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BUILDING RESILIENT CITRUS: INNOVATIONS IN VECTOR CONTROL

PUBLICATION OFFICE

P.O. Box 230
Visalia, CA 93279
P: (559) 738-0246
F: (559) 738-0607
www.citrusresearch.org

EDITORIAL STAFF

Marcy L. Martin, Executive Editor
Adriana Toste, Managing Editor
Melinda Klein, Ph.D, Science Editor
Meagan Iott, Associate Science Editor
Caitlin Stanton, Editorial Assistant
Ed Civerolo, Ph.D., Editorial Consultant

PUBLISHING AND PRODUCTION
Co-Publisher / Creative Director/ Graphic Designer

cribbsproject new media designs

Eric Cribbs
www.cribbsproject.com
graphics@citrographmag.com
(559) 308-6277

ADVERTISING
Eric Cribbs
graphics@citrographmag.com
(559) 308-6277

Advertising, business and production inquiries - call, email or write us at:
Cribbsproject
890 E. Tietan St., Walla Walla, WA 99362
P: (559) 308-6277 • F: (866) 936-4303
graphics@citrographmag.com

Editorial inquiries - call, email or write us at:
Citrus Research Board
P.O. Box 230 • Visalia, CA 93279
P: (559) 738-0246 • F: (559) 738-0607
info@citrusresearch.org
www.citrusresearch.org

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On the Cover: *The spring 2026 issue of* Citrograph *focuses on vectored diseases, with several articles examining recent advancements and research in this area. The cover showcases a citrus plant grown from tissue culture, a technique being implemented by the Citrus Clonal Protection Program to produce clean rootstock for therapy and plants for bio-indexing. These efforts further reduce the risk of disease and safeguard the long-term sustainability of California's citrus industry. To learn more, see "The Citrus Clonal Protection Program," by Georgios Vidalakis, Ph.D., on page 42.*

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THE MISSION OF THE CITRUS RESEARCH BOARD

Ensure a sustainable California citrus industry for the benefit of growers by prioritizing, investing in and promoting sound science.

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Citrus Research Board | 217 N. Encina St., Visalia, CA 93291
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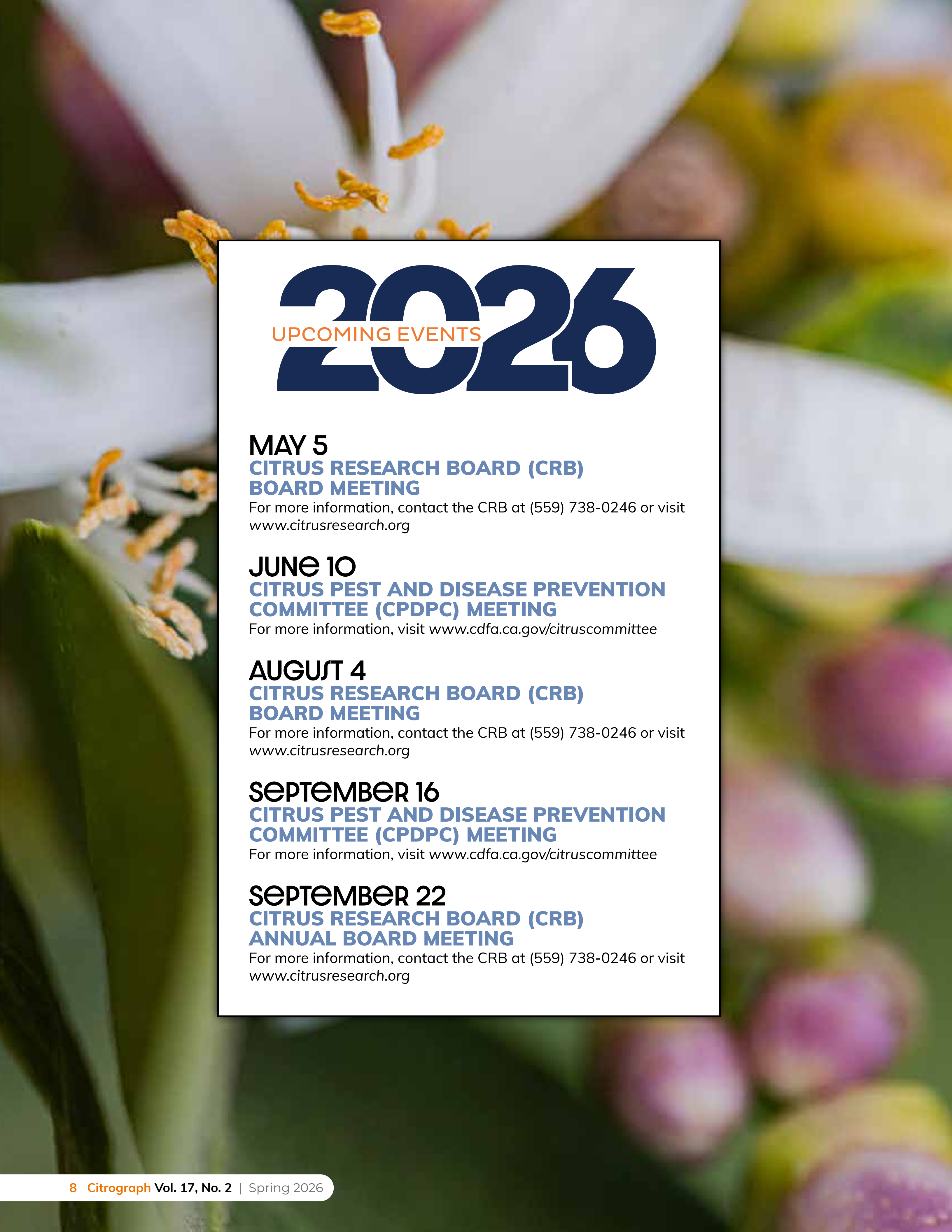

2026 UPCOMING EVENTS

MAY 5

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For more information, contact the CRB at (559) 738-0246 or visit *www.citrusresearch.org*

JUNE 10

CITRUS PEST AND DISEASE PREVENTION COMMITTEE (CPDPC) MEETING

For more information, visit *www.cdfa.ca.gov/citruscommittee*

AUGUST 4

CITRUS RESEARCH BOARD (CRB) BOARD MEETING

For more information, contact the CRB at (559) 738-0246 or visit *www.citrusresearch.org*

SEPTEMBER 16

CITRUS PEST AND DISEASE PREVENTION COMMITTEE (CPDPC) MEETING

For more information, visit *www.cdfa.ca.gov/citruscommittee*

SEPTEMBER 22

CITRUS RESEARCH BOARD (CRB) ANNUAL BOARD MEETING

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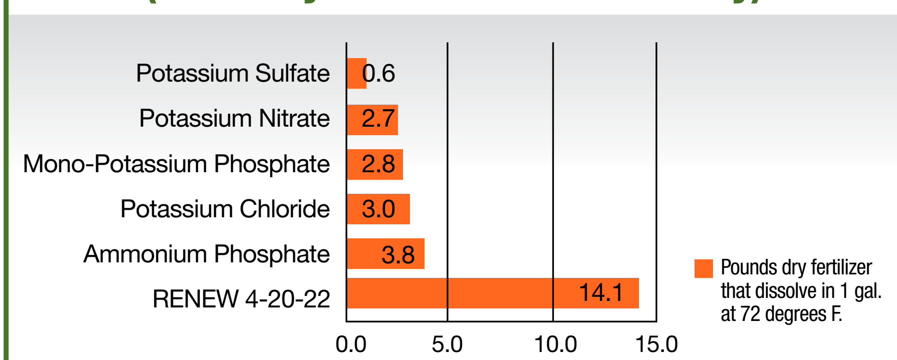


For more information including research results and scientific publications, contact;

Mark Brady, Western Marketing Manager, Plant Food Systems, Inc. (559) 731-1267

Plant Food systems

From the PRESIDENT'S DESK

Marcy L. Martin

Marcy L. Martin

For many years, the California citrus industry has remained steadfast in our commitment to reducing the threat of vector-borne diseases. Each time the threat of a new disease arises, our industry organizations put measures in place to slow its spread, conduct necessary research, and address regulatory issues. I encourage you to read the research articles in this issue of *Citrograph* to learn more about ongoing vector-borne disease research, how it affects California's citrus industry, and lessons we can learn from other growing regions.

As we continue through the harvest season, we are reminded how much our industry can change year over year. Evolving market demands, regulatory issues, and changing weather conditions affect our industry and agriculture as a whole. Across the state, growers have embraced these challenges through innovative practices and adaptability, from production to post-harvest. From intense desert heat to rainfall and frost warnings in the Central Valley, numerous factors influence bloom, fruit set, and harvest schedules. Weather remains unpredictable from year to year and can significantly impact pest pressures, as we've seen with citrus thrips populations increasing with warmer weather. These conditions reinforce the importance of being ready to respond to the needs of California citrus, as our industry has done for many years through proactive production practices. As always, the Citrus Research Board (CRB) is prepared to assist with new challenges that occur throughout the year.

Machine Learning for HLB Detection

The CRB and the Citrus Pest and Disease Prevention Program (CPDPP) have partnered for many years to manage pest and disease issues and develop solutions for problems affecting the industry. Over the last few years, CRB has supported a project using machine learning to predict huanglongbing (HLB) findings across California. This information can be used to more readily detect where HLB-infected trees are located, thereby increasing the efficiency of the California Department of Food and Agriculture's (CDFA) regulatory survey activities. Dr. Rob Clark and his research team built the model and have recently completed testing, demonstrating high accuracy in locating HLB-infected plants in Southern California. Now that testing is complete, CRB and CPDPP are working together to integrate this technology into the state's citrus program. The machine learning technology will provide a long-term, cost-effective way for CDFA to plan survey efforts and increase efficiency in locating HLB-infected trees. For additional information about this project, please visit page 30.

Grove-First Project

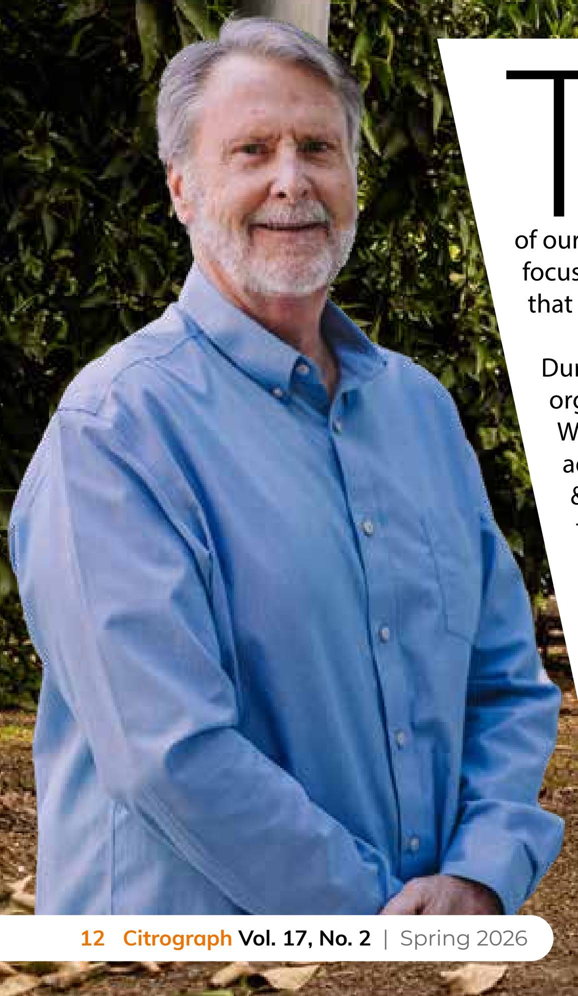
Our industry is lucky to have not yet felt the effects of HLB in commercial settings, but we can learn from research in other growing regions where the disease has had a devastating impact. Many growers in Florida have relied on direct trunk injection of the antibiotic oxytetracycline to treat trees infected with HLB. Dr. Michelle Heck of USDA-ARS developed the Grove-First program. This field-based screening program is streamlining the testing of a range of compounds, including both active ingredients and compounds that can improve the effectiveness of oxytetracycline. This study found that oxytetracycline and its formulations were most effective in preserving fruit quality. Still, the timing of the injection and the tree's starting condition are essential considerations for this therapy. Most importantly, the Grove-First project has evolved into a method to streamline commercially-available injection solutions for the treatment of vectored diseases. An in-depth look at this project can be found on page 48.

Despite the challenges, California citrus remains strong because of the innovative nature of the growers, researchers, and industry partners who continue to work together to protect and advance our industry. Each issue of *Citrograph* highlights the innovative research CRB is supporting to address longstanding and emerging diseases, pests, production issues, and more. We will continue to address the needs of California's growers through our research investments, ensuring the industry thrives well into the future.

***Marcy L. Martin serves as the president of the Citrus Research Board, based in Visalia, California. She is also the executive editor of* Citrograph. *For more information, contact* marcy@citrusresearch.org**

CHAIRMAN'S VIEW

Mark McBroom



The citrus industry continues to navigate a period of significant challenge and transformation. From disease pressure and weather variability to shifting market demands, the need for sound science and practical solutions for growers has never been greater. At the same time, innovation, collaboration, and resilience continue to be defining strengths of our industry. Over the past year, the Citrus Research Board (CRB) has remained focused on advancing research, supporting growers, and fostering partnerships that move citrus forward.

During the 2024-25 year, the CRB worked diligently to strengthen the organization's direction and ensure responsible use of industry resources. We focused our efforts toward supporting several key research projects and addressing grower concerns as issues developed in the field. Our Production & Post-Harvest Technology Research Committee concentrated its efforts to bring the Nanovel mechanical harvesting machine to fruition, which recently began field trials in California. These trials will provide practical feedback on the use of the harvester in our groves, allowing it to evolve to meet the industry's needs. Each of our committees relies on valuable feedback from industry members to direct and develop our research priorities. We heard concerns from growers about lemon pitting and established a project with our research partners to look at possible causes from every angle. This project will continue through the year as they analyze additional data to provide management recommendations. The CRB values feedback from the industry, as our mission revolves around prioritizing sound science that will keep California citrus profitable and sustain our industry for many years to come.

For several years, we have been developing our California Citrus Research and Field Trials (CRaFT) program, which has now expanded to nearly 6,500 acres. This program is working to keep Asian citrus psyllid (ACP) numbers low in participating groves with targeted detection methods including trap, tap, visual, and canine inspections. Consistent monitoring with these methods is part of the strategy toward maintaining ACP-free groves. This program will now focus on expanding into Ventura County, aiming to tailor management strategies to specific growing regions.

To provide a broader range of plant material for California's growers, CRB has been working with the United States Department of Agriculture (USDA) to develop a breeding program in the Central Valley. The USDA maintains a breeding program in Florida, but the varieties it produces are tailored to meet the needs of Florida's industry. By developing a comparable program in California, we can focus on new varieties to meet evolving market demands and improve production efficiency. Our research partners at the University of California, Riverside breeding program continue to test their varieties, as they will soon begin grower field trials to gather data in a production setting. These two breeding programs provide complementary work toward the collective advancement of the industry.

In addition to our research work, we hosted several events to bring information directly to the industry through research presentations. Our Growers' Educational Webinar Series, held each summer, provides growers with direct information and the convenience of attending from the office or the field. Our Post-Harvest Conference brought together leaders in post-harvest research to discuss new information on food safety, post-harvest diseases, market demands, and other relevant topics. Together, our research and outreach activities focus on developing and sharing field-based solutions that benefit growers.

Looking ahead into the following year, the Board remains committed to maintaining a strong and balanced research portfolio, collaborating with partner institutions and organizations, and communicating findings to the industry through our events and the *Citrograph* magazine. The CRB staff play a vital role in bridging the gap between the lab and the field. Throughout the year, staff members will continue to work closely with growers and researchers to facilitate project coordination, monitor progress, and ensure open lines of communication. Their efforts help translate research into practical field-based information and ensure that grower needs continue to inform research priorities. In addition, we will continue to work with our industry partners as the collective expertise of these alliances addresses shared challenges facing the citrus industry.

This year ahead will undoubtedly bring new challenges, but it also presents opportunities to build on the progress already underway. Through focused research, strong leadership, and collaboration across the industry, we can continue to strengthen the future of California's citrus industry. I am honored to continue serving as the CRB chairman for the 2025-26 fiscal year, and I am confident the CRB will continue to ensure an innovative and resilient citrus industry by addressing the needs of California's growers.

Mark McBroom serves as the chairman of the Citrus Research Board. For more information, contact
markm@bloomtobox.com

Challenges and Strategies in Protecting California's Agriculture from Pests and Diseases – *An Agricultural Commissioner's Perspective*

Melissa Cregan

California, renowned for its vast and diverse agricultural industry, faces constant threats from invasive pests and diseases. Protecting California from exotic pests and diseases is a collaborative effort involving U.S. Customs and Border Protection (CBP), the United States Department of Agriculture (USDA), the California Department of Food and Agriculture (CDFA), and County Agricultural Commissioners (CACs). CACs serve as the last line of defense by running programs for exclusion, detection, and eradication to safeguard California's $60 billion agricultural industry from potential damage and losses due to these invasive threats.

California's unique geography and substantial population make it particularly susceptible to the introduction of pests and diseases that pose a threat to agriculture. Key factors include the state's 141 miles of international border with Mexico, seven land border crossings, 12 seaports managing over 32% of the nation's trade, and nine international airports accommodating over 100 million passengers annually. California faces risks not only from international sources but also from pests and diseases originating in other states and territories.

In Fresno County, pest exclusion efforts are primarily concentrated on detecting pests within parcels handled by express shipping companies such as UPS, FedEx, and the U.S. Post Office. Annually, we examine approximately 60,000 packages transiting through these parcel terminals. To enhance efficiency and effectiveness, we employ two detector canine teams specifically trained to identify packages containing plant materials. These packages undergo inspection for pests and diseases and are destroyed if found to contain prohibited items. Last year, more than 4,700 packages were inspected, leading to the interception of 75 A-rated pests and 120 Q-rated pests. While some of these interceptions posed a low risk for establishment, others represented a very high risk.

Pest ratings are intended as aids to inform county agricultural commissioners and other interested persons as to a particular pest's environmental, agricultural and biological significance, as well as its importance to the general public and the action recommended by the CDFA to deal with the pest.

- "A" is an organism of known economic importance subject to state (or commissioner when acting as a state agent) enforced action involving eradication, quarantine regulation, containment, rejection, or other holding action.
- "Q" is an organism or disorder requiring temporary "A" action pending determination of a permanent rating. The organism is suspected to be of economic importance, but its status is uncertain because of incomplete identification or inadequate information.

