

ISPVP 2026 ABSTRACT BOOK

International Symposium for Plant Vascular Pathosystems
October 20–23, 2026 · South Padre Island, Texas

Contents

Wednesday, October 21 — Session 1: Vector Biology, Epidemiology, and Diagnostics

1. Leonardo De La Fuente — Keynote Speaker — **Adaptive strategies of a xylem-limited bacterium for survival in the plant vascular system** 4
2. Judith K. Brown — Invited Speaker — **Multimomics interrogation of psyllid vector-”*Ca. Liberibacter*” interactions in divergent co-evolved pathosystems** 5
3. Alisson da Silva Santana — Oral Presentation — **Spatial Distribution of the Corn Leafhopper (*Dalbulus maidis*) within Corn Fields in South** 6
4. Surender Kumar — Oral Presentation — **Development of a Cas13a-based Rapid, Grower-Friendly Detection Tool for Tomato Spotted Wilt Virus** 7

Wednesday, October 21 — Session 2: Molecular Host–Pathogen Interactions and Biotechnology

5. Baoye He — Invited Speaker — **Extracellular Vesicles as a Root-To-Shoot Communication System Triggered by Root Microbiota** 8
6. Pankaj Kumar Yadav — Oral Presentation — **Fungal sRNAs Target Plant Mitochondria to Suppress Host Immunity** 9
7. Maryam Khalilzadeh — Oral Presentation — **Role of the Membrane Contact Site Protein SYNAPTOTAGMIN A During *Candidatus Liberibacter asiaticus* Phloem Infection** 10
8. Chi-Kuan Tu — Invited Speaker — **A Multiplex CRISPR-Based Genome Editing Strategy to Identify Citrus Immune Regulators that Confer Resistance to Huanglongbing (HLB)** 11
9. Manikandan Ramasamy — Oral Presentation — **Hairy Root Platform Enables Antimicrobial Discovery Against Fastidious *Candidatus Liberibacter* species and Field Validation in HLB-Affected Citrus**..... 12

Thursday, October 22 — Session 3: Pathogenesis, Plant Physiology, and Disease Tolerance

10. Gitta Coaker — Keynote Speaker — **Spatial and Structural Strategies of Xylem- and Phloem-Colonizing Bacterial Pathogens** 13
11. Nian Wang — Invited Speaker — **Mechanistic dissection of *Candidatus Liberibacter* Triggered Chronic Immune Disease** 14
12. Amit Levy — Invited Speaker — **Fitting The Pieces into A Whole Picture: Physiology and Pathogenesis in Huanglongbing Disease** 15
13. Maryam Khalilzadeh — Oral Presentation — **HLB tolerance is associated with maintained carbon**

export despite callose accumulation	16
---	----

Thursday, October 22 — Session 4: Integrated Disease and Pest Management Strategies

14. Ute Albrecht — Invited Speaker — Integrating different Horticultural Practices to manage endemic Huanglongbing	17
15. Chuanyu Yang — Oral Presentation — Trunk Injection of Oxytetracycline for the Management of Citrus Huanglongbing and Its Effects on the Citrus Microbiome in South Texas	18
16. Flavia Campos Vieira — Oral Presentation — Identification and characterization of secondary metabolites of citrus-associated <i>Bacillus</i> spp. inhibitory to the Huanglongbing pathogen, ‘<i>Candidatus Liberibacter asiaticus</i>’ and citrus root pathogens	19
17. Waqar Shafqat — Oral Presentation — Optimization of Phenology-Based Oxytetracycline Trunk-Injection Timing to Suppress <i>Candidatus Liberibacter asiaticus</i> Titer and Improve the Health of HLB-Affected Citrus Trees	20
18. William Anderson — Oral Presentation — Engineering ihpRNAi Constructs for RNAi-Mediated Resistance to Grapevine Leafroll-associated Virus 3	21
19. Jacobo Robledo — Oral Presentation — Predicting tree-level yield in an endemic vascular pathosystem: stable eco-physiological, nutritional, and climatic predictors of ‘Hamlin’ orange under Huanglongbing	22
20. Jawwad Qureshi — Oral Presentation — Citrus tree protection and production against the citrus pest complex	23

Poster Presentations

21. Carmen Padilla — Poster Presentation — Spinach-Derived Antimicrobial Peptides as a Novel Strategy for Tolerance to Citrus Greening and Potato Zebra Chip Diseases	24
22. Ashley Zavala — Poster Presentation — Changes In Vector Feeding Behavior Arising from Application of Heat are Detectable Using Signal Analysis Techniques	25
23. Qiuni Yang — Poster Presentation — Investigating Pierce’s Disease (<i>Xylella fastidiosa</i>) Tolerance and Resistance in Historical Grapevine (<i>Vitis</i> spp.) Cultivars Developed by T. V. Munson ...	26
24. Damodar Pokhrel — Poster Presentation — Evaluation of trunk-infusion volume and site of application on the uptake of oxytetracycline (OTC) by HLB affected grapefruit (<i>Citrus × paradisi</i>) trees	27
25. Nancy Her — Poster Presentation — Lipopolysaccharide structure influences the biogenesis and cargo selection of <i>Xylella fastidiosa</i> outer membrane vesicles	28
26. Mia Acker — Poster Presentation — Complete genomes reveal metabolic differences between Lethal Bronzing Phytoplasma, co-recovered <i>Candidatus Uzinura</i>, and phloem-limited pathogens 29	
27. Aditya Kulshreshtha — Poster Presentation — Hairy Root Transformation in <i>Citrus medica</i> L.: An Efficient Platform for HLB Therapeutics Discovery	30

28. Junseong Kang — Poster Presentation — **Development of a Viability qPCR Assay for *Fusarium oxysporum* f. sp. *vasinfectum* Race 4** 31

Adaptive strategies of a xylem-limited bacterium for survival in the plant vascular system

De La Fuente, Leonardo ^{1,*}, R. Liu¹, N. Kaur¹, M.V. Merfa², M. KC¹, N. Potnis¹

¹Department of Entomology and Plant Pathology, Auburn University, Auburn, Alabama, USA.

²Department of Microbiology, University of Tennessee-Knoxville, Knoxville, Tennessee, USA.

*CORRESPONDENCE: Leonardo De La Fuente, lzd0005@auburn.edu.

Xylella fastidiosa is a xylem-limited plant pathogenic bacterium that is transmitted exclusively by xylem sap-feeding insect vectors. This pathogen is known to infect more than 700 plant species, although many of them do not develop symptoms. Pierce's disease in grapevine has been reported in the United States for approximately 150 years; however, in recent decades, this and other emerging diseases caused by *X. fastidiosa* have been detected in other parts of the world. Although historically most cases were recorded in the Americas, its presence has been detected in Europe and Asia over the past decade, most notably causing the devastating olive quick decline syndrome in southern Italy. This bacterium is one of the few known plant pathogens restricted to the xylem, which is why we are interested in understanding the molecular and microbiological features that allow it to successfully survive inside xylem vessels. We are studying the process of natural competence—the ability of a bacterium to acquire and recombine DNA fragments from its environment—because of its potential role in niche adaptation and in the evolution of *X. fastidiosa*. In the search for genes that determine host specificity, we have examined pseudogene composition across diverse genomes, demonstrating that genotypes differ in their generalist or specialist lifestyle. The in vitro growth of strains in media enriched with sap was used as an indicator to understand the host range of emerging pathogenic strains worldwide. Understanding the strategies *X. fastidiosa* employs to live exclusively in the xylem will help develop targeted approaches to attack vulnerable aspects of this pathogen to control the disease.

