantify the mutation rates at each target gene. We currently have a population of over 4000 transformed lines of Carrizo citrange and are genotyping these lines for mutations at target susceptibility gene loci. We are also assessing approaches to rapidly introduce the CRISPR/Cas9 machinery into other commercial cultivars through *in planta* transformation. As proof of concept, we have mutated three potential susceptibility genes simultaneously in Lisbon lemon and these lines are currently undergoing testing for HLB resistance. Our collection of citrus mutants will help to identify intervention targets most worthy of advanced field testing and commercialization.

Non-technical summary: We are assessing 1200 potential huanglongbing (HLB) susceptibility genes using a modified CRISPR/Cas9 approach where we mutate up to four genes simultaneously in Carrizo citrange, and then test the resulting modified plants for HLB resistance. We are also working on new transformation approaches to introduce these types of genetic modifications into other commercial citrus varieties.

Multiple approaches towards Huanglongbing tolerance

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Abstract: Huanglongbing (HLB) is a devastating citrus disease caused by the bacterial pathogen '*Candidatus* Liberibacter asiaticus' (CLas). Currently there is no cure for this disease. It is well known that the plant immune system is balanced by positive and negative regulators and can be boosted by adding positive regulators or removing negative regulators. We use this strategy in citrus to create long-term solutions to HLB. Thus far, we have transgenically overexpressed 20 positive immune regulators in sweet orange and grapefruit and found that overexpression of certain immune regulator(s) led to robust tolerance to HLB (tolerance refers to the capacity to endure the presence of CLas). We also employ a citrus tristeza virus-delivered RNA interference (CTV-RNAi) technique to silence a group of 44 negative regulators to identify targets for gene editing. Two CTV-RNAi constructs that induce strong HLB tolerance have been identified and more HLB tolerance-inducing constructs are expected to come soon. Our results demonstrate that robust HLB tolerance can be achieved by modifying the citrus immune system. We are editing the target genes identified in the CTV-RNAi screening to produce transgene-free HLB-tolerant citrus trees through mature tissue transformation. Furthermore, we have built highly efficient intragenic and microRNA vectors using citrus DNA sequences, which allows production of intragenic plants. We are using these vectors to modify the citrus immune system, producing intragenic trees that are tolerant to HLB. We are also generating transgenic and/or intragenic rootstocks expressing microRNA or hairpin RNA that can silence the target genes in the scion to create non-transgenic HLB tolerance. Finally, we continue to educate the citrus communities about the CTV- RNAi, gene editing, intragenesis, and microRNA technologies and their benefits to the citrus industry and consumers to help establish market acceptance for modern biotechnology-based citrus products.

Non-technical Summary: Transgenic sweet orange and grapefruit with robust huanglongbing (HLB) tolerance have been generated, demonstrating that HLB tolerance can be created in commercial citrus cultivars through genetic modification of the citrus immune system. Various approaches including both transgenic and non-transgenic methods are needed to ultimately solve the HLB problem.

A whole-plant physiological framework for HLB

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Abstract: Understanding plant dysfunction is key to formulating therapies and management practices in the face of persistent diseases. Whole-plant physiology is a valuable framework for understanding plant function and dysfunction. Because huanglongbing (HLB) impacts plant carbon transport, understanding both function and dysfunction of carbon fixation, transport, and allocation are key targets. We will draw from a series of studies that demonstrate important characteristics of how citrus plants fix, move, store and consume sugars, and how HLB impacts these characteristics. Citrus have a highly buffered transport system, and store sugars all along the transport pathway, possibly as a mechanism to circumvent their uniquely slow transport. The reduction in phloem transport speed caused by HLB is unique, and not seen under other conditions. Despite the systemic nature of the infection, impacts on phloem appear to be localized: transport speeds in the stem and starch accumulation in leaves are correlated with bacterial titers within the local tissues. Not only the quantity, but also the pattern of callose deposition appear to impact transport, with important differences between varieties. Carbon fixation on a surface area basis appears to be increased by HLB, but photosynthetic surface area is decreased, leading to the overall reduction of growth. Reducing the photosynthetic surface area that supplies a particular fruit does not immediately reduce fruit growth in HLB affected trees, though it does in uninfected trees. In general, sink, rather than source, activities appear to be more affected by HLB, indicating a strong local effect in rapidly expanding tissues such as root tips, new shoots, and fruits, particularly early in development. We expect this knowledge to contribute to the design of therapies and horticultural practices.

Non-technical summary: Understanding how huanglongbing (HLB) affect the production, movement, and use of sugars in the whole plant is essential in devising effective management. HLB does not have the expected effects on photosynthesis, but does affect transport and utilization in the sinks (fruits, new shoots, and roots). Effects are strongest where the bacteria are most abundant.

Citrus Under Protective Screens: A novel tool for managing Asian citrus psyllid-huanglongbing and citrus pest complex

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Abstract: Cultural, biological, and chemical pest control methods significantly reduce Asian citrus psyllid **(**ACP) populations in traditional open orchards; however, huanglongbing (HLB) continues to spread in the infected regions causing economic losses to the citrus industries. We conducted a multi-year investigation of advanced production systems such as Citrus Under Protective Screen (CUPS) and Individual Protective Covers (IPCs) for citrus production and protection against ACP and HLB. Only a few ACP adults were detected in CUPS except when damaged by hurricanes; however, no HLB-positive trees were detected. Other pests such as mites, citrus leafminer (CLM), scales, thrips, and mealybugs at low levels as well as several parasitoids and small predators attacking these pests were observed in CUPS. The citrus rust mite *Phyllocoptruta oleivora* and citrus red mite *Panonychus citri* were common pests in the CUPS. Ten species of predatory mites of the family Phytoseiidae useful for pest control were identified from CUPS. Several parasitoids attacking *Phyllocnistis citrella* (CLM) were found in CUPS and its populations were reduced by more than 80% compared to control. Infestation of Florida red scale (FRS) *Chrysomphalus aonidum* was observed on 12-13% of trees in CUPS and its parasitoid *Aphytis melinus* provided 20-25% parasitism. *Frankliniella bispinosa* thrips populations were high in the CUPS compared to the traditional open production system and induced scarring on grapefruit in both systems. IPCs on newly planted citrus trees provided significant protection against ACP-HLB for 3 years, translating to healthy plants and better yield. These structures also suffered some pest problems such as scales, mealybugs, and garden armyworms. Overall, CUPS and IPCs were effective in providing significant protection against ACP-HLB and bringing young trees into production with better health and higher yields than open production controls. The occurrence and functioning of beneficial organisms in CUPS suggest opportunities for strengthening biological control in these systems.

Non-technical summary: Citrus crops grown in Citrus Under Protective Screen (CUPS) and Individual Protective Covers (IPCs) production systems remained free of Asian citrus psyllid (ACP) and huanglongbing (HLB). The quality and quantity of fruit and health of the trees produced under these systems were superior to those in the control. The beneficial effects of these production systems on citrus trees lasted for years suggesting sustainability. CUPS and IPCs are useful for producing citrus free of ACP-HLB both for fresh fruit and processed fruit markets and are being implemented in citrus industries.

Trunk injection of oxytetracycline improves plant performance and alters the active bark and rhizosphere microbiomes in huanglongbing-affected citrus trees

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Abstract: Delivering oxytetracycline (OTC) by trunk injection to combat huanglongbing (HLB) can reduce bacterial titers of '*Candidatus* Liberibacter asiaticus' (CLas) and improve tree health, yield, and fruit quality. Microbial communities within plants and associated with the roots can play critical roles in soil and plant health, and it is not clear how injections of OTC will impact the microbiome within different plant tissues and thus other aspects of plant health. The goal of this field study was to determine the impact of OTC injections on the abundance, diversity, and composition of the active bark and rhizosphere prokaryotic and eukaryotic microbiome and how those microbiome impacts were related to tree physiology, fruit quality, and yield. Eight-year-old Valencia orange trees in a commercial citrus orchard in southwest Florida, USA, were injected with OTC in June 2022. Bark and rhizosphere samples were collected 3 days, 3 weeks, and 3 months after injection. RNA was extracted from the bark and rhizosphere samples and used for reverse transcription reactions. Amplicon sequencing was conducted on the cDNA with prokaryotic and eukaryotic primers. OTC injection had a significant impact on the prokaryotic microbial diversity in the bark and rhizosphere, but the timing of these impacts varied between sample types. While there were no significant impacts of OTC on the overall prokaryotic composition, there were significant increases in the relative abundance of specific prokaryotic genera with OTC injections in both the bark and rhizosphere, some of which were correlated with increased fruit yield, weight, and size. As expected, there were no significant impacts of OTC injections on the eukaryotic microbiome of the bark and rhizosphere. Overall, our findings suggest that OTC injections not only impact the titer of CLas, but also result in changes to the bark and rhizosphere prokaryotic microbiome that positively impact fruit yield, size, and quality.

Non-technical summary: Trunk injections of the antibiotic oxytetracycline (OTC) significantly reduced the total bacterial diversity of citrus bark and rhizosphere samples and the amount of '*Candidatus* Liberibacter asiaticus' (CLas)-titer in the plant tissues. However, OTC injections also increased fruit yield, size, and quality, and these increases were related with increases in some bacteria in the bark and rhizosphere. These results indicate that benefits in fruit yield, size, and quality may be due to other changes in the plant bacterial community beyond a reduction in CLas.

Needle-based, automated trunk injection system for HLB-affected citrus trees

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Abstract: Trunk injection is an alternative method to control citrus huanglongbing (HLB). However, conventional (manual) trunk injection methods are labor-intensive and time-consuming, resulting in high application costs and posing implementation challenges for commercial citrus growers. Therefore, an automated, drill-free system for injecting therapeutic materials into citrus trees to control HLB has been developed. The injection mechanism consists of a positioning arm mountable to a farm vehicle and an end effector for performing trunk injection. The system utilizes a metering pump with variable pressure and flow rate for injecting therapeutic materials into tree trunks. This novel technology was evaluated in commercial citrus orchards; the injected volume, applied pressure, and injection rate were measured, and differences based on the time of injection, diameter at the injection point, and irrigation status were examined. The injection was performed until the flow stopped. Injection duration ranged from 30 s to 72 s for all trees. The average injection rate per tree raged from 1.23 mL/s to 3.58 mL/s. The minimum and maximum volume injected were 53.9 mL and 243.6 mL, respectively, at 44 s and 68 s, and cut-off pressure ranged from 0.97 MPa to 2.01 MPa.

Non-technical summary: An automated trunk injection mechanism has been developed. The device does not require drilling the tree, can carry out trunk injection rapidly, and is mountable on existing farm vehicles to deliver therapeutic materials to citrus trees.

Using antisense oligonucleotides and oxytetracycline trunk injections to reduce '*Candidatus* Liberibacter asiaticus' titers in citrus trees, acquisition, and transmission by *Diaphorina citri* Kuwayama (Hemiptera: Liviidae)

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Abstract: Huanglongbing (HLB) is caused by the bacterial pathogen '*Candidatus* Liberibacter asiaticus' (CLas) and has decimated the $9.3 billion Florida citrus industry since its discovery in 2005. CLas is an obligate bacterial pathogen that multiplies in citrus trees and the insect vector, the Asian citrus psyllid (ACP) *Diaphorina citri* Kuwayama. Management of HLB has relied heavily on suppressing ACP populations with insecticides, and broad-spectrum antibiotic treatments are becoming more prevalent. The sustainability of these approaches has been questioned because of treatment costs and the possibility of resistance development. FANA ASOs are chemically modified synthetic single-stranded nucleic acid analogs that can modulate gene expression by enzymatic degradation of an RNA target. FANA ASOs can recognize and bind to specific RNA forms through complementary base pairing. Unlike the RNAi pathway, which requires the interaction of several enzymes to form the RISC complex, FANA single-stranded antisense oligonucleotides use RNase H-mediated RNA cleavage. Recent investigations indicate that trunk-injected oxytetracycline (OTC) effectively reduces CLas in mature citrus trees and improves yield parameters. We hypothesized that FANA oligonucleotides targeting CLas would reduce infection by silencing essential CLas genes with comparable effects to OTC. The results of field evaluations indicate that FANA ASOs and OTC reduce CLas infection in mature citrus trees and disrupt CLas transmission by ACP. However, the effectiveness of trunk-injected OTC was greater than that observed with FANA ASOs in initial field tests, suggesting that treatment dosage and formulation of FANA will require further optimization.

Non-technical summary: In Florida, management of citrus greening disease, or huanglongbing, has relied heavily on suppressing Asian citrus psyllid populations with insecticides. Recent studies suggest that trunk injections of antimicrobials effectively reduce '*Candidatus* Liberibacter asiaticus' in mature citrus trees and improve yield parameters. These outcomes suggest that antimicrobials targeting insect-transmitted plant bacteria may be valuable for integrated citrus greening management.

Citrus root mass response of oxytetracycline-injected trees in Florida

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Abstract: '*Candidatus* Liberibacter asiaticus' (CLas) moves downward in the phloem and infects roots soon after transmission into shoots. Before canopy symptoms appear, 30-50% of the fibrous roots are lost. Root lifespan is decreased from 9-12 months to 4 months. Short-term gains in root density do not sustain canopy and fruit growth in declining trees. Without aggressive management to reduce abiotic and biotic stressors, root loss can increase to 70-80%. In response to root loss, trees decline due to premature leaf drop, canopy dieback, enhanced susceptibility to *Phytophthora* and premature fruit drop. In the Spring of 2023, oxytetracycline (OTC) was commercially injected into millions of citrus trees in Florida to potentially stabilize or reverse HLB-induced decline. OTC injected into the trunk is well distributed in the canopy and root system. CLas titer in roots is reduced up to 120 days post-treatment. OTC-injected trees show signs of restoration of leaf flushes, root mass, canopy density, fruit size and quality. As part of a >3-decade Phytophthora surveillance program, Syngenta Crop Protection collects root-soil cores (20 cores/20 trees per 20-acre area) from commercial citrus blocks in all major production regions to quantify root mass and Phytophthora propagules. Industry-wide, the monthly root mass average increased 10% in June 2023 and 17% in July when compared to 2022. Root and tree health responses to OTC injection continue to emerge.

Non-technical summary: Oxytetracycline (OTC) trunk injection of citrus trees has reversed huanglongbing tree decline in Florida industry-wide. OTC reduction of '*Candidatus* Liberibacter asiaticus' titer in roots concomitant with an increase in root mass may serve as a leading indicator of restoration of canopy density, fruit quality and quantity.

Optimizing citrus bioindexing protocols necessary for Huanglongbing research using light manipulation in next-generation indoor farming systems

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Abstract: The Citrus Clonal Protection Program (CCPP) at the University of California, Riverside is responsible for introducing citrus accessions into California with potential huanglongbing (HLB) tolerance or accessions needed for HLB breeding and research. Such accessions are required to undergo therapy and stringent disease bioindexing testing under quarantine before distribution to scientists and other stakeholders. Bioindexing, although an invaluable biosecurity tool, represents a bottleneck in the introductory procedure, as much time is spent for potential symptoms of regulated graft-transmissible pathogens to develop on slow-growing citrus plant indicators (months instead of days for laboratory tests). Alternative agriculture techniques applying next-generation indoor vertical farming systems (IVFS) were used to optimize the CCPP bioindexing protocols. Light manipulation with different wavelengths of red (R) and blue (B) spectra from adjustable LEDs was tested for optimal viral symptom onset and expression on citrus indicators grown in IVFS. High percentages of R light (95% R, 5% B) induced rapid and severe symptom expression in several regulatory significant graft-transmissible viral pathogens for California, compared to standard greenhouse conditions. These alternative and sustainable agriculture practices employing IVFS can be used to reduce the time and materials needed in the required bioindexing step for HLB research materials to be introduced into California, and become available via the National Clean Plant Network to all citrus-producing states.

Non-technical summary: New indoor vertical farming techniques with light manipulation were used to optimize the required bioindexing procedures, to reduce time needed for huanglongbing research materials to move between states and subsequent release into the environment and the industry.

Present status of developing diagnostics, molecular characterization and novel management strategies for Huanglongbing (HLB) in India

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Abstract: Huanglongbing (HLB), or citrus greening disease, poses a major threat to the global citrus industry. This disease is caused by a phloem limited bacterium called '*Candidatus* Liberibacter asiaticus' (CLas) and is spread by psyllids. Unfortunately, the lack of effective long-term control measures for HLB makes it more challenging to manage. In recent years, significant progress has been made in detecting HLB using advanced molecular techniques like RPA and LAMP. These methods are praised for their rapid and precise detection capabilities. We developed a LAMP-based detection assay consisting of specific primer set designed to target the 16S rDNA region of Indian isolates CLas. Additionally, the HLB-RPA-LFA assay was also developed for on-site HLB detection. To further enhance detection accuracy, an RPA-LFA assay was developed, targeting the CLas five-copy nrdB gene. Our studies on characterization of CLas revealed greater diversity within CLas populations of India compared to Florida and Guangdong. In India, we found a wider range of patterns in tandem repeats at the genomic locus CLIBASIA_01645, including TRN copy numbers of 9, 10, 11, 12, and 13, with occasional instances of TRN2 and TRN17 patterns. Notably, northeastern samples exhibited TRN6 and TRN7 patterns, like South China. Bhutanese CLas isolates were also studied, showing classes II and III at the CLIBASIA_01645 locus, similar to Indian isolates from the northeast, and Term-A based on the CLIBASIA_05610 locus. In terms of management practices, several experiments assessed the effectiveness of antimicrobial compounds such as the 2S albumin protein from pumpkin seeds, Nano-ZnO nanoparticle, and inhibitor compounds like pimozide and clidinium bromide, engineered on the cystine binding receptor. The results demonstrated the efficacy of these compounds in suppressing CLas.

Non-Technical Summary: In conclusion, theabstract highlights India's efforts in combating huanglongbing (HLB) by utilizing advanced molecular detection techniques for 'Candidatus Liberibacter asiaticus' (CLas) and studying the diverse patterns in the Indian CLas population. Additionally, new methods of managing the disease using compounds such as protein inhibitors and antibacterial proteins and being explored, which have shown promising results.

In vitro culture and artificial inoculation of 'Candidatus Liberibacter asiaticus' (CLas) reveals a long-lasting low titer infection of CLas in citrus plants

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Abstract: *'Candidatus* Liberibacter asiaticus' (CLas) is one of the causal agents of huanglongbing (HLB), the most devastating disease of citrus worldwide. Due to the intracellular lifestyle and significant genome reduction, culturing CLas *in vitro* has proven to be extremely challenging. In this study, we optimized growth conditions and developed a semi-selective media (aka: CLas-growth medium or LG) based on the results of nutritional and antibiotic screening assays. Using these optimized conditions, we were able to grow CLas in the LG liquid medium with ~100-1000-fold increase observed, which peaked after 4-6 weeks and were estimated to contain 10^6-10^7 cells/ml. The cultured CLas bacteria remained in a dynamic state of growth for over 20 months and displayed limited growth in subcultures. The survival and growth of CLas was confirmed by fluorescence *in situ* hybridization with CLas-specific probes and expression of its metabolic genes. Growth of CLas in the optimized medium relied on the presence of a helper bacterium, *Stenotrophomonas malitophilia* FLMAT-1, that is multi-drug resistant and dominant in the CLas co-culture system. To recapitulate the disease, the co-cultured CLas was inoculated back to citrus seedlings via psyllid feeding. The results demonstrated a successful inoculation of the cultivated CLas back to psyllids and citrus plants. It is important to note that the cultivated CLas did cause HLB-like symptoms on most of the plants (88.9%) having a low CLas titer infection (Ct>33.0) that remained low even 3-5 years post inoculation in a greenhouse setting. Some of these plants only displayed HLB symptoms on one of the original inoculated branches and did not appear to develop systemic disease throughout the infected plant. These results provide new insights into factors that affect CLas growth *in vitro* and a system for improvement toward axenic culture and genetic modification of CLas.

Non-technical summary: We have developed a semi-selective medium for culturing '*Candidatus* Liberibacter asiaticus' (CLas) *in vitro*. Using this medium, we were able to growth CLas up to 10,000,000 cells/ml with the help of a multi-drug resistant bacterium, *Stenotrophomonas malitophilia* FLMAT-1. The co-cultivated CLas caused typical and non-typical huanglongbing diseases when they were inoculated back to citrus plants through feeding psyllids. The developed system provides a basic foundation for improvement toward axenic culture and genetic modification of CLas as well as a high-throughput screening for antimicrobial compounds.

CRISPR/Cas9-mediated Editing of *DMR6* and *SWEET1* Genes for Resistance to Citrus Canker and Huanglongbing

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Abstract: Engineering resistance to citrus canker and huanglongbing is vital for the citrus industries in the U.S. and in the world. Editing disease susceptibility (*S*) genes has resulted in increased resistance to bacterial and fungal pathogens in plants. In this study, we have applied this new strategy to citrus, edited *DMR6* and *SWEET1* in Carrizo citrange and 'Duncan' grapefruit, and evaluated the resistance of *dmr6* and *sweet1* mutants to citrus canker. Nine Carrizo and 'Duncan' *dmr6* mutants were generated. Most of the mutations were deletions or insertions that could cause frameshift in the coding region. Mutation frequency in these mutants ranged from 1.81% to 100%. Six Carrizo and 'Duncan' mutants with high mutation frequencies in *DMR6* showed strong resistance to citrus canker, reducing canker lesion size by 71.2% to 98.6% and *Xanthomonas citri* subsp. *citri* (*Xcc*) cell count by greater than 99% or 2.71 to 4.92 Log10 unit. Five 'Duncan' lines were generated for *SWEET1*. Mutations in these lines included one- or two-base deletions and one-base insertion; mutation frequency ranged from 1.43% to 94.73%. Two 'Duncan' lines with high mutation frequencies showed strong resistance to citrus canker and reduced canker lesion size by 71.2% to 98.2% and *Xcc* cell count by 99.99% (3.97 to 4.67 Log10 unit). 'Duncan' lines carrying *dmr6* or *sweet1* mutations accumulated more salicylic acid before and after *Xcc* inoculation. A number of *dmr6* and *sweet1* mutants with high mutation frequencies have been propagated and inoculated with '*Candidatus* Liberibacter asiaticus' to evaluate the mutants' resistance to huanglongbing.

Non-technical summary: Two disease susceptibility genes (*DMR6* and *SWEET1*) in Carrizo and 'Duncan' grapefruit have been edited and knocked out. A number of *dmr6* and *sweet1* mutants have shown strong resistance to citrus canker; evaluation of their resistance to huanglongbing is ongoing in Florida.

Finding HLB Tolerance for Commercial Citrus – From Expected and Unexpected Sources

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Abstract: As huanglongbing (HLB) threatens the Citrus industry, the UF/CREC Citrus Improvement Team has been evaluating all available citrus germplasm for HLB tolerance. The long-term solution for sustainable and profitable production in HLB-endemic areas is the development of tolerant or resistant varieties. Sugar Belle and Marathon show the best potential among tolerant fresh fruit mandarin selections. Triploid pink grapefruit-like hybrids C2-5-3 (approved for release) and C4-10-36 are showing improved HLB and canker tolerance, along with early maturity. Florida NFC (not from concentrate) orange juice is allowed to contain up to 10% juice from mandarin hybrids. We have identified several highly tolerant mandarin hybrids that produce juice with high Brix, flavor, and color, including 1859 (released), C4-10-42, C7-12-19, KE-9-15, and 6-2-55. These triploid hybrids were produced from interploid crosses using an HLB-tolerant diploid female parent, and all have great potential for improving the quality of NFC juice. We are finding a higher level of phenotypic diversity than expected among nucellar seedlings derived from somaclones of Vernia and OLL (Orie and Louise Lee) sweet oranges; some with enhanced HLB tolerance and earlier maturity have been identified and are being propagated for stage 2 trials. The ultimate solution to HLB is to develop rootstocks that mitigate the disease in any grafted commercial scion. Thousands of hybrid rootstocks were screened using the 'gauntlet' high-throughput screening method, and several promising hybrids have been identified. We also unexpectedly discovered what appears to be a 'deletion mutant' of commercial rootstock X-639 that is showing better HLB tolerance than the standard X639.
'Super-root' mutants from tissue culture micropropagation have also shown enhanced HLB tolerance in limited field trials. All these candidates are being propagated for large-scale replicated trials.

Non-technical summary: Distant citrus relatives are not the only good source of huanglongbing (HLB) tolerance, as we are identifying a surprising number of HLB-tolerant hybrids and mutants with more direct commercial potential among our genetically diverse citrus germplasm collection. Identified scion selections for fresh fruit and processing, and rootstock selections with good pedigree, are being propagated for replicated Stage 2 field evaluation.

Dissecting the genetic basis of HLB tolerance in large-scale breeding and evaluation trials

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Abstract: The rootstock determines citrus tree health, fruit productivity and fruit quality, including in huanglongbing (HLB) diseased groves. To develop and select rootstocks with enhanced tolerance to HLB, in 2006 the USDA Rootstock Breeding Program initiated a series of large-scale field trials to identify rootstock hybrids with HLB tolerance and/or resistance and superior overall field performance when grafted with a common sweet orange scion. These rootstock trials include over 10,000 individual trees and represent, to our knowledge, the largest systematic evaluation of genetically diverse citrus rootstocks. We will integrate genetic information for 378 new rootstock hybrids evaluated in these trials with field performance data to dissect the genetic basis of rootstock-mediated tolerance to HLB. Initial analysis of the earliest planted trials revealed that HLB tolerance in the susceptible sweet orange scion is genetically controlled by the rootstock. Pedigree-based estimates of heritability for traits related to tree size, fruit productivity, and fruit quality of the common sweet orange scion were substantial, with 46% of variation in cumulative yield across 46 next generation rootstock hybrids attributed to genetics. The most heritable traits were those related to tree size (65-73%). Fruit quality traits tended to have lower genetic control (4-33%). Combining large-scale field evaluation with intense selective pressure, like current levels of HLB disease in Florida, is ideal for rapidly identifying rootstocks with robust tolerance/resistance to HLB and initial genetic analysis provides strong support for the potential of selective breeding to enhance tolerance to HLB.

Non-technical summary: Relatively little is known about the genetic basis of huanglongbing (HLB) tolerance. We combine large-scale breeding and evaluation trials with genetic information to dissect the genetic basis of field performance in HLB infected groves.

Exploring the Feasibility of Australian Limes to Create Hybrid Rootstocks with HLB tolerance

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Abstract: Huanglongbing (HLB) has greatly affected the US citrus industry. Most commercially cultivated citrus scions and rootstocks are highly susceptible to this disease. This study aimed to harness the HLB resistance traits reported in some Australian lime species (specifically the finger lime and the round lime) and utilize this trait to create rootstocks with tolerance to HLB. A large population of Australian lime – citrus hybrids, developed in our citrus breeding program were assessed under field conditions in Florida. Several lines have been identified with enhanced tolerance to HLB, even after 8+ years in the endemic HLB environment. A specific subset of these hybrids was selected for clonal propagation, and the resulting cuttings were separated into two replicated groups to undergo assessment. The first set was grafted with HLB free 'Valencia' sweet orange budwood and the other was grafted with 'Valencia' budwood obtained from HLB-infected field trees (Ct value of 22.25 ± 1.11). Controls consisted of 'Valencia' on Swingle rootstocks. Our results indicated that 'Valencia' sweet oranges infected with HLB and grafted onto finger lime hybrids demonstrated a gradual reduction in the '*Candidatus* Liberibacter asiaticus' titer over time, compared to the control group. Additionally, we recorded elevated levels of several phenolic compounds in the experimental trees. Furthermore, we detected increased transcript levels of several defense related genes such as a PR-1 like, *Cs*LCR69, catalase, and glutathione S-transferase in these trees. 'Valencia' sweet orange grafted onto CFL1-98, also demonstrated improved growth and higher chlorophyll pigment production in the grafted scion. These findings indicate that utilizing the finger lime hybrids as rootstocks hold significant promise and a well replicated multilocation field assessment is planned to confirm greenhouse results and determine horticultural performance.

Non-technical summary: The huanglongbing (HLB) resistance trait in the Australian limes was utilized to develop HLB tolerant hybrid rootstock candidates. These hybrids outperformed Swingle rootstock when used as rootstocks in greenhouse tests and show promise as part of a robust integrated management approach to successfully grow citrus under endemic HLB.

Genomics-abled breeding for Huanglongbing tolerance in citrus

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Abstract: Huanglongbing (HLB) caused by '*Candidatus* Liberibacter asiaticus' is a major citrus disease affecting the citrus industry worldwide. Since most modern citrus cultivars are highly susceptible to HLB, breeding for resistance/tolerance is urgently needed. In this study, genome-wide association studies (GWAS), quantitative trait locus (QTL) mapping, and genomic selection (GS) were applied to breed for HLB tolerance. A total of 493 F1 hybrids from the cross of HLB-sensitive mandarin 'Monreal' and HLB-tolerant 'Khasi' papeda, with 113 randomly selected individuals grafted 3-4 times on 'Carrizo' citrange. Seedlings and grafted trees were established in the field under high-HLB disease pressure at USDA/ARS in Fort Pierce, FL in 2017. Each tree was evaluated for HLB responses and 11 different growth indexes in 2020 and 2021. Whole genome genotyping by GBS yielded a total of 13,171 high-quality SNP markers. GWAS and QTL mapping identified a major QTL on chromosome 6 (*QTL6*) with highest PVE of 21% for multiple traits. DNA markers and high-resolution melting analysis significantly differentiated citrus growth of 70 tested accessions. We evaluated the GS performance, which utilizes genome-wide markers to predict performance of non-phenotyped individuals. Self-rooted trees were used as training population to rank individuals in the testing population of grafted trees. Among GS models, Bayesian LASSO was the most predictive in most of the traits with predictive ability ranging from 0.15 to 0.54. GS-assisted selection of top-ranked individuals based on their genomic-estimated breeding values achieved comparable phenotypes to traditional phenotypic selection. In summary, this study identified a major QTL associated with citrus growth with HLB and developed DNA markers which can be effectively used for selection of future seedlings with improved HLB tolerance. Finally, we successfully demonstrated GS of complex traits that can be used for rapid genetic improvements of HLB tolerance in citrus.

Non-technical summary: Next generation breeding methods using genomics tools can facilitate breeding new citrus varieties with durable huanglongbing (HLB) tolerance. In this study, we developed and validated molecular DNA markers for accurate selection of new citrus seedlings with better growth traits and improved tolerance to HLB. This marker-assisted selection technology will significantly reduce the breeding cycle and will aid the breeders in rapid breeding for HLB tolerance in citrus.

Genome-assisted breeding to incorporate huanglongbing resistance in citrus.