In 2024, Fresno County teams intercepted three parcels containing citrus trees originating from Florida that tested positive for HLB. In 2025, our teams intercepted one parcel containing a tree infected with citrus canker, two parcels containing fruit infested with Caribbean fruit fly from Florida, and one parcel containing curry leaves from Los Angeles County infested with Asian citrus psyllid (ACP). Each of these interceptions represents a potential introduction of a pest or disease that could significantly impact the citrus industry in the San Joaquin Valley.

Unfortunately, we do not catch every parcel that contains plant material which may be infested with pests and diseases. With the increase in e-commerce, more parcels are being shipped from out-of-state and international sources than ever before. Additionally, there are only 17 detector dog teams working statewide, while data suggests that we need twice as many before we reach a point of diminishing returns. We simply do not have enough resources to catch everything.

Due to inadequate resources to fully prevent pests from entering the state, we must depend on detecting and eliminating the most severe threats before they become established. Pest detection programs aim to identify and eradicate these pests early. Our detection programs utilize over 2,200 insect traps across Fresno County to capture any exotic fruit flies that got past our exclusion efforts. In 2025, we detected a single peach fruit fly in North Fresno. Fortunately, further delimitation trapping showed no additional finds, so eradication was unnecessary. Many other parts of the state are not as fortunate, with several fruit fly quarantine and eradication efforts being carried out annually, particularly in the Bay Area and Los Angeles Basin.

Another detection program monitors ACP across the county. In September 2025, 10 ACP were found on a single trap at a North Fresno residence. A visual survey revealed ACP in all life stages at this site and an adjacent property. Both properties and surrounding areas were treated, and enhanced trapping is ongoing. No additional ACP have been found so far.

While acknowledging the value and successes of our pest prevention system is crucial, it is equally important to identify and evaluate its failures. In California, a new pest or disease is detected every 60 days. Although not all these pests have significant impacts, some, such as the Carpophilus beetle affecting almonds and pistachios, became well established before they were detected. This pest has been responsible for a reported 10-15% reduction in yield.

The citrus industry is particularly aware of the threats posed by pests and diseases. Although there is a robust coalition of government agencies coordinating efforts to prevent pest introductions, the funding for these programs remains insufficient and has not kept pace with rising costs over the years. Consequently, each year a greater financial burden falls on individual counties to either shoulder the costs or reduce program size, thereby increasing vulnerability and risk of pest and disease introductions.

Given these vulnerabilities and limited resources, it is crucial that the collaborative efforts between federal, state, and local agencies remain robust and vigilant. The intricate network of surveillance and immediate response protocols are designed to swiftly identify and combat any threats, ensuring minimal impact on the agricultural sector. Public awareness campaigns and educational programs also play a key role, encouraging the community to participate in safeguarding California's agricultural health by reporting suspicious activities and adhering to regulations.

Melissa Cregan is the agricultural commissioner/sealer of weights and measures at the Fresno County Department of Agriculture. For additional information, please contact **mcregan@fresnocountyca.gov**

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Protecting California Citrus from Vectored Pests and Diseases

Dahmoon Maeesomy

Inspiring the next generation of citrus scientists: Future scientist explores citrus pest and disease management tools at the CDFA outreach booth during a community event.

The Citrus Pest and Disease Prevention Division (CPDPD) at the California Department of Food and Agriculture (CDFA) leads statewide efforts to keep citrus thriving by preventing, detecting, and eradicating/containing invasive, vectored pests and diseases that can silently spread through orchards and neighborhoods before symptoms appear. Its mission centers on science-based regulations, rapid response, and community engagement to safeguard commercial groves and backyard trees, while preserving the industry's economic stability and agricultural heritage.

California faces sustained pressure from pest species capable of transmitting destructive pathogens. CPDPD's policies and regulations, aligned with federal partners, guide coordinated initiatives that emphasize early detection, containment, and education across residential and commercial settings. The program's vision is to keep California citrus resilient through collaboration, innovation, and outreach, focusing specifically on vectored threats that jeopardize both homegrown fruit and commercial operations.

Key vectored pests and diseases of concern include:

- Asian citrus psyllid (ACP) and huanglongbing (HLB): ACP spreads the bacterium that causes HLB, a fatal, incurable disease that can rapidly devastate trees and regions.
- Citrus Stubborn Disease: Spread by leafhoppers; reduces tree vigor and produces misshapen fruit.
- Citrus Leprosis Virus: Transmitted by Brevipalpus mites, causing ring-like lesions on fruit and leaves and diminishes market value.
- Citrus Variegated Chlorosis: Spread by sharpshooter insects; blocks water flow, leading to leaf scorching and fruit deformation.
- Citrus Tristeza Virus: Carried by several aphid species that are present in California; has caused the decline of millions of citrus trees worldwide.
- Citrus Black Spot: A fungal disease (*Phyllosticta citricarpa*) affecting all commercial varieties, with late-maturing cultivars and lemons being most vulnerable.
- Citrus Canker: A bacterial disease (*Xanthomonas citri ssp.*) that can cause defoliation, premature fruit drop, twig dieback, general tree decline, and blemished fruit.
- Sweet Orange Scab (SOS): A fungal disease that spreads via contaminated tools, insects, or rain splash, causing blemishes that affect marketability.
- Citrus Yellow Vein Clearing Virus (CYVCV): Transmitted by aphids and a whitefly species that are present and widespread in California.

HLB, SOS, and CYVCV have been detected in California, prompting targeted regulations to mitigate risk and prevent further spread. Consistency between CDFA and the United States Department of Agriculture's Animal and Plant Health Inspection Service (USDA APHIS) strengthens statewide protection and coordinated response against these threats.

Detection and response efforts are comprehensive. Multi-pest surveys in residential neighborhoods, commodity surveys in commercial groves, and trapping programs in both settings aim to detect insect vectors early and before populations become established. Confirmed HLB-infected trees are removed promptly to reduce transmission. Movement of citrus plants and fruit is regulated to limit risk pathways. Collaborations with USDA APHIS, the Citrus Research Board (CRB), County Agricultural Commissioners, and industry stakeholders increase the program's capacity for rapid, science-based action and consistent implementation across jurisdictions.

Outreach and education are also central to prevention. Public awareness campaigns encourage homeowners to inspect their citrus regularly and report suspicious insects or symptoms through the state's established channels, including the CDFA Pest Hotline at 1-800-491-1899. Digital outreach, bilingual messaging, and collaboration with University of California Master Gardeners have increased community engagement and reporting, improved the pace and accuracy of detection, and supported timely interventions.

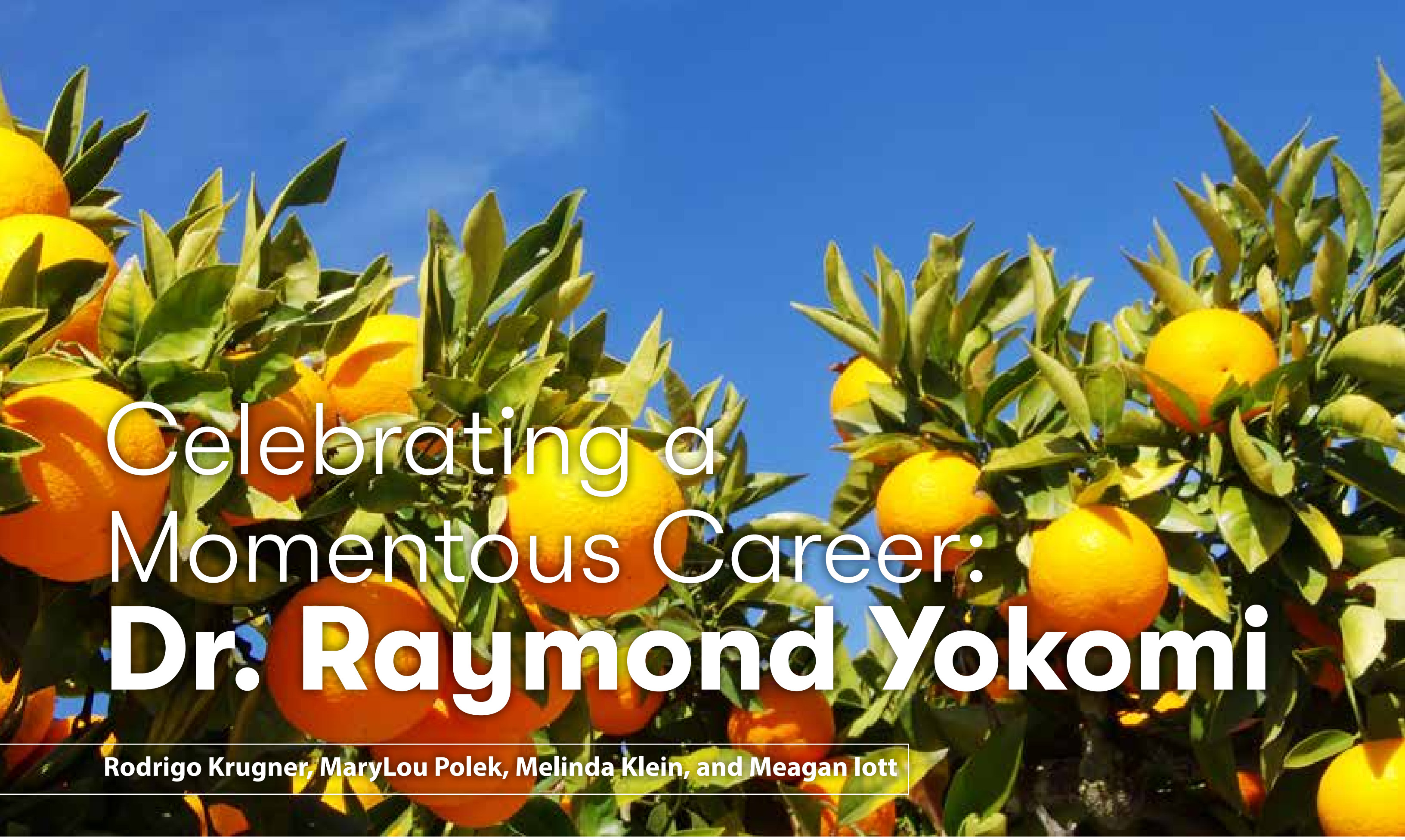
Zippy, the Mountain Mandarin Festival mascot, highlights citrus pest monitoring with a yellow panel trap—bringing awareness and smiles to the fight against invasive pests.

Community participation remains vital because many initial detections occur in residential areas. Residents should avoid moving citrus plants, leaves, or fruit between regions and follow local quarantine rules to limit artificial spread. Commercial growers, pest control advisors, and nurseries work with regulatory partners to adopt integrated pest management best practices. These actions protect homegrown fruit, commercial operations, and strengthen regional biosecurity by reducing opportunities for pest establishment and disease transmission.

Additionally, innovation and research continue to drive progress against vectored citrus diseases. CRB-funded and USDA-supported projects are advancing biological control of ACP using parasitoid wasps, improving trapping systems for more effective monitoring, and refining diagnostic methods for faster, more reliable disease confirmation. Research into resistant citrus varieties offers long-term promise for sustainable management, complementing short-term surveillance and control measures. CPDPD is also refining its response framework to anticipate future pest incursions, helping maintain California's leadership in citrus protection and ensuring readiness as threats evolve.

California's citrus industry represents billions of dollars in economic activity and supports thousands of jobs statewide. The combined efforts of CPDPD and its partners protect trees and the livelihoods, traditions, and communities connected to them. Continued vigilance, innovation, and collaboration are essential to preserve this legacy. By working together with growers, homeowners, researchers, and regulators, California can continue to thrive despite the challenges posed by vectored pests and diseases. Staying informed, adhering to regulations, and participating in community reporting and best practices are the foundation of a resilient citrus future in the state.

Dahmoon Maeesomy is an agricultural pest control supervisor for the Citrus Pest and Disease Prevention Division of the California Department of Food and Agriculture. For additional information, please contact **dahmoon.maeesomy@cdfa.ca.gov**



Celebrating a Momentous Career: Dr. Raymond Yokomi

Rodrigo Krugner, MaryLou Polek, Melinda Klein, and Meagan Iott

Dr. Raymond Yokomi recently retired from the United States Department of Agriculture – Agricultural Research Service (USDA-ARS) after 43 years of service advancing science in both entomology and plant pathology. His work for the California citrus industry was instrumental in providing growers with vital scientific information and management strategies for several key citrus diseases.

Dr. Yokomi was born and raised in Fresno, California. He attended Fresno City College and earned an associate degree in liberal arts before proceeding to the University of California, Davis, where he earned both his Bachelor of Science and Ph.D. degrees in entomology. He began his career as a post-graduate research entomologist at the University of California, Riverside, before taking a position as an assistant research scientist at the University of Florida, Lake Alfred. In 1982, Dr. Yokomi joined the USDA-ARS in Orlando, Florida, as a research entomologist, and in 1997, he returned to the San Joaquin Valley to accept a newly created position as a research plant pathologist with the USDA-ARS in Parlier, California. This position was created through the insistence and efforts by the citrus industry in California to address Citrus tristeza virus (CTV) and the potential invasion of its most efficient vector, the brown citrus aphid. Dr. Yokomi retired in January 2026.

"Dr. Yokomi brought the presence of a USDA-ARS research scientist with expertise in citrus vectors and pathogens to California. His research accomplishments connected molecular tools with practical applications for the protection of the California citrus industry and particularly, for the benefit of citrus growers."

– MaryLou Polek, Ph.D., Retired Plant Pathologist

During his career at the USDA-ARS in Parlier, Dr. Yokomi addressed graft-transmissible and vector-borne pathogens of citrus such as *Liberibacter sp.* (citrus greening), CTV, and *Spiroplasma citri* (citrus stubborn). His research focused on pathogen detection and molecular characterization, which filled key data gaps for better ongoing surveillance, early detection, and pathogen strain differentiation. His work led to improved, or in some cases, the development of entirely new, laboratory-based assays designed for rapid and accurate disease diagnoses.

Among his many accomplishments, Dr. Yokomi utilized cutting-edge molecular biology tools to understand the genomics of CTV. He developed specific and absolute pathogen diagnosis including robotic high-throughput nucleic acid extraction and purification for the detection of citrus pathogens. He also designed and developed PCR primers and probes to differentiate CTV strains. This information directed efforts in strain-selective eradication of CTV-infected trees in California. He was also the first scientist to detect resistance-breaking strains of CTV in California.

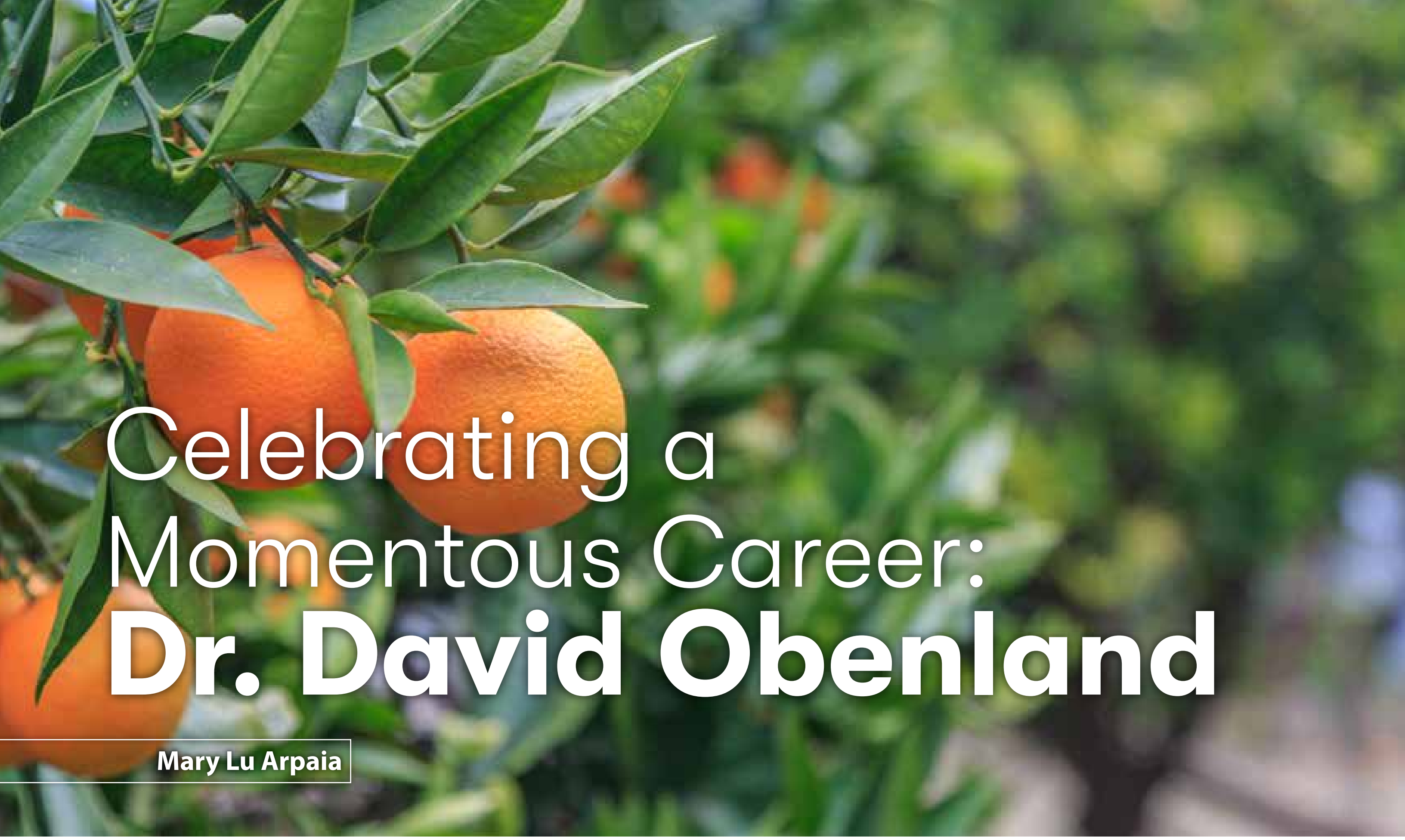
Dr. Yokomi and his team improved detection protocols for *Spiroplasma citri* and *Liberibacter* for regulatory and research purposes. He was a key member of the team at the USDA-ARS in Parlier that identified distinct populations of *Liberibacter* in Southern California, indicating separate introductions from different parts of the world.