Multiomics interrogation of psyllid vector-*Ca. Liberibacter* interactions in divergent co-evolved pathosystems

Judith K. Brown

School of Plant Sciences, The University of Arizona, Tucson, AZ 85721

CORRESPONDENCE:

J.K. Brown

jkbrown@arizona.edu

“Candidatus (Ca.) Liberibacter solanacearum” (CLso) is the causal agent of zebra chip and vein-greening disease of potato and tomato, respectively, and is transmitted by the potato psyllid (PoP) *Bactericera cockerelli* (Sulc.) in a circulative, propagative manner. Comparisons between the CLso-PoP and citrus greening pathosystem, *“Ca. Liberibacter asiaticus” (CLas)* and its vector, the Asian citrus psyllid (ACP), indicate they share some similar and unique biological characteristics. In both, *“Ca. Liberibacter”*-psyllid pathogenesis involves gut invasion, propagation, circulation, and salivary glands (SGs)-mediated acquisition, and results in disruption of physiological processes especially notable in the late-nymphal and teneral adult stages pre- and/or post-SGs invasion and/or subsequent acquisition. In ACP, CLas must be ingested by the earliest nymphal stages for efficient transmission by the ACP adults. For PoP also both immature and adult life stages can acquire and transmit CLso, post-ingestion. To begin to understand the organ-, cellular- and molecular mechanisms surrounding *“Ca. Liberibacter”* spp. invasion of the psyllid host gut and SGs-acquisition, parallel comparative transcriptomic, proteomic, yeast di-hybrid, and/or TEM analyses were undertaken. Functional genomics studies have focused on characterizing pathosystem-specific nuances of gut invasion, systemic infection/circulation, and SGs acquisition. In both pathosystems *“Ca. Liberibacter”* utilizes psyllid-host/vector endo-exocytotic pathways and cytoskeletal remodeling to invade, colonize, and exit the psyllid gut. New knowledge about the involvement of specific genes, pathways, and networks that connect organ-specific navigation will further the understanding of fundamental mechanisms and processes that govern vector-pathogen specificity among fastidious bacteria that have co-evolved with phylogenetically-divergent hemipteran (s.o. homopteran) vectors.

Spatial Distribution of the Corn Leafhopper (*Dalbulus maidis*) within Corn Fields in South

Texas

Alisson da Silva Santana^{1,2*} Ana Paula Santana Lima¹, Alberto D. Salinas¹, Cindy J. Rodriguez¹, and Anna V. Cantu¹

¹ Texas A&M AgriLife Research and Extension Center, Weslaco, TX, United States

² Department of Entomology, Texas A&M University, College Station, TX, United States

* Corresponding author: Alisson da Silva Santana (alisson.santana@ag.tamu.edu)

The corn leafhopper (CLH, *Dalbulus maidis*) (Hemiptera: Cicadellidae), is an important sap sucking pest of corn in the Americas and an emerging threat to corn production in the United States. In addition to direct feeding damage, CLH transmits several pathogens associated with corn stunt disease, including corn stunt spiroplasma (CSS), maize bushy stunt phytoplasma (MBSP), and maize rayado fino virus (MRFV). Understanding the spatial distribution of CLH within corn fields is important for improving pest monitoring, risk assessment, and management strategies. Therefore, this study evaluated the horizontal distribution of CLH within corn fields in South Texas. Leafhoppers were vacuum sampled along transects extending from the field edges toward the interior, with sampling points established at increasing distances from the field edge toward the center of the field. Sampling was conducted in 12 corn fields across the Rio Grande Valley of Texas. The spatial distribution of CLH showed an aggregated pattern, with higher densities concentrated near field edges and decreasing densities toward the field interior. These results indicate that CLH distribution within corn fields is not uniform and that field edges may represent important areas for early detection and monitoring. Understanding this spatial pattern may help improve scouting efficiency and inform sampling strategies for CLH and the pathogens it transmits.

Development of a Cas13a-based Rapid, Grower-Friendly Detection Tool for Tomato Spotted Wilt Virus

Surender Kumar^{1,2}, Kiran R. Gadhave^{1,2*}

¹ Texas A&M AgriLife High Plains Research and Extension Center, Canyon, TX 79015

² Department of Entomology, Texas A&M University, College Station, TX, 77843

* CORRESPONDENCE

Kiran R. Gadhave

kiran.gadhave@ag.tamu.edu

Tomato spotted wilt virus (TSWV), transmitted by western flower thrips, causes significant disease in several economically important crops, resulting in estimated billions of dollars losses worldwide. The widespread cultivation of resistant varieties has contributed to the emergence of resistance-breaking strains in several parts of the world. Since current nucleic acid and protein based tools are time consuming, expensive and require special equipment and expertise, there is an urgent need for reliable, rapid, and grower-friendly tools for TSWV detection and management.

In this study, we developed a simple, rapid and grower-friendly TSWV detection tool using a Cas13a-mediated detection platform. The developed method can detect TSWV within 15 minutes using only a portable fluorometer. Among the crRNAs designed against different viral genes, the crRNA targeting the N gene demonstrated higher sensitivity than those targeting the NSm gene, potentially due to the higher transcript abundance of the N gene. The developed assay was capable of detecting 100 pg of viral RNA comparable to routinely used nucleic acid-based detection methods. Importantly, the assay successfully detected TSWV from both purified and crude RNA preparations and was validated using samples from *Nicotiana benthamiana*, tomato, pepper, and thrips. The assay worked well in temperatures ranging from 30-40°C and can detect infection as early as 5 dpi, demonstrating its applicability in a suite of conditions. These findings demonstrate the potential of the Cas13a-based platform as a rapid, accurate, sensitive, portable, and user-friendly diagnostic tool for on-site TSWV detection, enabling timely disease diagnosis and management in the field.

Extracellular Vesicles as a Root-To-Shoot Communication System Triggered by Root Microbiota

Pei-Cheng Huang¹, Pankaj Kumar Yadav¹, Joseph Edwards¹, Baoye He^{1*}

¹ *Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX, United States*

***CORRESPONDENCE**

Baoye He

Beneficial root microbiota can promote plant growth in both colonized roots and distant aboveground tissues, but the mechanisms connecting root–microbe interactions to systemic plant responses remain poorly understood. Extracellular vesicles (EVs) selectively package and protect molecular cargo, suggesting that they may facilitate long-distance signaling through the plant vascular system. We developed a beneficial synthetic microbial community (SynCom) comprising root-associated bacterial strains originally isolated from switchgrass and selected for their reproducible plant growth-promoting activity. Inoculation with this SynCom promoted growth in maize and *Arabidopsis*. Importantly, SynCom inoculation also increased the abundance of plant EVs isolated from maize xylem sap, supporting a potential link between beneficial microbial perception and vascular EV production. Based on these findings, we propose that SynCom-derived signals are perceived by plant receptors and activate EV biogenesis, secretion, and selective cargo loading. The resulting EVs may enter the xylem and transport growth-promoting molecular cargo from roots to shoots. Ongoing work is identifying the microbial signals and plant receptors that regulate this response and characterizing SynCom-induced changes in the protein and small RNA cargo of maize xylem EVs. This research reveals a previously unexplored mechanism through which beneficial root microbiota may regulate plant vascular communication and systemic growth.