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Abstract: Sustainable citrus cultivation in the presence of the citrus huanglongbing (HLB) disease will require the development of disease-tolerant/resistant commercial cultivars. We have demonstrated that the Australian wild limes possess innate HLB resistance/tolerance traits that are heritable. For ten years we have conducted citrus breeding using *Microcitrus* as one of the breeding parents. Promising, HLB-resistant, F1 hybrids were utilized as parents to generate advanced hybrids of the second generation. We have developed genomic resources for four Australian limes and use genotyping to facilitate the selection of promising progeny from the breeding populations. The hybrids are challenged with the HLB pathogen in contained research greenhouses for evaluating disease tolerance followed by multi-location field trials. We utilize data generated from various approaches like disease response to HLB, taste evaluation, metabolomic profile, performance in the greenhouse and field, etc. to select breeding parents for conducting targeted crosses. The genotyping data conducted using genome-wide, single nucleotide polymorphism markers is useful in marker-assisted breeding and selection. The development of disease-resistant hybrids with acceptable fruit quality traits will provide financially sustainable solutions to HLB. The hybrids generated in our program through breeding will not require regulatory approvals and will be readily accepted by the public. We will discuss the status of the breeding program with about 2,500 hybrids in various stages of evaluation. Some advanced hybrids have disease resistance and fruit quality and are close to being acceptable to the industry. Confirmation of the two important traits- HLB resistance and acceptable fruit quality- is in progress.

Non-technical summary: The development of genetic resistance to disease will provide the ultimate solution for huanglongbing. We have over 250 hybrids of the advanced generation that are undergoing thorough evaluations for the selection of useful varieties for cultivar development.

Genome-wide association study identifies loci and candidate genes for HLB sensitivity in mandarin hybrids

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Abstract: Field trials in Florida have revealed large variation in severity of huanglongbing (HLB) symptoms in mandarin hybrid families within the UF-CREC breeding program. The mechanism of the HLB sensitivity among citrus varieties is complex and not clearly defined. In this study, we used a diverse collection of 462 mandarin hybrids from citrus breeding populations with an age range of 15-25 years. All trees have been exposed to HLB since 2008. We measured quantitative traits including HLB symptom severity, *'Candidatus* Liberibacter asiaticus' (CLas) titer, and seven morphophysiological traits. The mapping population was genotyped using an Axiom^{Tm} Citrus 56AX array, encompassing 58,433 SNP probe sets. Genome-wide association study identified 244 SNPs near or in 195 genes. There were no significant differences in regarding CLas titer, callose and H_2O_2 among three HLB severity groups in the validation sub-population. The low gene expression of plant innate immunity-related genes among the less sensitive genotypes may be a mechanism to avoid excessive plant immunity responses resulting in a systemic response that leads to cell death in the phloem tissue. The less sensitive genotypes may not be true tolerance due to high CLas titer and callose accumulation, but rather their ability to avoid excessive plant immunity response and directing energy resources for growth and development.

Non-Technical summary: Genome-wide association study (GWAS) can empower citrus breeders to identify tolerance genes and SNP markers. Molecular markers can be used to select huanglongbing (HLB)-tolerant citrus trees and to deliver HLB-tolerant cultivars from citrus breeding programs effectively.

Effectiveness of Asian citrus psyllid management in huanglongbing quarantine zones in residential Southern California

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Abstract: Residential citrus played a central role in the initial invasion of Asian citrus psyllid (*Diaphorina citri*; ACP) and, more recently, huanglongbing (HLB) incidence in Southern California. Attempts to slow HLB spread in the region rely on removal of infected trees, to limit inoculum supply, and vector control via insecticide treatments of infected neighborhoods and releases of the parasitoid *Tamarixia radiata.* To better understand the value of the vector control element, we analyzed more than five years of monitoring at 35 properties spread among three transects running through HLB treatment zones in residential areas of Southern California. The results show significant seasonal and interannual differences in flush availability, with peaks generally occurring in early Spring and lows in late Summer. All metrics of ACP abundance were significantly and positively affected by flush availability and differed significantly among citrus cultivars, with grapefruit being the most preferred or favorable. ACP abundance was also affected significantly by time-by-treatment interactions, with a near-absence of ACP on treated properties after Summer 2019. Parasitism averaged 13% with a peak at 50%, but was fairly uncommon due to a lack of available late-instar nymphs – especially in treated areas. Nonetheless, *T. radiata* has the potential to have larger effects as evidenced by significant declines in ACP abundance at sites with larger or more frequent releases, which may be indicative of substantial host-feeding by this parasitoid.

Non-technical summary: Surveys in huanglongbing quarantine zones in residential Southern California showed that insecticide treatments strongly suppressed populations of Asian citrus psyllid (ACP). Moreover, despite modest evidence of parasitism, *Tamarixia radiata* releases significantly reduced ACP abundance – likely due to host-feeding by this species.

Temperature-dependent index for ACP population dynamics

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Abstract: The development rate for Asian Citrus Psyllid (ACP) is strongly correlated with the accumulation of thermal time (development degree days) between lower and upper thresholds. Development rates for ACP in controlled environment experiments have been estimated independently by several groups of researchers in the past. Although they were successful in determining the biologically optimal temperature range for insect development (reporting lower and upper threshold estimates around 10°C (50°F) and 33°C (91°F)), to date there has been a lack of consistent methodology and field-ready translational application of these findings. We performed a summary analysis on 8 of these reports and created a generalized dataset of hourly temperature and developmental rates, classified by life stages. Seven nonlinear equations were used to describe the relationship between temperature and life stage transitions (6 transitions and oviposition). Seven additional piecewise linear functions were calculated to describe the temperature-mortality relationship for each life stage. All 14 models were designed to quantitatively predict the effects of broad temperature ranges that could occur in citrus orchards, including detrimental effects of high temperatures. The 14 models were combined in a projection matrix to calculate theoretical population development for several locations in California, based on hourly historical temperature data. A temperature-driven daily population growth index (DPGI) was computed by dividing each day's ACP population size by the previous day's. The model was compared with several sources of actual ACP population data, confirming that most commercial citrus areas are suitable for ACP development but with periods of winter and summer reduction in growth depending on temperature extremes during these seasons. The model can be a useful decision-making tool for growers, using localized temperature forecasting for near future ACP risk predictions. Additionally, the DPGI can be used as a risk mapping tool, serving as an objective geolocated parameter for ACP risk.

Non-technical summary: Environmental temperature influences ACP population growth and can thus potentially provide useful information on its management. This work proposes a model-based scoring system to evaluate how favorable a given day is for ACP development, based on localized temperature information. The scoring system is used to determine ACP fitness in a given area and season, and predict psyllid population variation in the near future, based on weather forecasting.

Incidence of '*Candidatus* Liberibacter asiaticus' in South Texas populations of Asian citrus psyllid and the nymphal parasitoid, *Tamarixia radiata*, on residential citrus

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Abstract: For US citrus growers, the Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is challenging to manage because it spreads huanglongbing (HLB) among residential citrus and from residential landscapes into nearby groves. The nymphal ectoparasitoid, *Tamarixia radiata* Waterston (Hymenoptera: Eulophidae), was introduced as a biocontrol agent for ACP throughout the US citrus belt because biological control is the most feasible approach for suppressing ACP populations and slowing HLB spread in urban areas. Acquisition of '*Candidatus* Liberibacter asiaticus' (CLas), the putative causal agent of HLB, usually occurs horizontally via ACP adults or nymphs obtaining the bacterium while feeding on infected plants or vertically as offspring from infected females. However, recent laboratory studies reported *T. radiata* acquires CLas during immature development on CLas-positive nymphs and ensuing female wasps can horizontally transmit CLas while probing healthy nymphs. *Tamarixia radiata* is now widely established in urban areas of South Texas and parasitism of ACP is, on average, 25-30% on residential citrus. However, it would diminish *T. radiata*'s benefit as a biocontrol agent if this parasitoid contributes to HLB spread. Presently, the extent of this phenomenon under field conditions is unknown. To address this information gap, we conducted a field evaluation of CLas acquisition by *T. radiata* in South Texas. During flush cycles from March to December 2022, we collected different life stages of *T. radiata* or ACP on flush shoots of HLB-symptomatic, residential citrus trees in the Lower Rio Grande Valley. Host plant and insect samples were tested for CLas using qPCR assays. We detected CLas among only 4% of *T. radiata* immatures or adults collected on CLas-positive trees. For South Texas, incidence of CLas acquisition by *T. radiata* appears to be very low, but additional studies are needed to determine whether the parasitoid can transmit CLas to ACP.

Non-technical summary: Biological control of Asian citrus psyllid by the introduced parasitoid wasp, *Tamarixia radiata*, slows the spread of citrus greening disease or huanglongbing (HLB) in residential citrus trees. Recent laboratory studies showed female wasps of *T. radiata* could acquire and transmit '*Candidatus* Liberibacter asiaticus' bacterium, which supposedly causes HLB, to healthy psyllid nymphs; thus potentially diminishing this parasitoid's benefits as a biological control agent. In South Texas, we found the likelihood of *T. radiata* acquiring this bacterium is very low on HLB-infected residential citrus trees and suggest its contribution to HLB spread is somewhat limited under field conditions.

Optimizing HLB Surveillance in Southern California through Adaptive Risk-Based Surveys

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Abstract: Citrus huanglongbing (HLB), transmitted by the Asian Citrus Psyllid (ACP), has emerged as a devastating threat to citrus-growing regions in California. The presence of HLB was initially identified in the Los Angeles residential basin in 2012. Subsequently, it rapidly established itself in urban neighborhoods, spreading across Orange, Riverside, San Bernardino, and San Diego counties. Timely detection of HLB is crucial to prevent its establishment and safeguard California's citrus industry. This study introduces a risk-based survey model designed to enhance evidence-driven decision-making for HLB surveillance. The model evaluates the effectiveness of surveys and deployment strategies to optimize data collection and impact. Within this risk-based framework, various factors and model components evolve as the epidemic unfolds, necessitating continuous updates to ensure data accuracy and model reliability. By conducting a retrospective longitudinal analysis of HLB occurrences spanning from 2012 to 2022, the predictive capabilities of individual risk factors were assessed. These factors include introduction risk from census travel patterns, ACP density, previous HLB-confirmed locations, citrus transportation corridors, citrus packinghouses, and farmers' markets. Notably, census travel risk emerged as a pivotal factor in the early detection of HLB during the epidemic's onset. Conversely, historical ACP density displayed a robust correlation with disease spread following its establishment. Consequently, promptly adjusting the weight assigned to each component within the comprehensive model construction becomes imperative to enhance predictive accuracy as the HLB epidemic evolves. By refining the risk-based model through real-time integration of dynamic factors, regulatory agencies can proactively allocate resources and implement adaptable surveillance strategies to effectively manage HLB outbreaks.

Non-technical summary: This study addresses the urgent threat of Citrus huanglongbing (HLB) in Southern California by introducing a risk-based survey model. The model assesses various factors like human-mediated introduction, Asian citrus psyllid density, and historical HLB detection data to optimize surveillance efforts, offering a proactive approach to managing HLB outbreaks in real-time.

Invasion and spread of Asian citrus psyllids and HLB in Florida

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Abstract: Most biological invasions are not observed directly unless there is reason to anticipate them. The initial invasion of Asian citrus psyllids and the bacterium, '*Candidatus* Liberibacter asiat-icus' (CLas), in Florida in the 1990s and early 2000s are no exception to this situation. The empiri-cal challenge is to pick up their trails as early as possible, identify the modes of spread, and hope-fully introduce interventions that may have some chance of slowing, stalling, and even reversing their progress.

We combine plausible speculation about both invasions with modeling of psyllid reproduction and local dispersal among orange jasmine (*Murraya paniculata*) plants in residential areas, natural psyl-lid dispersal in commercial citrus groves, and the appearance of huanglongbing symptoms anywhere from 2 - 6 years post infection. Based on a model of psyllid reproduction that incorporates infection status, egg laying and time-to-emergence of new generations of adult psyllids, windblown dispersal is sufficient to explain initial infestations and subsequent movement of psyllids in commercial groves bordering the Everglades in 1998-9. However, for the subsequent statewide expansion, we require a form of supply-chain modeling that deals directly with anthropogenic movement of plant material (fruit and propagative material) along major transportation corridors. Analogous spread of CLas infection followed the same pattern from late 2000 onward. However, tracking the invasion of CLas proved more difficult because 1) during its initial spread, the transmission mechanism was poorly understood and 2) orange jasmine plants themselves rarely are symptomatic or test positive for CLas even though the pathogen can be transmitted from parent psyllid to offspring at feeding sites. Our analyses support previous movement hypotheses based on observations of psyllid and CLas distribution in Florida and may improve exclusionary and mitigative practices for citrus grow-ing areas in earlier stages of infestation.

Non-technical summary: Mathematical models were employed to assess the relative importance of natural windborne spread and human-assisted movement of Asian citrus psyllids and huanglongbing on plant material (fruit, propagative material) along transportation corridors. Windblown movement probably was responsible for infections in commercial groves bordering the Everglades, but uninten-tional human-assisted movement played a large part in further statewide spread. These findings may improve management measures for citrus growing areas in early stages of infestation.

Optimization of early qPCR detection of '*Ca.* Liberibacter asiaticus' in Asian citrus psyllid sentinels and snapshots of pathosystem dynamics

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Abstract: Surveillance of homopteran insects such as psyllids and whiteflies as early-warning sentinels of plant pathogen outbreaks is ideal for early-detection of phloem-limited viruses or fastidious bacteria they transmit because they are often more readily detectable in an insect vector cohort than host plant, particularly woody or perennial species. Advantages include more rapid and efficient DNA isolation and greater yield of high-quality DNA. Toward qPCR optimization, Asian citrus psyllid (ACP) colonies were reared on *'Candidatus* Liberibacter asiaticus' (CLas)-infected and CLas-free *Citrus* spp. seedlings, and cohorts of 1, 3, 5, and 10 nymphal instars, at stages 1+2, 3, or 4+5 and adult color morphs (3) were collected. DNA was extracted using maceration in a tube (1.7ml) with bead-beating (15-20 2-mm beads), followed by DNA isolation with the Qiagen DNeasy blood and tissue kit. Total DNA was quantified by real-time qPCR amplification of RNR/WG (3-reps). Detection of CLas based on Cq values/genome copy number was most optimal for 4-5th instars (5/tube) and adults (5/tube). When the number of psyllids per tube was >5, Ct values for known positives decreased disproportionately. Analysis of nymphal instars 1+2 or 3 produced high rates of spurious negative results, regardless of individuals/tube. No failures to detect CLas from known CLas-infected cohorts occurred for 4-5th instars (5 or 10) and adults (3, 5, or 10). Among field locations sampled, the greatest number of ACP adults and ACP+CLas adults were collected (D-Vac sampling) in San Diego, Ventura, and Riverside/San Bernadino counties, respectively. Field-collected adult blue and orange color morphs (*vs.* white) yielded the majority of adult ACP-CLas positives from July 2021 – May2023.

Non-technical summary: Monitoring Asian citrus psyllid (ACP) for early-detection of potential *'Candidatus* Liberibacter asiaticus' (CLas) spread in citrus orchards can help guide risk assessment and recommendations when treatment to manage ACP is economically feasible. Adults and 4-5th instar nymphs were identified as the most reliable sentinels for early detection of CLas.

'Candidatus Liberibacter asiaticus' -phloem interactions in susceptible and tolerant varieties

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Abstract: Developing tolerant huanglongbing (HLB) cultivars is needed, but our understanding of the disease is still limited. We showed that in HLB infected trees, callose accumulates and plugs the phloem, and that this accumulation inhibits the translocation of sugars. These results explain many of the symptoms observed in the HLB trees but raised the question of how the bacteria can move in the occluded phloem. To explain this, we compared *'Candidatus* Liberibacter asiaticus' (CLas)-phloem interactions in the leaves and the seed coats, where the bacteria accumulate to high levels. In the infected leaves, both phloem occlusion and ROS levels increased, but phloem cells examined were for the most part bacteria-free. We found that that when bacteria are present, either in the leaves or seed coats, they reduced both callose and ROS levels, thus allowing their propagation and movement. Surprisingly, in the tolerant Sugar Belle (SB) mandarin, callose levels still increased after infection, but we found that the distance of a callose deposit to its closest neighbour was smaller in infected SB versus healthy while in HLB-affected susceptible Pineapple (PA) sweet orange it was higher. Moreover, SB exhibited less transversal plugging than PA, indicating a more localized response causing less phloem damage. Indeed, SB infected leaves showed a high increase in 14CO2 fixation and a moderate decrease in 14C-carbohydrate export whereas HLB-affected PA presented a large decrease of both parameters. An increased investment in xylem structure was also observed in the tolerant variety. Our results demonstrate a constant arms race between CLas and the phloem, and that during pathogen colonization, the bacteria can inhibit the plant phloem response, aimed to block bacteria movement, which allows it to move through the sieve pores. In tolerant varieties, a more localized accumulation of callose and higher xylem conductance may limit the bacteria while still allowing carbohydrate transport.

Non-Technical summary: Excessive phloem defense against *'Candidatus* Liberibacter asiaticus' (CLas), aimed to limit bacteria accumulation, also decrease sugar transport, and increase disease symptoms. Tolerant varieties respond to CLas more efficiently with less damage to sugar transport. These properties can help identify tolerant varieties and enable us to increase tolerance in susceptible varieties.

Engineering citrus disease resistance via transgene-free CRISPR genome editing

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Abstract: Citrus huanglongbing (HLB) presents an unprecedented challenge to citrus production in many regions especially Florida. Disease resistance is considered the most efficient, economic, and sustainable solution for disease control. Here, I will present our current progress in developing transgene-free citrus genome editing technology in the T0 generation and its application in generating disease resistant citrus. We have developed two different strategies for transgene-free CRISPR genome editing. The first utilizes Cas12a/crRNA ribonucleoprotein transformation of embryogenic protoplasts. The second strategy uses a co-editing strategy via Agrobacterium-mediated transient expression of cytosine base editor (CBE) to edit ALS to confer herbicide chlorsulfuron-resistance as a selection marker, Cas12a/crRNA for editing genes(s) of interest, and GFP for selecting transgene-free transformants. Both strategies have been successfully used to generate transgene-free, canker-resistant sweet orange lines. The entire process of RNP-mediated citrus genome editing, from transformation to grafting, takes about 10 months. The CRISPR technology significantly shortens the time needed to generate citrus varieties to 4-6 years from 20-30 years using traditional breeding. In addition, 97.4% biallelic/homozygous mutation rate was achieved for this transgene-free genome editing technology, indicating transgene-free CRISPR citrus genome editing technology is mature and efficient for implementation. The transgene-free CRISPR citrus genome editing technology is being used for generating transgene-free, HLB resistant/tolerant citrus varieties. Specially, HLB disease has been demonstrated to be a pathogen-triggered immune disease. The HLB pathogen '*Candidatus* Liberibacter asiaticus' (CLas) causes systemic and chronic immune responses, which subsequently lead to phloem cell death and HLB symptoms. The progress in editing the key genes responsible for the disease development will be presented.

Non-technical summary: Non-GMO CRISPR genome editing technology has been successfully developed which is highly efficient and significantly shortens the time needed to generate new citrus varieties. This technology has been successfully used to generate canker-resistant Hamlin sweet orange trees that have received regulatory approvals from USDA APHIS and EPA.

Transcriptional regulator inhibitors of *Candidatus* Liberibacter spp. as potential therapies for HLB

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Abstract: *Candidatus* Liberibacter spp. (e.g., CLas, CLso) are phloem-limited pathogens that cause diseases in several economically important crops, notably huanglongbing (HLB) in citrus and zebra chip (ZC) in potatoes. Previously, using *Sinorhizobium mellioti* as a surrogate host, multiple inhibitors of CLas transcription regulators were identified (Barnett et al. 2019). Whether these inhibitors inhibit *Candidatus* Liberibacter spp. in plant tissues and can be used to control disease remains unknown. Here, we evaluated the efficacy of these inhibitors in hairy root bioassays and *in planta* assays. Multiple molecules showed significant, dose-dependent, antimicrobial activity and attenuated *Candidatus* Liberibacter spp. transcription factor activity (LdtR, VisN, VisR) in the hairy roots bioassays. Further, foliar treatment of CLso-infected potato plants with the inhibitors showed attenuated symptoms, which correlated with reduced bacterial titers and transcription regulator activity. Similarly, CLas-infected citrus plants treated with the inhibitors showed a significant reduction of CLas titer compared to untreated controls. In conclusion, these *Candidatus* Liberibacter spp. transcription regulator inhibitors are promising candidates for the control of HLB and ZC diseases.

Non-technical summary: Currently, there are no effective curatives for huanglongbing (HLB) disease. Disease management is primarily done by limiting the spread of insect vectors and, most recently, by using bactericides. Here, we evaluated the potential of a new class of *Candidatus* Liberibacter spp. transcription regulator inhibitors as potential therapies to control HLB and zebra chip diseases.

Best foot forward: Australian efforts to stay in front of HLB

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Abstract: Huanglongbing (HLB) is not found in Australia, however industry and government have been proactive over many years preparing for its potential arrival. Australia has a formal biosecurity framework underpinned by policy, long-running government quarantine and surveillance programs, and an industry organisation (Auscitrus) supplying high health status propagation material. Importation of varieties with low commercial potential but a high smuggling risk has always been part of the Auscitrus program, but a new project has recently been launched to increase our understanding of the demand for new citrus varieties and make better informed decisions on which varieties are worth the investment required for importation. In recent years the peak industry body, Citrus Australia, has established a Citrus Pest and Disease Prevention Committee (CPDPC) to provide further guidance on exotic pest and disease preparedness within the industry. The CPDPC has helped to set a strategic direction for the industry, by identifying biosecurity priorities and resourcing options. Facilitated by the plant biosecurity organisation, Plant Health Australia, simulations have been held to identify issues that could impact on our collective ability to scale up a response and eradicate exotic threats to citrus. Many cross-sectoral collaborations, often facilitated by Plant Health Australia, have been developed over the years, which further strengthens our ability to respond effectively during an incursion. Research programs offshore improve our surveillance and diagnostic capability and our understanding of HLB and its vectors. Additional surveillance and education activities, branded 'CitrusWatch', are industry driven and government supported, to increase our chance of early detection in the event of a border breach. A particular focus has been placed on increasing awareness and checks in high-risk urban and peri-urban environments.

Non-technical summary: Huanglongbing **(**HLB) or its known insect vectors are not found in Australia. Industry and government are proactive in preparing for the disease to increase our chance of eradication and impact to industry. There are a multitude of industry and government organisations that collaborate to deliver activities in the areas of high-health propagation material, emergency response, research and development, pest surveillance and diagnostics, public education, policy and strategy. These collective efforts are furthering Australia's efforts to respond to HLB, although more work remains.

Living with African Greening

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Abstract: A disease referred to as African Greening has been observed in South Africa for nearly 100 years. Unusual symptoms of yellow branches and green fruit were reported in the late 1920's, that were linked to significant crop losses. The disease was named Greening in reference to the fruit symptom without knowing the aetiological agent. Pioneering research conducted in South Africa demonstrated that the disease was graft transmissible and vectored by *Trioza erytreae.* However, it took more than 50 years of research to identify the causative agent of African Greening as '*Candidatus* Liberibacter africanus' (CLaf). It has been detected in more African countries and is spread by infected planting material and the insect vector. Five CLaf subspecies have been identified in various Rutaceous species with one CLaf subsp. clausenae being the only subspecies for which a biovar was detected in citrus.
'*Candidatus* Liberibacter asiaticus' (CLas), is by far the most devasting of the three liberibacter species associated with huanglongbing (HLB). Although, CLas is steadily spreading across the world and is present on the African continent it has not reached South Africa yet. CLaf and its vector are heat sensitive that prevents the establishment of African Greening in unfavourable climatic regions. The disease has been successfully managed in regions deemed unsuitable for citriculture, due to established populations of CLaf and *Trioza erytreae*, through coordinated area-wide management strategies. In South Africa, research efforts are focused on the development of sensitive detection methods and monitoring strategies for HLB causing liberibacter species and their vectors. In this presentation and historical overview of African Greening will be presented as well as the current strategies used to manage the disease.

Non-technical summary: Citrus Greening, also known as a huanglongbing (HLB) is a devastating disease affecting citrus crops worldwide. It is caused by three species of bacteria; '*Candidatus* Liberibacter asiaticus' (CLas), 'Candidatus Liberibacter americanus' (CLam), and '*Candidatus* Liberibacter africanus' (CLaf). In South Africa, only CLaf is present and is associated with African Greening that produces milder symptoms compared to CLas. The development of sensitive detection tools to identifying these liberibacter species and their vectors are crucial for managing this disease.

Economic analysis update of orange groves in the State of Sao Paulo under the impact of the Huanglongbing

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Abstract: Brazil is the world leader in the production of orange juice. However, the Brazilian citrus industry is facing a considerable increase in the incidence of huanglongbing (HLB or Greening). HLB reduces productivity and can render trees unproductive, shortening the life span of orchards and threatening the economic viability of the investment. The significant increase of symptomatic trees has left growers skeptical about investing in renovation and new orchards in regions with high rates of the disease. Given the current good prices for oranges, the search for phytosanitary gaps within and outside the citrus belt, and even living with the disease while managing it to maintain production, have been the only options for growers and, at the same time, a great challenge. The objective of this study was to update economic viability calculations through the economic internal rate of return (IRR) in orange production in different HLB prevalence scenarios (L = low, M = medium and H = high), taking into account the Gompertz model to calculate the drop in productivity, and in the prices per box of oranges paid to the producer when harvested and transported to the processing industry (ranging from US$7.63 - BR$37.00 to US$9.69 - BR $47.00 (exchange rate of US$1.00 = BR$4.8512 on 08/10/2023). The sensitivity analysis showed that in the scenario of lower HLB pressure, the IRR varied from 10% to 24%, considering the price range proposed for the box of oranges, while in the high HLB pressure scenario, the IRR ranged from negative to 20%. Therefore, even under high HLB pressure conditions, but with reasonable prices, it is still worth investing in new plantings under the conditions proposed by the study in the citrus belt from Sao Paulo and Triangulo Mineiro

Non-Technical summary: Faced with the surprising increase in the incidence of huanglongbing (HLB) in the citrus belt, most growers are unsure about continuing to invest in orange orchards. Although prices should remain reasonable for the coming years due to the forecasted drop in production caused by HLB, economic feasibility studies considering disease pressure and scenarios for the price of a box of oranges are fundamental for growers' decision-making. The study showed that the internal rate of return (IRR), even under conditions of high disease pressure but with reasonable prices, is positive and can motivate growers to continue investing in orange production, contributing to meeting global demand.

Citations

ADAMI, A.C. de O. **Risco e retorno de investimento em citros no Brasil**. 151p. Dissertação (Doutorado em Economia Aplicada) – Escola Superior de Agricultura “Luiz de Queiroz”, Universidade de São Paulo, Piracicaba, 2010.

BASSANEZI, R. B.; BASSANEZI, R.C. **An approach to model the impact of Huanglongbing on citrus yield**. Proceedings of the International Research Conference on Huanglongbing. Orlando. 301-304p., 2008.

MIRANDA, S. H. G. de; ADAMI, A. C. DE O.; BASSANEZI, R. B. Economic impacts of Huanglongbing disease in Sao Paulo State. Association of Agricultural Economists (IAAE), Trienial Conference. **Poster...** Foz do Iguaçu. Paraná, 2012

Forecasting the Impact of Greening Disease on Orange Acreage and Production in Brazil

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Abstract: Brazil is the world's largest orange juice producer, with the citrus sector playing a important role in the Brazilian economy by generating employment and moving over 6.5 billion dollars annually. The industry faces a growing threat from huanglongbing (HLB or Greening). This disease has been causing significant damage, reducing production and leading to the eradication of orchards. Estimating the future of orange production and the extent of citrus cultivation in the next decade is important for investor decision-making, but studies in this regard are lacking. A forecasting model was proposed, taking into account the regional prevalence of Greening disease and citrus growers' investment expectations. The analysis was based on inventory data and crop estimates obtained from the Crop Estimate Survey (PES) conducted by FUNDECITRUS (Fund for Citrus Protection) for the citrus belt region – an area in the states of São Paulo and southwest Minas Gerais responsible for almost 80% of the country's planted area and production. The premises of the decision-making process took into account the intention of new plantings and the eradication of orchards according to the incidence of HLB in the region, based on the history of recent years, in addition to the drop in productivity of plants with HLB. The study's results indicate that over the next decade, there will be a significant reduction of 82,6 thousand hectares (-21,3%) in citrus cultivation area, resulting in a total of 305.1 thousand hectares. And, that production in five years will be down in the range of 249,0 (-20,0%) to 228,6 (-21,2%) million boxes, while over a period of ten years production will be down in a range of 203,7 (-34,6%) to 182,2 (-37,1%) million boxes, considering optimistic and pessimistic scenarios respectively.

Non-technical summary: These results have relevant implications in the global orange juice market and will guide decision-makers, mainly regarding investments in the sector in Brazil. The model can be updated annually in sync with the PES (Crop Estimate Survey) data updates. The reduction in orange area and production projected by the model in Brazil, the largest global orange juice producer, will lead to unfulfilled worldwide demand for juice consumption and consequently, a price increase over the next decade.

Citations

FUNDO DE DEFESA DA CITRICULTURA [et al.]. **Tree inventory of the São Paulo and westsouthwest Minas Gerais citrus belt: snapshot of groves in March 2023.** Araraquara,SP: Fundecitrus, 2023. 114 p. Available on site: <https://www.fundecitrus.com.br/pdf/pes_relatorios/2023_06_05_Tree_Inventory_and_Orange_Crop_Forecast_2023-2024.pdf>. Date access: 10th of August, 2023.

Tailoring the Silver Bullet: Holistic Management to Overcome Endemic HLB in Jamaica

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Abstract: Citrus management in Jamaica has evolved a lot in the last decade. Huanglongbing (HLB) is the most important disease of citrus in Jamaica, greatly reducing productivity and the lifespan of plantations that did not improve their production technology. HLB was confirmed in 2009, and its main impact has been a reduction of more than 50% of the area (from 10,000 to 5,000 hectares) and 65% of the general yields and an endemic infection environment. Despite the HLB infection levels (100%), trees in Bybrook Farm (St Catherine, 900 acres) of TWC have shown a positive response to the technical management carried out in the last 9 years. Great diversity in terms of scion/ rootstock combinations and a mosaic of soils required a scientific based understanding of the best genotype selection of the specific Jamaican conditions. An analysis based on historical yield and fruit drop data, soil conditions and the market determined that Valencia/ Cleopatra was the best choice. A new under screen nursery with certified budwood and seeds was built to produce trees for new plantings. Furthermore, drip irrigation became a corner stone for new plantings and revitalizing old productive groves. Asian citrus psyllid management (monitoring and control sprays) and a tailor made nutritional and crop protection program came together to finish rounding off the holistic plan to face HLB. The new holistic approach allowed TWC to improve 86% yields in old infected groves and achieved early production of more than 130 boxes/acre in year 3 and of more than 550 boxes/acre in 5-year-old new HLB-infected Valencia plantings.