More recently, a new citrus virus, citrus yellow vein clearing virus (CYVCV), was detected for the first time in the Western hemisphere, in Tulare and Hacienda Heights, California. As an authority in citrus virology, Dr. Yokomi delayed his retirement to conduct research on key traits of this virus, such as host range and mechanisms of transmission — information needed by regulators and the industry to respond to this new pathogen.

Dr. Yokomi advanced basic and applied citrus pathology research, becoming nationally and internationally recognized for his contributions and leadership. He was a member on panels for the National Academy of Science and for Florida's Citrus Research and Development Foundation and also served on numerous committees for both the Entomological Society of America and American Phytopathological Society.

Dr. Yokomi's scientific contributions have been fundamental in protecting and maintaining California's citrus industry. Following his retirement, Dr. Yokomi plans to enjoy his time with family and friends, travel, and serve as a volunteer at the USDA-ARS in Parlier to assist in research on emerging citrus diseases. We congratulate Dr. Yokomi on his industrious career and wish him a merry retirement.

Rodrigo Krugner, Ph.D., is a research scientist and research leader of the Crop Diseases, Pests, and Genetics Research Unit at the U.S. Department of Agriculture - Agricultural Research Service (USDA-ARS) San Joaquin Valley Agricultural Sciences Center in Parlier, California. MaryLou Polek, Ph.D., is the former research leader for the USDA-ARS Citrus and Date Germplasm Repository in Riverside, California. Meagan Iott is an associate research scientist at the Citrus Research Board (CRB) and serves as associate science editor of **Citrograph.** ***Melinda Klein, Ph.D., is the chief research scientist at the CRB and serves as scientific editor of*** **Citrograph.** ***For more information contact*** **research@citrusresearch.org**



Celebrating a Momentous Career: Dr. David Obenland

Mary Lu Arpaia

Dr. David Obenland at the United States Department of Agriculture – Agriculture Research Service (USDA-ARS) San Joaquin Valley Agricultural Science Center (SJVASC) in Parlier, CA retired in December 2025. Dr. Obenland had a long association with the California citrus industry, joining the USDA as a postdoctoral researcher in 1993 after working as a postdoctoral researcher at the Botanical Institute in Basel, Switzerland and the Kennedy Space Center in Florida. He was appointed as a research plant physiologist at the SJVASC in 1997. His contributions to the California citrus industry are varied but largely focused on the maintenance of fruit quality following harvest and the response to post-harvest quarantine treatments. He collaborated with industry and other researchers to gain answers for the citrus industry to optimize quality and secure market share through his research efforts, providing advice and collaboration with packinghouses and service companies as well as speaking at industry outreach events such as the Citrus Research Board (CRB) Citrus Post-Harvest Conference.

His early research focused on lemon chilling injury and the fruits' response to heat treatments and fumigation. This work highlighted the importance of fruit peel turgidity and subsequent damage susceptibility to hot water treatments and general fruit handling. At the same time, he initiated a career-long research program on examining post-harvest factors involved in citrus flavor and eating quality in response to varied quarantine treatments such as high-temperature forced air treatments, cold treatment, methyl bromide and phosphine fumigation, and irradiation. Most recently his work in this area confirmed the lack of phytotoxicity of phosphine fumigation on packed citrus, helping to pave the way for wide-scale adoption of this alternative fumigant.

As mandarins gained importance in California, Dr. Obenland shifted his attention to flavor quality. His work highlighted the importance of post-harvest conditions in the development of off-flavors in this citrus group with an emphasis on fruit maturity, waxing and holding temperature. This work illustrated the interaction of these factors on off-flavor development as well as differences between mandarin types. Most recently, he collaborated on a project concerning rind quality of 'Owari' satsuma mandarin.

Arguably, Dr. Obenland's greatest contribution to the California citrus industry was his involvement in the multi-year project initially funded by the CRB which resulted in the establishment of the California Standard. This research changed how the industry determined minimum maturity of navel oranges. This effort involved several years of data collection on the role of soluble solids content and titratable acidity and consumer acceptance; a Specialty Crop grant in collaboration with California Citrus Mutual building upon this data with consumer tests in Chicago and finally providing testimony to the California Department of Food and Agriculture to advocate with the industry for a change in the Code of Regulations in 2012. According to some in the industry, the outcome of this effort has bolstered and secured the California navel orange industry.

"Dave has been a generous and eager partner with the industry since I came on board with FMC/JBT in 1988. I remember his work looking at methods to assess freeze damage in the late 1990s and attending workshops to show industry how to use black light techniques to identify fruit of concern. Dave was the pioneer on the West Coast for using internal gas exchange as a method of measuring the respiration differences between different varieties of mandarins and the impact of post-harvest treatments on fruit quality. Our company used this work and the studies he did characterizing the peel of mandarins to assist in developing coatings that would be suitable for their unique characteristics. Anytime we had questions or needed support, Dave was always open to listen and help."
– Charlene Jewell, retired JBT Corporation

Dr. Obenland's contributions to the California industry have had a positive impact on our understanding of citrus fruit quality and the importance of the high-quality standards that characterize this industry. From a personal perspective, I will greatly miss working with Dr. Obenland. We have had a long and productive working partnership for many years, and I have greatly valued his friendship and steadfastness. Following his retirement, Dr. Obenland plans to enjoy his time hiking and traveling with his family. We congratulate Dr. Obenland on his impactful career and wish him a long and happy retirement.

***Mary Lu Arpaia, Ph.D., is a professor of extension in Department of Botany and Plant Sciences at the University of California, Riverside. For more information, contact* mlarpaia@ucanr.edu**

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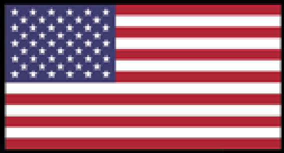

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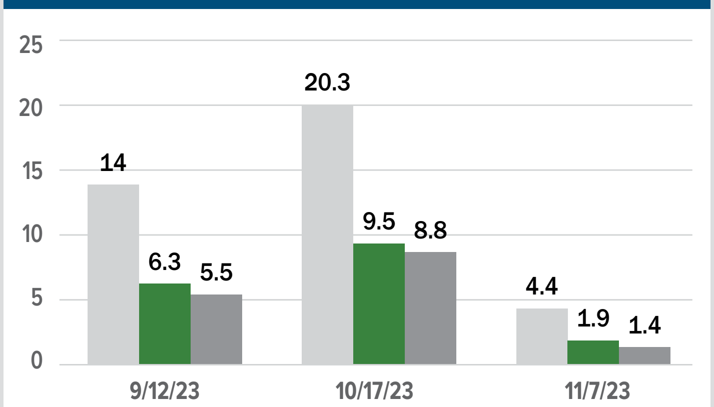
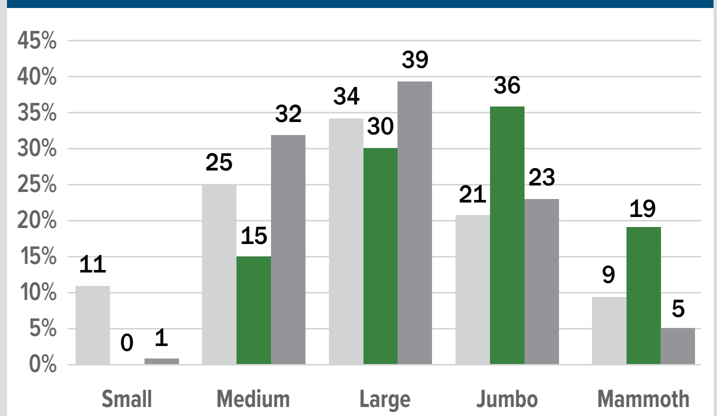
CITRUS – SUNBURN AND YIELD

Var. Miho Wase – Sawtooth Ag Research, Farmersville, CA 2023

Count of Sunburn-Damaged Fruit at 3 Evaluation Dates



Yield – % Grade Size



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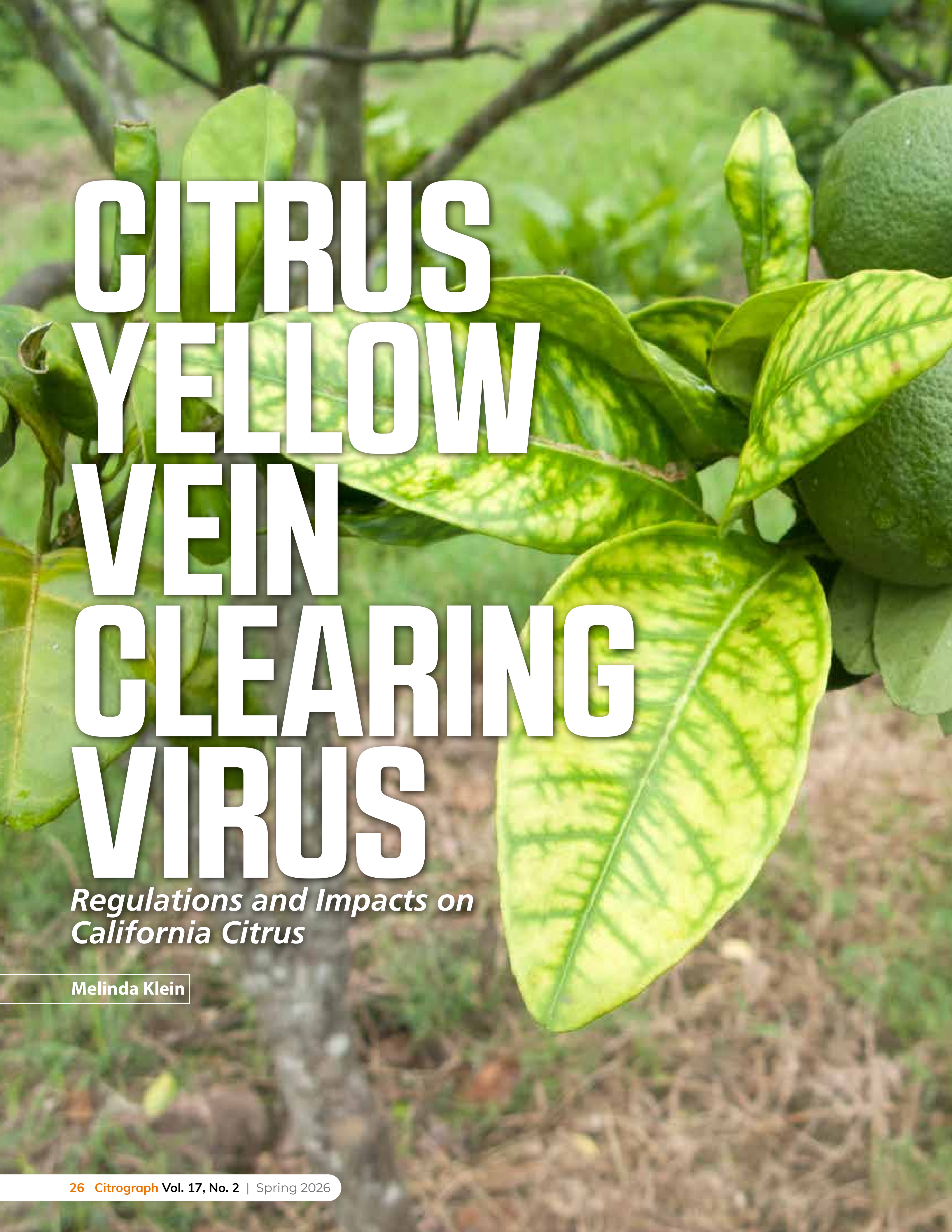

CITRUS YELLOW VEIN CLEARING VIRUS

Regulations and Impacts on California Citrus

Melinda Klein

California's citrus industry faces ongoing threats from invasive pests and plant pathogens that can undermine tree health, reduce yields, and disrupt domestic and international markets. The newest citrus pathogen detected in the state is Citrus yellow vein clearing virus (CYVCV). This virus has been shown to impact lemon and sour orange production in other citrus growing regions and can be spread mechanically, through grafting and by insect vectors. CYVCV has a global footprint, but a detection in the San Joaquin Valley in 2022 was the first appearance of this virus in the Americas. The discovery of CYVCV in California has prompted research efforts to understand the potential risk of this virus to the California citrus industry. It has also led to the development of quarantine measures. The quarantine guidelines for this viral pathogen are outlined below and compared with another citrus virus under regulatory control.

CYVCV Biology and Potential Impacts

CYVCV is an RNA virus that infects citrus and induces foliar symptoms including yellow veins or translucent vein clearing, leaf mottling, and leaf distortion or warping (Catara et al. 1993). Lemons (*Citrus limon*) and sour orange (*C. aurantium*), while not the only varieties affected, consistently exhibit the most pronounced symptoms and have been shown in other citrus producing regions to experience reduced vigor and declines in fruit yield and quality (Chen et al. 2014, Zhou et al. 2017). When California commercial varieties were surveyed for symptom development by Sun et al, this pattern remained true, with severe symptoms seen in Lisbon and Eureka lemons (2025); yield studies have not yet taken place in state. The expression of symptoms and disease severity appears to vary by both citrus variety and growing conditions, with higher temperatures reducing CYVCV symptom development and viral titer (Zhou et al. 2017, Yokomi et al. 2026). These heat-dependent effects look to be transient, as symptom severity and viral titer rebound when temperatures decrease, indicating suppression rather than elimination of infection.

Despite its name, the host range of CYVCV is not limited to citrus. Several herbaceous and weedy plant species have been identified as non-citrus hosts, with infections, when occurring, remaining localized rather than systemic. CYVCV has also been reported on wild grapevine (*Vitis vinifera*) in Türkiye, although experimental attempts to infect grape in state under controlled conditions have been unsuccessful (Afloukou and Önelge 2020, Sun et al. 2025). The presence of non-citrus hosts may complicate disease management by providing environmental reservoirs for the virus, but recent work suggests only localized infection and lack of systemic viral movement, (Yokomi et al. 2026) suggesting the risk from alternate hosts may be lower than originally feared.

CYVCV transmission can occur through two main pathways - physical transmission (i.e., grafting infected budwood or by contaminated tools and equipment) and insect-mediated transmission. CYVCV insect transmission has been shown elsewhere by several aphid species —including the spirea aphid (*Aphis spiraecola*), cowpea aphid (*A. craccivora*), and melon aphid (*A. gossypii*)—as well as the citrus whitefly (*Dialeurodes citri*) (Önelge et al. 2011, Zhang et al. 2018a, Zhang et al. 2018b, Afloukou et al. 2021). These vectors are widespread throughout California citrus growing regions. However, recent in-state trials have just shown transmission via citrus whitefly (Yokomi et al. 2026).

CYVCV Quarantines and Control Measures

In response to confirmed CYVCV detections, the California Department of Food and Agriculture (CDFA) established a State Interior Quarantine under Section 3447 of Title 3 of the California Code of Regulations. The following summary of quarantine regulations is intended for informational purposes only; the official regulatory text should be consulted to verify compliance requirements.

Under this regulation, a quarantine zone is designated when a single host plant is confirmed positive for CYVCV. CDFA, in coordination with local County Agricultural Commissioners (CACs), can then establish a quarantine area with a minimum one half mile radius surrounding the infested host. Once a quarantine is established, possession and movement restrictions apply to hosts and potential carriers of CYVCV. Hosts include citrus nursery stock, plants and plant parts, green waste, and fruit (excluding seed) of *Citrus* spp. and *Fortunella* spp., as well as any other articles deemed infested or exposed to infestation by the virus. Possible carriers of CYVCV include harvesting, processing, and hauling equipment when determined by CDFA or CACs to pose a risk of virus spread. There are exemptions to these restrictions – dead or dying plant material may be processed in an approved manner to eliminate CYVCV and moved directly to landfill or composting facilities within the quarantine zone. Commercially cleaned, graded, and packed host fruit, as well as citrus fruit for personal use (up to 25 pounds), are also allowed to move within and from the quarantine area.

Within quarantine areas, special permits may be issued to allow possession or movement of regulated nursery stock and plant materials. Alternatively, these materials may be maintained in a department-approved structure. All nursery stock offered for sale or distribution within a quarantine zone must be cleaned and/or treated to eliminate all live insect vectors of CYVCV to the satisfaction of CDFA or the CAC. Movement of host fruit, commodities or potentially infested articles from the quarantine area would require special permits; possible carriers of CYVCV would require treatment/cleanings to the satisfaction of CDFA or CAC prior to movement.

Potential CYVCV Impacts

CYVCV is part of CDFA's statewide citrus multipest survey, which allows for continued monitoring of this pathogen. Should additional detections expand or create new quarantines, commercial production could be more directly impacted. Current CYVCV regulations take a more expansive view of regulated materials than those associated with Citrus Tristeza Virus (CTV) quarantines, which restrict only the movement of citrus plants and propagative materials of common citrus genera (i.e. *Citrus, Fortunella, Poncirus*), their hybrids and more distant members of the Rutaceae (3 CA ADC § 3407). There are significant distinctions between CTV and CYVCV. CTV is far more widespread in California but has been extensively studied over the last 80 years, allowing industry and regulatory bodies the chance to shift to a more measured response. CYVCV regulations currently extend beyond plant material to include equipment and other possible carriers, reflecting concerns about mechanical and vector-assisted transmission pathways. This highlights the lack of information currently available on CYVCV transmission and disease severity that might be expected for California varieties and growing conditions.

Conclusion

CYVCV is the latest biotic threat to California's citrus industry. Continued research, surveillance, and collaboration among scientists, regulatory agencies, and industry stakeholders will be essential to address the risk from this pathogen for California citrus growers. Moving forward with a flexible, science-based regulatory framework that works to mitigate long-term risk without imposing disproportionate burdens on producers will be essential to safeguarding the productivity, economic viability, and sustainability of California citrus.

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***Melinda Klein, Ph.D., is the chief research scientist at the Citrus Research Board and serves as scientific editor for* Citrograph. *For more information, contact* melinda@citrusresearch.org**

USING MACHINE LEARNING TO SPOT HUANGLONGBING
IN HARD-TO-FIND PLACES

Robert Clark, Alex Blake, and Tim Farkas

The objective of this project was to use cutting-edge statistical techniques — machine-learning models — to forecast locations in California where plants infected with the 'Candidatus Liberibacter asiaticus' (CLas) bacterium could be found. Our project sought to use all available data on huanglongbing (HLB), the disease associated with CLas, and its environmental causes, to plan regulatory surveys in service of protecting the California citrus industry. With high accuracy, machine-learning models predicted the probability of finding CLas infected plants in locations across Southern California as part of the California Department of Food and Agriculture's (CDFA) regulatory survey activities in 2025. When tested in the field, CLas positive trees were found at several survey sites identified by the machine-learning model that were not originally scheduled to be surveyed, including several residential areas near commercial citrus. Automated data processing and cloud-computing services have been deployed, meaning that the machine-learning model is ready for adoption and use to plan surveys at low cost.