Fungal sRNAs Target Plant Mitochondria to Suppress Host Immunity

Pankaj Kumar Yadav¹, Baoye He^{1*} ¹*Department of Plant Pathology and Microbiology, Texas A&M University*

**CORRESPONDENCE*

Baoye He

baoye.he@ag.tamu.edu

Fungal pathogens such as *Botrytis cinerea* deliver small RNAs (sRNAs) into plant cells to silence host immune response genes through RNA interference in the plant cytoplasm. However, whether fungal sRNAs can also target plant mitochondria and regulate mitochondrial gene expression to suppress plant immunity remains unknown. In this study, we identified 11 distinct *B. cinerea* sRNAs that enriched in the mitochondrial fraction during infection by sRNA sequencing. To validate the fungal sRNAs identified in mitochondria, we performed stem-loop RT-PCR on RNA extracted from immunopurified mitochondria from infected plant tissue, and consistently detected high levels of fungal sRNA3, sRNA4, and sRNA5. These sRNAs remained detectable after enzymatic treatment to remove surface-associated RNAs, supporting their localization inside plant mitochondria. Gene expression analysis of mitochondrial genes revealed progressive downregulation after fungal infection, suggesting that fungal sRNAs may contribute to the suppression of gene expression in plant mitochondria. Our current efforts are focused on elucidating the molecular mechanisms underlying (1) fungal sRNAs-mediated suppression of mitochondrial gene expression, (2) suppression of plant immunity through fungal sRNA-mediated modulation of mitochondrial function, (3) the molecular mechanism of sRNA import into plant mitochondria. Collectively, our findings will provide evidence for a novel pathway of cross-kingdom sRNA mitochondrial regulation during fungal infection and lay the foundation for possible RNA-based disease management strategies.

Keywords: *Botrytis cinerea*, Cross-Kingdom Communication, Fungal sRNAs, Gene regulation, Mitochondria

Role of the Membrane Contact Site Protein SYNAPTOTAGMIN A During *Candidatus Liberibacter asiaticus* Phloem Infection

Chiara Bernardini^{1,2}, Stacy Welker¹, Donielle Turner¹, Christopher Vincent^{1,3}, Maryam Khalilzadeh^{1,3,*} and Amit Levy^{1,4}

¹*Citrus Research and Education Center, University of Florida, Lake Alfred, FL, 33850, USA*

²*Department of Agriculture, Food, Animal and Environment, Università degli Studi di Udine, Udine, Italy*

³*Department of Horticultural Sciences, University of Florida, Gainesville, FL, United States.*

⁴*Department of Plant Pathology, University of Florida, Gainesville, FL, United States.*

***CORRESPONDENCE**

Maryam Khalilzadeh

Email: mkhalilzadeh@ufl.edu

Citrus greening (or Huanglongbing; HLB) is a devastating citrus disease caused by the phloem localized gram-negative bacteria *Candidatus Liberibacter asiaticus* (CLAs). Gene expression and western blot analyses showed that SYNAPTOTAGMIN A (SYTA) is highly induced after CLAs infection. SYTA is a protein involved in the formation of ER-plasma membrane (PM) membrane contact sites (MCSs). MCSs are regions in which two membranes come into close contact without fusing. MCSs are characterized as sites for membrane trafficking and Ca²⁺ fluxes, being Ca²⁺ dependent, and resealing membranes after damage. Besides its role in the MCSs formation, SYTA also plays a role in the movement and replication of plant viruses, and plants with downregulated SYTA levels showed enhanced resistance to diverse viruses, including ones that are phloem limited. Here, we studied SYTA roles in the phloem and during CLAs infection. In *Arabidopsis*, SYTA::RFP co-localized with SUC2::GFP, confirming its presence in *Arabidopsis* sieve elements (SEs). In *syta-1* mutants, SEs often appeared damaged. The export of ¹⁴C-compounds from rosette leaves was higher in WT plants than in *syta-1* mutants. In citrus (*Citrus sinensis*), downregulation of *CsSYTA* levels by RNAi with the phloem localized *citrus tristeza virus* (CTV) delayed the onset and severity of HLB symptoms. Phloem plugging genes were downregulated, and the area of callose deposits was lower. We suggest that SYTA plays a role in maintaining the function of the SEs, and in delaying CLAs infection, probably by disturbing host-pathogen interactions.

A Multiplex CRISPR-Based Genome Editing Strategy to Identify Citrus Immune Regulators that Confer Resistance to Huanglongbing (HLB)

Chi-Kuan Tu^{1,2} and Kranthi K. Mandadi^{1,2,3}

¹Texas A&M AgriLife Research and Extension Center, Weslaco, TX, USA ²Dept. Plant Pathology and Microbiology, Texas A&M University, College Station, TX, USA ³Institute for Advancing Health Through Agriculture, Texas A&M AgriLife, College Station, TX, 77843, USA

*CORRESPONDENCE

Kranthi K. Mandadi

e-mail: kkmmandadi@tamu.edu

Huanglongbing (HLB), associated with the bacterium *Candidatus Liberibacter asiaticus* (CLas), remains the most significant threat to global citrus production, causing billions of dollars in economic losses worldwide. While current chemical therapies provide only temporary relief, long-term sustainability of the citrus industry relies on the identification of durable genetic resistance. Here, we establish a high-throughput functional genomics platform to rapidly identify and validate citrus immune regulators that confer resistance to HLB. This system integrates multiplex CRISPR-based genome editing with citrus transgenic hairy root induction for rapid gene function screening. To enhance editing efficiency, we employed a three-level Golden Gate Assembly (MoClo) strategy to construct polycistronic vectors capable of simultaneously targeting multiple genes. Visual reporters, including RUBY and GFP, enable non-invasive identification of successfully transformed tissues. Using bioinformatics and phylogenetic analyses, we systematically identified candidate immune regulators in the citrus genome potentially involved in HLB susceptibility or tolerance. Target-specific sgRNAs were designed for key regulatory gene groups, and genome editing was validated by Sanger sequencing. Quantification of CLas titers in edited hairy roots by qPCR revealed that knockout of several candidate genes significantly alters bacterial accumulation. Together, this integrated platform provides a powerful and scalable approach to dissect citrus/CLas interactions, accelerating the discovery of genetic targets for developing HLB-resistant citrus cultivars.

Hairy Root Platform Enables Antimicrobial Discovery Against Fastidious *Candidatus Liberibacter* species and Field Validation in HLB-Affected Citrus.

Manikandan Ramasamy^{*,1}, Chuanyu Yang², Sonia Irigoyen¹, Lakshmi Akhijith Pasupuleti²

Veronica Ancona² and Kranthi K. Mandadi^{1,3,4, #}

¹Texas A&M AgriLife Research & Extension Center, Texas A&M University system, Weslaco, TX, USA

²Citrus Center, Texas A&M University-Kingsville, Weslaco, TX 78599, USA

³Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX, USA

⁴Institute for Advancing Health Through Agriculture, Texas A&M AgriLife, College Station, TX, USA

* Presenting author

Corresponding author: kkmmandadi@tamu.edu

ABSTRACT

Candidatus Liberibacter species are phloem-limited, fastidious bacterial pathogens that cause economically destructive diseases, including citrus greening (Huanglongbing, HLB) and potato zebra chip (ZC). The inability to culture them axenically impedes the discovery and testing of effective therapeutics. To overcome this, we developed a *Rhizobium rhizogenes*-induced hairy root platform that supports the culturing of *Candidatus Liberibacter asiaticus* (CLAs) in citrus and *Candidatus Liberibacter solanacearum* (CLso) in potato, enabling scalable antimicrobial screening and functional studies of these pathogens. Screening over 220 chemically diverse libraries on this platform identified multiple compounds with strong anti-CLas activity, including small molecules and transcriptional regulatory inhibitors. Among these, C3, a naturally derived lead compound, was prioritized for field validation due to its strong efficacy and potential advantages over conventional antibiotics, such as oxytetracycline, including a reduced risk of promoting antibiotic resistance. In two-year trunk-injection trials with HLB-affected citrus orchards in Texas, C3 significantly improved canopy health, reduced disease severity, and increased fruit yield compared to untreated controls, demonstrating durable field efficacy and successful translation from laboratory discovery to practical application. This work establishes a comprehensive pipeline for culturing fastidious *Candidatus Liberibacter* pathogens, discovering and validating new therapeutics, and accelerating sustainable strategies for the management of vascular bacterial diseases.