Non-technical summary: Despite the huanglongbing (HLB) infection levels (100%), trees in Bybrook Farm (St Catherine, 900 acres) of TWC have shown a positive response to the technical management carried out in the last 9 years. The new holistic approach allowed TWC to improve 86% yields in old infected groves and achieved early productions of more than 130 boxes/acre in year 3 and of more than 550 boxes/acre in 5 year old new HLB-infected Valencia plantings.

A gap-free and haplotype-resolved lemon genome provides insights into flavor synthesis and huanglongbing (HLB) tolerance

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Abstract: The lemon (*Citrus limon*; family *Rutaceae*) is one of the most important and popular fruits worldwide. Lemon also tolerates huanglongbing (HLB) disease, which is a devastating citrus disease. Here we produced a gap-free and haplotype-resolved chromosome-scale genome assembly of the lemon by combining Pacific Biosciences circular consensus sequencing, Oxford Nanopore 50-kb ultra-long, and high-throughput chromatin conformation capture technologies. The assembly contained nine-pair chromosomes with a contig N50 of 35.6 Mb and zero gaps, while a total of 633.0 Mb genomic sequences were generated. The origination analysis identified 338.5 Mb genomic sequences originating from citron (53.5%), 147.4 Mb from mandarin (23.3%), and 147.1 Mb from pummelo (23.2%). The genome included 30,528 protein-coding genes, and most of the assembled sequences were found to be repetitive sequences. Several significantly expanded gene families were associated with plant–pathogen interactions, plant hormone signal transduction, and the biosynthesis of major active components, such as terpenoids and flavor compounds. Most HLB-tolerant genes were expanded in the lemon genome, such as 2-oxoglutarate (2OG)/Fe(II)-dependent oxygenase and constitutive disease resistance 1, cell wall-related genes, and lignin synthesis genes. Comparative transcriptomic analysis showed that phloem regeneration and lower levels of phloem plugging are the elements that contribute to HLB tolerance in lemon. Our results provide insight into lemon genome evolution, active component biosynthesis, and genes associated with HLB tolerance.

Non-technical summary: Lemon is tolerant to citrus huanglongbing (HLB). The gap-free and haplotype-resolved lemon genome will be benefit to citrus industry to identify the resistant gene and to improve the citrus against HLB by regenerating the phloem.

Comparative metabolic profiling of fruit juice from Australian limes and *Citrus* hybrids with *Microcitrus* parentage

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Abstract: The huanglongbing (HLB) disease can be mitigated if disease-resistant citrus varieties are developed. We reported resistance/tolerance to HLB in Australian native limes. Hybrids generated by crossing citrus with Australian native limes show various degrees of disease resistance; however, fruits of hybrids often possess undesirable fruit quality traits. We have conducted a systematic metabolomics study using the first generation hybrids, and the breeding parents. We analyzed fruit juice composition from selected plants using untargeted LC-MS and GC-MS. Specifically, we focused on fruit flavonoids, limonoids, and polyphenolics, coumarins, terpenoids, and volatile compounds, all of which influence the overall taste and palatability of the fruit. Metabolomic analysis revealed a distinct demarcation between the citrus and *Microcitrus* parents; their hybrids show a range of metabolic profiles. Certain off-flavors associated with hybrids can be correlated with the relative abundance of specific metabolites. Coumarins were present in relatively higher amounts (four to eight-fold higher) in *Microcitrus inodora* and Mexican lime compared to sweet orange and mandarin. In *M. australis*, the relative levels of coumarins were similar to commercial mandarins; nonvolatile terpenes were at higher levels compared to other cultivars in the study. We also identified specific flavonoids like nobiletin, tangeretin, naringenin, and hesperetin in the various samples. Commercial citrus cultivars (Washington navel orange, Fallglo mandarin) showed a higher flavonoid content compared to Australian native limes with levels ranging from two to nine-fold higher. In summary, our study provides valuable insights that can aid in the selection of novel hybrids for cultivar development based on the metabolic composition of fruit juice. The identification of disease-resistant hybrids with acceptable fruit quality characteristics represents a significant step towards addressing the challenges posed by HLB.

Non-technical summary: Metabolomic study of the fruit juice of breeding parents and the hybrid progeny will be useful in the selection of novel hybrids with acceptable fruit quality traits.

Cultivating Novel *Microcitrus* Hybrids as Scion for HLB Management in Disease-Endemic Growing Conditions

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The citrus huanglongbing (HLB) pathosystem presents one of the most significant challenges for plant disease management. The most effective and long-term management approach in HLB-endemic citrus-producing areas is the development of HLB-resistant or tolerant varieties. This project aimed to evaluate the in-field HLB resistance/tolerance and horticultural performance of four hybrids of Australian lime (*Microcitrus* spp.) grown as scions in Florida production environments. Field studies were initiated in the fall of 2021 at two different commercial production sites, one in Polk County and one in DeSoto County. Four different crosses of mandarin (*Citrus reticulata*) and *Microcitrus* spp. were propagated on two rootstocks, Carrizo (*C. sinensis* × *Poncirus trifoliata*) and US-942 (*C. reticulata* × *P. trifoliata*). 'Valencia' (*C. sinensis*) and 'Lisbon' lemon (*C. limon*) were included as scions for comparison. The experimental design was a randomized complete block design (RCBD) with 12 replications in Polk County and 9 replications in DeSoto County. The trees were regularly monitored for growth, health, and psyllid infestation in both locations. Growth evaluations include measurements of plant height and trunk diameter, along with monthly characterization of the new flush (number, length, and phenological stage), while disease evaluation involved monthly psyllid counts (adults, nymphs, and eggs), HLB symptom ratings, and biannual determination of pathogen titers. At both experimental sites and on both rootstocks, hybrid 2 ('Wilking' mandarin × *M. australasica*) performed better than the other hybrids, producing smaller trees, characterized by continuous and intensive new flush production, maintenance of healthy green leaves, significantly lower ACP colonization (adults and nymphs), and lower '*Candidatus* Liberibacter asiaticus' titer. In conclusion, our nearly 2 year- long field assessment suggests that hybrid 2 holds promise as a valuable breeding parent for a BC1 generation. Subsequent fruit evaluations will provide additional insights into this hybrid's organoleptic characteristics and other desirable traits.

Non-technical Summary: Genetic resistance or tolerance is the best strategy to sustain citrus production in the presence of huanglongbing (HLB). The use of Australian lime (*Microcitrus*) species as breeding parents to create novel HLB-resistant/tolerant scion hybrids with suitable fruit traits can potentially protect citrus industries from the devastating effects of HLB.

Current Knowledge of HLB Tolerance of Citrus Scion Varieties

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Abstract: Field research conducted in regions where huanglongbing (HLB) is endemic has produced published reports on which commercial citrus scion varieties have demonstrated tolerance to the disease when trees are mature. Knowledge of which varieties exhibit field tolerance to HLB is critical for the citrus industry, not only to identify which will permit economic citrus production where HLB infection is endemic, but also to determine which commercial citrus varieties might be appropriate parents for citrus scion breeding in order to possibly transmit tolerance to new hybrid progeny. This report summarizes published reports over the past 15 years of which commercial scion varieties have shown tolerance to HLB. For those commercial scion varieties with reported tolerance to HLB that have known hybrid parentage, this summary will provide an expanded list of possible varieties that might confer tolerance to HLB if used as a parent for citrus breeding or which could be re-evaluated for commercial citrus production.

Non-technical Summary: This report summarizes published reports over the past 15 years of those commercial citrus scion varieties and their parents that have shown tolerance to huanglongbing in field studies.

Different response of *PtPAO, CsBiP*, and *CsICS* transgenics sweet orange plants infected by '*Candidatus* Liberibacter asiaticus'

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Abstract: No resistance to '*Candidatus* Liberibacter asiaticus' (CLas), the causal agent of huanglongbing (HLB) is known within the commercial *Citrus* species, so far. Citrus engineered plants aiming to CLas and/or HLB resistance or tolerance have been researched worldwide by different strategies. In the present work we challenge transgenic sweet orange plants varieties 'Pinneaple' and 'Hamlin' overexpressing the genes *CsBiP* (immunoglobulin-heavy-chain-binding protein; 3 events), *PtPAO* (pheophorbide a oxygenase; 4 events), and *CsICS* (Isochorismate synthase; 4 events) against the CLas infection using by budding strategies. In the present work, we challenge the expression of HLB symptoms. All the transgenic lines were early obtained in the Citrus Research Center Sylvio Moreira. Fifteen reps of each transgenic event and of its no-transgenic varieties were propagated, and CLas-bud inoculated in the 8 months-old plants. The CLas transmission and colonization started at 120 days after inoculation (dai) following by analysis every three months until the 360 dai. In general, all the transgenic events were infected by CLas-inoculated by budding. All reps of *CsBiP* and *PtPAO* events were qPCR diagnosis positive for CLas at 360 dai, but the *PtPAO* 13A and 13B shown 100X minus bacteria compared to wildtype event. The events *CsBiP* 1A and 9BV shown 10X minus bacteria. Interesting results were obtained for the events *CsICS* 6-11 and *CsICS* 4-4 which shown 6.6% and 26.6% of infection, respectively. The CLas titer in these hybrids were 10 (*CsICS* 6-11) and 1000X (*CsICS* 4-4) less compared to wildtype Hamlin plants, with bacteria detected only at 240 dai and without HLB symptoms on the *CsICS* 4-4 event, possibly due to activation of systemic acquired resistance (SAR) in response to bacterial infection.

Non-technical summary: Engineering citrus plants have been used for '*Candidatus* Liberibacter asiaticus' (CLas) resistance. In the present work the plants showing the *CsBiP* (immunoglobulin-heavy-chain-binding protein; 3 events), *PtPAO* (pheophorbide a oxygenase; 4 events), and *CsICS* (Isochorismate synthase; 4 events) were challenge for CLas inoculation. Although all the events were susceptible to CLas infection, the events *CsICS* 6-11 and *CsICS* 4-4 shown lees rate of infection, minor CLas titer and no HLB symptoms after 360 days from inoculation.

Embryogenic Citrus Cell Lines for the Generation of Non-Transgenic HLB Resistant/Tolerant Citrus Varieties

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Abstract: Among the new plant breeding techniques, genome editing using CRISPR/Cas ribonucleoprotein complexes (RNPs) delivered to protoplasts have been successfully used to generate non-transgenic plants in a variety of crops with improved traits, including canker-resistant *Citrus sinensis*. To generate non-transgenic huanglongbing (HLB) resistant/tolerant citrus varieties using this technology, embryogenic cell lines of Tango and Daisy mandarins were first successfully established from nucellar and stigma/style tissues. Protoplasts were then isolated from embryogenic cells, using a sucrose-mannitol gradient technique, stained with fluorescein diacetate to assess their viability, and subsequently counted using a hemocytometer. Protoplast viability exceeded 98%, protoplast yields reached ~5-10 x 10 protoplasts/g of cells, and the first cell divisions and colony formation were observed 1-2 weeks after protoplast culture. A protocol for the transfection of protoplasts using polyethylene glycol was also tested using a plasmid carrying the green-fluorescent reporter gene , and green fluorescing cells were observed 24-48 hours after protoplast transformation. Ongoing efforts are focused on the delivery of RNPs to protoplasts, to knock down genes potentially involved in Citrus HLB susceptibility, and the regeneration of gene edited non-transgenic HLB resistant/tolerant Tango lines.

Non-technical summary: Initial protocols for the generation of non-transgenic huanglongbing (HLB)-resistant citrus mandarin plants through RNP-mediated gene editing have been established. These protocols can be more precise and efficient in the production of transgene-free genome-edited plants that are approved by USDA APHIS and exempted from EPA regulation.

Employment of an optimized construct for genome editing in *Citrus sinensis* aiming to obtain HLB-tolerant plants

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Abstract: Genome editing using CRISPR/Cas technology has emerged as a promising technique for developing pathogen-tolerant plants by silencing host key genes. In this context, strategies to enhance editing efficiency, such as employing codon optimization, promoters that boost sgRNA and Cas nuclease transcription and incorporating Kozak consensus sequences, should be considered. Thus, in this study we aim to implement the pCsMAD7U6 vector, developed by our research group, to enhance editing efficiency in *Citrus sinensis*. We seek to perform gene editing of sensitivity genes related to plant response against huanglongbing (HLB) to obtain disease-tolerant plants. Host genotyping, as well as design and selection of sgRNAs for two callose synthase (*CsCalS*) genes were carried out. In this way, Sanger sequencing for genotyping was performed for target regions of two varieties of *C. sinensis* (Hamlin and Valencia). Among the eight sgRNAs evaluated upon sequencing, only the target region of one sgRNA showed no mutation in any of the assessed citrus varieties. However, since it was necessary to select at least one sgRNA per gene, the sgRNA 273R, which exhibited a low frequency transition mutation (C→T) (1/22 colonies) in Valencia sequence, was also chosen. Following sgRNA selection, pCsMAD7U6 vectors were synthesized and used to transform *Agrobacterium tumefaciens* EHA105 strain. Subsequently, genetic transformation Hamlin and Valencia epicotyls was performed aiming to obtain edited shoots. A total of 2,316 explants were co-cultured with *A. tumefaciens*, from which 106 shoots have been obtained and will be analyzed to assess the editing efficiency and characterize indels. According to our previous results, we can conclude that genotyping of target regions is mandatory prior to sgRNA selection, ensuring annealing of sgRNAs to the genome target. Finally, by using the novel pCsMAD7U6 construct, it is expected to achieve high editing efficiency and possibly generate HLB-tolerant plants.

Non-technical summary: This work aims to implement optimized genome editing techniques developed by our research group to obtain huanglongbing (HLB)-tolerant citrus. Additionally, it emphasizes the importance of carefully preparing the experimental design for genome editing. We hope that by using these techniques we can enable market feasibility for sweet orange varieties that will not be affected by HLB, thereby benefiting both producers and consumers.

Harnessing the genetic potential of citrus species to develop new hybrids with enhanced tolerance to Huanglongbing

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Abstract: Most of the cultivated citrus varieties are primarily descended from 4 ancestral species: *Citrus maxima*, *C. medica*, *C. reticulata* and *C. micrantha* and many are highly susceptible to huanglongbing (HLB). Several HLB tolerant citrus relatives have been identified in recent years. We have developed and field tested HLB-tolerant hybrids of the *Citrus* species above with *C. australis*, *C. australasica*, *C. depressa, C. inodora* and *C. latipes* among others. Robust rootstock hybrids, both at the diploid as well as tetraploid level, have been produced using these species. At the tetraploid level, several protoplast fusion-derived finger lime tetraploids have remained HLB negative even after 8 years of growth in an HLB-endemic field environment. These are being used as parents in our breeding program. Several diploid hybrid rootstock candidates, especially pummelo hybrids with *C. latipes* have provided outstanding vigor to '*Candidatus* Liberibacter asiaticus'-infected 'Valencia' sweet orange scion with good soluble solids content. Several have remained PCR negative (both roots and leaves) for over 6 years. The profiling of volatile organic compounds (VOCs) and polar metabolites also indicated that the hybrids differ from their parents. Hybrids with *C. depressa* genetics have demonstrated robust tolerance to both abiotic and biotic stresses and have been extensively used to develop HLB tolerant rootstock candidates. The Australian limes (*C. australis*, *C. australasica*, and *C. inodora*) have also contributed to the development of both HLB-tolerant scions and rootstocks. Two recent scion releases (UF SunLime and UF RedLime) are both highly tolerant to HLB. Metabolic profiling on the finger lime leaves has indicated that they are significantly rich in several amino acids, organic acids, and sugars when compared to HLB susceptible cultivars. Altogether, development of these improved genetic resources should help the industry cope with endemic HLB.

Non-technical summary: A large population of huanglongbing (HLB) tolerant citrus selections have been developed and are being evaluated for HLB tolerance. These also provide valuable genes to develop the next generation of HLB tolerant citrus for profitable citriculture.

Increasing Citrus Resistance against Citrus Canker and Huanglongbing through Genome Editing

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Abstract: Citrus canker and huanglongbing (HLB) represent serious challenges to the citrus industry in the U.S. and worldwide. Addressing these challenges necessitates enhancing the resistance of citrus to these diseases. A powerful genetic approach for increased disease resistance involves using CRISPR/Cas to knock out specific and general disease susceptibility genes (*S* genes). The *CsLOB1* gene has been edited in its promoter or coding region, resulting in strong canker resistance. *CsLOB1* contains an effector binding element (EBE) in its promoter that binds to the transcriptional activator-like effector (TALE) PthA4 of *Xanthomonas citri* subsp. *citri* (*Xcc*), the causal agent of citrus canker. When the EBE structure is disrupted in citrus mutants, *CsLOB1* can't be activated by PthA4 and thus stops to respond to *Xcc*'s disease-causing mechanism and acquire resistance to citrus canker. Similarly, editing the coding region of *CsLOB1* has resulted in loss-of- function mutants that show resistance to citrus canker. The *CsSWEET1* gene also contains EBEs in its promoter and responds to *Xcc* infection; editing the coding region of *CsSWEET1* has led to strong resistance to citrus canker. The *DMR6* gene encodes a salicylate 3-hydroxylase that degrade salicylic acid, a key plant hormone for plant disease resistance. Knocking out the function of *DMR6* has resulted in resistance to citrus canker. The transcription factor gene *CsWRKY22* regulates citrus canker susceptibility; knocking out *CsWRKY22* has also conferred resistance to citrus canker. A number of genes in citrus are being targeted by genome editing for HLB resistance. Although progress has been slower, due to several complicated factors, the pace is picking up. In summary, measured progress has been made in augmenting resistance to citrus canker through editing of specific and general *S* genes, and significantly greater progress is expected in enhancing HLB resistance in the next 5 years.

Non-technical summary: Measured progress has been made in augmenting resistance to citrus canker through editing of specific and general *S* genes, and significantly greater progress is expected in enhancing huanglongbing resistance in the next 5 years.

Mechanisms Underlying Huanglongbing (HLB) Tolerance in the Finger Lime (*Citrus australasica*) and Its Hybrids

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Abstract: The finger lime (*Citrus australasica*) and its hybrids exhibit significant tolerance to huanglongbing (HLB). Understanding the underlying hypostatical mechanisms responsible for this tolerance is essential for developing effective strategies to combat HLB in other citrus cultivars. This study employed a comprehensive transcriptome analysis to unravel the mechanisms behind HLB tolerance in finger lime and its hybrids. Through a greenhouse assay involving HLB-infected budwood grafting onto healthy trees, we meticulously examined the response of finger lime and 'Valencia' sweet orange, a susceptible citrus cultivar, at the molecular level. Our investigation unveiled numerous differentially expressed genes (DEGs) encompassing diverse functional categories such as proteolysis, resistance (R) genes, signaling pathways, redox state regulation, peroxidases, glutathione-S-transferase, secondary metabolites, and pathogenesis-related (PR) proteins. Particularly noteworthy were the highly upregulated genes encoding cysteine-rich secretory proteins or Pathogenesis-related 1 (PR1-like) proteins in HLB-infected finger lime trees relative to both noninfected finger lime and infected 'Valencia' sweet orange. To further explore the generality of these findings, we assessed the expression of these upregulated genes in various commercial citrus cultivars and other Australian lime species grown in field conditions. Remarkably, all evaluated trees, with the exception of finger lime and the Australian desert lime (*Citrus glauca*), exhibited lower relative expression of these genes. Importantly, we also observed the same highly upregulated DEGs in several HLB-tolerant hybrids resulting from crosses between a pummelo and the finger lime within our breeding program. These findings strongly indicate the heritability of the HLB tolerance trait from finger lime and its potential for generating HLB-tolerant hybrids, offering a promising avenue for the citrus industry to combat this devastating disease.

Non-technical summary: This study sheds light on some of the mechanisms governing huanglongbing (HLB) tolerance in the finger lime and its hybrids, providing crucial insights for the development of HLB-tolerant citrus varieties to safeguard the citrus industry's future.

N13-32 Hamlin: a seemingly HLB tolerant sweet orange (*Citrus sinensis* L. Osbeck) somaclone

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Abstract: Huanglongbing (HLB) is the most important plant disease in citrus. This insect-vectored disease has devastated Floridian citriculture over the last several years, resulting in massive losses for growers, packers, and processors. 'Hamlin' sweet orange (*Citrus sinensis* L. Osbeck) is an early maturing variety that historically has been important to the Florida grower and the processing juice stream. However, the selection has a major problem because of its susceptibility to HLB, resulting in fruit drop, low total soluble solids content (TSS), high titratable acidity (TA), deformed and small fruit, tree decline, thin canopy, and low yields. There has been a concerted statewide effort to identify accessions with tolerance to HLB, with thousands of accessions under observation for better health under '*Candidatus* Liberibacter asiaticus' infection. A relatively new nucellar somaclone of 'Hamlin' sweet orange, dubbed 'N13-32', has shown strong tolerance to HLB at multiple locations in the State of Florida. Data were collected at two sites, one at the Citrus Research and Education Center City Block field trial and one at a private grower in Polk County. Data collected included number of fruit per tree, TSS, TA, sugar to acid ratio, yield in number of fruit per tree and fruit mass per tree, fruit drop, individual fruit diameter, individual fruit mass and drone metrics (canopy area, plant volume, plant health, and plant vigor). Using drone-mounted multispectral image sensors, plant health was measured using normalized difference red edge (NDRE) and vigor was measured using normalized difference vegetation index (NDVI). There were significant differences between the 'N13-32 Hamlin' somaclone and the conventional 'Hamlin' selection in both groves for several variables. The results indicated that N13-32 Hamlin may be a superior selection for tree health compared to 'Hamlin 1-4-1', the conventional 'Hamlin' clone most widely grown in Florida, USA.

Non-technical summary: In Florida, USA, sweet orange cultivars make up the bulk of orange juice production and they are typically susceptible to huanglongbing (HLB). There is a clone of 'Hamlin', which is an early maturing sweet orange, that appears to be tolerant to HLB, displaying a fuller canopy, greater health, and faster growth rate. This study evaluated this selection, dubbed N13-32 Hamlin, and conventional Hamlin for health, fruit quality and yield and determined that N13-32 may be a superior selection compared to the conventional Hamlin cultivar.

Navigating the Genetic Bottleneck of HLB: Exploring the Genomes of Australian Wild Limes for Development of Disease Resistance

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Abstract: The citrus industry needs a solution against the scourge of huanglongbing (HLB). Australian wild limes such as *Citrus australasica*, *C. inodora* and *C. glauca* possess valuable disease-resistant traits against HLB. To identify the resistant genes and understand the basis of resistance in the Australian wild limes, we developed high-quality, *de novo*, haplotype genome assemblies. The genome sizes range from 300 Mb (*C. inodora*) to 337 Mb (*C. australasica*) and 377 Mb (*C. glauca*). All three genome sequences were scaffolded into nine large, chromosome-scale scaffolds that constitute 86% – 91% of total genome. Synteny relationships of the three genomes showed variation in chromosomes 2, 3, 4, 6 and 9 when compared to *C. clementina.* Genome annotations identified 25,461 genes in *C. australasica*, 27,665 in *C. inodora*, and 30,067 in *C. glauca*. There were 668 *R*-genes in *C. australasica*, 404 in *C. inodora*, and 564 in *C. glauca*. We identified 47-56 Phloem protein2 genes and ~20 Callose synthase genes. In conclusion, the exploration of Australian wild limes has unveiled a treasure trove of genetic diversity and provides invaluable insights that hold the potential to understand HLB disease resistance.

Non-technical summary: We have sequenced three Australian wild limes known to have huanglongbing tolerance/resistance. The study helps to understand the basis of resistance and will facilitate development of resistant varieties for sustainable agriculture.

Efficient confirmation test of HLB '*Candidatus* Liberibacter asiaticus' (CLas) with confidence using a sensitive and highly specific quadruplex real-time PCR

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Abstract: The Plant Pathogen Confirmatory Diagnostics Laboratory (PPCDL) has developed and validated multiple diagnostic protocols for the detection of the bacteria associated with huanglongbing (HLB) in different regulatory settings. These protocols provide the scientific basis for regulatory action taken by PPQ to protect the US citrus industry. In certain regulatory scenarios, when federal confirmation is required, *'Candidatus* Liberibacter asiaticus' (CLas) detection may be challenging as the pathogen can occur at low concentrations and be unevenly distributed in plant tissues. This requires a highly specific confirmatory test, as sensitive as the screening real-time PCR test. The PPCDL utilized three verified CLas specific real-time PCRs to develop a quadruplex real-time PCR, which simultaneously detects three CLas targets - Ribonucleotide Reductase (RNR), 16S rDNA and Heat Shock Protein (HSP) sequences, plus a COX plant internal control. The combination of these targets provides high specificity, while maintaining the sensitivity of real-time PCR. The quadruplex PCR shows precision, repeatability, intermediate precision, linearity, analytical sensitivity, specificity, etc. comparable with the single-target assays. Testing Ct values of a sample will usually exhibit a relationship of RNR Ct< 16S Ct< HSP Ct in the titer linear range, indicating a relative quantification manner for the three CLas targets with individual 5/3/1 copies per genome, respectively. With the implementation of this protocol, conventional PCR and sequencing were not necessary for confirmatory testing and were removed from the PPCDL HLB confirmatory diagnostic workflow. This greatly reduces the cost and turnaround time of HLB diagnostics, allowing the PPQ Citrus Health Response Program (CHRP) and its stakeholders to make timely regulatory decisions to limit the spread and establishment of this devastating pathogen in US citrus production areas.

Non-technical summary: Early detection and eradication of citrus greening infected trees are one of the efforts in protecting citrus industry, as it eliminates inoculum source. USDA Plant Pathogen Confirmatory Diagnostics Laboratory (PPCDL) successfully developed a multiplex real-time PCR assay for confirmatory testing that efficiently detects three different targets of the endemic huanglongbing (HLB) *'Candidatus* Liberibacter asiaticus' and a citrus plant target simultaneously. Using this assay, PPCDL may detect very little quantity of HLB in a plant sample with confidence considering multiple pieces of evidence altogether.

Expecting the unexpected: '*Candidatus* Liberibacter africanus' detection using high-throughput sequencing data

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Abstract: Citrus greening or huanglongbing (HLB) is a highly destructive disease affecting citrus crops worldwide. '*Candidatus* Liberibacter asiaticus' (CLas), '*Candidatus* Liberibacter americanus' (CLam), and '*Candidatus* Liberibacter africanus' (CLaf) are the three species of bacteria associated with citrus greening. In South Africa, citrus greening is associated with CLaf, which has a milder expression of the disease compared to CLas. Detection and identification of liberibacter species is critical to the management of HLB and detection assays requires continuous evaluation to ensure specificity and sensitivity. Several indigenous liberibacter species and subspecies have been identified in South Africa that can complicate these assays and lead to false positive reports. One of these subspecies, 'Ca. L. africanus subsp. clausenae' (CLafCl) was first described in South Africa on indigenous *Clausenae anisata* trees. A biovar (bv.) of CLafCl (CLafCl bv. citrus) was shown to infect citrus and generate a false positive signal in some CLas assays. There is an immediate need for more genome information of various liberibacter species and subspecies infecting citrus to build a reference database primarily for the accurate identification of these liberibacter species and to facilitate further research and characterisation of this pathogen. In theory, high-throughput sequencing (HTS) of liberibacter infected specimens provides the ideal opportunity to identify and differentiate liberibacter species with increased sensitivity. In this study the aim was to apply HTS to sequence CLaf and CLaf subspecies from symptomatic plants to evaluate HTS as a detection tool and to add data to the liberibacter genome reference database. The results obtained from these metagenomic data sets illustrated the difficulties in sequencing liberibacters from plant material and shows the reality of what to expect in terms of read count and genome coverage.

Non-technical summary: Citrus greening is caused by three types of bacteria, including *'Candidatus* Liberibacter asiaticus' (CLas), *'Candidatus* Liberibacter americanus' (CLam), and *'Candidatus* Liberibacter africanus' (CLaf). Detecting and identifying these liberibacter species is crucial for managing the disease, especially considering the presence of indigenous liberibacter species that can complicate detection assays. To address this, high-throughput sequencing (HTS) was utilized to analyze CLaf and its subspecies from symptomatic plants, shedding light on the challenges of sequencing liberibacters from plant material.

Microbiomic evaluation of Asian citrus psyllids infected with '*Candidatus* Liberibacter asiaticus' from Mexico

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Asian citrus psyllid (ACP, *Diaphorina citri*), is the insect vector that transmits '*Candidatus* Liberibacter asiaticus' (CLas), the pathogen associated with citrus huanglongbing (HLB). In a previous study, CLas-infected ACP samples were collected from two states of Mexico with disparate climates, Yucatán (uc) and Baja California Sur (BCS). Two CLas genome sequences, one from each state, were acquired and characterized using Illumina sequencing technology. In this study, we further explored the microbiome (bacteriomes and viromes) in ACP. Sequence reads were classified using the Kaiju metagenomic classifier and confirmed with read-mapping to selected references. The bacteriomes of both ACPs were similar, characteristically with similar abundance of symbionts such as "*Ca.* Carsonella ruddii" and "*Ca.* Profftella armature". However, there were significant differences in their viromes. ACP-Yuc showed abundance of Pararnavirae that included a citrus endogenous pararetrovirus, whereas ACP-BCS showed abundance of Bamfordvirae that included some insect pathogenic viruses. Heunggongvirae virus was detected in both ACP-Yuc and -BCS. This may be related to the presence of Type 1 and Type 2 bacteriophages of CLas.

Non-technical summary: This research used microbiomic technique to acquire new information that helps understand Asian citrus psyllid and '*Candidatus* Liberibacter asiaticus' biology. Research outcomes could facilitate the development of effective huanglongbing management strategies to protect the citrus industry of the USA and Mexico.