A critical part of epidemiology is predicting where and when a disease may occur. In this project, we built a machine-learning model that processes a massive amount of data from public databases and remote sensing databases. These data sources correspond to known factors that influence progression of an insect-vectored disease like huanglongbing (HLB), such as human movement or weather. Once the model was built, our next goal was to ensure predictions are accurate. The gold standard for building trust in a disease forecast is ground-truthing[1]. Ground-truthing involves surveying a large number of sites predicted by the model as sites of disease to confirm accuracy. Just like in a hurricane weather forecast or an economic forecast, the only real way to know if you can trust the model is to see how well it performs in the real world.

Machine-learning models[2] belong to a family of approaches called artificial intelligence[3] (AI), which have many advantages over traditional mathematical epidemiological models. In a traditional approach, a team of subject-matter experts determine which factors that drive disease are most important. These are then used to predict where a disease is likely to be found or where it will be most severe. While this is a fine approach when data are limited, it has two drawbacks. First, the prediction process cannot be fully automated because researchers must manually enter information at each stage of the modeling process. Second, it is less likely to have mistakes of omission that a more sophisticated computer algorithm can avoid since it processes all the data. In our approach, we cast a wider net over many possibly important predictor variables and let the machine-learning process discover which variables are most important. We then used a testing dataset to continuously validate the models. New data can be remotely incorporated using cloud computing services without need for continual work from a researcher. Ultimately, the final machine-learning model is more flexible, accurate, and cheaper to maintain.

Over the past two years, EcoData scientists, supported by the citrus industry, have been building a machine-learning model that predicts locations of future HLB finds. The work has been a massive technical undertaking, where the team has processed data from a variety of sources including but not limited to cell phone movements, weather reports, state regulatory surveys, and public reports of host plants. The first full prototype was developed after the first year of the project, and the second year has focused on refining the work through data automation and building software to support long-term maintenance.

So, what does the machine-learning model *actually* predict? In short, the machine-learning model provides a probability that a CLas positive plant will be found when field teams conduct California Department of Food and Agriculture (CDFA) multi-pest survey activities and sample in a given location. It does not predict which locations across square mile grids (STRs[4]) will have HLB and which will not. To make this prediction, we are looking at historical data of CLas found in plant tissue as part of the regulatory surveys and projecting for the next round of surveys. For every STR in California, the model is asked: What is the chance a CLas positive plant will be discovered via field surveys and molecular testing of plant tissue over the next round of surveys? Most STRs outside the core area of Orange and Los Angeles counties have a probability of <1%, but if hundreds of STRs are searched, it becomes statistically guaranteed that there will be at least one found. The goal of our model validation activities is to verify whether these probabilities are correct.

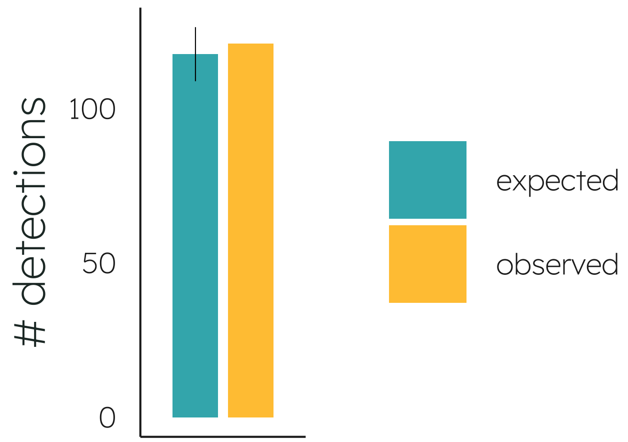

Figure 1: Expected number of HLB detections (118 ± 8.8) and observed HLB detections (121) from 2025 CDFA surveys. Expected detections were predicted using the EcoData machine-learning model using historical survey data through 2024 and based on the planned locations for CDFA surveys (provided to EcoData before surveys were conducted). Error bar shows bootstrapped 95% confidence interval.

The rubber meets the road with ground-truthing. A model can look impressive on paper, but then when it is used in the real world, it turns out it is not much better than guessing where new diseases may be found. We completed two forms of ground-truthing to validate our model. First, we looked at how the 2024 version of the model performed using the data from the surveys conducted in 2025. We were able to use AI to calculate the probability of finding HLB in every single STR searched by the CDFA as part of regulatory surveys in 2025. Second, we provided a list of locations the CDFA was not planning to survey to see if the machine-learning model could find HLB in areas that would have been missed. These were locations with relatively high likelihood of discovering new CLas positive trees in strategically important areas near quarantine boundaries or in residential areas in proximity to commercial citrus.

In the first type of ground-truthing, EcoData's model predicted that if the CDFA visited its planned sites in 2025, it would find 118 STRs with HLB. Most epidemiological models also account for environmental variation and uncertainty by giving a range of estimates, much like a hurricane forecast which shows a range of locations in which the storm could touch down. As long as the true results lie within that range, we know the predictions are reliable. So, to be more accurate, our model predicted a range of 110 to 127. The CDFA found 121 (**Figure 1**). That means that without knowing anything about the current year's environmental conditions, HLB finds in 2025, or any related data, our model was able to predict the future. Finally, despite the major strategic changes to the residential survey priorities over the last year and multiple changes to focus on field work to areas around commercial citrus, the model remained adaptable and useful.

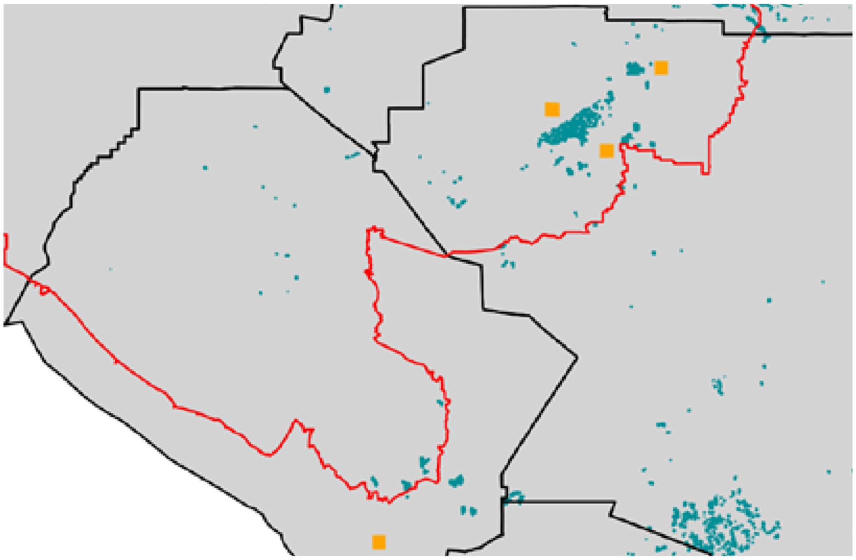

Figure 2: Map of the five locations with HLB detections (yellow cells) originating from the shortlist of locations recommended to the CDFA by EcoData in 2025. This shortlist consisted of locations that were not earmarked for surveys in 2025, but which were 1) within a designated 'citrus buffer' near commercial citrus groves (shown in blue) or 2) outside of existing quarantine zones as of January 1, 2025 (shown in red). Probability of HLB detection was predicted by the EcoData machine-learning model based on historical survey data through 2024. Map is cropped to Los Angeles, Orange, San Bernardino, Riverside, and San Diego counties, with county boundaries shown in dark grey. For the four locations with HLB found within existing quarantine zones, prior CDFA surveys had taken place in 2018, 2021, 2023, and 2024. For the location with HLB found outside existing quarantine zones (in Orange County), surveys were last conducted in January 2021.

In the second type of ground-truthing, we requested the CDFA Citrus Division to search a specific set of locations that they did not plan to visit. In addition to validating the model itself, we also wanted to verify if the machine-learning model was capable of finding HLB locations that would otherwise be missed, especially locations of extraordinarily high threat to the industry. We considered high-threat locations to be STRs outside the quarantine zone near commercial citrus. These are the "needle-in-a-haystack" locations since this is outside the area where HLB is prevalent, so the expected disease incidence is still very low — only fractions of a percent, but the consequences to the industry would be massive if these were left unchecked. How did the machine-learning model perform by this metric? We provided a list of 40 STRs that were not included in the initial residential survey plan for 2025 and asked that those sites be visited. CDFA was able to include 39 of those sites into their survey efforts, and five new STRs with CLas positive plants were found (**Figure 2**). These sites would have been missed this year if not for our model. Even with this small sample size, we demonstrated that the machine-learning model helps find HLB in locations that would have otherwise been passed over, increasing the threat of HLB to the industry.

Knowing that the machine-learning model works, growers may be wondering how it can be deployed to realize tangible benefits to the citrus industry. If utilized correctly, the platform we built ensures that the CDFA is being advised by all the data available to manage surveys more efficiently. The industry can be confident that manpower is not being wasted searching in areas where HLB will not be found in the current survey, and that newly emerging hot spots of HLB are found earlier. From a research perspective, the machine-learning model can be maintained at a lower cost because the most critical data sources come from publicly available databases or remote-sensing databases that only need to be accessed once a year or less. In short, this machine-learning platform not only supports survey efficiency and early detection of HLB, but also provides a long-term, cost-effective way to plan surveys that can aid in regulatory initiatives and industry research well into the future.

CRB Project #5300-215

Rob Clark, Ph.D., is the founder and principal data scientist of EcoData Technology LLC, a data science professional services company. Alex Blake, Ph.D., is a biological data scientist at EcoData Technology LLC and lead modeler on the HLB machine-learning project. Tim Farkas, Ph.D., is the director of data science at EcoData Technology LLC and lead engineer on the HLB machine-learning project. For additional information, contact **robclark@ecodata.tech**

Glossary

[1]**Ground-truthing:** The process of validating information collected indirectly by direct observations or measurements.

[2]**Machine-learning:** A statistical approach that uses algorithms to identify patterns and relationships in data and to build models that learn from past observations in order to predict future outcomes.

[3]**Artificial intelligence:** Computer systems that use algorithms to learn patterns from data and make predictions or decisions.

[4]**STR:** A mapping abbreviation that identifies land locations using the section, township, and range components of the Public Land Survey System, a system used in California for survey activities.

CRB-FUNDED FINAL RESEARCH REPORT

HOST RANGE AND TRANSMISSION OF CITRUS YELLOW VEIN CLEARING VIRUS CALIFORNIA ISOLATES

Raymond Yokomi, Ying Zhai, and Sydney Helm-Rodriguez

Project Summary

This research was conducted to determine how the newly introduced Citrus yellow vein clearing virus (CYVCV) affects popular California citrus cultivars. CYVCV was shown to multiply in a wide range of citrus cultivars. Lemon cultivars and sour orange typically showed strong symptoms, while the rest of the cultivars remained asymptomatic or had very mild symptoms of CYVCV. Growth measurements of infected citrus cultivars were variable but showed no trend of deleterious effects. In our experiments, CYVCV transmission was achieved only with citrus whitefly and only when pruning Citrus macrophylla *seedlings with CYVCV-contaminated clippers, but in both cases, transmission efficiency was low, 3.7% and 0.59%, respectively. Mechanical transmission of non-citrus herbaceous plants was demonstrated in inoculated leaves, but the infection was short-lived. Viral systemic movement away from inoculated leaves or to new growth did not occur. Therefore, these herbaceous plants were not considered hosts of CYVCV. Seasonal variation of virus titer and symptomology were observed in the greenhouse, and growth chamber experiments showed that daytime temperatures from 32 to 37°C reduced CYVCV titer and symptoms in Eureka and Lisbon lemons. Although these data were collected in the greenhouse, the results have relevance to how CYVCV may affect and spread in citrus in California.*

What is CYVCV?

Citrus yellow vein clearing virus (CYVCV) is an exotic citrus virus found in California. In a regulatory delimiting survey in 2022, 584 citrus trees of various cultivars were found infected with CYVCV in urban landscapes in Tulare (Abrahamian et al. 2024). CYVCV was also detected in one lemon tree in Hacienda Heights in late 2023 (Sun and Yokomi 2024). Reports from Turkey and China indicate that CYVCV has a wide range of citrus and non-citrus hosts and is vectored by both citrus aphids and the citrus whitefly and is mechanically transmissible (Zhou et al. 2017). In Anyue, Sichuan Province, China, CYVCV was mentioned to cause a 50-80% loss of lemon production and fruit quality (Zhang et al. 2019). The virus has a filamentous morphology and occurs in phloem and other cells in the citrus leaf (Loconsole et al. 2012). The effect of this virus on California citrus is unknown; therefore, the aim of our study was to assess how this pathogen will affect citrus cultivars of value to the California citrus industry. The specific research objectives of this research project were to determine: 1) the effect of CYVCV infection on various citrus cultivars, including orange, mandarin, tangelo, grapefruit, limes, and pummelo by graft inoculation, and some non-citrus hosts by mechanical and insect vector transmissions; 2) how temperature affects CYVCV titer and disease expression in lemons. In addition, we completed research on insect and mechanical transmission of the virus in citrus.

CYVCV Host Range

Citrus. Host range of CYVCV was evaluated in the greenhouse by graft transmission to different citrus cultivars and relatives obtained from commercial citrus nurseries in California (**Table 1**). All inoculated cultivars were successfully infected with CYVCV and supported active virus replication, as confirmed by RT qPCR[1] (**Table 1**). Eureka lemon, Allen Eureka lemon, 8A Lisbon lemon, and Sour orange exhibited typical severe virus symptoms of CYVCV including yellow vein clearing of the top leaf surface and water soaking appearance on the bottom surface of the leaf, along with some leaf distortion (**Figure 1A**). Periodic symptoms of mild vein clearing or flecking without water soaking appearance were observed in Barnfield Navel, Autumn Gold Navel, Kinnow, Shiranui, Owari, Minneola, Clemenules, and Persian lime, as shown by Moro Blood sweet orange (**Figure 1B**). These mild symptoms were inconsistent and would

Table 1. Citrus varieties graft-inoculated with Citrus yellow vein clearing virus (NCBI Accession Number OR037276.1) (Sun and Yokomi 2024) from Tulare, California. Infection status determined by RT-qPCR.

CULTIVAR	ROOTSTOCK	NOMENCLATURE	NO. POSITIVE/NO. TESTED
Autumn Gold Navel	Carrizo	*Citrus sinensis*	3/3
Barnfield Navel	Carrizo	*C. sinensis*	3/3
Cara Cara Navel	Carrizo	*C. sinensis*	3/3
Newhall Navel	Troyer	*C. sinensis*	3/3
Parent Washington Navel	Carrizo	*C. sinensis*	3/3
Olinda Valencia	Carrizo	*C. sinensis*	3/3
Moro Blood sweet orange	Carrizo	*C. sinensis*	3/3
Gold Nugget mandarin	Carrizo	*C. reticulata*	3/3
Kinnow mandarin	Volkermer	*C. reticulata*	3/3
Shiranui mandarin	Carrizo	*C. reticulata*	3/3
Tango mandarin	Carrizo	*C. reticulata*	3/3
Owari Satsuma mandarin	Carrizo	*C. unshiu*	3/3
Minneola tangelo	Carrizo	C. × tangelo	3/3
Clemenules clementine	Carrizo	*C. clementina*	3/3
Star Ruby grapefruit	Carrizo	*C. paradisi*	3/3
Chandler pummelo	Carrizo	*C. maxima*	3/3
Persian lime	Carrizo	*C. latifolia*	3/3
Nagami kumquat	Carrizo	*Fortunella margarita*	3/3
Australian finger lime	Carrizo	*Microcitrus australasica*	3/3
Eureka lemon	Carrizo	*C. limon*	3/3
Allen Eureka lemon	Flying Dragon	*C. limon*	3/3
8A Lisbon lemon	*C. macrophylla*	*C. limon*	3/3

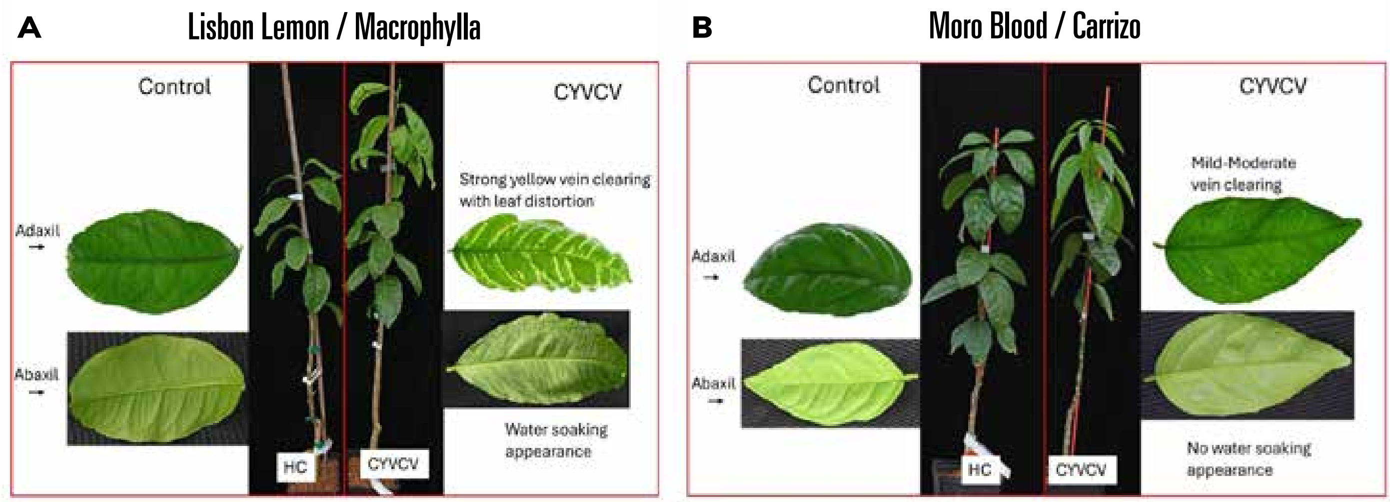

Figure 1. Citrus yellow vein clearing virus (CYVCV) symptoms observed in the greenhouse. A.) Strong symptoms in 8A Lisbon lemon/*C. macrophylla*. B.) Mild symptoms of slight vein clearing and flecking and in Moro Blood sweet orange grafted on Carrizo. HC = Healthy control; CYVCV= CYVCV infected plants.

disappear after a few days or a week. Most of the citrus varieties remained asymptomatic throughout the duration of the 18-month experiment. These data are in line with personal observations of many CYVCV-infected trees in Tulare dooryards, where only lemon trees showed typical strong symptoms while other cultivars were symptomless or showed occasional mild vein clearing, which faded away after a period of time. Greenhouse growth measurements of stem diameter, plant height, and foliar biomass of CYVCV-infected citrus varieties measured approximately 18-months post-infection showed no consistent trend of deleterious effects of CYVCV (**Figure 2**).