Spatial and Structural Strategies of Xylem- and Phloem-Colonizing Bacterial Pathogens

Charis Ramsing¹, Phoebe Sinner¹, Tianrun Li^{1,2}, Esteban Jarquin-Bolanos^{1,3}, Kerry Mauck⁴, Gitta Coaker^{1*}

¹ *Department of Plant Pathology, University of California, Davis, Davis, CA, United States*

² *Department of Biology, University of Oxford, Oxford, United Kingdom*

³ *Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA, United States*

⁴ *Department of Entomology, University of California, Riverside, Riverside, CA United States*

*CORRESPONDENCE

Gitta Coaker

Vascular tissues provide conduits for systemic colonization but also impose specialized spatial and nutritional constraints. Using spatiotemporal mapping of *Clavibacter michiganensis* infection in tomato, we show how this vascular pathogen escapes the xylem between lignin rings, enters the surrounding apoplast, and moves inside host cells, enabling cell-to-cell spread and access to plant nutrients. We also investigated phloem-colonizing, vector-borne pathogens through a broad survey of their secreted effectors. Although effectors often lack detectable sequence similarity, structural comparisons revealed a conserved effector supercluster shared across vector-borne bacterial pathogens of plants. Functional studies identified supercluster members that manipulate plant development. Consistent with this activity, *Candidatus Liberibacter solanacearum* alters tomato architecture and extends host longevity, suggesting that developmental and physiological reprogramming are a broad feature of infection by vector-borne pathogens. Together, these studies highlight both compartment specific invasion mechanisms and effector driven strategies enabling plant colonization that reshape host biology.

Mechanistic dissection of *Candidatus Liberibacter* Triggered Chronic Immune Disease

Nian Wang

Citrus Research and Education Center, Department of Microbiology and Cell Science, University of Florida, Lake Alfred, FL 33850, U.S.A.

Email: nianwang@ufl.edu

Citrus Huanglongbing (HLB) caused by the phloem colonizing bacterial pathogen *Candidatus Liberibacter asiaticus* (CLas) was suggested to be a pathogen triggered chronic immune disease. However, the genetic evidence and mechanistic understanding for such a disease model is lacking. Here, we show CLas triggers phloem cell death, reactive oxygen species (ROS) production and callose deposition in photosynthesis tissues, but little or none in non-photosynthesis tissues of citrus. We further demonstrate that CLas triggers ROS production in chloroplasts. Overexpression of *flavodoxin* (*Fld*), an electron shuttle, in chloroplasts reduced ROS production, cell death and HLB symptoms, but not phloem callose deposition induced by 26 CLas. Knockout, silencing, and overexpression of phloem callose synthase genes *CsCalS7a*, *27 CsCalS7b*, or *CsCalS7c* demonstrated that phloem callose deposition also caused phloem cell death. Trunk injection with 2-deoxy-d-glucose, a callose deposition inhibitor, reduced fruit drop and increased fruit yield of HLB symptomatic trees in field trials. Together, our data demonstrate that CLas-triggered chloroplastic ROS production and phloem callose deposition, which are typical immune responses, are the important factors for HLB disease development. Using tomato-*Ca. L. psyllaureus* (Lpsy) as a model, knockout of *Eds1* and *Pad4* but not *RbohB*, *Bik1* and *Sobir1* abolished ROS production, phloem callose deposition, cell death and disease damages caused by Lpsy. This study provides genetic evidence for *Ca. Liberibacter*-triggered immune disease.

Fitting The Pieces into A Whole Picture: Physiology and Pathogenesis in Huanglongbing Disease

Maryam Khalilzadeh, Rashmi Kumari, Dorcas Olufunke Alade, Zhonglin Mou, Christopher Vincent, Amit Levy

Amit Levy, Plant Pathology Department, Citrus Research and Education Center, IFAS, University of Florida, Lake Alfred, FL, 33850, United States.

Amitlevy@ufl.edu

Despite intensive efforts to characterize HLB disease, some aspects about its mechanism still remain elusive. In a recent review paper (Khalilzadeh et al., 2026), we proposed that although HLB is characterized as a phloem-associated disease, causing carbohydrate transport dysfunction, evidence indicates that its effects extend far beyond vascular obstruction. The disease involves complex disruptions in multiple interconnected physiological processes, beginning with phloem transport, carbohydrate allocation, and source-sink relationships and ending with photosynthesis, water relations, and hormonal regulation. In addition, while recent studies propose that HLB functions as a pathogen-triggered immune disease, in which the plant immune response is the primary reason of disease, other evidence indicates that HLB immune response is required for disease tolerance, which involves multiple interacting physiological and pathological processes. Building on this framework, this presentation will synthesize current knowledge of the pathological and physiological effects of HLB on citrus function and emphasize traits associated with HLB tolerance. Collectively, this framework supports an integrated view of HLB as a complex systemic vascular and physiological disease resulting from dynamic interactions among bacterial colonization, host defense responses, and disruption of whole-plant physiological homeostasis.

Khalilzadeh M, Kumari R, Alade DO, Levy A, Vincent C (2026) Fitting the pieces into a whole picture: physiology and pathogenesis in huanglongbing disease. *Physiological and Molecular Plant Pathology* 146: 103476

HLB tolerance is associated with maintained carbon export despite callose accumulation

Maryam Khalilzadeh^{1*}, Zhonglin Mou², Flavia Tabay Zambon³, Amit Levy⁴ and Christopher Vincent¹

¹*Department of Horticultural Sciences, Citrus Research and Education Center, IFAS, University of Florida, Lake Alfred, FL, United States.*

²*Department of Microbiology and Cell Sciences, University of Florida, Gainesville, FL, United States.*

³*Department of Horticultural Sciences, Indian River Research and Education Center, IFAS, University of Florida, Fort Pierce, FL, United States*

⁴*Department of Plant Pathology, Citrus Research and Education Center, IFAS, University of Florida, Lake Alfred, FL, United States.*

*CORRESPONDENCE

Maryam Khalilzadeh

Email: mkhalilzadeh@ufl.edu

Huanglongbing (HLB) disrupts phloem transport in citrus, causing carbohydrate accumulation in source leaves and reduced carbon movement to sink tissues. HLB-tolerant genotypes maintain better growth and productivity than susceptible genotypes, suggesting greater preservation of phloem transport during infection. To examine this, we measured the export of ¹⁴C-labeled sucrose from leaves in two pairs of citrus genotypes with contrasting HLB tolerance. In both pairs, tolerant plants exported more ¹⁴C-labeled sucrose than susceptible plants, linking sustained carbon export with HLB tolerance. Because callose accumulation is associated with phloem blockage in HLB-infected citrus, we next tested whether callose differences explained this contrasting export capacity. Surprisingly, the tolerant genotype accumulated more callose while maintaining greater carbon export, indicating that callose abundance does not necessarily reflect phloem impairment. We then tested whether callose contributes to reduced export in susceptible citrus by applying a callose inhibitor. Inhibiting callose formation increased ¹⁴C-labeled sucrose export, showing that callose limits transport in the susceptible background. Lastly, because susceptible genotypes harbor higher bacterial populations in sink tissues, we examined whether pathogen accumulation contributes to impaired sugar transport by simultaneously targeting callose and the pathogen. Pathogen presence in the sink was an additional transport-limiting factor. Together, these results show that both callose and pathogen contribute to impaired carbon export in susceptible citrus, but callose abundance alone does not explain differences in phloem transport among genotypes. Instead, HLB tolerance appears to be associated with the ability to maintain carbon export despite callose accumulation.