Molecule polymorphism of G-X-Y interruptions in Collagen Triple Helix protein of '*Candidatus* Liberibacter asiaticus'

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Abstract: '*Candidatus* Liberibacter asiaticus' (CLas), a prevalent strain associated with the most devastating disease of citrus huanglongbing (HLB) worldwide. Collagen-like proteins involved in a key aspect of pathogenicity, are an important source of genetic diversity in pathogenic bacteria. No genetic polymorphic analysis has yet been reported associated with collagen-like proteins in CLas. In the present study, 358 samples collected from China and 52 samples from USA were used to analyze the molecule polymorphisms of collagen triple helix protein (Cthr) of CLas. Divergent amplicons were got. The full length amplicon of 1652 bp covering gp245 ORF and the interspace region were got only from USA psyllid isolates. Sequences ranging from 941 bp to 1588bp were got both from Chinese and USA isolates. A typical 63-amino-acid terminal domain with a potential transmembrane helix domain, a central collagen-like region containing Gly-Xaa-Yaa (G-X-Y) repeats, and a 7-amino-acid carboxy-terminal domain were found. A total of 39 genotypes of Cthr proteins of Chinese isolates and 13 genotypes of USA isolates were characterized. Further analysis revealed both the N' and C'-terminals of CthP were always conserved. Yet hypervariable (Gly-X-Y) n repeats were found. Phylogenetic analysis indicated most of USA isolates including the representative UF506 strain were grouped into one independent clade indicating a complex origination. The rest were grouped with gene-variants from Jiangxi and Fujian. Yunnan isolates from China contains most gene-variants and differ greatly from all the other isolates from both China and USA. Based on our results, it can be concluded that the population of CLas contains different gene-variants which resulted in a complex citrus pathosystem for the study of interactions of phenotype and virulence. Molecule polymorphism analysis based on the Cthr protein of CLas will broaden the current knowledge of HLB population and lay a solid foundation for future research on pathogenicity.

Non-technical summary: We compared a Collagen-like protein in '*Candidatus* Liberibacter asiaticus' (CLas) from both Chinese and USA Isolates which may involve in a key aspect of pathogenicity. Divergent amplicons were got from different geographic originated samples from different hosts indicating a complex origination.

Multiplex qPCR detection of *Candidatus* Liberibacter spp. and *Spiroplasma citri*

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Abstract: *Candidatus* Liberibacter spp. and *Spiroplasma citri* (*S. citri*) are phloem-limited bacteria, causing the citrus diseases huanglongbing (HLB) and stubborn disease (CSD), respectively. CSD is endemic in California and has symptoms in citrus that can be easily confused with those of HLB. Therefore, a real-time multiplex quantitative polymerase chain reaction (qPCR) assay for the simultaneous detection of all *Candidatus* Liberibacter spp. (asiaticus, americanus, and africanus) and *S. citri* was developed and validated using different fluorescently labeled minor groove binding qPCR probes. The capacity of the multiplex qPCR assay in detecting the pathogens was compared to singleplex qPCR assays designed specifically for each pathogen. Pathogen isolates in citrus and psyllids from diverse geographical regions were used to validate the assays. Comparison of the pathogen load for each pathogen using singleplex and multiplex qPCR assays revealed no significant differences between the two assays in detection limits. Specificity was not affected by combining the two qPCR assays in a multiplex reaction. Optimizing the DNA extraction technique for citrus tissues and testing the quality of the extracted DNA using qPCR targeting the citrus cytochrome oxidase gene (COX) as a specific internal control generated better diagnostic assays.

Non-technical summary: Symptoms in citrus caused by *Candidatus* Liberibacter spp. and *Spiroplasma citri*, the causal agents of citrus greening (huanglongbing) and stubborn disease, respectively, can easily be confused visually. A multiplex qPCR-based testing protocol developed for the simultaneous detection of both pathogens, when co-existing, will streamline pathogen testing of citrus nursery stock and field surveys by replacing two separate singleplex assays, thus reducing time and labor without sacrificing sensitivity or specificity.

Nanometal reagent therapeutics suppress '*Candidatus* Liberibacter asiaticus', the causal pathogen of huanglongbing

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Abstract: Huanglongbing (HLB), also known as citrus greening, is one of the most devastating citrus diseases worldwide. HLB symptoms include yellowing of shoots, blotchy mottled leaves, corky veins, malformed and discolored fruits, premature fruit drop, and root loss, resulting eventually in tree death. It is caused by the psyllid-transmitted, phloem-limited bacteria '*Candidatus* Liberibacter asiaticus' (CLas). However, the detailed molecular mechanisms of CLas have not been fully understood yet. Oxytetracycline (OTC), an injected antibiotic, has now been approved for use in citrus to control CLas. The fact that growers are willing to treat trees individually changes how our team is screening for HLB solutions. Now, molecules are being evaluated by tree injection in the field alone and in combination with OTC. Work in the lab is done only in the context of field observations. To better understand how classes of unknown molecules work in the field, we developed a minimum inhibitory concentration (MIC) assay to assess molecule antimicrobial activities against *Agrobacterium tumefaciens*, a bacteria phylogenetically related to CLas. OTC standards are useful to benchmark assay performance. One class of molecules being evaluated includes metallic nanometer particles, including zinc oxide, magnesium oxide, and calcium oxide. This presentation will focus on detailing the MIC and other lab assays that are used to develop mechanistic data that may explain or support field observations during trunk injection. There is an urgent need to identify or develop inhibitor molecules to suppress or eradicate CLas from infected citrus plants.

Non-technical summary: Huanglongbing (HLB) is a lethal disease of *Citrus* species, caused by '*Candidatus* Liberibacter asiaticus' (CLas). We explore the use of oxytetracycline (OTC) in citrus as an antibiotic for CLas control in conjunction with previously untested molecules, like metallic nanoparticles. Our laboratory assays reveal treatments with potential and provide support in parallel to field observations, addressing the urgent need for growers to combat HLB.

Pathogenicity and transcriptomic analyses of two '*Candidatus* Liberibacter asiaticus' strains harboring different types of phages

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Abstract: '*Candidatus* Liberibacter asiaticus**'** (CLas) is one of the putative causal agents of citrus huanglongbing (HLB), a highly destructive disease threatening global citrus industry. Several types of phages had been identified in CLas strains and found to affect the biology of CLas. However, little is known about the influence of phages in CLas pathogenicity. In this study, two CLas strains, strain PYN and strain PGD, harboring different types of phages were collected and used for pathogenicity analysis in periwinkle (*Catharanthus roseus*). Strain PYN carries a Type 1 phage (P-YN-1) and PGD harbors a Type 2 phage (P-GD-2). Compared to strain PYN, strain PGD exhibited a faster reproduction rate and higher virulence in periwinkle: leaf symptoms appeared earlier and there was a stronger inhibition in the growth of new flush. Estimation of phage copy numbers by type-specific PCR indicated that there are multiple copies of phage P-YN-1 in strain PYN, while strain PGD only carries a single copy of P-GD-2. Genome-wide gene expression profiling revealed the lytic activity of P-YN-1 phage as evidenced by the unique expression of genes involved in lytic cycle, which may limit the propagation of strain PYN and lead to a delayed infection in periwinkle. However, the activation of genes involved in lysogenic conversion of phage P-GD-1 indicated it could reside within CLas genome as prophage form in strain PGD. Comparative transcriptome analysis showed that the significant differences in expression for virulence factors genes, included pathogenic effectors, transcriptional factors, Znu transport system and Heme biosynthesis pathway, could be another major determinant of virulence variation between two CLas strains. This study expanded our knowledge of CLas pathogenicity and provided new insights into the pathogenicity differences between CLas strains.

Non-technical summary: As one of the most common putative causal agents of citrus huanglongbing (HLB), **'***Candidatus* Liberibacter asiaticus**'** (CLas) have been found to be affected by the associated phage in its biology. Data from this study found that the possible lytic activity of Type 1 phage could limit the propagation of CLas strain and lead to the delayed infection in periwinkle. The heterogeneity in the transcriptome profiles, particularly the significant differences in expression of virulence factors genes, could be another major determinant of difference in virulence observed between CLas strains. These findings not only improved our understanding of CLas-phage interaction and CLas pathogenicity, but also provided new ideas in phage therapy for HLB.

ACP Biological Control in California

David Morgan and Grace Radabaugh

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Abstract: *Tamarixia radiata* (Hymenoptera: Eulophidae) has been used as a biological control agent of the Asian citrus psyllid (ACP) in urban California since 2011. Over the intervening period the release strategies to optimize the efficacy of this parasitoid have moved from establishment to dispersal, and finally to targeted release. These strategies are described, along with corresponding evidence for the success of each strategy including overwinter survival, establishment over a wide geographic area, significant area-wide reduction in ACP populations, and suppression of ACP populations in small, targeted areas. *Tamarixia radiata* has proven to be a valuable tool for managing ACP in California when used in conjunction with other control strategies.

Non-technical summary: Biological control agents play an important role in suppressing the Asian citrus psyllid in urban California. We describe optimal strategies for the use of these beneficial insects and show evidence of their efficacy.

Bacterial pesticidal proteins Mpp51Aa1 and Tpp78Aa1 are toxic to the Asian citrus psyllid, *Diaphorina citri*

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Abstract: The Asian citrus psyllid, *Diaphorina citri*, vectors the plant pathogenic bacterium that results in huanglongbing or citrus greening disease. In Florida USA alone, this disease has resulted in over US $8 billion in losses since 2005. The repeated application of chemical pesticides for *D. citri* management rapidly resulted in insecticide resistance. Bacterial pesticidal proteins (BPPs) are environmentally benign alternatives to chemical insecticides and have been successfully deployed against both lepidopteran and coleopteran pests. However, relatively few BPPs have been identified with sufficient toxicity against hemipteran species for practical use. Here, we report on the toxicity of 17 bacterial pesticidal proteins from four of the 15 different BPP structural groups against *D. citri.* These proteins were selected based on previously reported toxicity to other hemipteran species. Proteins were either expressed in 1) *Escherichia coli* and purified from inclusion bodies or via His tag-mediated affinity purification, or 2) Bt and purified from spore - crystal mixtures. Pesticidal proteins were initially screened for toxicity by feeding psyllids on a single dose of 200 or 500 ppm, and bioassays were conducted to determine LC_{50} values for proteins with significant mortality relative to the buffer control in the initial screen. The Bt proteins Mpp51Aa1 and Tpp78Aa1 were toxic to *D. citri* adults with LC_{50} values of 110.4 and 204 µg/ml respectively. Mpp51Aa1 and Tpp78Aa1 along with other reported *D. citri* - active pesticidal proteins may provide effective and environmentally benign tools for suppression of *D. citri* populations.

Non-technical summary: A bacterium commonly known as Bt produces insect-specific, pesticidal proteins. We have identified two new Bt proteins with toxicity against the Asian citrus psyllid, which plays an important role in the spread of huanglongbing or citrus greening disease that has devastated citrus production in Florida. These pesticidal proteins derived from Bt may provide additional tools to help mitigate citrus greening disease.

Biological Control of the Asian Citrus Psyllid, *Diaphorina citri*, in the Lower Rio Grande Valley of Texas Using the Ectoparasitoid, *Tamarixia radiata*

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Abstract: *Tamarixia radiata* Waterston (Hymenoptera: Eulophidae), a biological control agent of the Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), vector of the citrus pathogen, "*Candidatus* Liberibacter spp." has played a crucial role in reducing ACP populations in urban environments of citrus growing areas in Texas. The USDA APHIS PPQ S&T Insect Management & Molecular Diagnostics Laboratory developed effective methods for producing, releasing, and monitoring these beneficial insects. Since 2010, open releases of *T. radiata* reared by the laboratory were made in urban settings of the Lower Rio Grande Valley of Texas where plant tissue testing positive for Huanglongbing (HLB) had been detected. Since biological control releases began, *T. radiata* releases have resulted in a 93% decrease in ACP nymph populations, with only 2.91 nymphs per flush detected in 2023, compared to 43.11 nymphs per flush in 2010.

Non-technical summary: The introduction of *Tamarixia radiata*, a parasitic wasp that exclusively preys on the Asian citrus psyllid (ACP), has helped decrease ACP nymph populations by 93% in the Lower Rio Grande Valley of Texas. Scientists at the USDA lab have developed successful methods for rearing and releasing these beneficial insects. Since their introduction in 2011, *T. radiata* releases have led to an impressive 93% reduction in ACP populations greatly benefitting the citrus industry in the region.

'Candidatus Liberibacter asiaticus' transmission competence and biology of *Diaphorina citri* modulated by insect specific virus

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Abstract: *Diaphorina citri* (Heiptera: Liviidae) is one of the most important citrus pests. *D. citri* is the vector of the phloem-limited '*Candidatus* Liberibacter asiaticus' (CLas), the causal agent of the devastating citrus disease, huanglongbing (HLB). Several insect-specific viruses have been identified in *D. citri*, including the Diaphorina citri flavi-like virus (DcFLV). The role of DcLFV in *D. citri* biology and in the transmission efficiency of *C*Las by *D. citri* are still unknown. In this study, we determined the time of oviposition, number of eggs laid for each female and the total eggs laid by DcFLV-negative and DcFLV-positive *D. citri* females of California population (CA-*D. citri*). We evaluated the development duration of eggs to nymphs and nymphs to adults of DcFLV-negative and DcFLV-positive *D. citri* populations. Moreover, we investigated the feeding behavior of DcFLV-negative and DcFLV-positive *D. citri* adults on *Citrus macrophylla* plants quantifying the honeydew drops size (cm^2)/*D. citri*. In addition, we used DcFLV-negative and DcFLV-positive *D. citri* populations to determine if the DcFLV infection affect the CLas transmission to *C. macrophylla* plants. Our results showed that DcFLV infection delayed the overall biological development of *D. citri*. However, DcFLV infection did not affect the viability of CA-*D. citri*. Additionally, DcFLV-negative CA-*D. citri* produced more honeydew than DcFLV-positive *D. citri*, suggesting the differences in feeding behavior. Furthermore, the results showed better efficiency of CLas transmission with the DcFLV-positive than with the DcFLV-negative CA-*D. citri.* Our study provides insight into how DcFLV affects *D. citri* biology and how DcFLV could modulate CLas transmission by *D. citri*. This provides new knowledge and insights for potential strategies for HLB management.

Non-technical summary: Our results showed that the insect-specific virus (ISV), Diaphorina citri flavi-like virus (DcFLV), which was originally found in China and Florida, can modulate the *D. citri* biology and '*Candidatus* Liberibacter asiaticus' (CLas) transmission competence. This highlights the importance of the ISVs identified in *D. citri* and the potential of using them as one of the strategies for HLB control.

Cell lines derived from Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Liviidae)

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Abstract: The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is a pest of significant importance to global citrus production, particularly as the vector of a phloem-limited bacterium '*Candidatus* Liberibacter asiaticus' (CLas) that causes the fatal citrus disease huanglongbing or citrus greening. CLas is acquired as the psyllid feeds, replicates in ACP tissues, and persists throughout the life of the insect. The study of CLas has been hampered by the lack of a tractable *in vitro* culture system. As CLas replicates within psyllid tissues, we hypothesize that this bacterium also replicates in cultured ACP cells. In the current study, eleven ACP cell lines were produced in an optimized cell culture medium. Ten of the cell lines are composed of adherent cells with diverse morphology. By September 2023, six promising lines had been passaged between 10 and 25 times. These ACP cell lines will be maintained until a continuous cell line is established and used to study CLas replication.

Non-technical summary: We have established 11 cell lines derived from the Asian citrus psyllid (ACP), the host insect of the bacterium that causes huanglongbing or citrus greening disease. These cell lines provide a tool to test for replication of '*Candidatus* Liberibacter asiaticus' in ACP cells, which may lead to a better treatment paradigm for the devastating citrus greening disease.

Citation: Wu, Ke, Grace J. Ortgiesen, Cynthia L. Goodman, and Bryony C. Bonning (2023). Optimized Conditions for the Long-Term Growth of Primary Cell Cultures Derived from the Asian Citrus Psyllid, Diaphorina Citri (Liviidae: Hemiptera). In Vitro Cellular & Developmental Biology. Animal 59 (4): 235–40. https://doi.org/10.1007/s11626-023-00765-3.

Citrus in the Home Landscape

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Abstract: In an effort to reduce the reservoir of regional pathogen (‘*Candidatus* liberibacter asiaticus’) and vector (*Diaphorina* citri) buildup, Florida residents have been discouraged from having citrus in their residences for nearly a decade. However, with the current endemic status of huanglongbing (HLB) in much of Florida’s citrus growing regions and the availability of both new management tools and HLB tolerant citrus varieties, it is time to bring citrus back to home landscapes. In partnership with the Florida Master Gardener Volunteers (MGV), we have been evaluating management tools that can be used to support healthy young citrus trees for residential landscapes. As part of this project, all MGV participants attend an annual training, a quarterly check-in, and submit data monthly. Tools under evaluation include exclusion netting over trees, reflective ground cover, red kaolin clay particle films, and pesticides labelled for use on citrus in home gardens. To date, exclusion netting is the only tool that has effectively prevented *D. citri* access to citrus trees. Based on MGV data collection, HLB signs were highest in pesticide treated and reflective ground cover treatments. And less prevalent, though still present in the red kaolin and exclusion netting trees. These results are part of an ongoing study, and more information can be found at homecitrus.ifas.ufl.edu.

Non-technical summary: Management tools are currently being evaluated for use in home settings. To date, exclusion netting is the most effective option to prevent Asian citrus psyllid from accessing trees, reducing the exposure of young trees to the pathogen that causes huanglongbing.

Collection of California-adapted *Tamarixia radiata* lines to support ACP biological control program.

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Abstract: The Asian citrus psyllid (ACP), *Diaphorina citri*, was first reported in California in 2008. Introduction of *Tamarixia radiata;* the most widely used ACP biological control agent was initiated in 2010. After careful host range testing, USDA granted permission to mass produce and release *T. radiata* in California in 2011. Parasitoid rearing under optimized environments can lead to loss of fitness characteristics such as host finding ability, and their ability to survive and disperse under natural environmental fluctuations due to domestication. To prevent domestication of mass reared wasps and preserve the genetic diversity present in the wild, Pakistani *T. radiata* were maintained as 16 inbred lines at the University of California Riverside. The inbred lines were allowed to crossbreed to produce a genetically diverse composite source material for mass production. More than 28 million mass produced *T. radiata* have been released in California. Subsequent surveys indicated that *T. radiata* was established throughout southern California ranging from coastal to desert environments. After over a decade in captivity, it was considered more appropriate to replace the Pakistani lines with locally adapted lines collected from coastal, inland, and desert regions within California, and use them as source material for mass production program. Mass produced parasitoids from locally adapted source materials can enhance effectiveness of the *T. radiata* being released in the state.

Parasitized ACP nymphs were collected from residential areas where *T. radiata* was reported to be established and reared in the laboratory. A single female wasp emerged from each collection was used to start a new isofemale line. All the Pakistan lines have been replaced by fifteen California isofemale lines. Mitochondrial DNA analysis showed that the newly established lines had a high degree of genetic diversity. Four of the lines came from coastal environments, nine from inland areas, and two from desert regions. During the past year, 13,049 wasps were produced from the isolines that was further multiplied to 147,307 wasps as source materials for mass production programs.

Non-technical abstract: *Tamarixia radiata* imported from Pakistan and released for biological control of Asian citrus psyllid (ACP) has successfully established throughout southern California. Locally adapted *T. radiata* have been collected from coastal, inland, and desert regions of California and have been introduced into the rearing systems to replace the original Pakistani lines. Rearing and release of these California adapted strains will enhance the effectiveness of the ACP biological control program. During the past year, 13,049 wasps were produced from the isolines that was further multiplied to 147,307 wasps as source materials for mass production programs.

Diaphorina citri and contribution of natural enemies to its biotic mortality in different high-density citrus plantings

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Abstract: Citrus production has decreased drastically (>70%) since the advent of huanglongbing (HLB) in Florida in 2005. There is no cure for the disease yet. Several strategies, including high-density plantings, are being tested to increase profit in the early years of tree production. There are several unknowns for this new concept, including a lack of knowledge to understand the response of arthropods to these increasing plant densities, particularly Asian citrus psyllid (ACP) *Diaphorina citri* vector of HLB. We conducted experiments to investigate the influence of different planting densities on the populations of ACP and the contribution of natural enemies to its biotic mortality in different planting densities. The experiments were conducted on four-year-old sweet orange (*Citrus sinensis)* trees budded on the 'US-897' (Cleopatra mandarin x Flying Dragon *trifoliate* orange) citrus rootstock. The experiments used six planting densities at 447, 512, 598, 717, 745, and 897 trees per hectare. There was no significant effect of planting density on the tree's potential to produce young shoots, which ACP requires to develop and reproduce. However, more shoots accumulated with increasing plant density per hectare. There were 54-56% more shoots in the 897 trees per ha density than 447 trees per ha between 2021 and 2022. The shoot infestation with ACP immatures increased by 6-9% with the increase in planting density from 447 trees per ha to 897 trees per ha. Ants, spiders, lacewings, and ladybeetles were common in all densities. Natural enemies showed significant potential in suppressing ACP in all planting densities with no consistent significant differences in incidence. Biological control reduced ACP in all densities, reduction averaging between 49% and 99%.

Non-technical summary: Asian citrus psyllid (ACP) was common in all densities, and there was a positive relationship between the increase in shoot density and ACP infestation rate. Major predatory groups of arthropods, particularly ladybeetles, lacewings, and spiders, including several species, significantly reduced ACP populations across all densities. To maximize the average net return, citrus production in the future will need to make more effective use of the limited land and offer a faster return on investment; therefore, strategies such as high-density plantings are needed.

Don't shoot the messenger, sterilize it! Can the sterile insect technique provide a pesticide-free alternative for controlling Asian citrus psyllid in California?

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Abstract: Huanglongbing (HLB) is vectored by the Asian citrus psyllid (ACP), *Diaphorina citri*, an invasive sap-sucking insect that is already established in parts of California. Area-wide application of insecticides is the current strategy for managing the threat of ACP establishing in commercial citrus orchards, but this goes against the State of California's commitment to transitioning away from pesticides toward safer, sustainable pest control practices. In and around densely populated urban areas, successful management of ACP has been achieved via a classical biological control program based on mass-rearing and releasing the imported parasitoid, *Tamarixia radiata*. To further strengthen the biological control effort, we are evaluating the sterile insect technique as a potential sustainable method for controlling ACP. Herein, we show that ACP adults can be collected from a mass-rearing system, stored, irradiated and released in small cage, greenhouse experiments to successfully suppress the growth of "wild" populations of ACP by around 75%. We have determined effective irradiation doses and release rates (overflooding ratios). The next, and on-going phase of the project seeks to extend our evaluation of SIT to a more challenging environment provided by mature citrus trees held within secure field-cages. We will present and discuss our findings from a first round of field-cage experiments (conducted Summer/Fall 2023).

Non-technical summary: We are evaluating the sterile insect technique (SIT) for controlling Asian citrus psyllid (ACP). SIT is a bio-rational approach in which large numbers of ACP would be reared, sterilized by exposure to X-ray radiation, and then released into the field where they would compete with wild ACP for matings. Through a series of experiments, conducted in a secure, field-like environment, we hope to show that wild ACP that mate with an SIT-ACP produce no offspring (or a greatly reduced number) and, in this way, the wild population may be eradicated.

Effect of Three Commercial Mycoinsecticides on Asian citrus psyllid Populations in the Lower Rio Grande Valley, Texas USA

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Abstract: The use of commercial mycoinsecticides to manage the Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) has gained increasing popularity as the demand for alternatives to chemical pesticides continues to grow. Laboratory bioassays conducted at the USDA APHIS Insect Management and Molecular Diagnostic Laboratory revealed that pure spore suspensions of the commercial *Cordyceps fumosorosea* (FE 9901) and *Beauveria bassiana* (ANT03) exhibited greater efficacy in causing mortality among adult ACP when compared to the standard, *Cordyceps javanica* (Apopka 97). Further assessments were conducted in a mesocosm setting, using the commercial formulations of these fungi, (Isarid, BioCeres, and PFR-97 respectively), under the conditions of the Lower Rio Grande Valley in South Texas. The primary objective of the study was to assess the efficacy of the respective commercial formulations using the upper label rate against adult ACP. In our investigation, individual branches of citrus trees in an organically managed grove were enclosed within mesh sleeve cages and infested with 50 unsexed, adult ACP. The spray was conducted in triplicates, with branches and trees being randomly selected for inoculation and infestation. Spore deposition per cm^2 was calculated for each spray and each mycoinsecticide. ACP were collected 4 days after treatment, surface sterilized, and plated on selective water agar. Efficacy of treatments was measured using percent mycosis. Our findings showed that when applied at the high-label rate in field conditions, BioCeres WP demonstrated a notably higher infectivity rate compared to other treatments. Future studies will focus on multiple rates of BioCeres WP to pinpoint the minimum efficacious concentration required for effective ACP control.

Non-technical summary: The use of commercial mycoinsecticides for managing the Asian citrus psyllid (ACP) has become increasingly popular as an alternative to chemical pesticides. Laboratory bioassays found that the pure spore suspensions of the commercial products Isarid and BioCeres WP had a higher mortality rate on adult ACP when compared to the standard, PFR-97, and were therefore selected for further investigation. In a semi-field setting, BioCeres WP demonstrated the highest efficacy in controlling ACP, offering a promising solution for ACP management in citrus groves.

Effectiveness of biological control of Asian citrus psyllid from commercial citrus

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Abstract: Biological control has always been an essential component of citrus insect pest management in Florida, including *Diphorina citri*, also known as Asian citrus psyllid (ACP). Predators already present in Florida responded immediately to the ACP invasion in 1998. Fifteen years ago, feral populations of natural enemies such as ladybeetles, lacewings, and spiders were observed to cause a significant reduction of 90% or more in ACP populations. However, since the discovery of huanglongbing disease in 2005, chemical control of ACP increased significantly. *Tamarixia radiata* introduced from Taiwan and South Vietnam established in the citrus groves and its augmentation through the release of mass-reared populations from multiple countries including Pakistan, China, and Vietnam contribute to ACP mortality. In 2015-2016, releases of *Tamarixia radiata* in commercial citrus were observed to cause more mortality of ACP in the citrus blocks under organic pest management and untreated blocks compared to the blocks under conventional pest management. Recent studies from 2020-2022, evaluated the status of biological control of ACP in commercial citrus. The exclusion techniques were employed by protecting the developing colonies of ACP immatures from natural enemies against the colonies that remained exposed to measure the influence of biotic mortality in psyllid populations. Cohorts of protected and exposed colonies of ACP were evaluated in five commercial citrus groves. A reduction of 50-90% in nymphal colonies exposed to natural enemies was observed. Ladybeetles (*Curinus coeruleus*, *Harmonia axyridis*, *Olla v-nigrum*, *Cycloneda sanguinea*), lacewings, and spiders were common predators observed in the citrus groves. These findings suggest that there is still a significant role of biotic mortality in regulating the populations of ACP and contributing to integrated pest management. However, populations of natural enemies such as ladybeetles and lacewings were significantly reduced due to the high use of conventional insecticides for several years.

Non-technical summary: The natural enemies including predators such as ladybeetles, lacewings, and spiders as well as the release of parasitoid *Tamarixia radiata* provide significant reduction in Asian citrus psyllid populations in commercial citrus.

Impacts of area-wide treatments of ACP in urban citrus next to commercial citrus in Hemet CA

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Abstract: The purpose of this project was to evaluate the use of Area-Wide Integrated Pest Management (AW-IPM) for Asian citrus psyllid (ACP) control in urban citrus next to a commercial citrus production area. We evaluated the use of multiple control tools in an area-wide effort to assess if this control strategy could be effective to reduce the risk of ACP in urban citrus areas. Successful results of this demonstration project could help set the basis for a future buffer treatment program in high ACP- and high huanglongbing-risk areas adjacent to commercial citrus and may allow the replacement of insecticide treatments by other AW-IPM tactics in the event these treatments become unsustainable.

The specific objectives were to:

1) Evaluate the viability of using augmentative releases of natural enemies for ACP population control in urban citrus buffer zones next to commercial citrus.

2) Evaluate if sugar feeding ant control in urban citrus, where *Tamarixia* and other natural enemies are already present, can achieve the same or better results than augmentative natural enemy releases.

The area-wide insecticide buffer treatment rate ranged from 72-75% during 2019-2021 and there was a sharp decrease in ACP populations in inside pesticide buffer treatment compared to outside the buffer untreated areas of 90-99% demonstrating the effectiveness of these treatments. Adult ACP levels were lower in both the ant and biocontrol treatment areas compared to the control areas and were similar. There may be value in considering area-wide ant control as a viable ACP control tactic in areas where ACP natural enemies are present at likely lower cost than ongoing natural enemy releases.

Non-technical summary:

The results of this two-year project show that an Area-Wide Integrated Pest Management (AW-IPM) program of ant control, coordinated pesticide applications, and biocontrol releases reduced ACP populations in the urban buffer next to commercial citrus production areas ranging from 72-99% relative to adjacent no-treatment control areas over the course of the project. Area-wide ant control was as effective in reducing Asian citrus psyllid (ACP) populations as continued natural enemy releases and could be considered as an alternative to ongoing biocontrol agent releases to control ACP in urban citrus buffer areas next to commercial citrus.

Performance of metallic blue predatory ladybeetle *Curinus coeruleus* on Asian citrus psyllid *Diaphorina citri*

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Abstract: Citrus crops are threatened by several pests, affecting tree health and the economics of a successful production system. The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama, is a serious citrus pest and a vector of huanglongbing (HLB) disease. The metallic blue ladybeetle *Curinus coeruleus* is an important predator of several citrus pests including ACP. Temperature mediates trophic interactions, including the relationship between pests and predators. Functional response studies are important in determining the suitability of predators as biocontrol agents. We investigated the predation capacity and functional response of *C. coeruleus* larvae and adult foraging on *D. citri* nymphs at 28, 31, and 33°C. The predation capacity of 1, 2, 3, 4, and 5 *C. coeruleus* adults against 100 nymphs of *D. citri* was evaluated in plastic petri dishes. We evaluated densities of 1, 2, 4, 8, 16, 32 and 64 *D. citri* nymphs from young and mature instars against male and female adult *C. coeruleus*. The number of nymphs consumed by *C. coeruleus* was determined after 24 h. *Curinus coeruleus* predation effectiveness decreased in the presence of competition. Our finding demonstrated that the *C. coeruleus* predation rate declined with the increased density of *D. citri*. The predation rate of females was higher than males and with elevated temperatures. The implication of these results for biological control will be discussed.