Non-citrus plants. CYVCV was mechanically transmitted to herbaceous plants using purified virions (**Table 2**) or virus in crude sap from CYVCV-infected citrus leaves. However, virus multiplication was limited to inoculated leaves and never moved to new growth. This was further tested in cowpea and rainbow quinoa. Purified virions[2] or crude sap from CYVCV-infected lemon leaves were mechanically inoculated into one of the triplicate leaflets in cowpea, which resulted in an infection in the inoculated leaflet but did not move to the other leaflets or move systemically to other leaves or to new growth (**Figure 3A**). A similar result was obtained with rainbow quinoa, where infection was limited to the inoculated leaf (**Figure 3B**). In cowpeas, we noted that virus expression was highest on one day post inoculation (DPI) and subsequently decreased through 13 DPI, whereas virus expression in rainbow quinoa increased with each sample interval to 11 DPI. After 11 DPI in rainbow quinoa and 13 DPI in cowpea, there were no more inoculated leaves remaining on the plants to sample, therefore, the samples taken on 20 DPI were new growth. Additional experiments are needed to ascertain why virus expression in inoculated leaves increased with time in rainbow quinoa while titer decreased in cowpea but virus-induced stress or senescence may be involved. Since virus replication occurred only in inoculated leaves

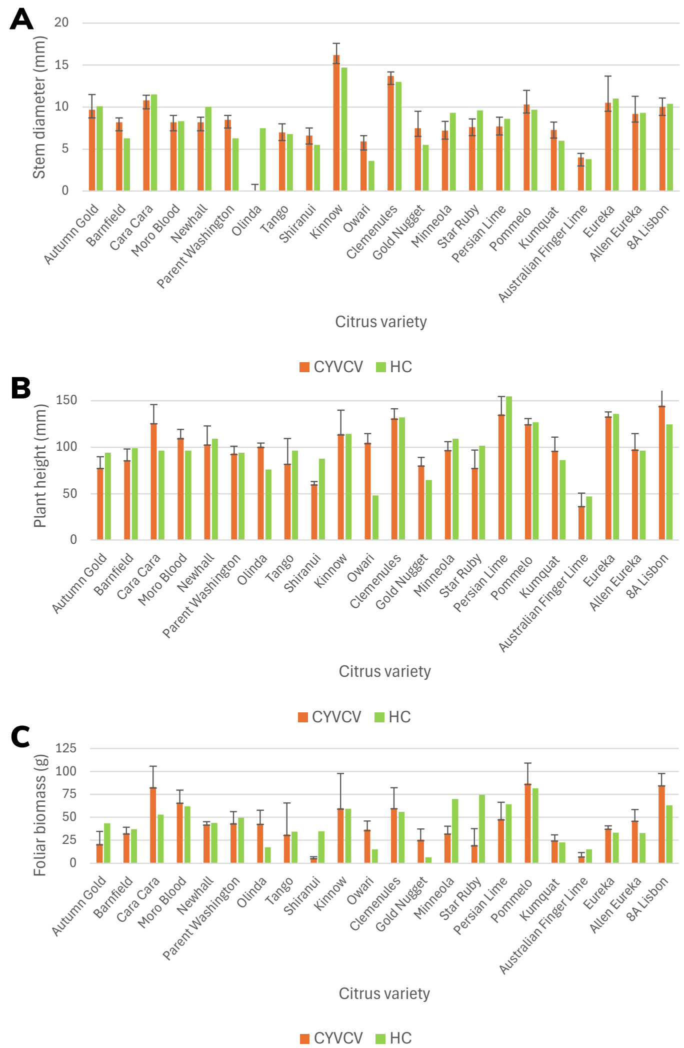

Figure 2. Greenhouse growth measurements of young citrus varieties infected with citrus yellow vein clearing virus (CYVCV). Measurements were taken on July 17, 2025, approximately 18 months after inoculation. Graph A represents stem diameter (mm). Graph B represents plant height (mm). Graph C represents foliar biomass (g). CYVCV= CYVCV infected plants; HC = Healthy control plants.

Table 2. Mechanical transmission of citrus yellow vein clearing virus to non-citrus herbaceous plants using purified virions. Infection status determined by RT-qPCR.

PLANT FAMILY	COMMON NAME	GENUS AND SPECIES	NO. POSITIVE/ NO. TESTED
Amaranthaceae	Rainbow quinoa	*Chenopodium quinoa*	5/5
Brassicaceae	Sweet alyssum	*Lobularia maritima*	7/10
Brassicaceae	Mustard greens	*Brassica sp.*	3/3
Fabaceae	Common bean	*Phaseolus vulgaris*	5/15
Fabaceae	Cowpea	*Vigna unguiculata*	9/15
Fabaceae	Kidney beans	*Phaseolus vulgaris*	2/10
Fabaceae	Sugar peas	*Pisum sativum*	8/10
Malvaceae	Mallow Rose	*Hibiscus moscheutos*	3/4
Ranunculaceae	Western Buttercup	*Ranunculus occidentalis*	5/10
Solanaceae	Hot pepper	*Capsicum frutescens*	1/1
Solanaceae	Sweet pepper	*Capsicum annuum*	4/10

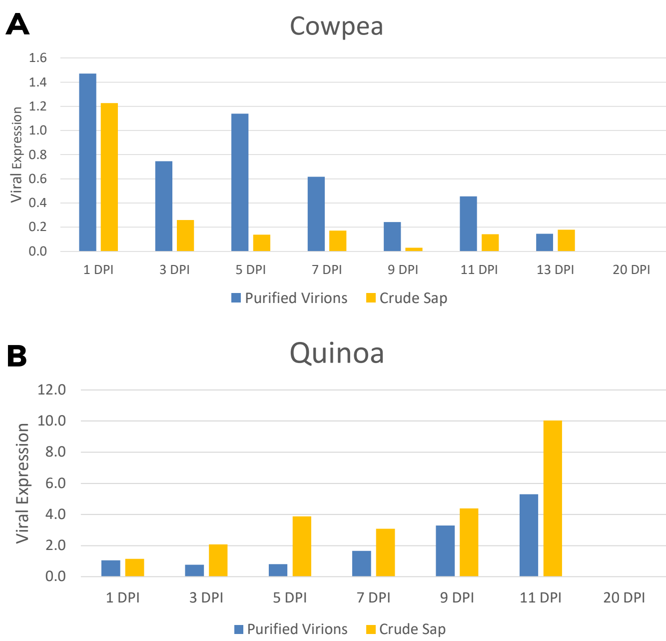

Figure 3. Viral expression of citrus yellow vein clearing virus (CYVCV) at two-day intervals in inoculated leaves of cowpea (A) and quinoa (B) after mechanical inoculation of purified virions or crude sap from CYVCV-infected lemon leaves with carborundum powder as an abrasive. DPI = days post inoculation. No virus was detected in non-inoculated leaves of cowpea so that data is not shown. Data shown at 20 DPI is from non-inoculated new growth of cowpea or quinoa plants.

Table 3. Results from insect transmission tests of citrus yellow vein clearing virus where insects were allowed to feed for 48-hours on virus infected lemon or sour orange leaves for virus acquisition and immediately transferred to *Citrus macrophylla* and Eureka lemon for a 48-hour feeding period for virus transmission. Insects were killed after 48 hours, and receptor plants were tested for the presence of virus after 30, 60 and 90 days by RT-qPCR.

INSECT VECTOR	TAXA	NO. TRANSMITTED/NO. TESTED
Citrus whitefly	*Dialeurodes citri*	1/27 (3.7%)
Woolly whitefly	*Aleurothrixus floccosus*	0/20
Citrus mealybug	*Planococcus citri*	0/60

and persisted for less than 20 days without movement to new growth in the greenhouse, these data suggest that these herbaceous plants are only temporary hosts of the virus and would have minimal consequences on disease distribution. This data updates our data on mechanical transmission of herbaceous plants where we reported no CYVCV transmission was obtained in common bean and cowpea in new growth measured from 7 to 33 DPI (Sun et al. 2025). In the same report, we also presented our evidence that mechanical transmission to CYVCV to grapevines was negative.

Insect vector transmission. In recent insect vector experiments, we obtained one CYVCV transmission out of 27 replications (3.7%) by the citrus whitefly using 75 to 100 insects per *Citrus macrophylla* or Eureka lemon receptor plant (**Table 3**). We also tested woolly whitefly and citrus mealybug as vectors, but no virus transmission was obtained (**Table 3**). The other vectors tested included spirea aphid, melon or cotton aphid, green peach aphid, and greenhouse whitefly, and all have so far failed to transmit CYVCV (Sun et al. 2025). We collected insects from CYVCV-infected trees (citrus red scale, *Scaphytopius* sp. leafhoppers, citrus mealybugs, etc.) and found some of them positive for CYVCV by RT-qPCR, but this represented transient presence of the virus in the mouthparts or gut rather than true vector competence. We have performed many vector tests with field-collected and lab-reared CYVCV-positive citrus mealybugs but have not yet obtained transmission.

Contaminated Clipper Transmission of CYVCV

We obtained mechanical transmission of CYVCV in the greenhouse by trimming branches of young *Citrus macrophylla* seedlings with virus-contaminated clippers at a low rate (0.59%), whereas no transmission was obtained with Eureka lemons (**Table 4**). Trimming was done three times at three-week intervals, and RT-qPCR test was conducted 82 and

189 days after initial trimming. This shows that tree-to-tree mechanical transmission of CYVCV can occur and may be a critical pathway for virus spread in an orchard without proper sanitation of pruning equipment.

Effect of Heat on CYVCV Titer and Symptomology

On May 14, 2025, Eureka and Lisbon lemon plants infected with CYVCV were divided into three different diurnal temperature regimes: 1) Greenhouse temperatures (32/21°C; high day/low night temperatures) and ambient lighting; and growth chambers programmed for a photoperiod of 16/8 hours with 2) moderate heat stress (32/27°C); and 3) high heat stress (37/32°C). A separate set of three Eureka and three Lisbon 'control' plants remained in the greenhouse. The experiment was conducted from May 14 to July 23, 2025, and the impact of these treatments was measured by relative viral expression and through symptom observation.

Relative viral expressions were calculated and transformed from RT-qPCR values to relative viral levels using a standard molecular approach comparing the treatment plants to the control plants within each sample date. After exposure of infected Eureka and Lisbon lemons to the different treatments, relative viral expression was lower 14 DPI (May 28, 2025) for the plants at the higher growth chamber temperatures compared to the greenhouse treatment plants (**Figure 4A** & **4B**). Comparative viral expression of plants shows a decrease in viral expression for all treatments compared to the greenhouse grown 'control' plants. This is most likely due to higher day time temperatures experienced by greenhouse grown plants in late May and early June. The lower viral expression between the greenhouse treatment plants and the greenhouse control plants could be due to spatial temporal differences within the greenhouse. (**Figure 4A** & **4B**).

Virus symptoms were recorded weekly in new growth for the 49-day period of the heat exposure experiment. Symptoms per plant were scored as the total of vein clearing severity (1-5) plus presence/absence (0/1) for each trait: leaf distortion, water soaking, and leaf cupping. The heat experiment was initiated on May 14, 2025, and on that date, all plants showed strong symptoms of CYVCV (**Figure 5A** & **5B**). Virus symptoms in Eureka and Lisbon lemon generally declined in weekly observations in the moderate heat stress and high heat stress treatments compared with those in the greenhouse during the 49-day heat experiment. An example of symptom differences in this experiment is seen in **Figure 6**.

The plants from both growth chambers (moderate and high heat treatments) were returned to the cooler greenhouse diurnal temperatures on July 2, 2025. A marked increase in relative viral expression was observed in the plants returning from the heat treatments after 14 days (results not shown).

Table 4. Mechanical transmission of citrus yellow vein clearing virus by trimming young citrus branches with virus-contaminated clippers. Experiment was initiated on May 14, 2025, and plants were trimmed in three-week intervals a total of three times. The results shown are 189 days following the initial trim. Infection status of plants determined by RT-qPCR.

VARIETY	NO. TESTED	NO. CYVCV	PERCENT TRANSMISSION
Eureka lemon	55	0	0
Citrus macrophylla	511	3	0.59

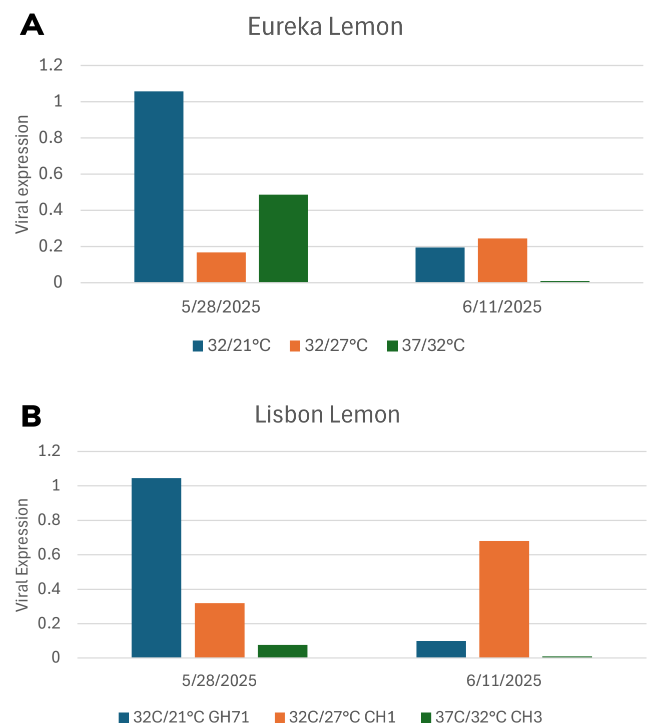

Figure 4. Reduction of relative citrus yellow vein clearing virus (CYVCV) titer in Eureka lemon (A) and Lisbon lemon (B) due to diurnal heat treatment. Plants were moved to temperature regimen on May 14, 2025, and virus titer was determined on day 14 and day 28. Difference between greenhouse treatment and greenhouse control could be due to spatial temporal differences within the greenhouse.

The resurgence of virus expression from hot to cooler temperatures could have epidemiological consequences in some hot temperature citrus-growing regions in California. For example, the Coachella and Imperial Valleys experience night-time temperatures equal or greater than 27°C (80°F) from June through September. Our greenhouse data predict that CYVCV symptom expression may be strongly suppressed during the hottest months in these regions if infected trees are present. However, as night temperatures drop in October–November, systemic titer and symptom severity would expect to increase. In contrast, Central Coast and San Joaquin Valley have cooler summer nights (rarely above 24°C (75°F)), and CYVCV is likely to remain symptomatic.

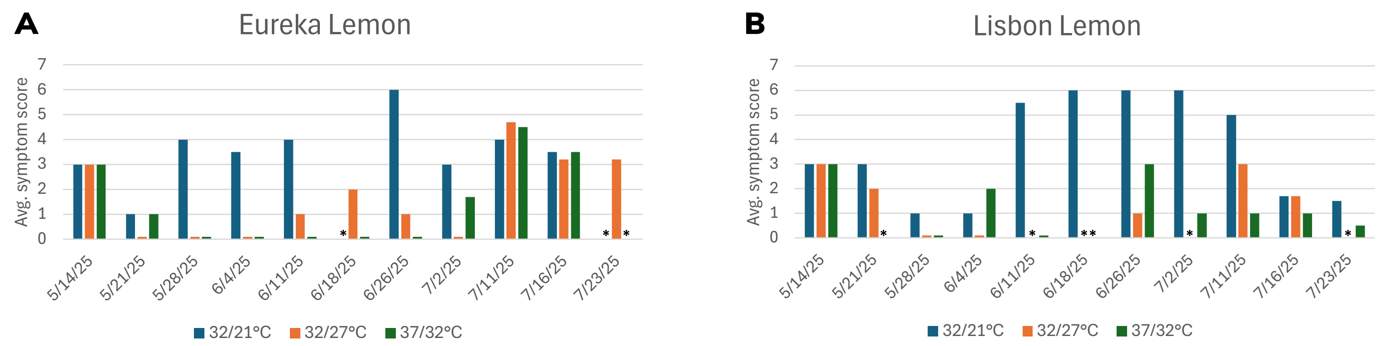

Figure 5. Weekly scores of visual symptoms of citrus yellow vein clearing virus (CYVCV) in Eureka lemon (A) and Lisbon lemon (B) in diurnal heat treatment experiments. The experiment began on May 14, 2025, when all plants were equally symptomatic. After 49 days, on July 2, 2025, plants were returned to the greenhouse. *=No data was collected when there was no young flush. Symptoms per plant were scored as the total of vein clearing severity (1-5), plus presence (1) or absence (0) of leaf distortion, water soaking, and leaf cupping.

Figure 6. Effect of increasing temperature on symptomology of CYVCV in Eureka lemon/Carrizo (A) and 8A Lisbon lemon/*C. macrophylla* (B) 28 days (June 11, 2025) after the start of diurnal treatments.

Conclusion

A broad range of citrus and citrus relatives were shown to be susceptible to infection by CYVCV, but serious disease symptoms were limited to lemons and sour orange. Virus titer and symptomology, if present, were reduced by daytime temperatures greater than 32°C (89.6ºF) for 16 hours. Among many insect vectors tested, only the citrus whitefly transmitted CYVCV at a rate of 3.7%. A low rate (0.59%) of mechanical transmission of the virus to citrus was achieved by pruning branches of young *C. macrophylla* seedlings with CYVCV-contaminated clippers, while no clipper transmission occurred in Eureka lemon plants. Mechanical transmission to non-citrus hosts was shown to occur, but infection was short-lived and limited to inoculated leaves of herbaceous plants. Systemic movement of the virus to new growth was absent and by 20 DPI, the virus was no longer detectable in cowpea and quinoa; hence, these herbaceous plants were not considered to be a host of CYVCV.