Integrating different Horticultural Practices to manage endemic Huanglongbing

U. Albrecht*, C. Tardivo, L. Nunes, G. Pugina

University of Florida/IFAS, Horticultural Sciences Department, Southwest Florida Research and Education Center, Immokalee, FL, USA

*Corresponding author: ualbrecht@ufl.edu

Citrus production in Florida has declined by more than 90% since the discovery of huanglongbing (HLB). In Florida, HLB is associated with *Candidatus Liberibacter asiaticus* vectored by the Asian Citrus psyllid (*Diaphorina citri*). Although HLB is the primary reason, hurricanes and freezes have exacerbated production losses. Novel management practices are needed to restore tree health and productivity and ensure continuity of an industry on the brink of collapse. One such practice is the use of individual protective covers (IPCs), which restrict vector access to the trees. In addition to reducing the need for insecticides, the covers reduce heat and radiation while creating a microclimate that promotes vegetative growth. As IPCs must be removed once trees become too large, complementary strategies are needed to ensure tree health and productivity. In 2022, the systemic delivery of oxytetracycline (OTC) by trunk injection was approved for HLB management in Florida. Field studies have demonstrated significant increases in production after only one injection and cumulative increases after two or more consecutive years of application. Moreover, OTC injections enhance fruit and juice quality, benefiting both the juice and fresh fruit market. Another strategy to promote citrus tree health and productivity is the use of rootstocks carefully selected for specific growing conditions and the desired influence on the scion. Here we present field-based evidence for the efficacy of integrating these practices to ensure the survival of an industry threatened by disease and environmental stress.

Trunk Injection of Oxytetracycline for the Management of Citrus Huanglongbing and Its Effects on the Citrus Microbiome in South Texas

Chuanyu Yang¹, Lakshmi Akhijith Pasupuleti^{1,2}, Raelene Mendez¹, Tejesh Chowdary Kolla¹, Kranthi Mandadi^{2,3,4} and Veronica Ancona¹

¹Citrus Center, Texas A&M University-Kingsville, 312 N. International Blvd, Weslaco, TX, 78599, USA.

²Texas A&M AgriLife Research and Extension Center, 2415 E. Bus. 83, Weslaco, TX 78596, USA.

³Department of Plant Pathology and Microbiology, Texas A&M University System, 2132 TAMU, College Station, TX 77843, USA.

⁴Institute for Advancing Health Through Agriculture, Texas A&M AgriLife, College Station, TX, 77843, USA.

Abstract:

Citrus Huanglongbing (HLB) caused by “*Candidatus Liberibacter asiaticus*” (CLas) is a serious disease for citrus industries worldwide. It leads to reduction of fruit yield and quality and eventually tree death. Currently, there are limited strategies available to mitigate HLB in citrus production. Recent studies have demonstrated that trunk injections of the antibiotic Oxytetracycline (OTC) can suppress CLas titers and improve field performance of HLB-affected citrus. A recent report from Florida suggests that OTC-induced alteration of the microbiome in HLB-affected citrus is associated with improved citrus health. Because climate and soil conditions affect the microbiomes of plants, in this study, we aimed to uncover the effect of OTC injections to the microbiome of HLB-affected citrus in South Texas. In the 2-years field trial, following OTC injection, the yield of HLB-affected citrus trees increased by 25.40% and 10.65% in 2022 and 2023, respectively. However, there was no significant difference in brix and acid in citrus juice between OTC-treated and untreated trees. Also, no OTC residues were detected in citrus leaf after 181 days post-injection. The composition of leaf endophytic, root endophytic and rhizosphere microbiomes of HLB-affected citrus were altered by OTC injection. Relative abundance of several beneficial bacteria in endosphere and rhizosphere were enhanced after OTC injection, which may be involved in enhancing citrus health. This study provides valuable information about trunk-injected OTC against HLB in South Texas and suggests that CLas-microbiome interactions can be exploited for developing strategies to enhance tree health and suppress HLB symptomology.

Identification and characterization of secondary metabolites of citrus-associated *Bacillus* spp. inhibitory to the Huanglongbing pathogen, ‘*Candidatus Liberibacter asiaticus*’ and citrus root pathogens

Flavia Campos Vieira^{1#}, Manikandan Ramasamy², Kranthi Mandadi^{2,3,4}, Mariana Bossi Esteves⁵, Amancio de Souza⁶, Macie Allen⁷, Carolina Ruiz Rivera⁷, Christopher Yang⁷, Katherine N. Maloney⁷, M. Caroline Roper¹

¹Department of Microbiology and Plant Pathology, University of California, Riverside, California, USA.

²Texas A&M AgriLife Research & Extension Center, Texas A&M University System, Weslaco, Texas, USA.

³Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas, USA.

⁴Institute for Advancing Health Through Agriculture, Texas A&M AgriLife, College Station, Texas, USA.

⁵Citrus Research Center “Sylvio Moreira” /Agronomic Institute - IAC, Cordeirópolis, São Paulo, Brazil.

⁶Metabolomics Core Facility, University of California, Riverside, California, USA.

⁷Department of Chemistry, Point Loma Nazarene University, San Diego, California, USA.

#corresponding author: Flavia Campos Vieira,

Huanglongbing (HLB) is a devastating citrus disease associated with the phloem-limited and uncultured bacterium “*Candidatus Liberibacter asiaticus*” (CLas). Currently, the most effective control strategy for this disease relies on trunk injection of oxytetracycline. Therefore, there is a need to find alternative solutions to complement or replace chemical control. From a previously established citrus-associated microbial collection, we screened 18 *Bacillus* isolates for antagonism against *Liberibacter crescens*, CLas, and citrus-associated *Phytophthora* and *Fusarium* spp., and further generated their genomic and metabolomic profiles. Phylogenetic analysis grouped the isolates into four species groups: *Bacillus velezensis*, *B. subtilis*, *B. pumilus*, and *B. cereus*. Among these, *B. velezensis* exhibited the strongest and broadest inhibitory activity, followed by *B. subtilis*. This enhanced antagonistic potential is likely associated with the greater number and diversity of biosynthetic gene clusters (BGCs) identified. Notably, CLas-inhibitory isolates included *B. velezensis* (CB21 and CB909) and *B. altitudinis* (CB86). Bacilysin and surfactin BGCs were conserved across all CLas-inhibitory isolates, suggesting a potential role in CLas suppression. Accordingly, surfactins were detected in bioactive fractions of *B. velezensis* (CB27 and CB909) and *B. subtilis* (CB893), supporting their likely contribution to bioactivity and inhibition to *L. crescens* growth in vitro, highlighting its potential as a candidate compound for CLas management. Overall, this study underscores the importance of microbial discovery, screening, and characterization as an effective strategy for identifying sustainable solutions to manage HLB.

Optimization of Phenology-Based Oxytetracycline Trunk-Injection Timing to Suppress *Candidatus Liberibacter asiaticus* Titer and Improve the Health of HLB-Affected Citrus Trees

Waqar Shafqat¹, Meryam Manzoor¹, Maryam Khalilzadeh¹, Amit Levy², Tripti Vashisth¹, and Christopher I Vincent¹

¹Department of Horticultural Sciences, Citrus Research and Education Center, University of Florida, 700 Experiment Station Road, Lake Alfred, FL 33850, USA

²Department of Plant Pathology and Citrus Research and Education Center, IFAS, University of Florida, Lake Alfred, FL 33850.