Non-technical summary: The metallic blue predatory ladybeetle is one of the species common in citrus groves even during hot summers when populations of other ladybeetles are low. Our research has shown that it is a good predator of Asian citrus psyllid (ACP) even with increasing temperatures that we tested. It also targets several other pests, particularly scales. We have already demonstrated its effectiveness against the Florida red scale. Therefore, conserving and augmenting populations of this species will be useful for areawide control of multiple pests of citrus particularly ACP and its vectored huanglongbing for improving citrus production under changing climate conditions.

Proper identification of psylloids: an important consideration in managing the threat of HLB and its vector

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Abstract: In South Africa, the indigenous *Trioza erytreae* (Del Guercio) (Hemiptera: Triozidae) is an important vector of citrus greening disease, *Candidatus* Liberibacter africanus. Currently, South Africa is faced with the threat of *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) which vectors Asian citrus greening or huanglongbing (HLB) following its establishment in East and West Africa. In South Africa, surveys for early detection of *D. citri* using mostly yellow sticky traps have been rolled out across the country. Higher surveillance efforts of the vector are implemented in areas bordering other southern African countries. The proper identification of psylloids in the early detection surveys is key to managing the threat of *D. citri* as well as HLB. In this study, we aimed at developing tools to correctly identify psylloid specimens collected in citrus environments in South Africa. The tools developed included both morphological and molecular methods. Psylloid specimens were collected using yellow sticky traps and sweep nets in and near citrus orchards in the north-eastern parts of South Africa. Sixteen triozid spp. and 18 *Diaphorina* spp. were collected in total and were identified using published and unpublished morphological dichotomous keys. DNA barcodes were generated for each specimen. Specimens of *T. erytreae* from different populations in South Africa were morphologically similar but differed in their nucleotide sequences in the cytochrome c oxidase subunit 1 mitochondrial gene. The *Trioza erytreae* populations in South Africa could be broadly grouped into five genetic clusters. Morphological identifications revealed subtle differences between *D. citri* and some indigenous *Diaphorina* species caught in citrus environments, but molecular techniques used could also clearly differentiate them. In addition, we highlight the need for host plant identification associated with psylloids in citrus environments.

Non-technical summary: Psylloids or plant jumping lice are a group of insects that contain two of the most devastating insect species causing substantial damage to crops. Proper identification of insect-carrying diseases is an important component in the management of diseases in citrus orchards and can help improve monitoring techniques to combat citrus greening.

Updates on classical biological control of Asian citrus psyllid in Arizona

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Abstract: The parasitoid *Tamarixia radiata* has been released in San Luis, Wellton, Ajo, and Nogales Arizona since 2019 in an effort to establish classical biological control of Asian citrus psyllid (ACP) (*Diaphorina citri*) in residential citrus and decrease risk of huanglongbing spread should the disease be introduced to Arizona. Each region receives 2000 *T. radiata*, shipped from the Citrus Research Board rearing system in California, once every four weeks, and agents are released at residential properties with one or more citrus trees. Monitoring of ACP density and *T. radiata* activity is conducted though visual inspection, tap sampling, yellow panel traps, and flush shoot sampling at separate monitoring sites at least 0.5mi from the nearest release sites. ACP life stages were mainly detectable only in spring in San Luis and Wellton but were found at varying densities year-round in Ajo and Nogales, which have milder summers. Adult *T. radiata* have been captured on panel traps at four monitoring sites in Ajo since 2021. Immature *T. radiata* were found parasitizing ACP nymphs on flush shoots sampled at a monitoring site in Nogales in 2022, and at two monitoring sites in Wellton and two in San Luis sampled in 2023. Gene sequencing of specimens collected from Ajo and Nogales confirmed that *T. radiata* in these samples belonged to haplotypes found among the colonies propagated in California. This suggests that *T. radiata* releases have led to establishment in these regions.

Non-technical summary: The wasp *Tamarixia radiata*, a natural enemy of Asian citrus psyllid (ACP), was released in four regions of Arizona to control ACP in dooryard citrus trees. Its presence at sites where it was not released suggest that is has successfully become established in these regions.

Utilization of ACP detector canine in the desert and coastal regions of California and Arizona improves HLB vector population monitoring and local eradication efforts

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Abstract:
Successful management of Asian citrus psyllid (ACP) populations requires information about the incidence and abundance of the vector. Strategies for keeping areas of citrus production free from '*Candidatus* Liberibacter asiaticus' (CLas) infections emphasize aggressive, coordinated insecticide applications to suppress ACP populations, or achieve local eradication. Finding ACP colonies on mature citrus trees is a difficult and time-consuming task for human scouts. The practical difficulty of the task often limits the number of trees which can be assessed and the time that can be spent on each tree, when resources are limited. Canine detection of a range of biological and non-biological targets, by scent, is now considered routine in the biosecurity domain. Given the possible much higher detection sensitivity of canines over human scouts, the use of canine surveillance for ACP in citrus has been investigated, particularly in connection with detection of ACP at low population densities and in evaluating the efficacy of ACP treatment programs. Canines have been trained successfully to detect the presence of ACP colonies in citrus trees, and extensive evaluations of their performance in coastal and desert regions of southern California and in Arizona have been conducted. Comparative cost analysis with federally funded canine detection programs showed that the cost of canine teams for ACP detection is comparable. Comparative observational studies involving canine teams and trained scouts from a Pest Control Advisor service, in which the canine teams and PCA scouts followed their own standard practices, showed that the canines scouted 4.5 times as many trees and located 11 times as many infested trees as the human scouts. At one location the increase in detection of ACP from use of canine scouting resulted in the owner switching to a more aggressive pesticide program that was correlated with a subsequent reduction in ACP numbers.

Non-technical summary:
Asian citrus psyllid detection canines are able to scout 4.5 times as many trees as trained scouts from pest control advisor services and locate 11 times more infested trees when population densities are low. Extensive evaluations of their performance and usefulness in coastal and desert regions of southern California and in Arizona have resulted in increased vector population monitoring and the identification of previously unknown breeding populations which assisted in localized eradication efforts.

Analysis of R-genes in Australian Limes in Comparison to Commercial Citrus Cultivars

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Abstract: Huanglongbing (HLB) is a devastating threat to the citrus industry, due to a lack of effective control measures and a lack of HLB resistant cultivars. Several species of Australian native limes have been recognized to have remarkable tolerance or resistance to HLB and are thus hypothesized to harbor unique genes that confer resistance against HLB. Therefore, identifying resistance (R) genes, which confer pathogen-specific responses, would help us better understand HLB pathogenesis and facilitate breeding for resistance in citrus. In this study, we identified putative R-genes in three Australian native limes (*Citrus australasica*, *C. inodora* and *C. glauca*) in comparison to two commercial cultivars *C. clementina* and *C. sinensis*. Our results showed there are 300-600 R-genes in the Australian limes, among which about half have either a Coiled Coil (CC) domain or a Toll-Interleukin receptor (TIR) domain. Despite their distinct evolutionary history, the total number and composition of R-genes in Australian limes are largely similar to those in *C. clementina* and *C. sinensis*. Phylogenetic analysis indicates R-genes from all the five genomes are largely grouped together according to R-gene types. R-gene clusters occurred in many of the same chromosomal locations, however, important differences were identified that indicate the potential presence of unique R-gene clusters in Australian native limes. Analyses in motifs, mutations and synteny revealed several distinct evolutionary patterns in Australian native limes, relative to their cultivated counterparts. This study shed light into the structure and organization of R-genes in Australian native limes and will thus potentially assist development of resistant varieties.

Non-technical summary: Plant genes designated as ‘R’ genes are generally associated with disease resistance. We have identified different groups of R genes in Australian wild limes. This study will be useful in understanding resistance and development of huanglongbing resistant citrus cultivars.

Analyzing Huanglongbing-Resistant Pummelo from India

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Abstract: There is no appreciable resistance to huanglongbing (HLB) disease reported in cultivated citrus. We have identified a pummelo variety resistant to citrus HLB from the Gonikopal area in the Coorg district of Southern India. HLB has devastated the once thriving Coorg mandarin industry in this area where the disease has been present for nearly a hundred years. While most citrus types were affected by HLB in the area, some native pummelos showed no HLB symptoms and continued to grow well and produce healthy trees in spite of both the disease and the vector being present in the area. Real-time PCR analysis conducted in 2013 showed the absence of the HLB-associated bacterium, '*Candidatus* Liberibacter asiaticus' (CLas) in the tested pummelo tree, but the presence of the pathogen in the surrounding Coorg mandarin trees. A clone of this pummelo, "Cariappa" was imported at the USDA Repository under valid permits in 2013 and maintained under quarantine. After therapy followed by testing the plant material for the absence of multiple citrus pathogens, the accession was released from quarantine in 2019. In general, most pummelo varieties are highly susceptible to HLB. We have inoculated the putative HLB-resistant "Cariappa" and a susceptible Siamese pummelo variety with two isolates of CLas, "Hacienda Heights" (HH) from California and "Psy62" from Florida in contained research facilities in Riverside. Tissue from these plants was subjected to transcriptome analysis. The symptomatology of inoculated plants and analysis of transcriptome studies will be discussed. Possible uses of "Cariappa" pummelo for breeding novel HLB-resistant varieties will be addressed.

Non-technical summary: Naturally occurring trees resistant to citrus huanglongbing (HLB) will provide clues for understanding the mechanism of resistance to the disease. We are studying gene expression in an HLB-tolerant/resistant pummelo variety from India. The results will be useful for the identification of the candidate resistance genes; the information will be useful in developing solutions for HLB.

Assessing the Effect of Propidium Monoazide on Sequencing the Viable Endophytic Microbiome in Huanglongbing-affected Citrus

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Abstract: Citrus huanglongbing (HLB) is one of the most destructive diseases for the citrus industry worldwide. It is associated with the unculturable and phloem-limited bacteria '*Candidatus* Liberibacter asiaticus' (CLas). Several studies suggest that the Citrus endophytic microbiome plays a role in HLB progression. Metagenomic analysis is widely used for deciphering citrus endophytic microbiomes. However, this approach can not differentiate DNA from dead or live bacterial cells. Propidium monoazide (PMA) treatment prior to DNA extraction has been effective at excluding DNA from dead CLas cells, but there are no reports about using this technique for deciphering the viable microbiome in HLB-affected citrus trees. In this study, 16S rRNA amplicon sequencing was applied in conjunction with PMA (PMA-seq) to assess the effect of PMA on microbial communities in HLB-affected citrus trees after treatment with the broad-spectrum antibiotic Oxytetracycline (OTC). The results indicated that PMA had no significant effect on the number of OTUs, Shannon index, and Simpson index, although more OTUs were found under PMA treatment. It also found that only the relative abundance of the phylum *Proteobacteria* was reduced by PMA treatment. In contrast, the relative abundance of the other phyla, such as *Acidobacteriota*, *Actinobacteria*, *Actinobacteriota*, *Bacteroidota*, *Desulfobacterota*, and *Firmicutes*, were enhanced by PMA treatment. In this work, 146 bacterial species were identified, and only the relative abundance of 21 in 146 species, including CLas, was significantly lower in PMA-treatment sample portions. In addition, the relative abundance of CLas was reduced by OTC in both PMA and without PMA treatment, similar to the quantitative-PCR result. Although PMA-seq can detect viable and non-viable microbes (such as CLas), it cannot accurately quantify viable taxa in a complex community. Our findings suggest that the implementation of PMA-seq may result in skewed bacterial community analyses.

Non-technical summary: The meta-genomic approach is useful for understanding microbes associated with '*Candidatus* Liberibacter asiaticus' (CLas) in citrus, which is beneficial for developing eco-friendly strategies against huanglongbing (HLB). Due to meta-genomic is a DNA-based method, this approach can not differentiate DNA from dead or live bacterial cells, which can disrupt the Meta-genomic data. In this work, the effect of the Meta-genomic approach combined with PMA treatment on the viable microbiome in HLB-affected citrus was assessed, it found that PMA-seq can detect viable and non-viable CLas, but cannot accurately quantify viable taxa in a complex community.

CRISPR-Cas12a RNP-mediated editing of RBOHD in navel oranges

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Abstract: Citrus huanglongbing (HLB) is a devastating disease caused by the phloem-limited bacterium '*Candidatus* Liberibacter asiaticus' (CLas). HLB-infected citrus plants exhibit excessive reactive oxygen species (ROS) production that causes oxidative stress and tissue damage. ROS production is mediated by the NADPH oxidase Respiratory Burst Oxidase Homologue D (RBOHD), which is regulated by phosphorylation and ubiquitination. In this study, we aimed to generate non-transgenic navel orange varieties with reduced RBOHD activity and ROS levels using the CRISPR-Cas12a ribonucleoprotein (RNP) complex for genome editing. We prepared embryogenic citrus cell suspensions from callus cultures and isolated protoplasts by enzymatic digestion. We are working to deliver the RNP complex, consisting of LbCas12a protein and a crRNA targeting the RBOHD gene, into the protoplasts by polyethylene glycol (PEG) transformation. In addition to confirming the editing of RBOHD, we will measure levels of ROS in edited protoplasts compared to wild-type controls. We expect the RNP-mediated editing of RBOHD will reduce ROS production in the protoplasts, suggesting a potential strategy for reducing oxidative stress in HLB-infected citrus plants.

Non-technical summary:
We are working to develop non-transgenic navel orange varieties that have lower levels of oxidative damage in response to infection with '*Candidatus* Liberibacter asiaticus' and, potentially, enhanced tolerance to huanglongbing disease.

Diffusible Signal Factor (DSF) acts as an elicitor in citrus defense response and increases tolerance to Huanglongbing

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Abstract: The diffusible signal factor (DSF) represents a well-known class of quorum sensing (QS) molecules produced by diverse Gram-negative bacteria, mediating intraspecies, interspecies, and inter-kingdom communication. In this study, we developed two commercial varieties of sweet oranges, Hamlin and Pineapple, overexpressing the *rpfF* gene responsible for DSF synthesis. These plants have previously demonstrated tolerance to Citrus Canker and Citrus Variegated Chlorosis, which can be attributed to the 'pathogen confusion' caused by the overproduction of DSF, disrupting bacterial communication. Since 2019, a field trial was conducted, involving 10 clones of GM (genetically modified) plants for each variety, along with their respective controls, consisting of 10 clones of wild-type (WT) for each variety. The field trial is in a region of the São Paulo State, Brazil, characterized by the highest incidence and severity of huanglongbing (HLB). Analysis of HLB incidence and severity in GM and WT plants were conducted at three to six-month intervals for three years. To analyze disease progression over time, symptom severity data were employed to calculate the area under the disease progress curve (AUDPC). GM clones exhibited significantly lower AUDPC compared with WT, indicating a reduction in disease evolution over the time. These results suggest, as recently verified in other crops, that DSF may act as an elicitor, activating plant immune response. This hypothesis was reinforced by the hypersensitivity reaction observed in *Nicotiana benthamiana* infiltrated with extract from transgenic plants, but no reaction with WT ones. Our findings advanced the understanding of DSF signal-mediated communication and provide a new insight for the citrus bacterial diseases control.

Non-technical summary:

This work aims to use a molecule produced by some bacteria and reproduce it in citrus plants and promote tolerance/resistance against the main diseases. This molecule has shown promising results in controlling three citrus diseases, with emphasis on huanglongbing (HLB). Therefore, we hope that the use of this molecule, regardless of the production/ application method, will be an alternative for the management of HLB, thus helping the entire citrus supply chain.

Effects of HLB on citrandarin H-222 as a rootstock for 'Valencia' canopy

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Abstract: Information regarding the use of citrandarins (*Poncirus trifoliata* x *Citrus sunki*) as rootstock for citrus canopy under '*Candidatus* Liberibacter asiaticus' (CLas) infection is limited, although their tolerance as canopy is well-established. In this study, we investigate the impact of CLas inoculation on 'Valencia' citrus canopy and its effects on the citrandarin H-222 used as rootstock, as well as interstockes between Swingle citrumelo rootstock and 'Valencia' canopy. Additionally, a treatment with Swingle rootstock was considered. After 360 days post-inoculation, the cycle threshold values (CT) for CLas in the canopy samples ranged from 15.89 to 22.72 (indicating a high CLas titer), with no statistically significant difference observed regardless of the rootstock variety or interstock used. However, CLas positive sample was detected in only one of tested repetition in the root system of H-222 during this period (CT average 37.92), while all samples from Swingle's root system tested positive for CLas (CT ranging from 27.88 to 31.33). Despite the absence of CLas detection in H-222, there was a statistically significant reduction in the development of root system (both volume and mass) compared to treatments with uninfected 'Valencia' canopies. This reduction was stronger in the Swingle root system, suggesting that canopy infection leads to root damage, even in the absence of CLas in the root system. However, it's worth noting that the damage to the root system of H-222 was significantly less severe compared to that observed in the Swingle rootstock. Through multivariate hierarchical analysis (using Ward's statistic), which considered variables such as stem diameter, root volume and mass, and CLas infection rate, H-222 as a rootstock for CLas-infected 'Valencia' was clustered together with the uninoculated plants. Consequently, based on the variables analyzed, it can be concluded that the H-222 rootstock was less affected by the CLas-infected scion compared to the Swingle rootstock.

Non-technical summary: The aim of this work was to investigate the effect of bacterium '*Candidatus* Liberibacter asiaticus' (CLas) when present the sweet orange 'Valencia' canopy graphed on the rootstocks citrandarin H-222 (previously selected for its tolerance to CLas) and Swingle citrumelo. The root system of H-222 compared to Swingle citrumelo, as determined by root volume and mass, exhibited significantly lower susceptibility to the presence of CLas in the canopy ($P<0.05$), additionally, multiplication of CLas was weakly observed after 360 days of experimental evaluation in only one of repetition of the H-222 rootstock. We believed that the tolerance of citrus rootstock to CLas and the associated symptoms represents a crucial piece of the puzzle in the managing of huanglongbing.

Effect of rootstocks on fruit quality, yield, incidence, and severity of infection of '*Ca.* L. asiaticus' (CLas) in scion varieties of sweet oranges

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Abstract: Huanglongbing (HLB), also known as citrus greening disease, is the most destructive citrus disease worldwide. The causative bacterial species in the state of São Paulo, Brazil, is '*Candidatus* Liberibacter asiaticus'. Preliminary studies demonstrate that some citrandarins, which are hybrids of Sunki mandarin and *Poncirus trifoliata*, are tolerant to HLB under field conditions. Thus, we believe that scion varieties grafted onto citrandarins may exhibit some tolerance as well. This work aimed to evaluate the behavior of two scion varieties ('Pera' and 'Valencia' sweet oranges) grafted onto six different rootstocks [citrandarins (7, 70 and 299), Rangpur lime (*Citrus limonia*), Swingle citrumelo (*C. paradisi* x *P. trifoliata*) and *P. trifoliata* cv. Rubidoux] in the face of natural HLB infection under field conditions. The experiment was implemented in 2017, in a randomized block design, with six replications. The evaluations carried out were incidence and severity of HLB, height, diameter, canopy volume, yield, and fruit quality. The incidence of HLB in all treatments was 100%, but severity differed between treatments. Scion varieties grafted onto Rangpur lime showed the greatest severity compared to the other rootstocks; it was not possible to observe differences in plant development between treatments; and the yield and yield efficiency of 'Rubidoux' and citrandarins 7 and 70 were highlighted when the scion variety was the 'Valencia' sweet orange.

Non-technical summary: The aim of this work was to investigate the behavior of two scion varieties ('Pera' and 'Valencia' sweet oranges), grafted onto six different rootstocks in the face of natural huanglongbing infection under field conditions. We conclude that rootstocks 'Rubidoux' and citrandarins 7 and 70 proved to be more tolerant than Rangpur lime, which is reflected in higher yield, especially with 'Valencia' sweet orange as scion.

Endophytic microbiome in healthy and Huanglongbing-infected citrus plants cultivated in the Central Highlands, Vietnam

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Abstract: Huanglongbing (HLB) is one of the worst diseases of citrus trees in the world. It is caused by bacterial species in the genus Candidatus *Liberibacter,* which spreads through the tree canopy, causing decline and then death of the tree. The only way to manage citrus HLB is to control insect vector population. HLB is also one of the most serious infections in citrus grown in Vietnam. In this initial work, we report the change in endophytic microbiome in healthy and HLB-infected citrus cultivated in the Central Highlands of Vietnam. Root samples of healthy and HLB-infected citrus grown in Buon Don district, Dak Lak Province, were collected. The genomic DNA of endophytic microorganisms was isolated using the DNeasy PowerSoil Pro kit (Qiagen, Germany). Shotgun metagenomic libraries were prepared using the NEBNext dsDNA Fragmentase, NEBNext Ultra II DNA Library Prep Kit (NEB, USA). Prepared libraries were sequenced using the Illumina MiSeq platform (2 × 150 PE). Bcl2fastq was used to demultiplex raw basecall sequences. Raw basecall sequences were demultiplexed using Bcl2fastq. Adapters, primers, and low-quality sequences were removed using the Trimmomatic 0.39 and Cutadapt 2.10. Clustering and dereplication of reads into amplicon sequence variants were carried out using the q2-dada2 plugin and QIIME2 pipeline 2020.8. Taxonomic analysis of the endophytic microbiome was performed using QIIME2, which was aligned with the SILVA SSURef reference database. The functional profile of endophytic microorganisms was predicted using the PICRUSt2 2.3.0-b and MetaCyc databases. The obtained data will provide an understanding and comparison framework of the diversity and functionality of endophytic microbiome in healthy and HLB-infected citrus for further studies concerning HLB disease management.

Non-Technical Summary: Several endophytic bacteria are beneficial to their host by enhancing plant growth while helping to defend against pathogens. By comparing the endophytic microbiome of healthy citrus plants with those infected by huanglongbing disease, we hope to identify any variations in bacterial diversity and functionality. Understanding these differences could provide valuable insights into the mechanisms by which endophytic bacteria contribute to plant health and disease resistance.

Evaluation of Microcitrus-Derived Hybrids for ACP Colonization and HLB Resistance in Florida

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Abstract: At the Queensland Department of Agriculture and Fisheries, the breeding program used native Australian citrus species (*C. australasica*, *C. australis*, *C. garrawayi*, and *C. inodora*), collectively referred to as Microcitrus, and cultivated *Citrus*. Commencing in 1998, this program aimed to introduce desirable Microcitrus traits into cultivated citrus, resulting in hybrids with pedigrees ranging from 12.5% to 100% Microcitrus. Recent findings suggest that specific Microcitrus accessions may exhibit resistance to huanglongbing (HLB), a disease associated with '*Candidatus* Liberibacter asiaticus' (CLas). To assess this resistance, we conducted tests on these Microcitrus-derived seedlings in a Florida field site where HLB and its vector, the Asian Citrus Psyllid (*Diaphorina citri*; ACP), are prevalent. Seeds were received in August 2015 and grown into field-ready seedling trees at the USHRL greenhouse. In August 2018, these trees were planted in a randomized layout at the Fort Pierce USDA grove, comprising 53 Microcitrus-derived populations alongside grafted Clementine and Hamlin standards. During 2020 and 2021, a subset of these plants underwent 24 assessments for ACP colonization, focusing on periods of abundant citrus flush and active *D. citri* presence. Data collected included observations of eggs, nymphs, and 5th instars, indicating *D. citri*'s ability to complete development on the plant. Statistical analysis assessed variations in egg and nymph abundances concerning scion and date, revealing differing nymph counts across dates and scion types, with Hamlin and Arrufatina X 09Q048 displaying the highest nymph counts. Additionally, all trees were evaluated for CLas levels in October 2021. The study encompassed seedling development, field planting, and standard citrus production practices, with minimal pesticide usage to determine the tolerance of Australian-derived hybrids to HLB.

Non-technical summary: Researchers in Ft. Pierce, Florida evaluated Microcitrus-derived hybrids that were created in in Queensland, Australia for tolerance to huanglongbing. These hybrids varied in performance in the 2020 and 2021 seasons and many were not as tolerant as native Australian citrus species.

Genetic engineering strategies for HLB resistance/tolerance based on overexpression and gene silencing

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Abstract: Biotechnological strategies are essential for the sustainable management of plant diseases. In recent years, genetic engineering approaches encompassing transgenesis and genome editing based on CRISPR/Cas systems showed promising results for disease management in citriculture. However, despite the existence of modern molecular tools, there are still no huanglongbing (HLB)-resistant sweet orange varieties developed. Therefore, this study aims to obtain genetically modified plants using two strategies: overexpression of a *Poncirus trifoliata* endochitinase B (PtChiB) through classical transgenesis and silencing of the *CsSEOc* host sensitivity gene through CRISPR technology. Previous studies performed by our group have demonstrated that these genes are associated with resistance and plant response/symptoms to HLB, respectively. Hence, for classical transgenesis, we have designed three expression vectors for overexpression of *PtChiB* genes, two of them harboring a phloem-specific promoter (RTBV) and one harboring a constitutive promoter (FMV). We have also designed two expression vectors for genome editing based on the pDIRECT_23A plasmid backbone for the overexpression of SpCas9 (pCsCas9U6) and eErCas12a (pCsMAD7U6) – the engineered and royalty-free MAD7 nuclease – and their optimized sgRNAs, both for targeting *CsSEOc* gene. Epicotyls from Hamlin and Valencia varieties and/or from Carrizo citrange were subjected to *Agrobacterium*-mediated transformation with these gene constructs. PCR-positive shoots were obtained and grafted/micrografted onto Rangpur lime or Carrizo citrange rootstocks. We have successfully regenerated transformed shoots for both FMV:ChiB and RTBV:ChiB constructs with 0.5% and 3.0% of transformation efficiency, respectively. pCsCas9U6 and pCsMAD7U6 vectors yielded transformed shoots for *CsSEOc* targeting with up to 1.7% and 4.9% of transformation efficiency. Transgenic seedlings are being screened for gene editing and triggered indels will be characterized through sequencing. Thus, we expect to demonstrate the usefulness of our molecular strategies so that we successfully regenerate at least one transformed and/or gene-edited citrus variety for each construct, concurrently displaying HLB resistance/tolerance.

Non-technical summary: This work aims to address the high impact of huanglongbing (HLB) in citrus industry worldwide by using modern tools of biotechnology, filling gaps concerning the absence and urgent need for HLB-resistant/tolerant varieties of sweet orange. We expect that the results provided by our research could greatly aid growers to deal with the most devastating citrus disease and counterbalance huge losses existing in economy and orange juice supply to consumers.

Informing gene discovery efforts through dissection of gene-regulatory architecture of pathogen susceptibility

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Abstract: Genetic improvement of citrus cultivars is a promising, long-term solution for protecting the citrus industry from the devastating effects of huanglongbing (HLB) disease. Genetic improvement of citrus rootstocks is of particular interest since rootstocks have been shown to impart tolerance or resistance to their grafted scion under infection by a range of diseases, including HLB. Here, we study the transcriptional response to HLB infection using a parent-offspring trio of rootstocks to understand how hybridization of citrus and its wild relative *Poncirus trifoliata* may produce novel patterns of gene expression in an HLB-tolerant rootstock hybrid. To accomplish this, we germinated seeds of 'Cleopatra' mandarin (*C. reticulata*), 'Flying Dragon' (*P. trifoliata*), and their HLB-tolerant, F1 hybrid 'US-897'. Seedlings were genotyped using competitive allele-specific PCR markers (KASP) to ensure they were derived from nucellar embryos. These true-to type plants were then caged with 20-25 Asian Citrus Psyllids (*Diaphorina citri*) that were exposed to '*Candidatus* Liberibacter asiaticus' (CLas), the presumed causal agent of HLB. One month after psyllid exposure, plants were tested for levels of CLas in leaf tissue using qPCR. The plants were then pruned to initiate new branches, and sampled at 18, 21, and 25 days post bud-initiation for construction of RNA-seq libraries. Future work will involve the comparison of parental and offspring transcriptomes to understand how hybridization produces novel patterns of gene expression.

Non-technical summary: In this study we infected a commonly used rootstock hybrid 'US-897' and its parents with the bacteria that causes huanglongbing (HLB). By comparing the response to pathogen infection between parents and their offspring, we can identify genes that may be important for improving tree performance during HLB infection.

Initial Host Response of Sensitive and Tolerant Citrus Varieties to '*Candidatus* Liberibacter asiaticus' (CLas) Transmitted by Asian Citrus Psyllid (ACP)

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Abstract: The citrus industry is one of the economic backbones in the State of Florida. However, citrus huanglongbing (HLB, or citrus greening disease) has caused over 85% loss in citrus production since it was first reported in Florida in 2005. Previous studies demonstrated immediate transcriptomic-level changes in response to feeding by *'Candidatus* Liberibacter asiaticus' (CLas)-free healthy Asian citrus psyllid (ACP), but responses were delayed against CLas-carrying infective ACP in HLB-sensitive Valencia sweet orange (*Citrus sinensis*; VAL) during a 10-day-post-inculation (dpi) period. In the current study, similar transcriptomic profiling was advanced to a 2-dpi window, specifically at 2-, 12-, 24- and 48-hour-post-inoculation (hpi), between two differently HLB-responding citrus varieties: HLB-sensitive Valencia sweet orange (VAL) and HLB-tolerant LB8-9 Sugar Belle® mandarin (*C. reticulata*; SB). The results revealed distinct patterns of differentially expressed genes (DEGs) in VAL and SB in response to CLas: DEGs of SB indicated a gradual and stable increase throughout the 2-dpi period. By contrast, DEGs of VAL are characterized by a minor fluctuation from 2-hpi to 12-hpi followed by a major burst from 24-hpi to 48-hpi. In response to CLas-inoculation, VAL showed a burst of DEGs at 48-hpi, whereas DEGs in SB increased significantly at an earlier 24-hpi. On a transcriptomic level, a delayed response to CLas-inoculation was observed in VAL compared with SB. More down-regulated DEGs were differentiated in VAL than in SB, and the initial responsive DEGs in SB were related to plant response to stimulus, catalytic activity, and transferase activity.

Non-technical summary: This transcriptomic study aimed at investigating the complete set of RNA transcripts produced by the corresponding genome and identifying differentially expressed genes in response to very early stages of '*Candidatus* Liberibacter asiaticus' infection. It captured as many DEGs as possible and facilitated understanding the biological pathways that lead to different huanglongbing-tolerance levels in different citrus varieties.