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Glossary

[1]**RT-qPCR:** Real Time Reverse Transcription Polymerase Chain Reaction: A variant of PCR that is used to quantify amounts of RNA target sequence in the sample for the purpose of identifying the presence and relative quantity of the target species.

[2]**Virion:** Complete, infective form of a virus outside of a host cell.

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Raymond Yokomi, Ph.D., and Ying Zhai, Ph.D., are research plant pathologists. Sydney Helm Rodriguez is a biological science technician. All work was conducted at the U.S. Department of Agriculture-Agricultural Research Service-San Joaquin Valley Agricultural Sciences Center in Parlier, California. For more information, contact **ray.yokomi@usda.gov**

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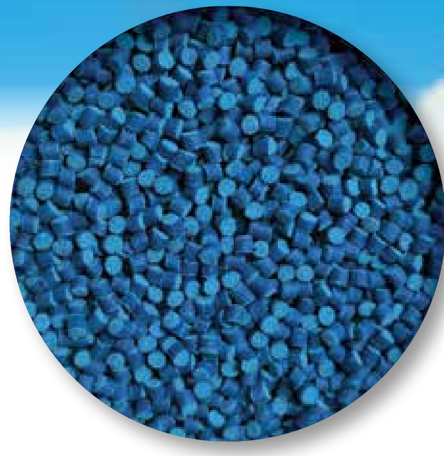

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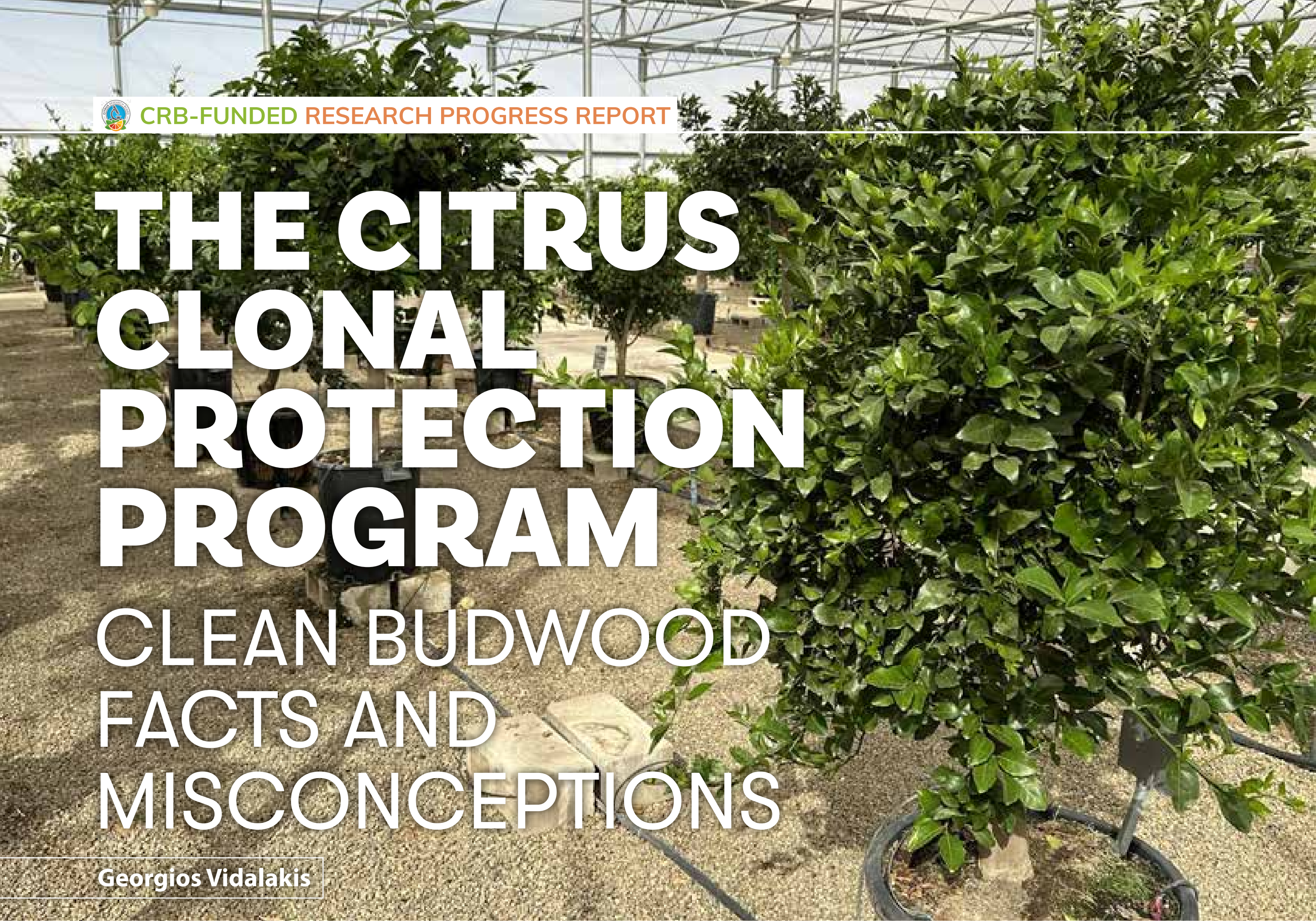

CRB-FUNDED RESEARCH PROGRESS REPORT

THE CITRUS CLONAL PROTECTION PROGRAM

CLEAN BUDWOOD FACTS AND MISCONCEPTIONS

Georgios Vidalakis

Summary

The Citrus Clonal Protection Program (CCPP) safeguards California's citrus industry by providing growers, researchers, and the public with pathogen-tested propagation materials. This report summarizes activities from October 2024 through September 2025 and addresses some misconceptions about clean budwood. Activities from October 2023 through September 2024 were reported previously (Vidalakis 2025).

In recent years, California's citrus industry has faced unprecedented challenges. From huanglongbing (HLB), and other diseases, to climate pressures and shifting consumer demands, the CCPP has remained on the front line of defense, combatting misconceptions and ensuring citrus production remains sustainable. One common misconception is that clean plant programs matter only during disease outbreaks, yet CCPP routinely intercepts infected budwood introductions. Another misconception is that, "if a variety looks healthy, it must be healthy," even though many graft-transmissible pathogens move silently in budwood that is symptomless. Through rigorous detection, therapy, and quarantine protocols, CCPP ensures that only disease-free, high-quality citrus materials enter California and remain available to commercial and non-commercial users.

From October 2024 to September 2025, CCPP received 79 varieties, therapied 83, initiated indexing for 44, and released 50 (**Table 1**). A common misconception is that all quarantine programs and regulations are alike. In reality, California's requirements—such as the California Department of Food and Agriculture's (CDFA) Nursery Stock Pest Cleanliness Program—are among the strictest nationally, and United States federal standards are among the highest globally. CCPP's systems-approach and specialized infrastructure meet these stringent expectations and ensure that every variety entering and released through the program complies with the world's most rigorous clean-plant standards.

Another misconception is that any budwood from anywhere is fine to use, and pathogens are only a concern in trees, not budwood, because of their natural modes of transmission. In fact, testing of the 79 introductions detected 11 pathogens in 30 accessions from eight countries (Brazil, China, Japan, South Korea, Spain, Switzerland, Australia and Italy) and the United States, including citrus viroids, citrus tatter leaf virus, citrus mosaic sadwavirus, citrus tristeza virus, and *'Candidatus* Liberibacter asiaticus', the bacterium associated with HLB. Viroids and tatter leaf virus threaten trifoliate type rootstocks, the dominant rootstocks in California, and specific viroid variants can severely affect mandarins—one of the state's most valuable citrus. Exotic pathogens like citrus mosaic sadwavirus reduce yield and fruit quality, particularly in mandarins and satsumas. Although tristeza is endemic in California, aggressive stem-pitting strains not found in the state can severely damage oranges and grapefruits regardless of rootstock.

Of the 50 varieties released from quarantine and confirmed negative for known graft-transmissible citrus pathogens under CCPP's comprehensive variety index (VI) protocols, 14 became available to the industry, researchers, and the public (**Table 2**). The misconception that a single molecular lab test can declare budwood pathogen-free, or that biological indexing is outdated, overlooks the complexity of citrus pathogens. Molecular tests detect only known targets; mutated variants and unknown agents can evade detection.

Table 1. Number of citrus accessions at different stages of the CCPP introductory process from October 2024 through September 2025.

ACCESSION TYPE*	NUMBER OF ACCESSIONS			
	RECEIVED	THERAPIED	VARIETY INDEX	QUARANTINE RELEASE
Proprietary	22	16	4	2
Public Domain	20	28	14	11
Breeding Programs	30	34	8	3
Re-index	4	4	14	20
Research Material	1	0	1	14
Specific Agreement	2	1	3	0
Total: 256	**79**	**83**	**44**	**50**

**Proprietary: Accessions where the requestor funds therapy and testing costs for exclusive access to the produced budwood source in perpetuity. Public Domain: Accessions available to any interested party for the cost of the budwood unit. Costs of therapy and testing are funded through CCPP's operational budget. Breeding Programs: Therapy and testing of breeding program accessions is funded through the CCPP's operational budget. If the introduction of accessions is part of a funded research project, such as the U.S. Department of Agriculture (USDA)-National Institute of Food and Agriculture (NIFA) or USDA-HLB-Multi-agency Coordination Group (MAC), budgeted research funds are used to offset costs of therapy and testing. Re-index: The re-therapy and pathogen testing of lost or older accessions previously maintained within the CCPP field collection, funded through CCPP's operational budget. Research Material: Accessions from research programs or visiting scholars. Costs of therapy and testing are offset by related research projects or scholarships. Specific Agreement: Accessions receiving therapy and testing under additional agreements with UC Riverside such as material transfer agreements and exclusive rights agreements. These agreements may include payment of cleanup fees by requestors and limited periods of exclusive availability for requestors.*

Table 2. Public domain citrus varieties available at the CCPP and accessions that can be requested from the University of Florida breeding programs, which completed the variety index (VI) from October 2024 through September 2025.

VI	VARIETY	CITRUS TYPE	ORIGIN†	Accession Type
1056	Arvin Pummelo	Grapefruit & Pummelos	Willits & Newcomb Nursery	Public Domain
1476	P2014-175-14 (Misterioso)	Australasian Lime & Hybrid	USDA-ARS-NCGRCD	Public Domain
1477	P2014-175-19 (Maroon Gem)	Australasian Lime & Hybrid	USDA-ARS-NCGRCD	Public Domain
1481	P2014-176-38 (Ruby My Dear)	Australasian Lime & Hybrid	USDA-ARS-NCGRCD	Public Domain
1488	P2014-176-36 (Rubirosa)	Australasian Lime & Hybrid	USDA-ARS-NCGRCD	Public Domain
1492	P2014-176-14 (Toots)	Australasian Lime & Hybrid	USDA-ARS-NCGRCD	Public Domain
1518	Vanoni Macrophylla	Rootstocks	UCR-CBP	Public Domain
1561	Black Twig Lime	Lime	Fruitmentor	Public Domain
1562	Citrus leiocarpa hort. ex Tanaka Koji orange (CRC 3147)	Mandarin – Other	UCR-Givaudan Citrus Variety Collection	Public Domain
1576	Daidai double calyx sour orange (CRC 656)	Sour Oranges	UCR-Givaudan Citrus Variety Collection	Public Domain
1607	Bingo	Mandarins	UF	Breeding Programs*
1627	Croxton Grapefruit	Grapefruit & Pummelos	Triple Bee Nursery	Public Domain
1630	S11x50-7-16-6	Rootstocks	UF	Breeding Programs*
1669	A+HBPxWhite1-13-39	Rootstocks	UF	Breeding Programs*

†Origin types: CCPP: Citrus Clonal Protection Program; USDA: United States Department of Agriculture; ARS: Agricultural Research Service; NCGRCD: National Clonal Germplasm Repository for Citrus and Dates; UF: University of Florida; UCR: University of California, Riverside; CBP: Citrus Breeding Program.
**Accessions available upon request to the University of Florida. Some accessions may be under evaluation or patented.*
For more information on specific varieties, contact the CCPP at ccpp@ucr.edu. For real-time updates on citrus accessions at the CCPP, visit https://ccppdms.ucr.edu/ccppdms/upcoming_varieties.

CCPP therefore uses VI for multiple diagnostic layers, including universal viral RNA detection, high throughput sequencing, biological indexing, and annual post-release monitoring of budwood source trees. Biological indexing remains essential because some diseases lack identified causal agents, and new molecular assays require symptom-expressing reference material for validation. For decades, bioindexing has detected pathogens before sequencing technologies existed, and it remains indispensable today. In citrus, no single test is sufficient; protection comes from combining molecular diagnostics with traditional symptom-based indexing.

During the reporting period, CCPP executed 1,079 orders totaling 83,763 buds from 804 users for 384 VIs. Commercial users ordered the bulk of the budwood at 64,722 buds compared to 14,158 buds for non-commercial users (with remaining buds distributed to international users and research institutions). However, the number of orders made by non-commercial users was nearly ten times more than commercial users (952 vs. 98) and accounted for the majority of purchasers (758 vs. 31).

From the top 10 citrus types distributed, Valencia oranges were the top choice, accounting for 24 percent of budwood orders, followed by navel oranges at 22 percent and mandarins at 19 percent. The other seven citrus types (specialty fruit, limes, grapefruit, blood oranges, other lemons, pummelos, and rootstocks) collectively accounted for 35 percent (**Figure 1**).

From the top ten citrus varieties requested by commercial users, Fukumoto Navel was the most popular, accounting for 18 percent of the budwood orders, followed by Olinda Nucellar Valencia (Mulholland) at 14 percent, Cutter Nucellar Valencia and Olinda Nucellar Valencia (McEwen) each at 11 percent, and Parent Washington Navel and Tango each at 10 percent. The remaining four varieties (Flame Grapefruit, Campell Nucellar Valencia, Atwood Navel, and Persian Lime) collectively accounted for 26 percent (**Figure 2**).

From the top 10 citrus varieties requested by non-commercial users, Shiranui Mandarin was the most popular, accounting for 19 percent of the budwood orders, followed by Cara Cara Navel at 14 percent, Gold Nugget Mandarin and Australian Finger Lime each at 11 percent, and Frost Owari Satsuma at 10 percent. The remaining five varieties (Bearss Lime, Tarocco #7 Blood Orange, Yuzu Ichandrin, Improved Meyer Lemon, and Valentine Pummelo Hybrid) collectively accounted for 35 percent (**Figure 3**). For more detailed data regarding the CCPP's comprehensive budwood introduction and distribution activities, please download the full CCPP Annual Budwood Reports at ccpp.ucr.edu/about/index.html#report.

The misconception that non-commercial users pay unfairly more for budwood overlooks how heavily the citrus industry has subsidized the program for decades. The original price of $0.75 per bud, set in 1984, remained unchanged for 35 years because commercial growers and nurseries supported CCPP through their respective marketing-order assessments, offsetting true operational costs. Full cost analysis showed that producing one pathogen-tested bud costs $5.20, not including therapy, quarantine testing, or infrastructure

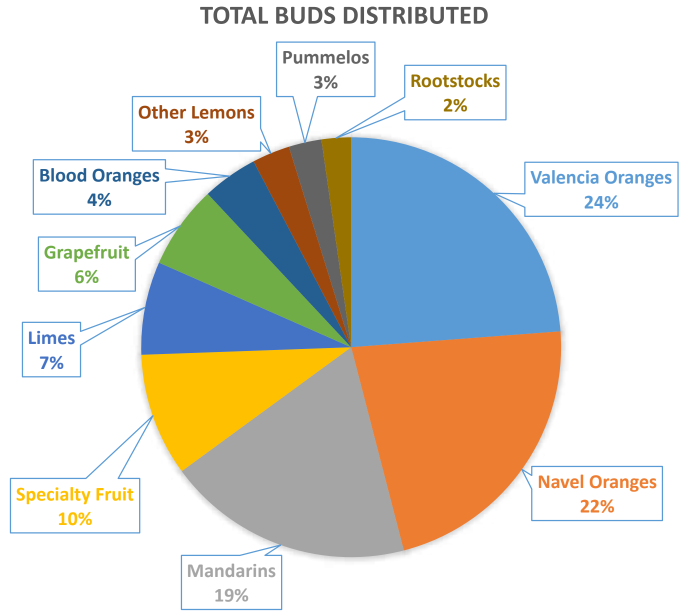

Figure 1. Total budwood distribution for the top 10 citrus types from October 2024 through September 2025. Specialty fruit includes papeda, kumquat, Australasian lime, citron and their hybrids. All other types include limettas, limequats, sour orange and hybrids and other sweet oranges.

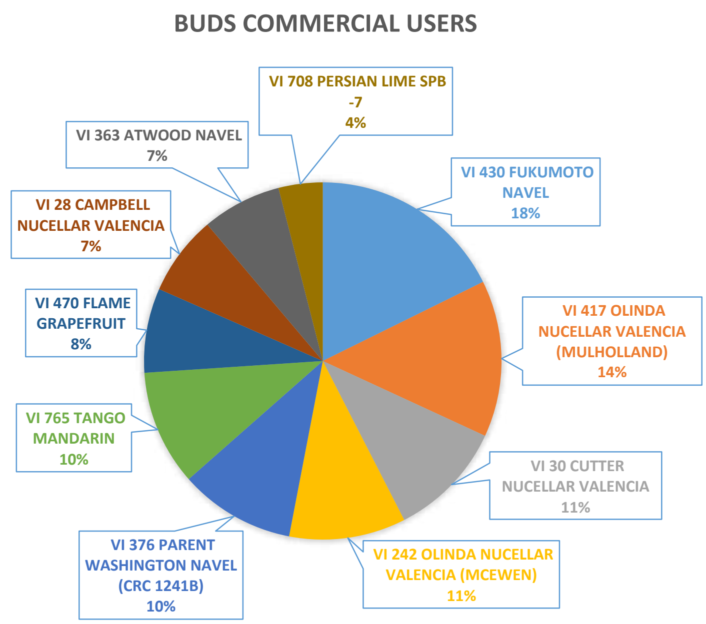

Figure 2. Distribution of budwood among the top 10 citrus varieties requested by commercial users from October 2024 through September 2025.

CCPP specialist, German Villalba-Salazar, working on the inarches of the 1958 Batchelor Memorial Tree at UC Riverside. The tree was inarched by the CCPP in May 2022 to stop and reverse its decline.