Oxytetracycline (OTC) trunk-injection suppresses *Candidatus Liberibacter asiaticus* (CLas) and mitigates HLB-related declines in citrus health and productivity. The efficacy of OTC trunk injection may depend on phenological timing, as vascular activity and source–sink dynamics vary across citrus developmental stages. We hypothesized that OTC injections applied at different times of the year to target key phenological stages would differentially affect growth, fruit yield, and the quality of HLB-affected trees. To test this phenological approach, we injected the 7- to 8-year-old HLB-affected ‘Valencia’ sweet orange (*Citrus x sinensis*) with OTC at a dose of 0.55g/tree. The targeted phenological stages include flowering, fruit set, vegetative shoot growth & fruit drop, summer root & fruit growth, fruit development, and fall root growth & fruit ripening. A water-injected treatment was included as the control. We completed one year of the phenological injection cycle in 2025 before harvesting and obtained preliminary findings about plant growth and fruit data. Results showed that trees injected at the fall root & fruit ripening stage increased plant canopy volume, root growth, and yield, and reduced pre-harvest fruit drop. Plants injected during vegetative growth & fruit drop also show a positive effect on plant canopy volume and fruit yield. Tree injections at the fruit ripening & fall root growth stages improved fruit quality by increasing total soluble solids. In total soluble solids decreased with the length of the time from injection to harvesting. Phenological injections did not suppress the CLas titer after one complete year of injections, as observed in the seed coat vasculature two months after harvesting. Results suggest that injections during the fall root-growth and fruit-ripening stage may support phloem transport and source-sink carbon allocation more than other phenological stages, thereby improving root function, fruit retention, quality, and plant performance. Continued evaluation during the second annual cycle is needed to determine whether repeated applications produce consistent improvements in CLas suppression, canopy recovery, and productivity.

Keywords:

Engineering ihpRNAi Constructs for RNAi-Mediated Resistance to Grapevine Leafroll-associated Virus 3

William Anderson¹, Surender Kumar², Lorenzo Rossi¹, Olufemi Alabi³, Kiran Gadhave², Andrej Svyantek^{1†}

¹*Department of Horticulture Sciences, Texas A&M University, College Station, TX, United States*

²*Department of Entomology, Texas A&M University, College Station, TX, United States*

³*Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX, United States*

*CORRESPONDENCE

Andrej Svyantek

†andrej.svyantek@ag.tamu.edu

Grapevine leafroll disease (GLD) is a disease of grapevine caused by several associated viruses. The most widespread and damaging of these associated viruses is grapevine leafroll-associated virus 3 (GLRaV-3) which poses a severe threat to global viticulture. Estimated losses from GLRaV-3 are as high as \$900/acre per year over a 25-year production lifespan. To engineer broad-spectrum resistance, hairpin RNA interference (hpRNAi) constructs targeting GLRaV-3 were designed. Three 100-bp viral regions selected for high predicted RNAi activity were concatenated into a 300-bp target cassette. Multiple sequence alignments against fully sequenced GLRaV-3 isolates confirmed high target conservation: 48 isolates shared 100% identity in at least one region (and 98–99% in others), while 25 additional isolates exhibited 92–98% identity across multiple regions. Two PDK-intron-spliced hpRNA constructs were created: a conventional sense/antisense design and an innovative variant featuring C-to-T substitutions in the sense arm. This modification takes advantage of G:U wobble base pairing to avoid intrinsic self-silencing and improve construct stability which is especially important in long-lived perennial crops. Both constructs are currently undergoing evaluation via transient expression assays in GLRaV-3-infected grapevine tissues. Future work to obtain stably transformed grapevines, assess greenhouse performance trials, and evaluate systemic siRNA graft-transmissibility may deliver producers new options for rootstock-mediated protection across scion genotypes.

Predicting tree-level yield in an endemic vascular pathosystem: stable eco-physiological, nutritional, and climatic predictors of 'Hamlin' orange under Huanglongbing

Jacobo Robledo^{2,3,4}, Bikas Basnet^{1*}, Karen Garrett^{2,3,4}, Taylor Livingston¹, Tripti Vashisth^{5*}, Amit Levy^{6*}

¹*Citrus Research and Education Center, Institute of Food and Agricultural Sciences, University of Florida, Lake Alfred, FL 33850, USA*

²*Plant Pathology Department, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL, USA*

³*Global Food Systems Institute, University of Florida, Gainesville, FL, USA*

⁴*Emerging Pathogens Institute, University of Florida, Gainesville, FL, USA*

⁵*Department of Horticultural Sciences, UF/IFAS, Citrus Research and Education Center, University of Florida, Lake Alfred, FL 33850, USA*

⁶*Department of Plant Pathology, UF/IFAS, Citrus Research and Education Center, University of Florida, Lake Alfred, FL 33850, USA*

***CORRESPONDENCE**

Amit Levy

amitlevy@ufl.edu

Huanglongbing (HLB), caused by the phloem-limited bacterium *Candidatus Liberibacter asiaticus* and transmitted by the Asian citrus psyllid (*Diaphorina citri*), is now endemic in Florida citrus and drives high year-to-year variability in tree-level yield, making prediction difficult. Using HLB-endemic 'Hamlin' orange groves, we tested whether yield predictors are stable and transferable across years and orchards. From 2019–2021 data, 161 predictors were reduced to 49 by removing correlated variables, then screened with nine feature-selection algorithms. Eight of 25 candidates were chosen by more than 6 algorithms: canopy index (CI), canopy volume, foliar B, Ca, and Zn, cumulative solar radiation during fruit cell division, and peak vapor pressure deficit and daily maximum temperature variance during fruit maturation. A LightGBM model trained on 2019–2020 reached $R^2=0.56$ (RMSE=44.80 lb tree⁻¹) on held-out 2021 data. Prediction accuracy tracked year-to-year shifts in predictor distributions: training on 2019 alone failed for 2021, whereas adding 2020 reduced the shift and restored accuracy. Errors were largest for the highest- and lowest-yielding trees. Applied to an independent grove (2024–2025), performance fell as distributions shifted, but retraining with 2025 trees gave mean $R^2=0.32\pm0.30$ across ten random draws; CI had the highest SHAP importance. A compact set of phenotypic, nutritional, and weather predictors can estimate tree-level yield under endemic HLB, but reliable transfer to new years and groves will require training across broader environmental variation and local adaptation, an essential step toward yield-forecasting and decision-support tools for managing this endemic vascular pathosystem.

Citrus tree protection and production against the citrus pest complex

(Based on discussion with Dr. Nian Wang for a 30-minute oral presentation)

Jawwad A. Qureshi

Professor of Entomology, University of Florida Entomology and Nematology Department, Southwest Florida Research and Education Center, Immokalee, FL, United States

CPRRESPONDENCE

Jawwad A. Qureshi

The citrus pest complex dominated by the Asian citrus psyllid (ACP), *Diaphorina citri*, vector of ‘*Candidatus liberibacter asiaticus*, continues to cause significant losses to citrus industries. Over the last two decades, we evaluated cultural, biological, and chemical methods of pest control to manage ACP in citrus produced for both processed and fresh fruit markets. We demonstrated the effectiveness of Individual Protective Covers (IPCs) in protecting young trees against ACP and huanglongbing (HLB) during the early years, and of the Citrus Under Protective Screen (CUPS) in bringing large acreages of citrus into production and sustaining them. CUPS and IPCs were effective at bringing young trees into production, with better health and higher yields than open production systems, without ACP-HLB. However, both systems suffered incursions of other important pests, including mealybugs, scales, mites, citrus leafminer, thrips, and armyworms. In addition to demonstrating the effectiveness of IPC and CUPS against the ACP-HLB complex, we have found biological and chemical solutions for managing pests, such as mealybugs, scales, and mites in both covered and uncovered systems. These include the potential of biological control in protected environments for pests such as scales, mites, and mealybugs, as well as the effectiveness of mycoinsecticides and conventional insecticides in managing mealybugs and psyllids. Findings from the above studies will be discussed.