Members of Sieve Element Occlusion gene family can interact with a complex genetic network on HLB-infected *Citrus sinensis* plants

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Abstract: The Sieve Element Occlusion (SEO) gene family encodes a structural protein (P-protein) in phloem vessels. After injuries, this protein is translocated to sieve plates and stops the sieve flow as a defense mechanism. Previous studies by our group have demonstrated an up-regulation of several genes that may be associated with huanglongbing (HLB) symptoms, including the *SEOc* gene. However, molecular features of these genes in *Citrus sinensis* are still unknown, as well as how they can be regulated or interact within the pathosystem. Hence, our goal is to characterize the *CsSEOc* gene and its encoded protein, including inferences of a possible genetic network based on databases for protein-protein interaction (PPI). MBS and ABRE are among the cis-regulatory elements found on PlantCARE, being responsive to gibberellic acid and abscisic acid, respectively. Two functional domains typical of the SEO family were found on SMART and NCBI CDD: an N-terminal and a C-terminal domain. The predicted protein on ProtParam has a molecular weight of 57.15 kDa, theoretical isoelectric point of 5.57, instability index of 50.43 (unstable), GRAVY value of -0.185 (hydrophilic) and location in the cytoplasm (WoLFPSORT). The P-protein from *C. sinensis* shows high structural similarities with the P-protein from Arabidopsis and tobacco, particularly in its N-terminal and C-terminal domains, as observed through 3D modeling (PyMOL). Results from the String platform showed interesting interactions of a homologous P-protein from Arabidopsis with transcription factors and receptor-like kinases involved in phloem developmental processes. Additionally, it revealed interactions with CalS7 and PP2A proteins, which are important in the context of HLB disease. These *in silico* results encouraged us to validate these findings. Hence, we designed a strategy for obtaining *CsSEOc* mutants using different CRISPR/Cas systems to confirm these findings in citrus and generate HLB-tolerant plants, whose transformed candidates are being regenerated in greenhouse.

Non-technical summary: The purpose of this study is to contribute to a better understanding of molecular mechanisms associated with huanglongbing (HLB) through bioinformatics analyses. The findings open up the possibility of developing genetically modified plants resistant or tolerant to HLB, which would make a significant contribution to integrated disease management.

Priming commercial citrus cultivars by overexpression of a systemic acquired resistance key regulator confers robust tolerance to Huanglongbing

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Abstract: Huanglongbing (HLB) is a devastating citrus disease caused by '*Candidatus* Liberibacter asiaticus' (CLas). It is well known that CLas infection triggers callose hyperdeposition and reactive oxygen species (ROS) overeraccumulation, which eventually lead to phloem collapse and nutrient blockage, resulting in severe disease symptoms in the commercially grown citrus varieties. Most rootstock varieties exhibit high tolerance to HLB, however, grafting commercial varieties on the rootstocks does not provide disease tolerance. So far common strategies for controlling HLB symptom development or the spread of the disease involve foliar application and/or trunk injection of antimicrobial agents, and production of transgenic and edited citrus varieties. Transgenic overexpression of the *Arabidopsis thaliana* NPR1 (*At*NPR1), a key regulator of systemic acquired resistance (SAR), in 'Duncan' grapefruit and 'Hamlin' sweet orange has been shown to provide robust tolerance to HLB in the greenhouse and the testing field with high disease pressure. However, the mechanism underlying the tolerance has not been studied. Here, we compared CLas-induced early responses including callose deposition and ROS accumulation in the transgenic lines and the wild-type (commercial) cultivars. Surprisingly, unlike the significantly induced callose deposition and ROS accumulation in the wild type, we found increased basal levels of callose, which remained unchanged upon CLas infection, and dromatically reduced ROS accumulation in the *At*NPR1-plants. Furthermore, *At*NPR1-plants exhibited limited phloem expansion and increased xylem fibers, a common intrinsic trait in tolerant plants enhancing water uptake. Our results indicate that priming the commercial cultivars with the SAR regulator *At*NPR1 enhances the capability of preventing callose and ROS overaccumulation as well as phloem expansion to suppress HLB disease symptom development, resulting in robust HLB tolerance.

Non-technical summary:
The heightened susceptibility in commercial citrus varieties to huanglongbing (HLB) likely represents a tradeoff for achieving higher yield. However, this can be addressed by priming the commercial cultivars through *At*NPR1-overexpression enhancing their tolerance to HLB.

Rapid Selection and Evaluation of Citrus Bud-Sports with Resistobiome for HLB Resistance/Tolerance

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Abstract: Since no huanglongbing (HLB)-resistant citrus cultivar is available in the world, selection of elite bud-sports with HLB-resistance becomes a much more appealing breeding approach, especially in HLB-epidemic regions. In this study, we have selected and evaluated more than 30 bud-sports and volunteer seedlings from commercial citrus varieties in the past eight years in Florida. After greenhouse and field trials with high HLB disease pressure, we have identified several improved HLB-resistant/tolerant lines. Although at year 4 to 5 all the trialed plants in the field were almost 100% infected, the selected grapefruit lines had 20 to 30 percent higher yields and equal or better fruit quality than their maternal or sibling plants. Meanwhile, we developed new methods for rapid screening and evaluating the bud-sports for HLB resistance/tolerance, which include a host PR protein-derived ELISA-based screening. We also identified a set of newly selected or genetically modified citrus varieties for graft-based evaluations. From the genomic and transcriptomic comparisons of these bud-sports, we have identified a special microbe, and several genes and pathways that might be associated with the improved resistance/tolerance. We will discuss the molecular mechanisms underlying the improved HLB-resistance, especially how the resistobiome plays a role in the improved HLB resistance/tolerance.

Non-technical Summary: In the past eight years, we have selected and evaluated more than 30 bud-sports and volunteer seedlings from highly huanglongbing (HLB) endemic fields of commercial citrus varieties in Florida. After greenhouse and field trials with high HLB disease pressure, we have obtained several improved HLB-resistant/tolerant lines, and identified a beneficial microbe associated with the improved HLB resistance/tolerance. The bud-sport and a beneficial microbe work together as citrus resistobiome for HLB resistance.

Discovery of nodule-specific cysteine rich peptides that block psyllid acquisition of '*Candidatus* Liberibacter asiaticus'

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Abstract: Nodule-specific cysteine-rich peptides (NCRs), encoded in the genome of the Mediterranean legume, *Medicago truncatula* Gaertn. (barrelclover) are known to regulate plant-microbe interactions. A subset of 183 were synthesized to identify NCRs with activity against the unculturable vascular pathogen associated with citrus greening disease, '*Candidatus* Liberibacter asiaticus' (CLas). We evaluated the peptides in a screening pipeline involving three distinct assays: a bacterial culture assay, a CLas-infected excised citrus leaf assay, and an assay to evaluate effects on bacterial acquisition by the nymphal stage of hemipteran vector *Diaphorina citri.* A subset of NCRs inhibit both CLas growth in citrus leaves and CLas acquisition by *D. citri*. As no therapies or peptides currently exist to block CLas transmission by the insect vector, these findings reveal NCR peptides as a new class of plant-derived biopesticide molecules to control citrus greening disease.

Non-technical summary: We discovered a group of plant peptides called nodule-specific cysteine-rich peptides (NCRs) in the genome of a leguminous plant that can combat the unculturable vascular pathogen associated with citrus greening disease, '*Candidatus* Liberibacter asiaticus' (CLas). Through a series of experiments, we found that some NCRs can inhibit CLas growth in citrus leaves and prevent its acquisition by the insect vector, *Diaphorina citri*. These findings suggest that NCR peptides could serve as a promising new type of plant-derived biopesticide to control citrus greening disease, for which there are currently no effective therapies.

Genome-wide association study reveals genes regulating vector competency of *Diaphorina citri*, insect vector of Huanglongbing (HLB)

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Abstract: Genome-Wide Association Studies (GWAS) have revolutionized scientific insight and the potential to examine complex diseases. Analysis of single nucleotide polymorphisms (SNPs), across an entire genome, identifies novel loci linked to a wide range of traits of both agricultural and medicinal significance. In this study, we performed a multi-population GWAS on 500 adult *Diaphorina citri*, the insect vector of '*Candidatus* Liberibacter asiaticus' (CLas), to investigate the genetic components of diverse acquisition phenotypes. *D. citri* adults were collected from four citrus groves in southeast Florida, USA, and the CLas acquisition phenotype for each insect was estimated using the absolute titer of CLas, as measured by qPCR. Haplotyping was performed with GATK, and imputation was performed with BEAGLE to increase the number of markers available for analysis. The R-package rTASSEL then examined the imputed-markers to identify hundreds of SNPs significantly associated with acquisition phenotypes. A high-quality reference genome, assembled with PacBio long reads, allowed for accurate SNP calling and loci association. Furthermore, mitochondrial haplotyping of nearly 700 *D. citri* mitochondrial genomes uncovered globally distributed haplotypes, as well as haplotypes within local Florida populations. This study provides novel insight into the genetics underlaying vector-biology and promotes further research to identify therapeutics which prevent insect-vector disease acquisition and transmission.

Non-technical summary: Our genome-wide association study of 500 individual *Diaphorina citri* adults revealed mutations correlated with pathogen '*Candidatus* Liberibacter asiaticus' (CLas) acquisition. We then examined the genetic regions around these mutations to uncover loci revealing the mechanisms by which *D. citri* acquire CLas. Statistically determined candidate loci can now become targets of novel therapeutics to reduce huanglongbing acquisition and transmission rates of *D. citri* adults, decreasing disease incidence in citrus crops.

Co-occurrence analysis of root-associated microbial community members reveals associations with '*Candidatus* Liberibacter asiaticus' infection status

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Abstract: Following a two-year long monthly survey for '*Candidatus* Liberibacter asiaticus' (CLas) using DNA samples from feeder roots of previously healthy grapefruit trees grafted on sour orange rootstock, we found that CLas spread rapidly among trees (~70% of trees became huanglongbing [HLB]-positive within a year). Yet, after two year ~10% of trees remained pathogen-free despite growing in the same field under the same agricultural practices. This unexpected finding prompted us to investigate the microbial community in the rhizosphere of the citrus feeder roots. Using DNA samples collected during the two-year study, we conducted 16S metagenomics sequencing on trees that were diagnosed as HLB-positive within 4 months after the onset of the survey (Group E) and trees that remained HLB-free (Group H). The Chao 1 and Shannon entropy metrics showed that Group H had higher microbiota richness and diversity than the samples in Group E. At the phylum level, Actinobacteria and Proteobacteria were the predominant bacterial phyla, comprising > 93% of total bacterial phyla, irrespective of HLB status. The co-occurrence network analysis captured 48 associations among 21 nodes in Group E and 83 associations among 23 nodes in Group H. Network clustering of Group E and H revealed that the major difference between Group E and H was the presence/absence of Streptomycetaceae that displayed co-occurence or mutual exclusion with other bacterial families in the network. The root DNA fractions provided a unique opportunity to examine the bacterial association network in the citrus feeder root system with differential HLB disease status.

Non-technical summary: Microbial communities can influence plant health and the success of pathogen invasions. After observing an unexpected "resistance" to infection among Citrus trees, we examined the microbial communities associated with trees susceptible and "resistant" to infection by '*Candidatus* Liberibacter asiaticus', the bacteria that causes huanglongbing disease. By looking at co-occurrence of groups of bacteria, we found that the main difference between susceptible and "resistant" trees was in the presence/absence of Streptomycetaceae bacterial communities.

Effect of silicon on the quality of juice from orange trees infected with HLB

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Abstract: Greening causes loss of productivity and quality of juice from infected orange trees. The greater the severity of the infection, the greater this loss. The reduction in brix and other components of the juice harms the flavor and value of the final product.

The present study aims to carry out revitalizing soil management, with the introduction of potassium rock powders, with a high concentration of silicon. One of the functions of Silicon is to catalyze metabolic reactions and improve the photosynthetic efficiency of plants. In view of this, infected orange plants, "Pera", grafted on citromelo swuingle, were fed with different doses of Silicon, respectively, 0 (T1) 300 (T2), 375 (T3), 450 (T4) and 600 (T5) kg of Silicon per hectare. The parameters evaluated were Productivity, and the quality parameters, vitamin C content, Brix, Ratio, TSS, Fruit weight, Acidity and IT. The results revealed that plants subjected to treatment with 375 kg of Silicon per hectare showed higher productivity and better quality parameters when compared to the control treatment, except for the vitamin C content, which presented a higher value in the T4 treatment. The other treatments did not differ from each other for all other parameters evaluated.

Non-technical summary: Given the advance of greening in all citrus producing regions, and the lack of effective control of the disease, the use of rock minerals appears as a mechanism to minimize the harmful effects of the infection.Because they are rich in silicon, which element has several metabolic functions in plants, we observed a slowdown in the progression of the disease in infected plants, as can be shown in the results obtained in the present study.

Huanglongbing severely impairs yield of marketable fruit of Ponkan mandarin trees grafted onto several rootstock varieties

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Abstract: Brazil recorded the fifth largest production of mandarin-type fruit in the world in 2021. Rangpur lime and Swingle citrumelo are the most used rootstocks for Ponkan mandarin, the major variety in Brazil, and main cultivated areas are under the presence of the huanglongbing (HLB) disease. In order to determine whether alternative rootstocks could improve tree performance, we evaluated the HLB severity and damage on the production and fruit quality of Ponkan mandarin trees grafted onto 25 rootstock varieties. Trees were planted in February 2016 in Bebedouro, Northern São Paulo State, in rainfed conditions at 5.0 m x 2.0 m tree spacing. The insect vector, the Asian citrus psyllid (ACP), was controlled by insecticides drenches (0-3 years) and sprays (fortnightly since planting). Assessments were performed from 2021 to 2023 on three harvest seasons. The severity index was relatively high for most evaluated trees, presenting over 60% of the canopy affected. Although some low-vigor inducing rootstocks presented a lower mean severity index (~40%), this was possibly related to a later infection. Moreover, the amount of unmarketable fruit, that is, showing small size and typical HLB symptoms, comprised 46% of the production for symptomatic trees in average. The proportion of red nose fruit raised from 4.25% in 2021 to 22.21% in 2023 of the total fruit load, demonstrating the havocking consequences of HLB. Symptomatic fruit decreased weight and soluble solids by 48% and 25%, respectively, and increased the acidity by 42% in relation to asymptomatic fruit collected from asymptomatic trees on 11 selected rootstocks. Since all rootstocks evaluated were severely damaged by HLB, growers of Ponkan mandarin should focus on preventive control measures and selecting the most productive rootstocks, which included Tropical Sunki mandarin, Indio citrandarin, Volkamer lemon and the BRS Bravo hybrid among others.

Non-technical summary: Ponkan mandarin is highly sensitive to huanglongbing (HLB), and grafting onto rootstock varieties with different genetic background does not significantly decrease the disease severity and damage. Consequently, growers should focus on preventive measures to manage HLB more satisfactorily, while breeding programs may obtain new Ponkan-like mandarins with improved disease resistance. In the meantime, more productive and locally adapted rootstocks should be prioritized to optimize economical return.

Impact of Huanglongbing (HLB) on Source-Sink Dynamics and Photosynthesis in Citrus

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Abstract: Understanding how sink organs and photosynthesis respond to huanglongbing (HLB) can aid the development of effective disease management strategies. Healthy plants dynamically co-regulate photosynthesis and sink demand for carbon, and these dynamics are mediated by phloem translocation. HLB alters carbon availability leading to sugar hyperaccumulation in the source and along phloem pathway. We hypothesized that HLB would limit the coordination of sink activity and photosynthesis. To assess the impact of HLB on source-sink dynamics, we used HLB-affected and -unaffected 'W. Murcott' trees grown under protective screens, and to assess the impact of source-sink dynamics, we manipulated source-sink dynamics manually. We girdled each branch to separate them from alternate sinks, removed leaves at 0%, 50% or 75% of the leaves on the branch and left one fruit on each branch. We kept a control treatment with no defoliation or girdling. We used gas exchanged methods to quantify the maximum rate of carboxylation and electron transport rate in carbon fixation while the sink carbon demand was quantified by measuring carbon availability, accumulation and utilization in the fruit. Varying source:sink ratio did not significantly affect photosynthetic activity in both HLB-affected and unaffected trees and HLB did not reduce the photosynthetic parameters. Defoliation reduced fruit growth rate in healthy trees, which had lower growth, but were only affected at most extreme defoliation rate (75%). Thus, HLB impacts sink activity and source-sink transport more than photosynthesis. Although HLB impacts sink growth, it limits the effect of source:sink variation on sugar as sink carbon demand remains relatively stable. We hypothesize that this is due to the buffering effect of sugar hyper-accumulation in the stem phloem.

Non-technical summary: Huanglongbing does not dramatically affect photosynthesis but reduces growth of fruits and interrupts the coordination of carbon fixation and consumption by fruits.

A new compartment model for HLB dynamics

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Abstract: Compartment models have played an important role in predicting the development of huanglongbing (HLB) over the last few decades and continue to be used to support disease management decision making. For the last decade in particular, new information has been produced about the biology of the pathogen-vector-host interactions, but has not found its way into formulated models of Asian Citrus Psyllid (ACP) or HLB dynamics. We present early results from a new systems dynamics model for ACP specifically intended to include features of the biology of the pathogen-vector relationship which were not known when the first generation of epidemic models were developed. Compared with the early models was developed we now know, among other things: that '*Candidatus* Liberibacter asiaticus' (CLas) is more efficiently acquired by juvenile psyllids than adults; that adults can acquire CLas but are significantly less effective as vectors compared with individuals who acquire CLas as juveniles; that CLas multiplies in its vector as well as its plant host; that infected adult females can give birth to infected offspring at a low rate; that the uninfected offspring of uninfected females can acquire CLas by co-feeding with infected individuals independently of the infection status of the citrus host, overall. All of these details indicate that a standard SEIR compartment model is unlikely to account for the biology of the vector-pathogen-host interaction in a realistic way, unless much of the biological detail is unimportant to the broad emergent properties of epidemic dynamics. The assumption that the effects of fine details are washed out in the broad sweep of the underlying biological "rules"of the system expressing themselves is, *prima facie*, less realistic the more marginal for ACP and CLas reproduction is the local system. We discuss this in relation to the new formulation of the pathogen-vector-host relationship in the model.

Non-technical summary: Existing compartment models for huanglongbing follow standard epidemiological assumptions about the disease status of trees and the role that the vector plays in transmission of the pathogen. Some key features of the biology of the system are not usually included in these models and this may have important consequences for how we plan disease management. Our new model is an attempt to examine this issue.

Alternative Tissue Sampling for Improved Detection of '*Candidatus* Liberibacter asiaticus'

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Abstract: Early detection and prompt response are key factors in the eradication of huanglongbing (HLB) in California. Currently, qPCR testing of leaf tissue guides California Department of Food and Agriculture (CDFA) in the removal of infected trees. However, because of asymptomatic infection, the uneven distribution of '*Candidatus* Liberibacter asiaticus' (CLas) in infected trees, and the difficulties of finding symptomatic foliage in the dense canopy, selecting the best leaves to sample, when mature trees may have more than 200,000 leaves, is a major hurdle for timely detection. The goal of this study was to address this issue by testing alternative tissues that might improve the CLas detection rate. Using two years of field data, we evaluated old and young leaves, peduncle bark of fruit, and feeder roots for the presence of CLas. Quadrant-peduncle (Q-P) tissue sampling consistently resulted in better CLas detection than any other tissue type. Q-P samples had a 30% higher qPCR positivity rate than quadrant-leaf (Q-L) samples. No significant seasonal patterns were observed. Roots and single-peduncles had similar detection rates; both were higher than single leaves or Q-L samples. If symptoms were used to guide sampling, 30% of infected trees would have been missed. Based on these findings, CDFA has implemented an alternative sampling procedure by including fruit peduncles and feeder roots in the routine sampling of high-risk trees. The impacts of improved sampling on the epidemiology of HLB are discussed.

Non-technical summary: Optimizing the tree sampling protocol will help contain the spread of huanglongbing and protect the multibillion-dollar citrus industry in California, by increasing the rate of detection and leading to earlier removal of infected trees. The goal of this study was to test the relative accuracy of different tissue types sampled under real-world field conditions, to see if using tissues other than mature leaves might improve the '*Candidatus* Liberibacter asiaticus' (CLas) detection rate. Quadrant-peduncle (Q-P) tissue sampling was found to consistently result in better CLas detection than any other tissue type.

Effect of eradication and maintenance of Huanglongbing-symptomatic trees in the edge strip on disease progress and yield of sweet orange blocks

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Abstract: Asian citrus psyllids carrying '*Candidatus* Liberibacter asiaticus' (bacterialiferous ACP) that came from outside citrus orchards play an important role in huanglongbing (HLB) epidemic, mainly in orchards with frequent ACP control. This immigrant bacterialiferous ACP population concentrates in an edge strip of up to 200 m from the orchard's periphery and is the cause of the higher HLB incidence in this area. Hence, we hypothesized that HLB progress in internal blocks would not be influenced by the maintenance of HLB-symptomatic trees in the border strip when effective ACP control is in place. This work compared the effect of eradication *versus* maintenance of HLB-symptomatic trees in the orchard edge strip on the ACP population in traps, the percentage of bacterialiferous ACP, the HLB incidence, and the fruit yield. Three experiments were conducted over 3 to 4 years: experiments 1 and 2 in 4 year-old orchards and experiment 3 in an 8 year-old orchard. Four plots of 200 m × 200 m were marked on orchard edge: two border plots located up to 200 m from the edge (B1 and B2), and two central plots afterwards, from 201 to 400 m from the edge (C1 and C2). Symptomatic trees were maintained in B1 and eradicated in B2. Symptomatic trees in central plots were eradicated in experiments 1 and 2; and maintained in experiment 3. Vector control was performed every 13 to 20 days. In all experiments, ACP population, bacterialiferous ACP incidence, cumulative HLB incidence, and accumulated yield in B1 were statistically similar to B2. Furthermore, central plots were statistically similar to all variables. Cumulative HLB incidence at border plot was 24.5% at experiment 1, 4.6% at experiment 2, and 53.4% at experiment 3. The maintenance of symptomatic trees in the first 200 m from the edge of the orchard did not contribute to increased HLB incidence within the orchard in the short to medium term when effective ACP control is in place.

Non-technical summary: The non-eradication of huanglongbing (HLB)-symptomatic trees in the edge strip of up to 200 m from the orchard's periphery associated with effective vector control did not contribute to increased HLB dispersion in the interior of the orchard. This approach avoided the costs of tree eradication and replanting in the short to medium term.

Effect of scion–rootstock combinations with contrasting vigor on flush shoot dynamics, natural population of the Asian citrus psyllid, and huanglongbing incidence

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Abstract: Citrus tree vigor is regulated by the rootstock. It may affect vegetative flushes, which are related to the Asian citrus psyllid (ACP) dispersal within the orchard, contributing to the dissemination of the bacteria associated with huanglongbing (HLB). Our hypothesis is that less vigorous scion–rootstock combination could have a significant effect on HLB management. An experimental trial was set with the early season Hamlin sweet orange (HSwO) grafted onto vigorous (Swahi Tropical mandarin dwarfing (Flying Dragon trifoliate orange, FDTrO) rootstocks to investigate the influence of contrasting scion–rootstock combinations on flush shoot (FS) dynamics and natural ACP population within the orchard. Eight blocks of 184 and 136 trees were planted in Cordeirópolis (São Paulo State) at a spacing of 6.0 × 1.5 – 2.0 m for SkTM and FDTrO, respectively. Trees were rainfed and not pruned, and ACP was controlled with insecticides. Assessments of FS dynamics (V1-V3 flush stages) and ACP capture were performed fortnightly from September 2022 to August 2023 (from 10 to 20 months after planting, MAP). Tree growth was assessed 16 MAP. The number of HLB-symptomatic trees and HLB incidence were assessed over time. Vigorous trees grew two-fold higher than dwarfed ones with height of 1.52 m and canopy volume of 0.62 m^3. Dwarfed trees had shorter FS (28±0.8 cm) than vigorous trees (54±2.2 cm). Canopy volume is important for the HLB management as the contact area for ACP feeding and reproduction is substantially reduced in dwarfed trees. FDTrO also led to the lowest FS frequency (0.193 plants with V1-V3 flushes/day) resulting in lower ACP abundance over time (0.503 ACP/trap), whereas SkTM had a FS frequency of 0.239 plants with V1-V3 flushes/day and an ACP abundance of 0.984 ACP/trap. The number of HLB-symptomatic trees and cumulative HLB incidence was higher for the SkTM (88 trees and 64%) than the FDTrO (33 trees and 18%), respectively.

Non-technical summary: Rootstock plays a key role on the scion variety performance, because several horticultural traits of the grafted tree may be affected. Under this context, we investigated the effect of scion–rootstock combinations of contrasting vigor on flush shoot dynamics, Asian citrus psyllid (ACP) population and huanglongbing (HLB) incidence. Our results indicated that dwarfing graft combinations might contribute to HLB management, as the number and length of flush shoots, and ACP abundance are reduced over time. Furthermore, less vigorous scion–rootstock combinations may reduce HLB cumulative incidence under ACP control.

Huanglongbing incidence is influenced by either the scion or the rootstock varieties under the control of the Asian citrus psyllid

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Abstract: Brazil is the largest producer of sweet orange and its processed products since the late 1980's. The huanglongbing (HLB) disease was reported in the Brazilian citrus belt in 2004, being mainly associated with '*Candidatus* Liberibacter asiaticus' (CLas) which is transmitted by the Asian citrus psyllid (ACP). Previously, our research group observed that truly dwarfing rootstocks were related to a lower cumulative incidence of HLB for the Valencia sweet orange. Herein, we broadened for evaluation of four scion varieties (the Pera and Folha Murcha sweet oranges, Persian lime and Ponkan mandarin) grafted onto 25 rootstocks varying from highly vigorous to dwarfing ones. Trees were planted in February 2016 in Bebedouro, Northern São Paulo State, in a rainfed orchard at 5.0 m × 2.0 m tree spacing. ACP was controlled by insecticides drenches (4 applications per year up to the third year) and fortnightly sprays. All trees were visually scouted for HLB symptoms and symptomatic tissues were sampled for qPCR detection of CLas since 2016. Seventy-nine months after planting, the HLB cumulative incidence on Folha Murcha sweet orange trees (~5%) was about half of that on Pera sweet orange and Persian lime, and 6-times lower than on Ponkan mandarin, in general. Regarding to the rootstock type, only trees grafted on dwarfing genotypes such as the Flying Dragon trifoliate and a few citrandarins presented a slower disease progress (<5%), with lemon and mandarin-type rootstocks showing notably higher disease incidence (~20% in general). Within each scion variety, the same rootstock behavior was observed. However, since 2021, ACP populations increased substantially in São Paulo citrus belt, as corroborated by the much higher ACP abundance in yellow traps monitored every 20 days on the perimeter of the experimental area. Consequently, HLB incidence in 2023 increased across all genotypes evaluated, which emphasizes the relevance of the regional management irrespectively of the scion and rootstock varieties currently available.

Non-technical summary: Although all commercial citrus varieties are susceptible to huanglongbing (HLB), some scion and rootstock combinations demonstrated a lower cumulative incidence over seven years of planting in condition that the Asian citrus psyllid (ACP) population was under control. This finding is probably related to the flush shoot dynamics lowering ACP presence or the risk of infection, even though the implicated mechanisms need further investigation. Planting strategies based on specific scion/rootstock combinations can benefit the HLB management, i.e., by concentrating disease dissemination at the orchard edge or reducing the amount of spray volume.

Taking cover – keeping young citrus trees protected from huanglongbing with exclusion netting

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Abstract: The citrus industry in Florida has been devastated by huanglongbing, a destructive citrus disease, caused by '*Candidatus* Liberibacter asiaticus' and vectored by the Asian citrus psyllid, *Diaphorina citri*. Current management of young replanted trees relies on protection from psyllids with frequent insecticide applications, an unsustainable practice that has led to insecticide resistance in *D. citri* and increased costs of grove management. Citrus growers are exploring non-insecticidal options such as exclusion netting over trees, reflective ground cover, and red kaolin clay particle films, although the effects of these treatments have never been fully evaluated or compared. We tested the efficacy of these treatments in reducing *D. citri* densities on young citrus trees against a grower standard control (monthly insecticide application) as well as their effects on other citrus pests and plant pathogens. Over a three-year period, the exclusion mesh successfully excluded psyllids, with less than 1% of trees developing huanglongbing, only after damage to the netting from two hurricanes in 2022. Trees in this treatment also showed the lowest rate of citrus leafminer infestation and citrus canker. Tree health was also greatest under the exclusion netting, with the largest canopies and trunk diameters among the treatments. However, trees with exclusion netting were still susceptible to spider mites, scales and mealy bugs, sooty mold, and greasy spot, suggesting that trade-offs exist with this tool. While these covers appear to be a promising tool for protecting young citrus trees from huanglongbing and establishing healthy canopies, citrus growers need to monitor for other pests and pathogens that can thrive within the closed mesh environment created by the covers.

Non-technical summary: Exclusion netting can stop young reset citrus trees from getting huanglongbing (HLB) by eliminating the feeding of the Asian citrus psyllid. Trees infected early in their life often die and rarely reach profitability. Exclusion netting was the most effective defense against HLB and allowed excellent canopy growth when compared to monthly insecticides, reflective ground cover, and red kaolin clay films, but other pests and diseases will need management within the nets.

A cost-benefit analysis of dwarfed citrus trees in high-density plantings as a mitigation strategy against Huanglongbing economic losses

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Abstract: Huanglongbing (HLB) is a devastating disease causing substantial economic losses, thus demanding a comprehensive reassessment of citrus production strategies. Existing HLB management strategies often escalate costs for citrus growers (e.g., frequent insecticide applications). Moreover, reduced land availability, water scarcity, and increased labor costs collectively elevate growers' overall costs. Therefore, it is crucial to identify strategies that increase revenue per citrus orchard and mitigate these increasing costs.
High-density citrus plantings can increase revenue, mitigating overall production costs and lost revenue from declining trees. Fundamental to achieving sustainable high-density plantings are dwarfed trees, made possible through viroid TsnRNAs. TsnRNA-dwarfed citrus trees show a significant canopy volume reduction, allowing for high-density plantings, and lowering fruit harvest and pest inspection costs since smaller canopies enable easy ground-level access to the entire tree. While not doubling orchard yields, the reduced costs ultimately result in higher revenues for growers, hedging the risk of economic losses due to HLB-affected trees.
We analyzed the potential economic benefits offered by dwarfed citrus trees through a cost-benefit analysis of an experimental block of TsnRNA-dwarfed navel orange trees planted in 1998 at the UC ANR Lindcove Research and Extension Center in Exeter, CA. Key components of this analysis include production costs, harvesting, pest inspection labor costs, and yield revenue. The findings of this analysis shed light on the economic viability and sustainability of using dwarfed citrus trees at the commercial level for citrus orchard profit maximization and slowing negative HLB effects.