CCPP tissue-culture-produced citrus plant.

CCPP specialist, Adriana Lara Brigido, explaining the shoot-tip grafting process to international visitors at the Rubidoux Quarantine Facility.

CCPP mini fruit display at Lindcove REC during a meeting.

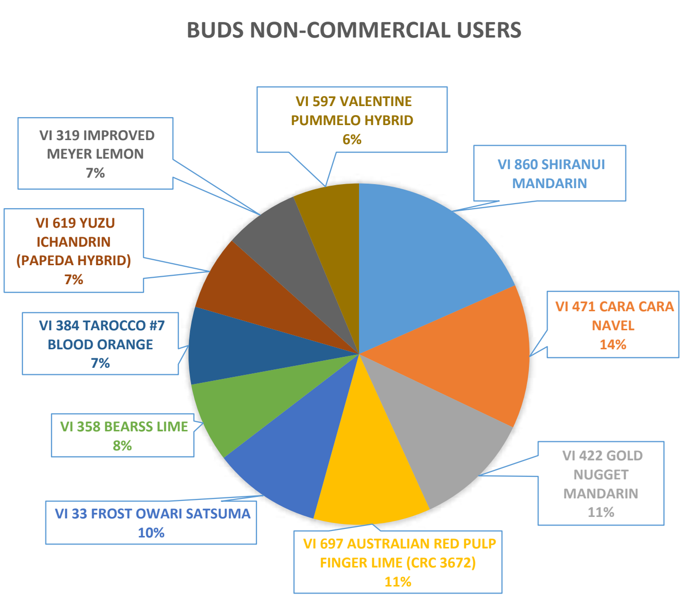

Figure 3. Distribution of budwood among the top 10 citrus varieties requested by non-commercial users from October 2024 through September 2025.

investments. Thanks to annual industry contributions, the bulk of which comes from the Citrus Research Board, CCPP continues to offer budwood below cost, ensuring affordable, high-quality materials for all users.

Extension and outreach remains central to CCPP's mission, with staff participating in 135 activities including presentations, workshops, field days, and direct stakeholder support. These efforts maintain the exchange of research-based information with scientists, growers, and the public. Despite the misconception that programs like CCPP are simple service operations that do not require research, the opposite is true. Research-driven advances in diagnostics, therapy, and quarantine—combined with extensive outreach—are what enable CCPP to protect the citrus community at every level.

The CCPP helps keep California at the forefront of citrus production by providing certified, pathogen-free propagation materials that reduce the risk of diseases and support healthier, longer-lived, more profitable orchards. Newly introduced varieties—such as seedless mandarins, redfleshed oranges, lemons, grapefruits, and disease-tolerant rootstocks—allow growers to diversify and meet evolving market demands. Although some misconceptions suggest that regulations slow clean-plant work, it is plant biology—not paperwork—that dictates the pace. Therapy is not routine; flush and meristem development vary widely. Pathogen titers fluctuate, and indicator plants require time to show symptoms. Even with improved controlled environment agriculture capacity, biological bottlenecks remain the true limiting factors. Continued access to pathogen-tested citrus, supported by rigorous science and quarantine, is essential for the long-term viability and competitiveness of California's—and the nation's—citrus industry.

CCPP experiment with sweet orange seeds to determine the optimum water content for storage and germination. Seeds are placed in a single layer on trays for air-drying; subsamples are collected in weighing trays to calculate water loss; and temperature and relative humidity are monitored via wireless sensor.

CRB Research Project #6100

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***Georgios Vidalakis, Ph.D., is a professor and University of California extension specialist in Plant Pathology with the Department of Microbiology and Plant Pathology at the University of California, Riverside and also serves as the director of the Citrus Clonal Protection Program. For additional information, contact* vidalg@ucr.edu**

CCPP germination of trifoliate rootstocks in the Modular Plant Growth Unit at the Rubidoux Quarantine Facility.

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Grove-First Update
SCREENING INJECTABLE TREATMENTS
to support tree health in HLB endemic areas

Michelle Heck, Lukas Hallman, Randall Niedz, Cody Estes, Nabil Killiny, Mark Ritenour

A photo of 10-year old Valencia oranges in a Phase 1 Grove-First screen. This treatment resulted in treatment sectoring, where one side of the tree displayed phytotoxicity and leaf drop, and the other side of the tree showed an improvement. Such field observations following injection screening help the team prioritize treatments advancing to Phase 2 and 3 trials.

Summary

Florida citrus growers continue to operate under the heavy burden of huanglongbing (HLB), a disease that has devastated their industry by reducing yield, fruit quality and the economic stability of the entire commercial citrus value chain. The California citrus industry has not yet felt these effects, but the disease is expanding in Texas and other citrus growing states. In Florida, growers have turned to direct trunk injection of the antibiotic, oxytetracycline. They demonstrated the economic feasibility and safety of injections and rapidly scaled up the new approach across the state. We developed the Grove-First program, now entering its third full year of multi-site, multi-treatment field screening and validation, to rapidly test for safe, available and affordable alternatives to oxytetracycline and combination therapies that will improve the efficacy of oxytetracycline. This article summarizes the work to date and proposes a framework for the industry to prioritize further field testing by grouping treatments into four categories based on their regulatory status and projected cost to commercial delivery.

What is Grove-First and What Does It Do?

Grove-First is an iterative, three-phase, on-farm pipeline that advances treatments from small-scale screens of three to six trees randomized within a block (Phase 1) to multi-row validation on commercial farms (Phase 2) and ultimately, testing at multi-acre scale (Phase 3, Heck et al. 2025). Across the last two seasons, the team evaluated more than 350 treatments with 44 treatments in Phase 2 trials involving approximately 2,100 trees in 2025. Candidate treatments advance through each successive phase only if they meet predefined performance benchmarks, ensuring that resources are focused on treatments with consistent, field-relevant benefits. Descriptions of the parameters used to advance a treatment through each phase are highlighted in **Table 1**. Candidate treatments being tested in Grove-First include registered antibiotics, non-antibiotic alternatives, biopesticides and plant-health promoting chemistries (Heck et al. 2025). We developed the pipeline to answer a single question with scientific rigor: What injectable treatments have the potential to restore economic profitability to citrus production in Florida?

Our latest, yet unpublished, datasets are now large enough for us to evaluate patterns with confidence. Many injectable treatments perform as well or better than oxytetracycline in terms of foliage response. But very few of these perform much better than oxytetracycline under the screening conditions when measuring effects on fruit quality **(Figure 1**). The latter might be achieved with further optimization or repeated treatments. Alternatively, additional screening is needed to identify more treatments that bring internal fruit quality back to pre-HLB levels. The growers in Florida need options now. Thus, state-wide engagement will be key to further screen, validate and optimize promising therapies from Phase 1 and 2 trials this year.

Our data shows that 25 treatments consistently improved canopy health, with six having increased yield and six having enhanced fruit quality under commercial conditions relative to injection with commercially available oxytetracycline formulas. Antibiotics, including oxytetracycline and specific combinations of other chemistries with oxytetracycline, remain top performers in Phase 1 trials for fruit quality. This confirms observations already emerging from the literature and grower observations. Grove-First now provides the robust, replicated evidence base needed to contextualize these results for regulators, economists, agrichemical companies and policymakers.

Our team has made important observations about trunk injection biology that appear to be universal across sites, citrus varieties and injectable treatments. These are:

1. ***Timing matters.*** Trees injected with higher doses of antibiotics and other treatments perform better over time when injected during the cooler months. Higher doses result in a more curative effect. Some branch dieback may occur, but if the injections are done in the cooler months, trees usually rebound.

2. ***The tree's starting condition predicts overall response.*** Severely compromised trees show slower gains. Trees that are moderately diseased respond earlier and more strongly. Healthier trees with less disease appear to show less of a response, although we expect this is due to the narrow range in which we rate the trees for their overall health. For example, a tree rated with a top score of "healthy" at the beginning of the season cannot improve beyond our top score of "healthy." As trees improve in their size and health at our test sites, we will adjust our scoring metrics to incorporate a broader range of tree conditions.

3. ***Ongoing research has informed ideal injection type and tip size.*** The TJ Biotech FlexInject™ device is the most commonly used commercially available injector. The device comes with two tip sizes. The small tip uses a 13/64" drill bit while the large tip uses a 17/64" drill bit. Our team is now using small tips for all injections, even on larger trees. We have not observed uptake issues using smaller tips, and trunk damage is very minimal or not observed.

Table 1. Overview of Grove-First Field Evaluation Framework (summarized from Heck et al. 2025). OTC = oxytetracycline

EVALUATION STAGE	HORTICULTURAL DATA COLLECTED IN THE FIELD	CRITERIA TO ADVANCE
Phase 1	• Tree health index • Trunk diameter • Canopy density • Stem angle • Yield • °Brix • Acidity • Fruit weight • Percent juice	• Treatment performance equal or better than OTC in two or more horticultural measures • Regulatory readiness • Grower feedback • Availability • Cost
Phase 2	• Yield • °Brix • Fruit weight • Percent juice	• Better than OTC in most categories • Grower feedback • Regulatory readiness
Phase 3	• Canopy density by drone imaging • Yield • °Brix • Fruit weight • Percent juice	• Phase 2 data • Discussions with: - Regulatory Agencies - Growers - Agrichemical companies

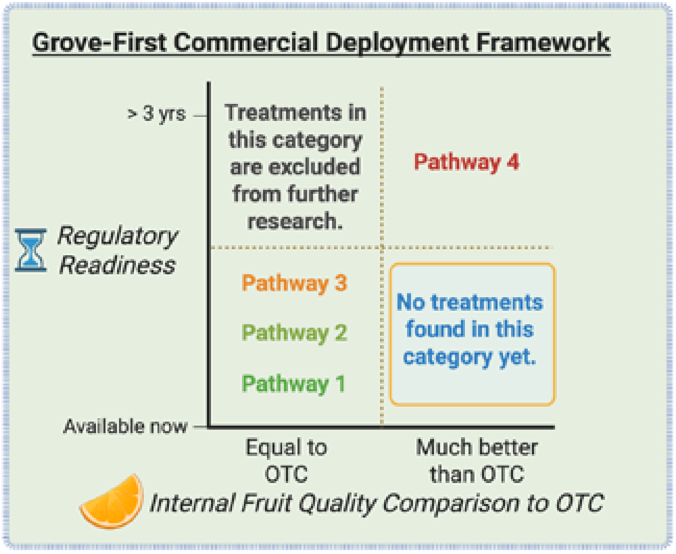

Figure 1. The Grove-First commercial deployment framework classifies injectable treatments for HLB management in citrus based on two key dimensions: regulatory readiness, indicating the estimated time to commercial availability and internal fruit quality. Pathways are color coded for clarity. Pathway 1 (green) represents treatments available now for grower testing and optimization; Pathway 2 (light green) represents treatments with limited supply but long-term potential due to regulatory readiness; Pathway 3 (orange) requires a label extension to be used for citrus injection; and Pathway 4 (red) demands a full EPA registration with an extended timeline.

4 ***Oxytetracycline residue levels are below regulatory thresholds.*** The residue levels of active ingredients, like oxytetracycline, depend on dose, timing and application method, so evaluating residue is important to understand whether changes in oxytetracycline formulation will change how the active ingredient persists in the tree. Our team tested different oxytetracycline combinations to improve treatment efficacy. In our trials, oxytetracycline residues remain below detection thresholds or 10 parts per billion in the juice samples of all tested formulation permutations.

In 2025, a central focus was using regulatory readiness to define the practical pathways that determine whether a treatment can become an economically viable injection treatment (**Figure 1**). These pathways use our biological results and regulatory status to reveal which treatments can be tested more expansively now in commercial production, which treatments need label changes, and which require a new pesticide registration.

1 **Pathway 1** treatments are immediately usable for further optimization in commercial production settings. These treatments include mixing oxytetracycline in the tank with various surfactants to improve systemic uptake or homobrassinolide (obtained from Repair Corporation), a plant growth regulator. Other examples include ProVida (Terra Nova Research and Development, Inc.) which is a microbial formulation and potassium phosphate. We are not claiming these treatments are ready for large-scale commercial operations but rather scaled up testing under commercial conditions (Phase 2 testing). Based on our findings, these treatments are now being incorporated into the Florida Citrus Research and Field Trial (CRaFT) program. Through Florida CRaFT, Florida growers will be able to participate in research to optimize performance and validate our findings in smaller-scale trials (on the order of a few rows randomized in a block). Data will be incredibly useful to provide information to other growers for more wide-scale use.

2 **Pathway 2** treatments are supplier-dependent but would be immediately usable once more widely available. Pathway 2 treatments include curry leaf extracts (Aglave et al. 2025) and FRC249 (Heck et al. 2025), an iron-rich fertilizer developed by a grower. Our team has small quantities available for trials with Florida growers, if there is an interest.

3 **Pathway 3** treatments require a label extension to be used for citrus injection and include tea tree oil (Heck et al. 2025). A company, SummitAgro, has been trialing their registered tea tree oil-containing product by injection to determine whether a label extension is feasible.

4 **Pathway 4** treatments will take the most time and investment because they require a new Environment Protection Agency (EPA) pesticide registration. These treatments, including tetracycline, doxycycline, and others, show some of the highest biological performance, including improvements in yield and fruit quality.

Before the Grove-First project, commercial consideration was relatively unstructured and/or not always a determining factor when making research investment decisions upfront. Now, the Florida industry has a clear, evidence-based framework (**Table 1**) that identifies what growers can adopt immediately and clarifies which treatments are worth regulatory investment by collecting data that matters to economic profitability. The framework also supports manufacturers in prioritizing production pipelines and gives growers options to test and optimize other treatments besides or in addition to oxytetracycline. Finally, it provides regulatory agencies with the field data needed for decision making and gives growers, citrus grower organizations, and policymakers a shared roadmap. Grove-First has emerged as a decision engine that accelerated the delivery of injectable therapies to the Florida growers. If the core principles of Grove-First, namely phased evaluation in groves for large treatment effects on tree horticultural characteristics and partnership with growers to conduct research under real world conditions, are adopted by other HLB research groups, our citrus research community has the potential to rapidly advance solutions that provide economic benefit to growers under HLB pressure. All state-specific regulations must be examined to expand Grove-First testing beyond Florida and/or involve crop-destruct research depending on the chemistry being used. It is also important to note that no pesticidal claims are being made for any treatment under study, as Grove-First does not consider mode of action, but focuses solely on improving horticulture of trees in HLB-endemic areas.

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Michelle Heck, Ph.D., and Randall Niedz, Ph.D., are career scientists in the USDA Agricultural Research Service. Lukas Hallman, Ph.D., is a postdoctoral associate, and Mark Ritenour, Ph.D., is a professor in the Department of Horticulture at the University of Florida Indian River Research and Education Center. Nabil Killiny, Ph.D., is a professor in the Department of Plant Pathology at the University of Florida Citrus Research and Education Center, and Cody Estes is a Florida citrus grower and the director of the Grove-First Research Initiative. For more information, please contact Michelle Heck at **Michelle.Heck@USDA.gov**


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A VIRUS-BASED DELIVERY PLATFORM FOR THERAPEUTICS AGAINST HUANGLONGBING

Anne E. Simon

Scientists rely on data, not destiny. Research outcomes are never certain; they emerge from experimentation and analysis. Over the past eight years, my work — from discovering an obscure citrus virus to conducting field trials — has advanced a potential therapeutic approach for huanglongbing (HLB) grounded in evidence, engineering, hard work, and a bit of luck.

The first step on my journey was the discovery of a database entry by Dr. Georgios Vidalakis at University of California, Riverside for citrus yellow vein-associated virus (CY1), a Southern California virus identified once in the 1950s in a limequat tree. Simply put, CY1 should not exist. It is missing nearly all of the components necessary to make it a functional virus, and yet it is still able to infect plants. Since CY1 is symptomless when infecting citrus, the thought was that it might be useful as a delivery vehicle for therapeutics to treat or prevent HLB, the devastating bacterial disease threatening citrus worldwide.

Tiny antimicrobial peptides that are naturally found in plants and can efficiently kill bacteria, as well as small interfering RNAs (siRNAs) that can target and destroy components in plants that bacteria need to survive, are well-studied solutions to HLB and other plant diseases. The central problem for use of these potent therapeutics is delivering them to sites within trees where the bacteria reside and at a cost that does not bankrupt growers. Viruses are uniquely capable of doing this, as they can migrate throughout an infected tree generating the therapeutic products and can remain for the life of the tree. CY1 seemed like the perfect virus delivery vehicle because it does not harm citrus, is incapable of escaping from infected trees, and is so tiny that engineering is easy. These insights led me and my brother, Dr. Rafael Simon, to co-found Silvec Biologics, Inc., a company devoted to using plant viruses for delivery of therapeutics into trees and other plants.

Two significant obstacles remained before CY1 could be used as a delivery vehicle: How to get engineered CY1 back into citrus and how to keep CY1 and other viruses from discarding the therapeutic-containing segments engineered into their genomes. Leveraging my lab's experience in viral RNA genome structure, we developed a breakthrough new approach to retain small inserts in viruses for years or even decades instead of days— an essential requirement for using CY1 as a delivery vehicle for citrus. What we had discovered was that every portion of a virus RNA genome has evolved to fall within a precise thermodynamic range with specific properties necessary for replication by viral enzymes. Want to add a new appendage to a virus? Just make sure that it has the same properties as natural virus structures, and the virus is perfectly content to treat it as belonging and not something to be discarded (Jang et al. 2025).

At the time, we thought that just the mere presence of CY1 in HLB trees might prove beneficial, as viral infections are known to ramp up plant defenses, making it harder for other pathogens to infect. This is a phenomenon known as systemic acquired resistance. Furthermore, we discovered that CY1 has a unique ability compared to other viruses. It can bind with the protein responsible for clogging the phloem in HLB-affected trees — a protein called phloem protein 2 (PP2), which is a major contributor to HLB yield losses (Ying et al. 2024, Kim et al. 2014). By interacting with CY1, our thinking was that there would be less PP2 available to block the pores through which sugars travel from leaves to roots. To test this possibility, collaborator Dr. Michelle Heck with the United States Department of Agriculture-Agricultural Research Service in Ithaca, New York, introduced the bacteria causing HLB into both healthy and CY1-infected Mexican lime trees using psyllids, and the initial results were promising. After a few months, HLB trees infected with CY1 appeared much healthier than trees containing just the HLB bacteria. But two months later, trees that initially seemed healthier now appeared far sicker and contained 10 times more bacteria than trees infected by just the bacteria (**Figure 1**). As such, we discovered CY1 should not be used as a delivery vehicle. Fate had delivered a crushing blow.