Spinach-Derived Antimicrobial Peptides as a Novel Strategy for Tolerance to Citrus Greening and Potato Zebra Chip Diseases

Carmen Padilla¹, Sonia Irigoyen¹, Manikandan Ramasamy¹, Mona B. Damaj¹, Michelle M. Dominguez¹, Denise Rossi¹, Renesh H. Bedre¹, William O. Dawson², Choa El-Mohtar², Michael S. Irey³ and Kranthi Mandadi^{1,4,5*}

¹Texas A&M AgriLife Research and Extension Center, 2415 East US Highway 83, Weslaco, Texas 78596, USA

²Citrus Research and Education Center, University of Florida, IFAS, Lake Alfred, Florida, USA

³Southern Gardens Citrus, U.S. Sugar Corporation, Clewiston, Florida, USA

⁴Department of Plant Pathology & Microbiology, Texas A&M University, 496 Olsen Blvd., College Station, Texas 77840, USA

⁵Institute for Advancing Health Through Agriculture, Texas A&M AgriLife, College Station, TX, USA

*Corresponding author:

Citrus greening (Huanglongbing) and potato zebra chip are among the most devastating diseases impacting citrus and potato production globally. These diseases are caused by fastidious, phloem-limited, and unculturable bacteria belonging to the ‘*Candidatus Liberibacter spp.*’ group, which are transmitted by insect vectors. Defensins, small (~40–50 amino acids) and cysteine-rich antimicrobial peptides, play a crucial role in the innate immunity of plants, animals, and insects. They exhibit broad-spectrum activity against bacterial and fungal pathogens. In this study, we explored the potential of expressing spinach-derived defensins in citrus and potato to confer resistance to diseases caused by *Ca. Liberibacter spp.* Since these bacteria cannot be cultured *in vitro*, we initially assessed the antimicrobial activity of two spinach defensins, SoAMP1 and SoAMP2, using an *ex vivo* microbial hairy root system. Both peptides significantly reduced levels of *Ca. Liberibacter spp.* in citrus and potato hairy roots compared to the control group.

The spinach defensin disrupted bacterial cell membranes, increasing their permeability and leading to bacterial death, as evidenced by *in vitro* cytotoxicity assays using the culturable surrogate *Liberibacter crescens*. Furthermore, heterologous expression of SoAMP1 and SoAMP2 in citrus and potato through genetic engineering or viral vector-mediated delivery significantly enhanced plant tolerance to *Ca. Liberibacter spp.* infection. This improvement resulted in a substantial increase in the yield of infected plants. These findings suggest that spinach defensin peptides hold promise as crop protection tools for managing citrus greening, potato zebra chip, and potentially other related diseases.

Changes In Vector Feeding Behavior Arising from Application of Heat are Detectable Using Signal Analysis Techniques

Ashley Zavala, Timothy K Martin, Annette Saucedo Padron, Thomas M Chappell

Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX, United States

Understanding vector feeding behavior reveals the impact of its variation on disease transmission and inoculum dynamics. Electropenetrography (EPG) is a technique through which feeding patterns and processes of piercing-sucking insects can be observed. EPG data can be analyzed by classifying behaviors to quantify occurrences, describe average durations of behaviors (e.g., salivation, ingestion), and compare statistical results between groups of interest. In this study, investigated factors involved concern of bioclimatic variables, beginning with heat. Controlled thermal regimens were used to observe effects on feeding. The first systems investigated were the *Aphis gossypii* on cotton plants and *Ferrisia virgata* on pepper plants. Applications of continuous wavelet transform (CWT), a signal processing method, was utilized to differentiate instances of feeding events from the EPG data for the purpose of studying environmental factors influencing vector feeding behavior. Preliminary results suggest that treatment affects the frequency (Hz) and there may be a correlation with heat influencing how the vector feeds. There is also variation within types of behavior, potentially adding information to counts and durations of those types of occurrences. Especially where external stimuli may not affect that an organism is feeding, but rather how it feeds. The combined EPG-CWT framework provides a basis for linking feeding dynamics to inoculum kinetics and pathogen transmission. Applying this approach to transmission assays will enable the observation of how feeding behavior influences pathogen acquisition and transmission efficiency across vector species and environmental conditions making this development of quantitative analytical methods useful for prediction based on EPG.

Investigating Pierce's Disease (*Xylella fastidiosa*) Tolerance and Resistance in Historical Grapevine (*Vitis* spp.) Cultivars Developed by T. V. Munson

Qiuni Yang¹, Venkateswara Rao Kadium¹, Andrej Svyantek^{1*}

¹*Department of Horticultural Sciences, Texas A&M University, College Station, TX, United States*

***CORRESPONDENCE**

Andrej Svyantek

andrej.svyantek@ag.tamu.edu

Pierce's disease (PD) of grapevines (*Vitis* spp.) is a gram-negative, xylem-limited bacterial disease caused by *Xylella fastidiosa*. PD is an economically important disease limiting grapevine production in many growing locations, including parts of Texas, USA. Management of PD is strictly prophylactic; incurring costs from infected vine removal and vector control. Development of durable genetic resistance to PD remains a key strategy for sustaining grape production in high PD pressure environments. Thomas Volney Munson's historical grapevine breeding efforts, conducted in Denison, TX, USA from the late 1800's through the early 1900's, incorporated diverse *Vitis* species with adaptation to abiotic and biotic pressures of the Southern United States. These cultivars retain substantial genetic variation and remain potential sources of PD resistance for contemporary grapevine breeding efforts. In this study, PD-related symptoms were rated under natural field conditions in a genetically diverse grapevine collection of 47 Munson-bred cultivars. Symptoms, including leaf margin scorch, green-islands, and 'matchstick' petioles, were collectively scored on a 0–4 severity scale across three assessments in the summer of 2026. Substantial phenotypic variation in PD severity was observed among the evaluated grape genotypes, suggesting this collection may harbor valuable genomic variance for PD survival. Building upon these phenotypic evaluations, breeding population creation is on-going to incorporate surviving grapevines as parents in breeding efforts and to develop segregating populations for future analyses. The seedling populations developed will be used to support new phenotypic, marker-assisted, and genomic selection strategies in the development and delivery of new grapevine cultivars with novel PD tolerance and resistance traits.

Evaluation of trunk-infusion volume and site of application on the uptake of oxytetracycline (OTC) by HLB affected grapefruit (*Citrus × paradisi*) trees

Damodar Pokhrel, Luca Scolari, Veronica Ancona*

Citrus Center, Texas A&M University-Kingsville, 312 N. International Blvd, Weslaco, TX, 78599, USA.

*CORRESPONDENCE

Veronica Ancona

Huanglongbing (HLB) is one of the most destructive diseases affecting citrus worldwide. In the US, the disease is caused by the phloem-limited α -bacterium *Candidatus Liberibacter asiaticus* (CLas). Trunk injection of small volumes (20 mL) of highly concentrated antibiotics while successfully suppress HLB presented two main side effects, the appearance of severe phytotoxicity symptoms and the unbalanced distribution of the chemical remedy throughout the canopy. In this study, an alternative approach (gravity-based trunk infusion system) with larger volumes of lower concentrated commercial OTC formulate was tested. A three factors experimental design was implemented, with two volumes (150 and 1000 mL), three sites of application (North, South and North-South) and three treatments (OTC solution, an acidified solution and water). The experiment was performed on 132 HLB-positive 'Rio Red' grapefruit trees in an orchard located in South Texas. Initial results showed that the site of application did not affect uptake. However, the amount of OTC uptake depended on the volumes applied. Trees uptake a maximum volume of 1000 mL with an average of 53% for the 1000 ml treatment. Trees receiving the 150 ml infusion treatment showed a 90% volume uptake. Furthermore, the preliminary results on CLas titers showed no effect of OTC treatment within a month post treatment. Analysis of OTC in leaves is under progress to model the antibiotic distribution throughout the canopy to assess the effect of volume and infusion site. Additional agronomic traits will be evaluated along the growing season to assess the efficacy of this approach.