Non-technical summary: Huanglongbing (HLB) inflicts significant economic losses on the citrus industry. Dwarf citrus trees enable high-density plantings, offering potential economic benefits by maintaining yields while reducing overall orchard management costs, and hedging the risk of losses due to HLB. We performed a cost-benefit analysis to assess the economic benefits of dwarfed citrus orchards as a mitigation strategy against HLB losses.

A summary of HLB testing in California from 2008 – 2023 by the California Department of Food and Agriculture

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Abstract: Huanglongbing (HLB) is a fatal disease of citrus associated with the bacterium '*Candidatus* Liberibacter asiaticus' (CLas) and vectored by the Asian citrus psyllid (ACP). In 2008, ACP was first detected in San Diego, California, which triggered ongoing statewide risk-based surveys and lab testing for CLas. Thousands of ACP and host plants were collected monthly throughout the year and tested using real-time PCR (qPCR) targeting the CLas Ribonucleotide Reductase (RNR) and 16S rRNA genes. Sampling of high-risk trees was modified to include peduncle and root tissues which improved detection by about 18%. Detections showed no host preference or seasonal pattern. qPCR Ct values ranged from 15-38 with an average of 27. Symptoms ranged from classic blotchy mottling and yellowing of leaves, to reduced leaf size and absence of fibrous roots with no visible leaf symptoms, even in leaves with high bacterial titer. Lime was the most reported infected host. Preliminary genotyping data on 169 California CLas isolates show distinct groupings suggesting different origins of introduction. Since the first HLB positive tree detection in 2012 from Los Angeles (LA) County, 6,388 additional residential positive trees have been detected for removal in southern California, with 70% from Orange County. Currently, the HLB quarantine zone has expanded over 2,000 square miles to include 72 cities and 5 counties: LA, Orange, Riverside, San Bernardino, and San Diego. To date, HLB has not been detected in commercial citrus groves. The timely detection and immediate removal of HLB infected trees, in conjunction with ACP control, are key elements to lowering the inoculum level in the field, slowing the spread of HLB, and protecting California's commercial production of citrus.

Non-technical summary: To date, 6,388 huanglongbing (HLB) positive trees have been confirmed in southern California. The detections have expanded the HLB quarantined zones over 2,000 square miles to include 72 cities and 5 counties: LA, Orange, Riverside, San Bernardino, and San Diego. Statewide plant and Asian citrus psyllid (ACP) testing is conducted throughout the year, with detections showing no seasonal pattern. To date, HLB has only been detected in landscapes, not in commercial groves. The CDFA's timely detection and immediate removal of HLB infected trees, in conjunction with ACP control, are key elements to lowering the inoculum level in the field, slowing the spread of HLB, and protecting California's commercial production of citrus.

References

Hajeri, S., S. Olkowski, L. Kumagai, N. McRoberts, R. Yokomi 2023. Alternative Tissue Sampling for Improved Detection of *Candidatus* Liberibacter asiaticus. *Plants* 2023, *12*(19), 3364; https://doi.org/10.3390/plants12193364

Zonghe, Y., B. Kasiborski, L. Kumagai, M. Nakhla, J. Rascoe, 2019. Development of routine multi-locus genotyping methods to characterize Huanglongbing (HLB) findings in California. In: 6th International Conference on Huanglongbing (IRCHLB VI) 2019, Riverside, CA.

Dai, Z., F. Wu, Z. Zheng, R. Yokomi, L. Kumagai, W. Cai, J. Rascoe, M. Polek, J. Chen, X. Deng 2018. Prophage Diversity if *Candidatus* Liberibacter asiaticus Strains in California. In: In: APS/ICPP Abstract/Poster of Presentation. Boston, MA. July 2018.

Kumagai, L.B., C.S. LeVesque, C.L. Blomquist, K. Madishetty, Y. Guo, P.W. Woods, and S. Rooney-Latham, J. Rascoe, T. Gallindo, D. Schnabel, and M. Polek 2013. First Report of *Candidatus* Liberibacter asiaticus Associated with Citrus Huanglongbing in California. Plant Disease 97(2): 283.1.

Are new chemical treatments for Huanglongbing cost-effective? Some evidence from Texas

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Abstract: Since its detection in 2005, huanglongbing (HLB) is now endemic in most U.S. citrus growing areas. Affected trees suffer gradual losses in yield and fruit quality, causing significant economic losses for growers. A new line of research involving new molecular compounds has shown promising results in laboratory tests by reducing the concentration of bacterial titers associated with HLB. The main objective of this study is to assess the economic feasibility of four chemical treatments, including oxytetracycline, for the control of HLB at the field level. It is imperative to evaluate their economic viability to ensure their broad adoption by growers. A stochastic bioeconomic model is used to assess the cost-benefit of the proposed treatments. The model is divided into two interconnected modules. The biological module simulates the spread of HLB at the orchard (incidence) and tree (severity) levels under different management practices. Complementary, the economic module uses the output generated by its biological counterpart to evaluate the long-term economic feasibility of each treatment under different market conditions. The field experiment was conducted in Texas on an 11-year-old grapefruit orchard. Affected trees were grouped into plots with similar severity levels and their trunks were injected with the different compounds. Preliminary results indicate that new treatments could increase yield by 48%-56% and extend the productive life of trees by 12 years compared to the control (conventional insecticide management). Production improvements translate to higher net present value of up to $5,671/ac and are expected to ripple through the supply chain. No significant differences in terms of projected net returns were observed between the control and the oxytetracycline treatment. The new compounds under evaluation could represent a viable option to improve the sustainability of the citrus industry worldwide. Furthermore, our economic evaluation framework can be extended to a wide range of HLB control alternatives.

Non-technical summary: This study uses a probabilistic bioeconomic model to evaluate the economic feasibility of four new chemical treatments, including oxytetracycline, for the control of huanglongbing at the field level. Preliminary results indicate that new treatments could increase yield by 48%-56% and extend the productive life of trees by 12 years compared to the control. Production improvements translate to higher net present value of up to $5,671/ac.

Citrus yellow vein associated virus field evaluation for applications as an anti-pathogenic expression vector

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Abstract: A novel citrus-infecting sub-viral RNA species was reported recently from University of California, Riverside named citrus yellow vein associated virus (CYVaV) with potential applicability in targeted virus-induced gene silencing (VIGS) systems. CYVaV has traits that make it suitable for developing an expression vector that could be potentially used against citrus pathogens such as the Huanglongbing-associated bacteria i.e., a small genome, sustained and systemic infectivity in citrus, mild to no effects on infected citrus, and being phloem-limited. This project focuses primarily on understanding CYVaV biology with regards to CYVaV-driven impacts on commercially popular citrus varieties as well as exploring potential transmissibility via natural modes (pollen, seed, and aphid vectors). In 2020, a field trial was established in Riverside, CA, comprising 12 replicated commercial citrus varieties, with trees of each variety subjected to graft-inoculations with a wild-type CYVaV isolate. This has been followed by symptom monitoring and routine diagnostic screening of the entire trial for CYVaV by RT-qPCR. CYVaV was not only detectable by RT-qPCR in leaves and budwood, but also in pollen from infected trees, thereby prompting in-field hand-pollination experiments investigating pollen transmissibility of CYVaV in Spring 2022. The resulting fruits were rigorously tested for CYVaV presence. Spring 2023 produced the first harvest from this field trial, with fruit yield and quality assessments conducted for graft-inoculated and untreated trees across all 12 varieties. We have initiated CYVaV aphid transmission bioassays with preliminary data indicating CYVaV uptake during phloem-feeding by prominent virus vectoring aphid species. This ongoing project is critical for generating the knowledge required to support regulatory approval proceedings to enable transitioning of CYVaV-based VIGS technologies from the laboratory to large-scale deployment in commercial citrus orchards.

Non-technical summary: Researchers at the University of California, Riverside reported a new citrus-infecting RNA species, citrus yellow vein associated virus (CYVaV) in 2021. CYVaV could be used in potential expression vectors targeting critical citrus pathogens. This project investigates CYVaV-driven effects on popular citrus varieties and potential CYVaV natural transmissibility with the goal of understanding CYVaV biology and generating data supporting regulatory proceedings to approve use of CYVaV-based anti-pathogenic systems at a commercial scale.

Combining Individual Protective Covers and Oxytetracycline Injection as an Integrated Strategy for Managing HLB

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Abstract: Worldwide, citrus production is threatened by huanglongbing (HLB), associated with phloem-limited *Candidatus* Liberibacter spp. This study investigates the potential of using individual protective covers (IPCs) and systemic delivery of oxytetracycline (OTC) through trunk injection as an integrated HLB management strategy. One-year old, greenhouse-grown 'Valencia' sweet orange (*Citrus sinensis*) trees on two different rootstocks were transplanted to a field site in southwest Florida, USA, in December 2020. Half of the trees were covered with IPCs. The other half of the trees were also covered with IPCs, but these contained 4 holes large enough to allow psyllid entry ("open" IPCs), while maintaining a similar growth environment as the "closed" IPCs. The open IPCs were temporarily removed during the main flushing period to further promote psyllid infestation and *Ca.* L. asiaticus (CLas) infection. Trees under open IPCs became infected within 4-6 months after planting while trees under the closed IPCs remained non-infected. After 18 months (July 2022), IPCs were removed from all trees, and a subset of infected and healthy trees was excavated to assess above- and below-ground biomasses. Infected and HLB-symptomatic trees that had been grown under the open IPCs exhibited significant biomass reductions of 19.5% aboveground and 36.8% belowground. Fibrous roots experienced the largest reductions (49%) despite considerably lower CLas titers compared to leaves throughout the study. The remaining trees were left to grow without covers. By April 2023, all trees, including trees previously grown under closed IPCs, were infected with CLas. In May 2023, half of the trees from each IPC treatment were injected with a commercial OTC formulation and the other half remained non-injected to assess the efficacy of integrating IPCs and OTC injections for HLB management. It is anticipated that previously covered, healthy trees will remain healthy and productive after OTC injection.

Non-technical summary: Growing young citrus trees under individual protective cover during the first two years of field establishment ensures that they remain free of huanglongbing (HLB). Combining this strategy with the systemic delivery of oxytetracycline after removal of the cover may provide a viable, integrated management strategy for citrus production under high HLB pressure.

Connecting Research to Get Better Huanglongbing Management Results

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Abstract: The Coordination Network program, designed to support better communication among USDA-funded researchers and stakeholders, addresses the national stakeholder need for a comprehensive source for science-based huanglongbing (HLB) management recommendations and USDA-funded research findings. National Institute of Food and Agriculture-Emergency Citrus Disease Research and Extension Program (NIFA-ECDRE) has identified a need to better identify knowledge/research gaps so that research and extension priorities can be designed to address them. Our project has two main focusses. The first focus is database tool development for stakeholders to access critical information about USDA-funded research findings to make connections leading to practical and sustainable HLB management solutions. We are working with NIFA-EDCRE/ and will engage the National Agricultural Research, Extension, Education, and Economics Advisory Board (NAREEE) citrus subcommittee to develop tools to better assess research gaps and develop future research priorities. As part of this project, we are working to develop a common vocabulary to help researchers communicate across disciplines and facilitate data searches. Additionally, terms commonly used by growers will be included to facilitate their searches. We are in the process of finding commonalities among the various sources of report summaries to build the database. Projects and refereed papers within the database will contain a non-technical research synopsis that highlights the key findings and the practical outcomes. Our second focus is the development of high-quality extension products from current and future findings. We will assemble regional focus groups of growers to determine the greatest needs and how tools should be designed to reflect regional differences. An easily searched web platform containing decision-aid tools to guide HLB-management will be delivered to citrus stakeholders. As part of the program, we will assess the extension message effectiveness and monitor for research and extension gaps.

Non-technical summary: We are developing a web-based tool to allow growers to search for USDA-funded research findings and read non-technical summaries of the work. The summaries will be focused on the practical outcomes of the research paper or research project. Additionally, we will be designing decision-aids to help make huanglongbing management decisions based on the stated needs of growers in the major citrus production regions.

Copper nanoparticle translocation and accumulation in citrus plants depends on nanoparticle surface charge

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Abstract: Chemicals from anthropogenic sources have been used in agriculture for centuries protecting against crop losses, controlling vector-borne disease, and enhancing overall plant health. While metals have played a role in these overarching effects, metal-based nanoparticles present a more precise means to attain comparable outcomes. With the advancement in nanotechnology, using nanoparticles in food and agriculture can improve crop yield due to increased stress tolerance, pathogens suppression, and improved micronutrient delivery. Similarly, copper nanoparticles (CuNPs) have attracted attention worldwide due to their broad-spectrum antimicrobial activity. Elemental copper is a key micronutrient playing a vital role in plant growth and development. While the small size of CuNPs facilitates absorption by plants, nanoparticles often elicit different biological responses than their un-assembled counterparts. This study evaluated the effects of CuNPs, with distinct surface charges, on plants as part of ongoing work to develop therapeutics for agriculturally relevant diseases, including huanglongbing (HLB). CuNPs with various surface charges were applied to citrus plants via foliar exposure and soil enrichment. Nanoparticle translocation and accumulation in the root, shoot, and leaves were monitored via ICP-MS and TEM. Physical attributes such as root and shoot length were measured in addition to biochemical components such as chlorophyll content. CuNP accumulation was greatest in leaves compared to roots, with minimal accumulation detected in the fruit. Overall accumulation was highest for negatively charged CuNPs in the foliar spray method and neutral CuNPs in the soil amendment method. Foliarly applied NPs can spread through the entire plant, reaching other plant compartments, such as the shoots and roots, and eventually exude into the rhizosphere soil. The downward translocation indicates phloem transport as the dominant route of NP distribution. These findings aid in elucidating the fate and physiological effects of CuNPs in fruit-bearing crops, thus supporting the development and application of nanomaterial-based therapeutics for HLB.

Non-technical summary: With the advancement in nanotechnology, using nanoparticles in agriculture can improve crop yield. We found that copper nanoparticles have antimicrobial properties and can spread through the entire plant after being applied to the leaves. The finding aids in understanding how nanoparticles can be used as a therapy to combat huanglongbing.

Defending Citrus Orchards: Novel Approaches to HLB and Canker Management Using New Plant Defense Inducers

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Abstract: Huanglongbing disease (HLB; citrus greening), caused by '*Candidatus* Liberibacter asiaticus' (CLas) and citrus canker, caused by *Xanthomonas citri* subsp. *citri* (Xcc) has devastated the citrus industry in Florida, USA, and continues to threaten other production areas worldwide. Unfortunately, effective control or preventive measures for HLB are currently not available, and managing citrus canker is challenging, primarily relying on copper applications, which carry the risk of soil over-accumulation and the emergence of copper-resistant Xcc strains. There is a dire urgency to pioneer innovative therapies for sustained protection of citrus orchards against these diseases. This study explored potential preemptive strategies to combat HLB and canker by using foliar applications of new plant defense inducers (PDIs). In the greenhouse, three initial foliar sprays of three proprietary synthetic PDIs, P2, P5, and P31 (500 ppm), were applied on two-year-old, healthy citrus trees (n ≥60), a week before exposure to CLas-infected Asian citrus psyllid (ACP), followed by monthly foliar applications (100 ppm). Remarkably, the treatments effectively delayed CLas infection and HLB symptoms for 6-10 months compared to water-treated controls. The PDIs maintained low bacterial titers in the treated trees (average Ct value ~30), while the water-treated control trees had high bacterial titers (Ct values ~23). In separate greenhouse experiments, a foliar spray of PDIs, P5, P26, or P31, or a soil drench application of Actigard (acibenzolar-S-methyl; 2g/tree) significantly reduced leaf canker lesions relative to water-treated control. Additional field trials conducted with a foliar spray of P31 combined with a single trunk injection of oxytetracycline (OTC) significantly reduced CLas titers when compared to trees that received OTC injections alone or water-injected and non-injected control trees. The disease prevention mechanism and potential use of the P31 alone or combined with OTC is currently being investigated for simultaneously managing HLB and citrus canker in the affected orchards.

Non-technical summary: The novel plant defense inducers P5 and P31, which are preparatory synthetic compounds and not currently labeled for use in citrus, showed promising effects managing both huanglongbing (HLB) and citrus canker without phytotoxicity when applied as foliar sprays in the greenhouse. The two compounds are currently being investigated for simultaneously managing HLB and citrus canker in the affected Florida orchards. These treatments can potentially be cost-effective and non-toxic solutions for integrated management of HLB and citrus canker in the future, potentially ensuring a more stable and sustainable citrus production industry.

Effects of compost on fibrous root health and physiology of four citrus rootstocks grafted with 'Valencia' orange growing in southwest Florida

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Abstract: Fibrous root loss is one of the early consequences of huanglongbing (HLB). In Florida where HLB is endemic, growers must also cope with the challenges of producing citrus on sandy, low organic matter and low nutrient-holding capacity soils. HLB has no cure, but different cultural practices, such as the use of soil amendments and HLB-tolerant rootstocks, may help reduce its consequences. In this study we investigated the effects of compost amendments on fibrous root health and physiology of four different rootstocks. The experiment was established on 20 acres of commercial citrus grove in southwest Florida in 2019 in a split-plot design. Compost was applied biannually at 5 tons/acre by broadcasting, and the control plots were left untreated. Four rootstocks, US-802, US-812, US-897, and X-639, grafted with 'Valencia' (*C. sinensis*) scion were used. Fibrous roots were collected to determine *'Candidatus* Liberibacter asiaticus' (CLas) titers, respiration rate, and morphological features. By November 2022, all trees were CLas-infected. Compost application did not change the CLas titers of the fibrous roots, but differences were detected among the rootstocks. Compost affected the soil physicochemical properties and increased the fibrous root respiration rate. Differences in fibrous root diameter and length were identified among the rootstocks but not between compost- and untreated plots. An untargeted GC TOF-MS analysis was conducted to determine fibrous root metabolic responses. Among the chemically known metabolites, proline, phosphate, and aspartic acid were significantly up-regulated, and putrescine and maleic acid were down-regulated in compost-amended plots. Significant differences among rootstocks were also detected and were related to their genetic backgrounds. Thus far, differences in yield and fruit quality were associated with the rootstock cultivar but not the soil amendment. Overall, our findings suggest that compost can alter the fibrous root physiology, which may ultimately increase stress tolerance and help mitigate the negative effects of HLB.

Non-technical summary: Compost amendments did not enhance tree growth and cropping during the first two years of field growth under high huanglongbing (HLB) pressure. Compost did however induce fibrous root physiological changes that may enhance their ability to tolerate HLB-induced stress and improve tree growth and productivity in the longer-term. Significant differences in root physiology and early cropping were found among rootstocks, confirming the importance of rootstock selection for citrus production.

Enhancing the qPCR detection of CLas with the use of bacteria RNA and a synthetic DNA internal standard

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Abstract: The associated agent to huanglongbing (HLB), '*Candidatus* Liberibacter asiaticus' (CLas), is detected by quantitative polymerase chain reaction (qPCR). In qPCR, unique regions of the pathogen's DNA are amplified to detectable levels. Some CLas DNA regions are transcribed into RNA at higher levels than others. By reverse transcribing these RNAs back into DNA, the number of DNA molecules of interest can be increased in a given sample. Our work shows this approach results in fewer cycles of amplification needed to detect CLas. In addition, the presence of plant or other inhibitors can lead to false negative CLas results, as they can hinder or delay the amplification of the targeted DNA. To assess the level of inhibition of a CLas qPCR reaction, we designed an internal standard that can be added to the reaction. The internal standard is a synthetic DNA molecule similar to the pathogen yet distinctive enough to be detected by itself. Because of this similarity, the detection of the standard will respond to inhibitors in the same way as the qPCR detection of the pathogen. Thus, when the internal standard is not detected, the test can be categorized as inconclusive instead of negative, thus reducing the rate of false negatives.

Non-technical summary: Our improvement of the qPCR detection of '*Candidatus* Liberibacter asiaticus' increases the confidence with which citrus growers can make decisions to mitigate the effects of huanglongbing on their operation.

Evaluating the antibacterial potential of distinct size populations of stabilized zinc nanoparticles for the treatment of Huanglongbing

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Abstract: Engineered nanoparticles are deliberately and precisely synthesized to exploit unique properties conferred by small size and high surface area for use in agricultural, biomedical, and environmental applications. While these physical properties dictate functionality, they can also have various implications in biological systems, both intended and unintended. Cellular uptake is influenced by both particle size and charge. The size distribution of agglomerated nanoparticles tends to broaden after internalization within an organism, potentially compromising their efficacy, particularly in targeted delivery applications. Zinc-based theragnostic agents are gaining popularity in biomedical science for their antibacterial properties, and for drug delivery and imaging purposes, neutrally charged surface stabilized zero-valent zinc nanoparticles (ZnNP) were selected for this study. Four synthesis methods were tested to produce distinct size populations of polymer coated ZnNP, all of which utilized water as the solvent to promote sustainable and green chemistry. In the United States, citrus crops have been threatened by the Asian citrus psyllid (*Diaphorina citri*) which incubate the gram-negative bacterium *Candidatus* Liberibacter that induces huanglongbing (HLB). Because a fastidious gram-negative bacterium causes HLB, the antibacterial activity of the ZnNP was tested in two agriculturally relevant, culturable, gram-negative bacteria strains: *Escherichia coli* and *Rhizobium rhizogenes*. The incubation temperature was also altered to assess bacterial growth and susceptibility to the ZnNP. As the diameter of ZnNP decreased, the bacterial growth also decreased. This trend was similarly observed at the lower incubation temperature, but the overall antibacterial effects of the ZnNP were decreased. These results further enhance the ability to utilize nanomaterials as a tool for pest/pathogen control.

Non-technical summary: In this study, researchers investigated nanoparticles to address challenges in agricultural infectious diseases. They found that nanoparticles are antibacterial, easily ingested by psyllids, and distributed through the citrus plants. The specially designed nanoparticles are valuable in controlling pests and offer promising solutions for the future of healthy agricultural systems.

Expediting the introduction of novel citrus cultivars via alternative temperature manipulation techniques

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Abstract: This study employs an alternating temperature treatment approach to rapidly establish pathogen-free budlines from potentially huanglongbing (HLB) tolerant or resistant scions and rootstocks, which have been evaluated in Florida fields. The study included Sweet Oranges: nucellar seedlings derived from somaclone exhibit unexpectedly high genetic diversity, and mandarin hybrids focus on seedless triploids. Many of these hybrids show promise for the fresh market and juice blending, coupled with HLB tolerance. Furthermore, we included rootstock hybrids, which undergo screening for their ability to mitigate HLB in susceptible scions. In accordance with Florida regulations, mother plants for replicated field trials must be free of disease. Consequently, we have developed a protocol that allows for the rapid generation of legal, pathogen-free budwood, crucial for advanced replicated Stage-II trials conducted at multiple locations. This protocol alternates between extreme temperatures restricting pathogen replication and optimal temperatures for plant growth and pathogen activity. The success of this treatment is attributed to the plant's rapid recovery of RNA synthesis machinery upon transitioning from high to optimal temperatures, while RNA viral pathogens require at least 12 hours to recover RNA synthesis. For citrus, the alternating temperature treatment involves cycling plants in a growth chamber between four hours at 42°C and four hours at 25°C over a period of 3 to 4 months, allowing new flushes to emerge and mature. While the treatment does not cure infected plants, it facilitates disease-free growth from HLB and CTV infected plants, as confirmed by qPCR, ELISA, and RT-PCR bioassays. Implementing this protocol will enable the generation of pathogen-free budlines from promising HLB-tolerant scions and rootstocks, suitable for replicated field trials. Scions and rootstocks demonstrating tolerance to HLB in these trials will be made available to farmers, ultimately aiming to provide pathogen-free, high-quality scions and rootstocks that are HLB-tolerant, supporting sustainable and profitable citrus production while reducing tree decline and crop losses.

Non-technical summary: The overall goal of this study is to greatly accelerate the delivery of pathogen-free budlines from Florida field-tested huanglongbing (HLB) resistant/tolerant scions and rootstocks by using an alternating temperature treatment. The study is an elevated risk/high reward that would enable HLB tolerant/resistant germplasms from different citrus genetic backgrounds, which have been developed by conventional breeding (not genetically modified or CRISPR manipulated genomes), to serve as resources for breeding programs and for dissecting the genetic basis of resistance to HLB. The results of this study would expedite large multi-site field testing in Florida necessary to determine the true value of HLB resistant/tolerant scions and rootstocks.

Exploring the Origins and Future of Citrus with Outreach Videos

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Abstract: With recent advances in understanding the origins of citrus cultivars, and the looming threat of huanglongbing (HLB), there is a unique opportunity to educate the public about topics in plant genetics through the citrus story, and contextualize the contributions of newer technologies to current and future citrus production. We have created a series of outreach videos exploring the origins of citrus cultivars, taking the viewer on a "tasting trek" of citrus genetic diversity in UC Riverside's Givaudan Citrus Variety Collection. The viewer follows us from the field to the lab and back again, meeting citrus experts along the way who help make sense of the complex history, culture, and genetics of these fruit trees. The current threat of HLB is introduced, and how breeding with citrus relatives and bioengineering are used to address this challenge, and finally, how the diversity of this collection is being protected for the future. The brief expert interviews in the main narrative also serve as introductions to more in-depth extended interviews published side-by-side. By weaving together field and lab footage with studio photography of diverse fruits, we believe we have created a unique format that can be extended to include more discoveries and developments from the citrus research community into the future. This project was developed in collaboration with Science for Citrus Health, a project of UC Agricultural and Natural Resources, with funding from NIFA award 2018-70016-27412, anonymous donors, and Biology Fortified, Inc. Videos will be available on UCANR's YouTube channel, at https://www.youtube.com/user/UCANR and at https://ucanr.edu/sites/scienceforcitrushealth/.

Non-technical summary: How have modern genetics tools helped us to understand the origins of oranges, lemons, and limes, and how are these tools being used to help in the struggle against huanglongbing, which threatens citrus production worldwide? We created a video "tasting trek" of the Givaudan Citrus Variety Collection at UC Riverside to show the diversity of citrus fruits, explain their origins and history, and introduce modern techniques being used to safeguard citrus for the future.

GA*A*NTRY technology to enhance Symbiote effectiveness against '*Candidatus* Liberibacter asiaticus'

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Abstract: This research presents a novel plant biotechnology for the rapid genetic improvement of crops. Although single genes have been important in engineering herbicide and pest tolerance traits, future improvements of complex traits like yield, nutritional quality and disease resistance will require the introduction of multiple genes. The GA*A*NTRY (Gene Assembly in *Agrobacterium* by Nucleic acid Transfer using Recombinase technologY) system as a flexible and effective system for stably stacking multiple genes within an Agrobacterium virulence plasmid Transfer-DNA (T-DNA). The system uses unidirectional site-specific recombinases *in vivo* and an alternating selection scheme to sequentially assemble multiple genes into a single transformation construct. GA*A*NTRY's capabilities were demonstrated though production of 10 cargo sequences sequentially stacked together to produce a 28.5 kilobase pair T-DNA, which was used to generate transgenic dicots, *Arabidopsis*, and potato. Approximately 89% of the events identified using the dual antibiotic selection screen exhibited all introduced traits. A total of 57% of the tested lines carried a single copy of the selection marker transgene located near the T-DNA left border and none of the plant tested contained sequence from outside the T-DNA. The process was repeated with an 11 cargo 37.4 kb T-DNA in the monocot, rice. These results demonstrate that the GA*A*NTRY is a powerful, yet simple to use, new tool for transgene stacking, plant synthetic biology and the generation of high quality genetically engineered plants. Currently, this technology is being applied toward combating huanglongbing by stacking multiple antimicrobial peptides (AMPs) for production in Symbiont technology. Symbionts are small clusters of plant cells that produce a gene of interest. Our team is testing the hypothesis that Symbionts expressing GA*A*NTRY-stacked AMPs will reduce '*Candidatus* Liberibacter asiaticus' titer and mitigate disease symptoms.

Non-technical summary: This research introduces a new plant biotechnology called GA*A*NTRY, which allows for the efficient stacking of multiple genes in crops to improve complex traits like yield, nutritional quality, and disease resistance. GAANTRY was successfully used to create transgenic plants with stacked genes in both dicots (Arabidopsis and potato) and monocots (rice), demonstrating its versatility and effectiveness. The technology is now being applied to combat huanglongbing by stacking antimicrobial peptides in plant Symbionts to potentially reduce disease severity.

Generating *de novo* transcriptome assemblies of Australian native lime species using various tissue types and its use as a reference for transcriptome data analysis

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Abstract: We conducted RNA-seq using various tissue types of three huanglongbing (HLB)-resistant Australian native lime species, *Citrus australasica*, *C. inodora,* and *C. glauca* to generate *de novo* transcriptome assemblies. RNA-seq yielded ~67 to 81 million (M) reads for *C.* different tissues. *De novo* assembly of quality filtered reads generated 252,646 contigs for *C. australasica*, and 282,079 and 283,961 contigs, respectively, for *C. inodora* and *C. glauca*; average lengths were 1,985bp (N50 =3,641bp) for *C. australasica*, 1, 948bp (N50=3,772bp) for *C. inodora* and 1,832bp (N50=3,654bp) for *C. glauca*. BUSCO analysis showed scores of 93%to 95%. After removing redundant contigs, TransDecoder predicted ca. 141,000 ~167,000 ORFs from the *de novo* assemblies. Functional annotation of these predicted ORFs showed that ca. 65.6% of *C. australasica* ORFs, and 61.9% and 65% of *C. inodora* and *C. glauca* ORFs, respectively, were annotated. Among them, 81,000 ~ 90,000 sequences were assigned to the GO category of Biological Process and ~8000 sequences were involved in "Defense Response to Other Organisms" (GO:0098542). The *de novo* assemblies were used as a reference to analyze the RNA-seq data obtained from 4 HLB-tolerant and 4 HLB-susceptible F1 hybrids with Australian native lime parentage. The preliminary data revealed that both HLB-tolerant and -susceptible hybrids expressed different sets of genes involved in pathogen detection, hypersensitive response, and response to ROS. This study examined the sequence variation of these genes between HLB-tolerant and -susceptible hybrids, to evaluate any potential relevance to HLB disease response phenotype.

Non-technical summary: We have generated information about gene expression in three Australian native limes. The study is useful for understanding gene expression in huanglongbing-tolerant and susceptible hybrids.

Improving growth and yield of HLB-infected citrus by Fe application at field experiments in Florida

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Abstract: Huanglongbing (HLB)-infected citrus trees often show chlorotic symptoms. It is also reported that HLB-infected trees suffer from micronutrient deficiencies in elements such as Fe, Zn and Mn[1)], and that HLB-infection symptoms are found in alkaline soils rather than acidic soils in the same area[2)]. The foliar application of iron material, which is easily absorbed as ferrous ion across the transmembrane of the leaf cell, recovered the chlorotic symptoms and HLB-infected citrus growth[3)]. These findings suggest that HLB-infected trees have reduced iron bioavailability and that efficient supply of iron nutrition recovered the growth against the infection.

We here report on a field experiment that indicates that foliar application of iron material improves the growth of HLB-infected trees in Florida, where almost all citrus trees are infected with HLB. Continuous foliar application of Fe material for 2.5 years increased the yield by around 70% compared to no application of Fe. We also showed that the HLB-tolerant cultivar has stronger enzymatic activity in response to Fe deficiency compared to HLB-sensitive cultivars. Fe is thought to be translocated in the phloem as a complex of nicotianamine, which is a natural metal chelator involved in micronutrient homeostasis in higher plants.

Non-technical summary: In summary, we hypothesize that HLB infection by '*Candidatus* Liberibacter asiaticus' causes Fe deficiency, and continuous application of nutritional Fe material which is easily absorbed into citrus leaves will recover the growth of HLB-infected trees. In field trials, foliar application of Fe material increased yield by around 70%, demonstrating the potential for iron treatments to extend the commercial viability of infected trees.

1) Y. Masaoka et al. (2011) Jpn. Agric. Res. Q., **45**, 269-275

2) H. Inoue et al (2020) Jpn. Agric. Res. Q., 54, 21-29

3) H. Inoue et al (2020) Int. J. Mol. Sci., 21, 4033

Innovative Strategies for HLB Control: A Multifaceted Approach using CTV Vectors

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Abstract: The citrus tristeza virus (CTV) vector can be a potent delivery platform against huanglongbing (HLB) due to its host-friendly attributes: non-symptomatic in most citrus; absence of tree-to-tree transmission; precise phloem-specific delivery; and robust long-term stability. Silvec Biologics, Inc., a company specializing in virus-induced gene silencing (VIGS) vectors, is now commercializing a first-generation (Gen1) product in partnership with US Sugar, UF/IFAS – the developer of the CTV vector, and Texas A&M – the developer of the natural spinach defensin anti-microbial peptide (AMP) which has been shown to improve HLB-induced yield declines, but does not prevent acquisition of the disease. This Gen1 product has garnered USDA approval for application across all Florida counties, with Silvec presently poised for an EPA PRIA application. In pursuit of an enduring HLB solution, Silvec and its collaborators are crafting a second-generation (Gen2) CTV product with a multifaceted approach to improve host resistance to HLB. Gen2 is designed to act synergistically on multiple levels, by: (*i*) suppressing bacteria propagation by expressing improved antibacterial defensins and anti-bacterial siRNAs to inhibit proliferation of '*Candidatus* Liberibacter asiaticus'; (*ii*) enhancing host resistance by delivering siRNAs that target host-susceptible genes and enhance innate resistance; (*iii*) alleviating disease symptoms by expressing siRNAs that target host genes implicated in symptom development; and (*iv*) controlling HLB spread by targeting transmission-essential genes of the Asian citrus psyllid. By synergizing these strategies, Silvec aims to revolutionize HLB control and provide a holistic solution to combat this disease.

Non-technical abstract: A non-transmissive version of citrus tristeza virus that is asymptomatic in most trees has been engineered to express an antibacterial spinach peptide which has been demonstrated to enhance yields in huanglongbing infected trees. The product can be seamlessly distributed to nurseries and growers by inoculating mother trees to transmit it to all progeny. The product has received USDA regulatory approval for use in all Florida counties and an application is under review by the US EPA.

Micronutrient therapies for improved citrus production in an endemic HLB era

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Abstract: Boron (B), manganese (Mn), and zinc (Zn) uptake are evident in the leaves as a constituent of photosynthesis and other plant body-building mechanisms for growth and development. Primary (nitrogen (N), phosphorus (P), potassium (K)) and secondary (calcium (Ca), magnesium (Mg), sulfur (S)) macronutrients are also critical for maintaining fruit yields, tree canopy and increasing root density. We investigated the availability of micronutrients (B, Mn, and Zn) to citrus trees through modified application methods and rates along with balanced primary and secondary macronutrients (N, K, Ca and Mg). Consistently greater fruit yields (350-500 boxes per acre), high root densities and denser canopies were observed with increased and elevated micronutrients over 3 to 5 years of studies. This indicates that sustained therapeutic strategies are available for improved citrus production despite the endemic huanglongbing environment in Florida and around the world.

Non-technical summary: Elevated micronutrient levels up to about 2x-4x the current recommendations increased fruit yields and root density. Optimal ratio of N to K and also elevated Ca and Mg increased tree size, juice quality and also fruit yield.

Mitigation of Huanglongbing: Implications of a biologically-enhanced nutritional program on yield, pathogen localization and host gene expression profiles

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Abstract: Huanglongbing (HLB, citrus greening disease) is the most destructive disease affecting citrus production and is primarily linked to the gram-negative, insect-vectored, phloem-inhabiting α-proteobacterium, '*Candidatus* Liberibacter asiaticus' (CLas). With no effective treatment available, management strategies have largely focused on the use of insecticides in addition to the destruction of infected trees, which are environmentally hazardous and cost-prohibitive for growers, respectively. A major limitation to combating HLB is the inability to isolate CLas in axenic culture, which hinders in vitro studies and creates a need for robust in situ CLas detection and visualization methods. The aim of this study was to investigate the efficacy of a nutritional program-based approach for HLB treatment, and to explore the effectiveness of an enhanced immunodetection method to detect CLas-infected tissues. To achieve this, four different biostimulant-augmented nutritional programs (P1, P2, P3 and P4) were tested on CLas-infected citrus trees. Structured illumination microscopy (SIM) preceded by a modified immuno-labeling process and transmission electron microscopy (TEM), were used to show treatment-dependent reduction of CLas cells in phloem tissues. No sieve pore plugging was seen in the leaves of P2 trees. This was accompanied by an 80% annual increase in fruit number per tree and 1,503 (611 upregulated and 892 downregulated) differentially expressed genes. These included an MLRQ subunit gene, UDP-glucose transferase, and genes associated with the alpha-amino linolenic acid metabolism pathway in P2 trees. Taken together, the results highlight a major role for biostimulant-amended nutritional programs as a viable, sustainable and cost-effective option for HLB management.

Non-technical summary: The current findings and the bENP program will assist growers in the mitigation of huanglongbing (HLB, citrus greening), which has devasted the citrus industry for over a century. The bENP program will also assist in year over year yield enhancements which otherwise is affected by HLB.

New Pathogen Testing Tools for Safe Movement of Citrus Germplasm in the Huanglongbing (HLB) Era

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Abstract: Many novel citrus cultivars and hybrids are developed by researchers to generate huanglongbing (HLB)-tolerant/resistant varieties. It is essential to test the new varieties in various agro-climatic conditions to ensure durable disease resistance/tolerance. Since citrus is susceptible to many graft-transmissible viral and bacterial pathogens, it is imperative to conduct thorough bud wood testing before the movement of citrus germplasm to different citrus-growing regions in the United States. We have developed a citrus pathogen testing array capable of analyzing plant extracts for the presence of 26 common pathogens. The qPCR-based array can be completed in 90 minutes for the detection of 26 pathogens. We have confirmed the array results using next-generation sequencing of transcripts from selected test plants. In addition, we have successfully used these tools to characterize pathogens in over 1000 trees including the inventory of positive control plants maintained at the Germplasm Repository, accessions held in quarantine as well as trees in the field in the Riverside Givaudan Citrus Variety Collection. The pathogen array is designed as a modular test; the primers and probes used for the detection of specific pathogens can be replaced by reagents for other pathogens relevant to the geographical area.

Non-technical summary: Breeding for huanglongbing resistance requires testing of plants in multiple locations which in turn requires movement of germplasm. We have developed advanced tools to rapidly test for multiple citrus pathogens and facilitate the safe movement of germplasm.

Nutrition is a Critical Part of the Equation for Maximizing HLB Tolerance

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Abstract: Huanglongbing (HLB) disease causes severe secondary and micronutrient deficiencies in '*Candidatus* Liberibacter asiaticus' (CLas)-infected trees, and the deficiencies are twice as great in the roots than in the scion portion of the tree. NPK levels remain relatively normal. These deficiencies must be corrected for normal vascular function and tree health, as necessary to maintain adequate yields of high quality fruit.
Optimal threshold levels of each nutrient required for adequate tree health in CLas-infected trees are clearly different than those of non-infected trees, but still have not been determined. Maintaining optimal nutrient ratios is also important in HLB+ trees. Use of Controlled Release Fertilizers (CRF) is an excellent way of providing constant elevated levels of secondary and micronutrients to tree roots year-round. However, growers must be aware that many available CRF products have only the NPK poly-coated, and not the secondary and micronutrients; these products do not work to correct the deficiencies in HLB+ trees. We have already proven that selected CRF can be used at the time of planting for establishing healthy, vigorous trees. We have found that use of CRF with coated secondary and micronutrients can also significantly improve the health of producing HLB+ trees, stop fruit drop, and increase yields and fruit quality. However, such CRF products are expensive, especially for use with mature trees. Thus our current efforts are focused on blending selected CRF products with traditional dry fertilizers to improve the economics. We are now testing a 4 application soluble dry fertilizer program where the 2nd and 4th applications contain CRF components with enhanced polycoated secondary and micronutrients. So far, tree health has been quite impressive, with even highly susceptible scion/rootstock combinations showing improved health. We will report on our results after the first year of testing this approach on multiple scion/rootstock combinations of trees that were not grown with covers or treated with antibiotics.

Non-technical summary: Secondary and micronutrient deficiencies in huanglongbing (HLB)+ trees, especially in the roots, must be corrected to restore vascular function as necessary for adequate tree health, yield and fruit quality. Use of Controlled Release Fertilizer (CRF) products containing polycoated secondary and micronutrients is an effective way to correct these deficiencies. We will report on preliminary results from testing a 4 soluble dry fertilizer program where the 2nd and 4th applications contain CRF products, on HLB+ trees.

Pathogenic Potential of HLB-Associated *Fusarium* Species

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Abstract: *Fusarium* spp. are associated with dry root rot, wilt, dieback and decline in citrus. Trees weakened by stressors, such as other pathogens, can be more susceptible to invasion of *Fusarium* spp. We observed that an increase in relative abundance of several *Fusarium* taxa in citrus roots was positively correlated with an increase in severity of huanglongbing (HLB). This suggests that HLB disease progression results in trees becoming more susceptible to *Fusarium*. Our working hypothesis is that *'Candidatus* Liberibacter asiaticus' (CLas) infection weakens trees and makes them more vulnerable to root invasion by soilborne pathogens including *Fusarium* and *Phytophthora* spp., leading to the rapid fibrous root loss associated with HLB. To begin testing this hypothesis, we isolated and sequenced the genomes of 18 strains of *Fusarium* from the roots, leaves, and stems of citrus under high and low HLB pressure. We screened select *Fusarium* isolates for their pathogenic potential on two common citrus rootstocks, Carrizo citrange and Swingle citrumelo, as well as on S-1 citron. Results indicated that while root-associated isolates from the *Fusarium solani* and *Fusarium oxysporum* species complexes were pathogenic on these citrus species, *F. oxysporum* and other *Fusarium spp.* isolated from leaves and stems were not. Our ongoing work is focused on the relationship between HLB severity and the invasion of pathogenic isolates of *Fusarium* and *Phytophthora* spp., and their potential to exacerbate fibrous root decline in citrus as a disease complex.

Non-technical summary: *Fusarium* spp. isolated from roots, leaves, and stems of huanglongbing (HLB)-impacted citrus were screened for pathogenicity, and those originating from roots were found to be pathogenic. *Fusarium* spp. increase in roots as HLB disease progresses, and may speed fibrous root decline in infected trees. Understanding interactions between HLB and other pathogens will enable the development of management strategies that include all contributors to HLB-related citrus decline.

Plant growth regulators for improving hormonal imbalance in Huanglongbing-affected sweet orange trees

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Abstract: Huanglongbing (HLB), a devastating disease of citrus, is reported to affect growth traits of citrus trees, such as tree height, number of shoots/trees, shoot length, internodal length, and leaf size compared to healthy trees. HLB-affected trees have fewer leaves/shoot, and greater leaf drop compared to healthy trees. HLB-affected citrus leaves showed high accumulations of the defense hormones salicylic acid (SA) and abscisic acid (ABA) and low accumulation of the growth promoting hormones such as auxin and gibberellins compared to healthy trees. In other words, a trade-off of growth to defense response can be seen in HLB-affected trees. In past few years we have conducted several studies demonstrating the improvement in vegetative and fruit growth with exogenous application of gibberellic acid. Herein we present the results of a trial where a combination of growth promoting plant growth regulators (PGRs) were applied to improve the growth and productivity of HLB-affected 'Valencia' trees. As compared to control, a combined treatment of cytokinin+auxin+GA3 showed statistically significant increases in number of new sprouts, average number of leaves/branch (38%), and average leaf area (40%). Upon further investigation of molecular mechanism leading to these changes, a significant decrease in expression of *lipoxygenase*, *Respiratory burst oxidase homolog*, and some other disease response related genes such as *Jasmonic acid methyl transferase* and *MYC2* were observed in PGR combination treatment suggesting that the use of combination PGR treatment reduced the oxidative stress and improved the hormonal balance in HLB-affected citrus. A reduction in *Phloem protein 2-B15* expression in PGR treatments suggest a reduction in phloem plugging thus possibly improving carbohydrate translocation to various sink tissues. Overall, our results suggest that PGRs can improve the hormonal balance of HLB-affected trees, thereby improving growth and productivity of HLB-affected citrus.

Non-technical summary: The huanglongbing (HLB) trees suffer from a growth trade-off for elevated plant defense response. Preliminary results suggest GA applications reduce oxidative stress, improve plant defense responses, re-adjust the hormonal balance, and improve phloem biology. In the presented research a combination of growth promoting hormones was applied to evaluate the performance of treated HLB-affected trees. The PGR application resulted in improve of growth attributes of HLB-affected trees by reducing oxidative, biotic, and abiotic stress and improving carbohydrate translocation in HLB-affected sweet orange.

The effect of price, practice costs, and risk perception on the decision to adopt ACP and HLB control practices in an established California citrus grove

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Abstract: Designing strategies to motivate personal protections from collective environmental risk, like huanglongbing (HLB) in California, are challenging given coordination may be conditional on beliefs in other growers' actions and confidence in the scientific information about the risk, and limited data on HLB in California commercial citrus groves. To observe how beliefs in other grower participation in area-wide coordinated insecticide spraying for the Asian citrus psyllid (ACP), the vector for HLB, and confidence in scientific information affect the success of alternative ACP spray strategies to protect California citrus groves from Huanglongbing, we simulate economic decision-making using an agent-based model (ABM) that integrates ACP population dynamics, HLB incidence, citrus production, with economic decision-making under HLB risk perception, belief in other grower cooperation, and confidence in scientific information. Using randomly drawn samples from the model parameter ranges, we simulate simulated economic experiments to observe how growers adjust their decisions based on changes in beliefs in other growers' spraying decision and scientific information about HLB spread. We observe that a grower is more likely to coordinate insecticide spraying as they believe more and more other growers are participating. However, when they believe most other growers are coordinating their spraying, they cooperate less, leading to faster HLB spread. Also, increased confidence in scientific information lowers HLB spread. As such, strategies to combat incurable infectious disease, like HLB, require simulation modeling that provides strategy guidance before a risk is actualized.

Non-technical summary: Designing strategies to protect California citrus groves from huanglongbing (HLB) are challenging given growers' risk perception, belief in other growers and their confidence in scientific information about the risk. Compounding the challenge is limited data on HLB in California commercial citrus grove. As such, strategies to combat incurable infectious disease, like HLB, require simulation modeling that provide strategy guidance before a risk is actualized.

The potential cost-effectiveness of ACP and HLB control strategies in a newly planted California citrus grove

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Abstract: Multiple Southern California counties have recently experienced steady increases in huanglongbing (HLB)-infected residential citrus trees. Moreover, two Asian citrus psyllid (ACP) infected with '*Candidatus* Liberibacter asiaticus' have been confirmed in commercial citrus groves in two Southern California counties raising concern about HLB spread there. Taking lessons learned from Florida's experience combating HLB, we evaluate the economic efficacy of insecticide spraying for ACP and rogueing of HLB-infected trees on a representative newly planted California Navel orange grove. To do so, we rely on a simulation model using data for ACP migration and HLB severity from an existing agent-based model that is combined with cost and returns data taken from University of California cost and return studies and California County Agricultural Commissioner Reports. We compare the profits over a 20-year period for each practice with varying degrees of spray efficacy and rogueing monitoring frequency and removal thresholds based on HLB severity. We also conduct sensitivity analysis by evaluating varying insecticide efficacy rates, Navel orange prices, and yield per acre based on distributions derived from the past 5 years of data. The preliminary results show that, in most cases, spraying and rogueing do not generate positive profits unless spray efficacy is at least 90% effective, and for rogueing, citrus prices and yields are close to the upper bounds of their distributions, reaffirming the need for new technologies to combat HLB.

Non-technical summary: The California commercial citrus industry faces a growing threat from huanglongbing (HLB). Taking lessons learned from Florida's experience and strategies to combat HLB, we evaluated coordinated insecticide spraying for Asian citrus psyllid and rogueing of infected trees on a representative California Navel orange grove using a simulation model. We found that, in most cases, spraying and rogueing do not generate positive profits, reaffirming the need for new technologies to combat HLB.

The roles beliefs in neighbors' behavior and confidence in scientific information play in area-wide coordinated spraying strategies for Asian citrus psyllid, the vector for Huanglongbing

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Abstract: Designing strategies to motivate personal protections from collective environmental risk, like huanglongbing (HLB) in California, are challenging given coordination may be conditional on beliefs in other growers' actions and confidence in the scientific information about the risk. To observe how beliefs in other grower participation in area-wide coordinated insecticide spraying for the Asian citrus psyllid (ACP), the vector for HLB, and confidence in scientific information affect the success of alternative ACP spray strategies to protect California citrus groves from huanglongbing, we simulated economic decision-making using an agent-based model that integrates ACP population dynamics, HLB incidence, citrus production, economic decision-making under HLB risk perception, belief in other grower cooperation, and confidence in scientific information. Using randomly drawn samples from the model parameter ranges, we simulated economic experiments to observe how growers adjust their decisions based on changes in beliefs in other growers' spraying decisions and scientific information about HLB spread. We observed that a grower is more likely to coordinate insecticide spraying if they believe more and more other growers are participating. However, when they believe most other growers are coordinating their spraying, they cooperate less, leading to faster HLB spread. Also, increased confidence in scientific information lowers HLB spread due to greater grower participation in area-wide coordinated spraying.

Non-technical summary: Designing strategies to protect California citrus groves from huanglongbing (HLB) are challenging given growers' risk perception, belief in other growers, spraying behavior, and their confidence in scientific information about HLB risk. Through simulated economic modeling, we found that growers are forego area-wide coordinated Asian citrus psyllid (ACP) spraying when they believe most other growers are participating in area-wide coordinated spraying, increasing the spread of HLB, and that greater confidence in scientific information resulted in greater participation in area-wide ACP spraying that slowed the spread of HLB.

Trunk injection of commercial oxytetracycline for HLB management in young Grapefruit trees using a novel injection system

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Abstract: Huanglongbing (HLB) is considered one of the most devastating diseases to the global citrus industry. It has drastically reduced the yields in Florida, especially for grapefruits whereas the forecast for the 2022-2023 season is expected to be the lowest production since the 1911-1912 season. The disease is associated with the bacteria '*Candidatus* Liberibacter asiaticus' and vectored by the Asian citrus psyllid (*Diaphorina citri*). The pathogen resides in the phloem of infected trees making control of the disease difficult. Recently, in Florida, trunk injections of oxytetracycline (OTC) have been approved for the treatment of HLB-affected citrus trees. In this study, 3-year-old 'Ruby Red' grapefruit trees grafted on UFR-17 were injected with either of two different OTC formulations or with distilled water (control) once or twice during the 2023 season. The first injections occurred in February 2023 and the second injections occurred in June 2023 using a novel delivery system that does not require drilling and targets the outer ring of vascular tissue under the bark. Trees injected once received 75 mg of the active ingredient (OTC-HCL), and trees injected twice received 150 mg of the active ingredient. Tree uptake of OTC formulations was measured 2 and 7 days after each injection and leaves were collected to measure OTC residues 7, 14 and 30 days after each injection. Trees were monitored at the time of initial injections and monthly for disease index (DI) and canopy volume. The rootstock injection site of each tree was monitored for external damage. Preharvest fruit drop, yield, and fruit quality will be measured prior to or at the harvest. Tree uptake 7 days after injection of each OTC formulation was greater than 90% for all treatments in the first injection and was 100% for all treatments in the second injection. Visual improvement in tree health was observed in trees that received an OTC treatment independent of the OTC formulation that was used at 2, 3 and 4 months after injection. The rootstock injection site showed more external damage in trees treated with OTC compared to control.

Non-technical summary: Huanglongbing (HLB) is a devastating citrus disease that has drastically reduced the yields in Florida, especially for grapefruits. In this study, we injected oxytetracycline (OTC), in young 'Ruby Red' grapefruit using a non-drilled based system to evaluate the relationship among to different OTC brands, injection frequency and the improvement in tree horticultural characteristics.

Understanding and Engineering Symbiont Physiology with *Arabidopsis* Cell Identity Lines and Post Transcriptional Gene Silencing

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Abstract: Symbiont technology, which uses the ability of the soil-dwelling bacteria *Agrobacterium tumefaciens* to induce a mass of plant cells directly connected to plant stems, was developed as a means to treat systemic plant diseases, such as citrus greening, with continuous production and vascular delivery of therapeutic biomolecules to the affected host. This technology is new and little is known about the cellular biology, rapid growth, and organization of the host-derived cells that comprise the Symbiont. The *Arabidopsis thaliana* SAND lines help us understand the organization and function of Symbiont cells by specifically marking cells with similar transcriptional profiles to well-studied cell types. Our data show strong parallels in marker expression and cellular organization between Symbionts and root primordia. Efficient and controlled export of large macromolecules such as peptides and RNA is key to advancing the technology. Post transcriptional gene silencing in Symbionts can help us manipulate expression of specific proteins to improve export. Results show that silencing in Symbionts is efficient and genes can be turned off by expression of hairpins. Targets under study include genes involved in plasmodesmata function and cell signaling. Work is ongoing to test whether silencing signals can be exported and move systemically.

Non-technical summary: Symbiont technology is a platform to help plants fight off harmful diseases, such as citrus greening, by giving them therapies directly to the vascular tissue. Ensuring therapies continuously get to the right place in a citrus tree is vital to commercial delivery. Our research shows that Symbionts resemble plant roots during their development. This information will help us engineer Symbionts that express and export therapeutic molecules into the tree.

Use of engineered Symbiont tissue for the *in vitro* production of novel therapeutic molecules

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Abstract: Symbiont technology is a method of engineering plant tissue by agrobacterium transformation to generate a persistent but not self-sustaining structure that can produce complex biologically active molecules and export them into the plant. We have adapted this technology to produce hormone independent in vitro plant tissue cultures to rapidly produce therapeutic molecules in quantity. Trials were conducted to identify an efficient source of transformable donor plant tissue; including multiple species (*Nicotiana benthamiana*, *N. tabacum*, *Lactuca sativa*, *Daucus carota*, *Helianthus annuus*, *Solanum lycopersicum*, and numerous *Citrus spp*.), tissue types (imbibed seeds, seedlings, young whole plants, immature and mature leaves), and growth conditions (in vitro, controlled growth chamber, and enclosed greenhouse). In vitro produced *Nicotiana* spp. tissue was found most suited for both transformation and down-stream uses. Transformed plant tissue was selected using antibiotic resistance and hormone independence traits conferred by the base symbiont plasmid as well as by visual and fluorescent markers and then maintained on either solid media or liquid media in a temporary immersion system. Molecules produced in the system can be recovered directly from the tissue or by exporting into the liquid culture media. Purification can be facilitated by including an affinity tag to the product when possible. Both growth methods have been demonstrated to produce high quantities of functional products. Proof of concept and quantification was completed using a fluorescent MCherry fusion protein. The system was used to produce a functional binding anti-covid nanobody. This method of production is suitable for experimental purposes and potentially scalable to industrial use.

Non-technical summary: Making organic molecules for experimental and therapeutic purposes is difficult to do economically. To address this, we have adapted Symbiont technology used to deliver molecules to plants and turned it into an in vitro molecule producing system. This method of production is economically viable and potentially scalable to industrial use.

Using Citrus tristeza virus (CTV) to identify anti-microbial peptides that limit '*Candidatus* Liberibacter asiaticus' (Clas) replication

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Abstract: The citrus industry in Florida is facing the debilitating challenge of huanglongbing (HLB) disease associated with infection by '*Candidatus* Liberibacter asiaticus' (CLas) bacteria. There are challenges in working with HLB: *in vitro* and *in vivo* screening of therapeutics that target the bacteria directly are hindered by the lack of pure culture for CLas, phloem tissue tropism makes bactericidal applications very challenging, and the recalcitrant nature and lengthy time required to produce transgenic citrus trees. CTV colonizes phloem tissue where the CLas bacteria reside without interacting synergistically in mixed infections in citrus trees. CTV-T36 field isolates as well as the CTV-T36 infectious clone-initiated infections are systemic but avirulent in tested host ranges and could be engineered to modulate the overexpression of an extra gene directly into the citrus phloem for many years. Thus, CTV overexpression vectors are used to target the bacteria directly by modulating the expression of small antimicrobial peptides (AMPs) ranging in size from 10 to 70 amino acids. We have screened the HLB phenotype of sweet orange sets infected with CTV vectors expressing more than 100 AMPs independently. CTV-AMP protected plants are not immune to HLB disease. Few CTV expressed peptides provide tolerance when compared to control plants. When AMPs are successful under high HLB + Asian citrus psyllid inoculum pressure, CTV-AMP protected plants reveal the HLB phenotype on some branches; however, unlike control plants that cease to grow and later die, CTV-AMP protected plants recover and have new growth free of HLB phenotype despite being Clas positive by qPCR. Due to the small size of AMPs which will be expressed from CTV for very long periods of time, this approach will not only provide a screening tool but will also provide a bridge to fill the time gap until a permanent resistant plant is developed.

Non-technical Summary: Antimicrobial peptides kill bacteria. Since the '*Candidatus* Liberibacter asiaticus' (CLas) bacteria is located within the phloem, it is very difficult to reach by spray application. However, when an antimicrobial peptide is added as an extra gene cassette into Citrus tristeza virus, it will be delivered to the phloem tissue of citrus where it kills the CLas bacteria, mitigating the effects of huanglongbing disease.

Using Citrus tristeza virus (CTV) to limit ACP survival and reproduction on citrus

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Abstract: A major contributing factor to the rapid spread of huanglongbing (HLB) disease is the insect vector, Asian citrus psyllid (ACP). ACP populations have flourished in Florida since 1998. Calendar insecticide spraying programs to control ACP failed to prevent the spread of HLB to all commercial groves, resulting in an infection rate of almost 100% of trees in Florida. Further, insecticide resistant ACP populations are emerging. An environmentally friendly approach is to exclusively target ACP populations via RNA interference (RNAi). Truncated RNA of 20 ACP mRNA target sequences were introduced into CTV independently or in combination. Upon infection of citrus by CTV, the virus replication will produce an abundant amount of dsRNA and truncated RNA that will trigger the RNAi machinery of the plant to produce small 21-23 RNA pieces. Different stages of ACP will acquire it from citrus while feeding, which triggers the ACP RNAi machinery to eliminate its mRNA. We successfully identified several ACP targets that almost completely prevent ACP from establishing on citrus. For example, control plants will have 200-300 progeny adults emerging from 25 input psyllids allowed to mate for 14 days, whereas CTV-RNAi protected plants will have few if any progeny. The drawback of CTV delivered RNAi technology is that it fails in some bioassay replicates. Another environmentally friendly approach to control ACP is the use of modified pesticidal proteins expressed by *Bacillus thuringiensis* bacteria. Using CTV overexpression vectors, we identified one protein that greatly affects ACP reproduction on citrus seedlings. We believe the two technologies will synergistically interact to prevent the establishment of ACP on citrus and are working to combine the delivery of the two technologies into a single plant.

Non-technical summary: The efficient spread of '*Candidatus* Liberibacter asiaticus' bacteria by its insect vector is a major contributor to the huanglongbing epidemic. Thus, preventing Asian citrus psyllid (ACP) establishment on citrus will limit acquisition and spread of the bacteria. We use Citrus tristeza virus RNAi and overexpression vectors to specifically target ACP phloem diet to limit its reproduction.

Viroid TsnRNA-IIIb reduces citrus apical shoot growth and overall canopy size. Can it help citrus growers manage HLB?

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Abstract: 'Transmissible small nuclear Ribonucleic acids' (TsnRNAs) are viroid RNA species that modify tree performance in specific citrus hosts. TsnRNA-IIIb can reduce the canopy volume of navel orange trees on trifoliate rootstock (approx. 50%), increase yield per canopy volume, and concentrate fruit in the optimum canopy zone for faster harvest times without affecting fruit quality. To observe the long-term effects of TsnRNA-IIIb on canopy volume, a survey of navel orange tree growth was conducted in an experimental block planted in 1998 at the UC ANR Lindcove Research and Extension Center, Exeter, CA. Apical vegetative shoot growth of TsnRNA-IIIb-treated trees was reduced by almost 20% compared to the non-treated controls. Dwarfed trees are fundamental for high-density plantings, which can be critical to meeting challenges posed by disease spread, water shortages, farmland reduction, and increasing labor costs. Furthermore, high-density plantings of dwarfed trees could play a role in mitigating the cost and enhancing huanglongbing (HLB)-management. To assess the commercial potential of this technology, we began a comprehensive analysis of the main horticultural practices, including pest inspections and fruit harvest, to determine empirically whether these practices require less time in dwarfed trees resulting in reduced costs. Comprehensive fruit quality analyses and water-use efficiency assessments of the dwarfed trees were also performed. Lastly, we conducted a cost-benefit analysis of TsnRNA-IIIb dwarfed trees. Findings from these assessments will be presented and their implications for HLB management discussed.

Non-technical summary: High-density plantings of dwarfed citrus trees with small canopies will be advantageous for different elements of huanglongbing (HLB) management. Canopy geometry and density affect psyllid colonization, life cycle, and bacteria transmission as well as HLB surveys and tree sampling for laboratory testing. Finally, high-density plantings of dwarfed citrus trees can offer an economic advantage and are excellent candidates for citrus production under protective structures.