Lipopolysaccharide structure influences the biogenesis and cargo selection of *Xylella fastidiosa* outer membrane vesicles

Nancy Nou Her¹ and Caroline Roper¹

¹Department of Microbiology and Plant Pathology, University of California, Riverside, CA 92507 USA

Xylella fastidiosa (*Xf*) is a prolific producer of outer membrane vesicles (OMVs) that bleb from the membrane and carry cargo that influences plant-microbe interactions. The mechanism of what determines OMV architecture and cargo loading is largely unknown. Lipopolysaccharides (LPS) comprise a major part of the outer membrane (OM) and influence the architecture and biophysical properties of the cell surface. Because OMVs derive primarily from the OM, we hypothesized that changes to LPS may impact OMV architecture and cargo. To test this, we characterized the physical structural properties of OMVs derived from a *Xf* mutant with a truncated LPS O-antigen (Δwzy) and compared this to OMVs from wild type *Xf*. LPS truncation incurred changes in OMV architecture, namely size and surface charge. We also characterized proteinaceous cargo within OMVs derived from these strains. PERMANOVA analysis indicated that LPS structure accounts for 46% of the variation in OMV protein profiles ($p=0.004$). Specifically, while WT and Δwzy OMV proteomes shared 181 core proteins, the Δwzy OMV proteome had a 38% increase in protein diversity that included unique putative virulence factors absent in the WT OMVs. The cell surface of Δwzy is more negatively charged compared to WT. We speculate that truncating the O-antigen removes steric bulk and increases negative charge in the LPS that promotes OM curvature leading to differential protein entrapment during blebbing. This suggests that LPS plays a role in membrane fluidity and surface charge during OMV vesiculation influencing OMV cargo loading and potentially, host-pathogen interactions.

Complete genomes reveal metabolic differences between Lethal Bronzing Phytoplasma, co-recovered *Candidatus Uzinura*, and phloem-limited pathogens

Mia Acker¹, Paula Rios Glusberger¹, Carrie Harmon², Eric W. Triplett^{1*}

¹*Department of Microbiology and Cell Science, University of Florida, Gainesville, FL, United States*

²*IFAS Plant Diagnostic Center, University of Florida, Gainesville, FL, United States*

***CORRESPONDENCE**

Eric W. Triplett

Deep metagenomic sequencing of lethal bronzing-infected date palm (*Phoenix dactylifera*) recovered two high-coverage, single-contig bacterial genomes: the lethal bronzing-associated “*Candidatus Phytoplasma aculeata*” (502.3 kb; 244x coverage) and a “*Candidatus Uzinura*” sp. (338.9 kb; 174x coverage). To our knowledge, this is the first co-recovery of a bacterial plant pathogen and insect endosymbiont from diseased plant tissue. The high-quality genomes provided a novel chance to investigate any new metabolic functions that could act as therapeutic targets. We evaluated both the individual and combined metabolic potential of the two new genomes alongside phloem-colonizing pathogens “*Ca. Liberibacter asiaticus*,” and *Spiroplasma kunkelii*, and related culturable bacteria *Liberibacter crescens* and *Acholeplasma laidlawii*. The combined metabolic potential of the new genomes was included to better understand phloem biology and potential for interaction. Comparative genomics showed the characteristic extensive erosion of amino acid, cofactor, vitamin, and terpenoid biosynthetic capabilities among phloem-limited pathogens, contrasting with greater metabolic capacity in the culturable relatives. Compared to the previously available Texas Phoenix palm phytoplasma genome, the new “*Ca. P. aculeata*” genome contains additional annotated functions associated with transcriptional regulation, and protein homeostasis (NusB, GrpE, and HrcA) and membrane efflux through a multidrug ABC transporter. The “*Ca. Uzinura*” genome exhibited broader predicted energy, amino acid, and carbon metabolic capabilities than the only characterized member of the genus, also encoding both a Tat and putative Sec protein translocation systems. Altogether, these findings expand the genomic characterization of lethal bronzing phytoplasma and reveal the unexpected co-occurrence of a putative insect endosymbiont within diseased palm tissue.

Hairy Root Transformation in *Citrus medica* L.: An Efficient Platform for HLB Therapeutics Discovery

Aditya Kulshreshtha¹, Manikandan Ramasamy¹, Sonia Irigoyen¹, Carmen S. Padilla¹, Chi-Kuan Tu^{1,2}, Kinnie Laughlin¹, James Borneman², Kranthi K. Mandadi^{1,3,4*}

¹Texas A&M AgriLife Research and Extension Center, Weslaco, TX, United States

²Department of Microbiology & Plant Pathology, University of California, Riverside, Riverside, CA, United States

³Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX, United States

⁴Institute for Advancing Health Through Agriculture, Texas A&M AgriLife, College Station, TX, United States

*CORRESPONDENCE

Kranthi K. Mandadi

Huanglongbing (HLB) is the most destructive citrus disease, presumably caused by the fastidious bacterium *Candidatus Liberibacter asiaticus* (CLas). Identifying effective therapies for HLB is challenging due to the inability of CLas to grow in a defined growth medium and the recalcitrance of citrus to stable genetic transformation. In this study, we established an efficient hairy root transformation system in citron (*Citrus medica* L.) for rapid genetic and antimicrobial studies. Citron explants showed up to 100% survival and readily induced transgenic hairy roots, with transformation efficiencies ranging from 25% to 73%. We demonstrated the applicability of this system for genome editing, transgene assessment, and evaluation of antimicrobial activity, with the overall goal of developing HLB-tolerant citrus. Collectively, this platform provides a rapid and versatile approach for functional characterization of citrus genes and screening candidate therapeutics for HLB.

Development of a Viability qPCR Assay for *Fusarium oxysporum* f. sp. *vasinfectum* Race 4

Junseong Kang, Madison Issacs, Huan Zhang, and Won Bo Shim*

Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX, United States

***CORRESPONDENCE**

Won Bo Shim

wbshim@tamu.edu

Accurate quantification of viable *Fusarium oxysporum* f. sp. *vasinfectum* race 4 (Fov4) is important for studying pathogen survival, stress responses, and disease development in cotton vascular system. Conventional qPCR detects pathogen DNA from both viable and nonviable cells and therefore may overestimate pathogen abundance and threats. In this study, we evaluated a viability qPCR (vPCR) assay using PMAxx to selectively quantify Fov4 cells with intact membranes. PMAxx treatment conditions were optimized to suppress amplification of DNA from cells with compromised membranes while allowing detection of viable cells. Defined mixtures containing different proportions of viable spores and spores killed by heat were used to examine the relationship between qPCR signal and fungal viability. PMAxx treatment substantially reduced amplification from nonviable spores, while Ct values increased consistently as the proportion of viable spores decreased. Each twofold reduction in the viable spore proportion resulted in an increase of about one Ct, demonstrating a strong relationship between qPCR signal and fungal viability. The assay was further applied to Fov4 exposed to several abiotic stresses to determine whether vPCR could detect changes in fungal viability due to stress. Cotton seedlings inoculated with Fov4 were also analyzed to evaluate the applicability of PMAxx vPCR for detecting viable Fov4 in infected plant tissues. These results support PMAxx vPCR as a rapid method for estimating viable Fov4 cell threshold without the need for culturing and demonstrate its potential for studying pathogen survival and disease development in cotton.