Or maybe not. Citrus tristeza virus (CTV) was another virus being optimized by Dr. William Dawson and his colleagues at the University of Florida for its potential use to introduce and distribute gene-based therapeutics in citrus plants (El-Mohtar and Dawson 2024, Folimonov et al. 2007). This mild version of CTV was selected due to it not causing disease in citrus and containing several alterations in a viral protein that hinders CTV's ability to be acquired by aphids and transmitted to new trees (Shilts et al. 2020, Padilla et al. 2025). Using funding from Southern Gardens Citrus, a subsidiary of U.S. Sugar, CTV was developed to deliver a type of anti-microbial spinach peptide known as "defensin" (Padilla et al. 2025). Although initial results were promising, results from longer term field trials did not meet expectations. With regulatory approval almost completed, Silvec Biologics saw an opportunity and worked with U.S. Sugar to obtain a sub-license for the CTV-defensin vector.

While CTV is the world's largest plant virus and notoriously difficult to engineer, this huge size also means that the virus is capable of maintaining inserted segments better than other viruses. Despite this, the defensin delivered by CTV was undetectable in both infected trees and laboratory plants (Silvec Biologics, unpublished results). My lab at the University of Maryland had identical problems detecting a different anti-microbial peptide in plants when using CY1 as a vector. We explored the hypothesis that the problem might be that peptides as a whole accumulate poorly, as they are rapidly degraded by cellular enzymes. Researchers

Figure 1. The impact of co-infection with CY1 and *Candidatus* Liberibacter asiaticus (*C*Las) is shown six months after infection. The citrus on the top was infected only with *C*Las while on the bottom, citrus was infected with both *C*Las and CY1.

investigating defensins for killing bacteria in humans have also been struggling with the same problem. These researchers found several ways of increasing the lifespan of defensins and other antimicrobial peptides inside organisms, including increasing their size, which significantly improved peptide accumulation and efficacy in animals (Tan et al. 2018). When this same approach was applied by Silvec to the spinach defensin, the amount generated from CTV increased many times over. One year after grafting with CTV carrying one of the stabilized defensins, greenhouse trees with significant HLB symptoms appear healthy and virtually symptom-free (**Figure 2**).

Thanks to our collaboration with the Rucks Citrus Nursery in Florida, we have many variations of the stabilized defensins introduced through CTV infection being propagated in both Hamlin and Valencia mother trees. Field trials grafting these CTV into 5- to 6-year-old HLB-affected trees were initiated in July 2025. In November 2025, young HLB-free greenhouse trees containing CTV and stabilized defensins were also planted at several locations in central Florida. Since the Florida CTV T-36 strain we are using is strikingly similar to mild California strains, we are working to obtain permits for trials in California. Environmental Protection Agency (EPA) registration is nearly finished for the original defensin product and should be expedited for the stabilized-defensin versions. Meanwhile, as we await the field trial results, we are busy engineering additional anti-bacterial and anti-fungal protections into the CTV vector.

Figure 2. The impact of grafting CTV carrying the stabilized spinach defensin on citrus infected with huanglongbing (HLB) is shown one year after grafting. The citrus on the left is grafted with CTV without the stabilized spinach defensin, while the citus on the right is grafted with CTV with the stabilized spinach defensin.

These past five years have been filled with high points (making CY1 into a vector that can retain inserted segments for years) and low points (CY1 making it easier for other pathogens to infect). But when one door closes, another can open. If we had not tried to make CY1 work, we would never have realized the untapped potential of the CTV vector. Will CTV and current stabilized defensins prove to be the elusive silver bullet? While hoping the answer is yes, additional options are already being explored by my lab and Silvec, and this research will continue until HLB is no longer the scourge of the citrus industry.

Author's Note: I would like to express my sincere gratitude to the Citrus Research Board, United States Department of Agriculture – Emergency Citrus Disease Research and Extension, United States Department of Agriculture – Huanglongbing Multi-Agency Coordination Group, National Science Foundation, and the Florida Citrus Research and Development Foundation, for their generous funding and support during this journey.

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Anne E. Simon, Ph.D., is a professor of Cell Biology and Molecular Genetics at the University of Maryland College Park and chief scientist at Silvec Biologics, Inc., in Gaithersburg, MD. For additional information, contact **simona@umd.edu**


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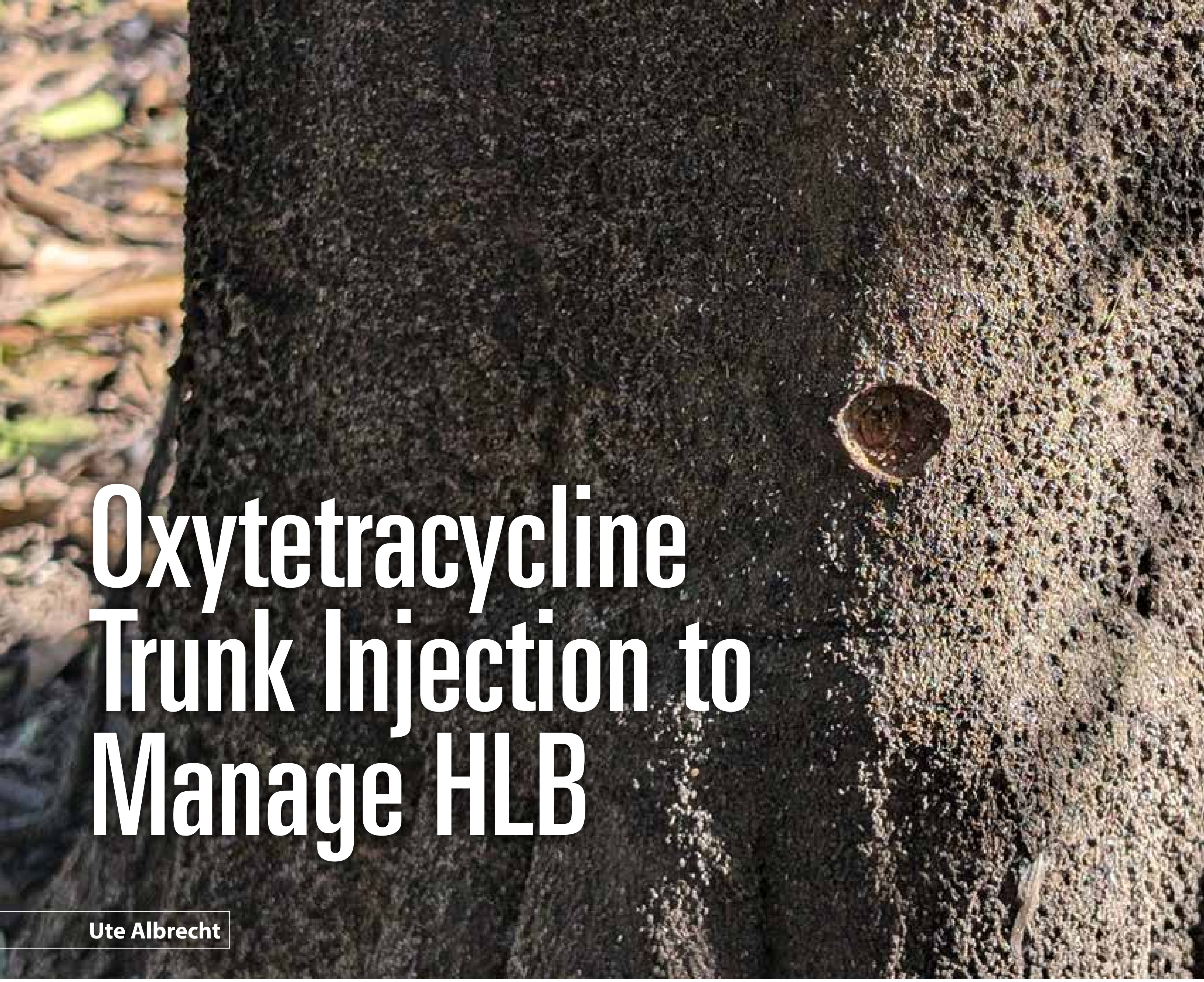

Oxytetracycline Trunk Injection to Manage HLB

Ute Albrecht

Summary

Huanglongbing (HLB) or citrus greening is threatening citrus industries worldwide and has especially ravaged Florida. It is the main reason for the more than 90 percent decline of this formerly iconic industry. One reason for optimism has emerged recently, namely the delivery of the antibiotic oxytetracycline by trunk injection. The results from numerous field studies document that one annual application of this antibiotic can increase yield and fruit quality significantly, with cumulative effects after two or more years. Florida growers are now implementing this strategy to bring back production while waiting for a genetic solution.

Figure 1. Injection of oxytetracycline into the trunk of a citrus tree in Florida. The swollen black tube inside the plastic bottle contains the oxytetracycline solution.

What is Trunk Injection?

Huanglongbing (HLB) is associated with the phloem-colonizing bacteria *'Candidatus* Liberibacter asiaticus' and is therefore a systemic disease. This means that the bacteria are dispersed throughout all organs of an infected tree, including leaves, fruits, twigs, branches, trunk, and even roots. Although most of the popular rootstocks used in Florida are inherently tolerant to HLB, most commercial scions are not, rendering the grafted tree vulnerable to the disease's impact. The systemic distribution of the HLB-associated bacteria makes it difficult, if not impossible, to target them by foliar administration of chemicals, as these do not effectively penetrate the leaf cuticle and are not translocated to the rest of the tree. However, chemicals can also be introduced into the tree by trunk injection, which delivers them directly into the tree's vascular system. Initially, an injected chemical enters the xylem, the part of the vascular system that transports water and dissolved minerals from the roots to the leaves. This process is primarily driven by transpiration. The injected chemical can then be translocated throughout the tree with the transpiration stream and may enter the phloem, the part of the vascular system that is associated with carbohydrate transport and harbors the HLB-associated bacteria. The chemical properties of the injected chemical determine its capability to move in the xylem and phloem and to translocate between the two tissues. One important advantage of using trunk injection for delivery of crop protection chemicals is that it reduces spray drift and run-off and therefore, the negative impacts on the environment.

Trunk Injection of Antibiotics for Commercial HLB Management

In Florida, the delivery of antibiotics by trunk injection was investigated in the 1970s and '80s to treat 'lethal yellowing' of coconut palms, a disease that is associated with phytoplasmas, which is a type of plant-pathogenic bacteria that also reside in the phloem. It is still the main method to control this disease. During that same time, trunk injection of antibiotics was also explored in South Africa and other countries to manage HLB, but despite positive results, it was not explored for large-scale management.

Large-scale use of a therapy that requires drilling a hole into the trunk, inserting an injection device, and waiting for the antibiotic solution to be taken up, seems daunting. But because of the devastation HLB has wreaked in Florida, trunk injection of antibiotics was re-explored, and in 2022, delivery of oxytetracycline HCl (OTC) by trunk injection was approved for commercial use in Florida (under a FIFRA 24(c) special local needs label). Most growers have now adopted the practice and streamlined the process (**Figure 1**), rendering it

Figure 2. Comparison of oxytetracyline (OTC)-injected and non-injected 'Valencia' orange trees in a Florida citrus grove after two years of OTC injection.

a viable option for HLB management under Florida's growing conditions. The applied dose of OTC varies based on the tree size. For mature trees, it usually ranges from 0.55 g to 1.1 g per tree, with a maximum of 1.65 g per year. Estimated costs of injection are $1 to $1.70 per tree, including labor, and many trees have now been injected for three consecutive years. Although Hurricane Milton caused a major setback in the 2024-25 production season, the consensus is that tree health has improved because of the injections, along with productivity and fruit quality (**Figure 2**).

OTC Trunk Injections Improve Yield and Fruit Quality

We have conducted numerous research studies to explore the efficacy of OTC administered by trunk injection under commercial production conditions. In all studies, one year of application (i.e., one single injection) produced yield increases of 20 to 30 percent or more. Importantly, after two consecutive years of OTC injection, yield improvements were cumulative, often exceeding 70 percent or more (Albrecht et al. 2025a; Tardivo et al. 2025; other unpublished results). OTC injections not only increase productivity but also enhance fruit quality attributes, like fruit size, peel color, and juice total soluble solids (TSS) content. The month in which injections are performed can modulate these effects (Albrecht et al. 2025b; other unpublished results). Injections performed in spring or early summer, when the fruits are still small, increase the fruit size more than injections performed in late summer or early fall. In contrast, late injections improve the juice TSS content more than early injections but increase OTC fruit residues (see below).

While fruit and juice quality improvements are relatively consistent (10 to 20 percent on average), yield improvements are more variable and depend on the production environment, tree age, the tree health status at the start of injections, and other factors, such as the rootstock cultivar. The example in **Figure 3** shows rootstock responses of 'Valencia' orange trees in an ongoing large field trial (>4,000 trees) after two consecutive years of injection (unpublished results). While the response after the first injection in 2023 was overall moderate, it was considerably larger after the second injection. Rootstock responses ranged from 17 percent for UFR-4 to 36 percent for US-942 in year 1, and from 57 percent for Carrizo to 103 percent for US-942 in year 2. The large response in year 2 is especially remarkable, as Hurricane Milton caused considerable fruit drop at that location in October 2024. For more results, see Tardivo et al. (2025).

Antibiotic Residues in the Fruits

In all our studies, OTC residues in the fruits were below the maximum residue limits when injections were completed by the end of June (Albrecht et al. 2025a; other unpublished results). When injections were performed during August and September, fruit residue levels increased, even with an interval of six or more months between injection and harvest (Albrecht et al. 2025b; other unpublished results). Thus, the

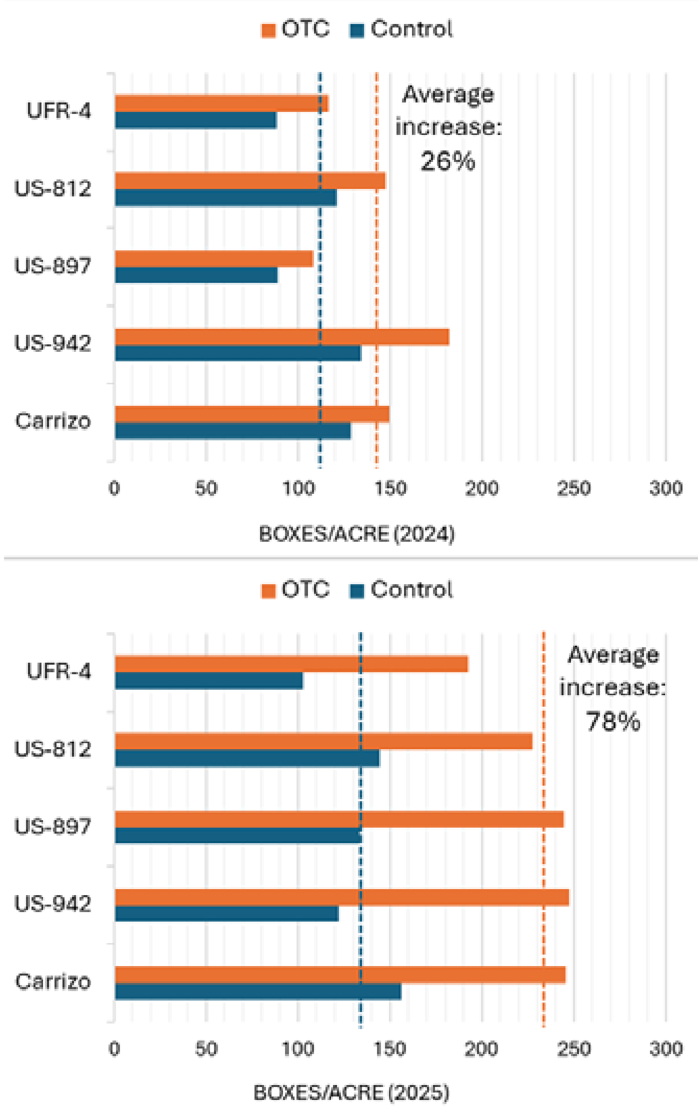

Figure 3. Boxes of fruit per acre of 'Valencia' orange trees grafted on different rootstocks without (Control) and with oxytetracycline injection treatment (OTC). The dashed lines indicate averages across rootstocks. Trees were planted in 2015 and received one injection in April 2023 and another in May 2024. One box equals 90 lbs. (40.8 kg) of oranges. Note that Hurricane Milton caused extensive fruit drop (approximately 30 percent) at this location prior to harvest in 2025. The trial was conducted in Polk County, Florida.

larger the fruit size at the time of injection, the higher the residues. The maximum allowed OTC residue level is 0.01 parts per million. Injections after June increase the risk for the residues to exceed this limit. These observations refer to studies involving sweet oranges.

Other Concerns

Aside from the additional costs, another drawback of injecting trees is the injury it causes to the trunk. In general, trees are effective at sealing wounds to prevent the spread of decay. However, chemicals such as OTC exacerbate trunk injuries, often resulting in bark cracking and discoloration of the wood. In addition, injection of OTC or other chemicals can produce leaf yellowing and other phytotoxic effects in the canopy, though these are usually temporary. The positive effects have thus far outweighed the negative effects, and with proper administration, negative effects can be minimized (Albrecht et al. 2025c).

There are concerns regarding the impact of the antibiotic on other microbes living in the tree or the rhizosphere. Our research has shown that OTC can indeed change the diversity and abundance of certain microbes colonizing the tree, but this effect is temporary. Interestingly, the increased abundance of certain microbes with potential plant-beneficial effects was correlated with increases in yield and fruit quality (Castellano-Hinojosa et al. 2024). We also found that a one-time injection does not increase the number of tetracycline resistance genes of the resident bacteria (unpublished results). Regardless, other therapies are needed to rotate with or replace OTC, to ensure continued efficacy until genetic or other solutions become available to manage HLB.

Looking Ahead

In conclusion, in areas where HLB is endemic, the systemic delivery of OTC by trunk injection is an effective tool to bring back tree health and increase productivity. However, protecting trees from the disease vector remains to be of utmost importance. For new plantings, this can be accomplished by using individual protective covers (IPCs). The combined use of IPCs, OTC trunk injection, and other good practices might be a sustainable path forward to rehabilitate Florida's citrus industry. In areas where HLB is not yet widespread, targeted OTC injections might help control inoculum levels to prevent the devastation that occurred in Florida.

Funding Acknowledgment

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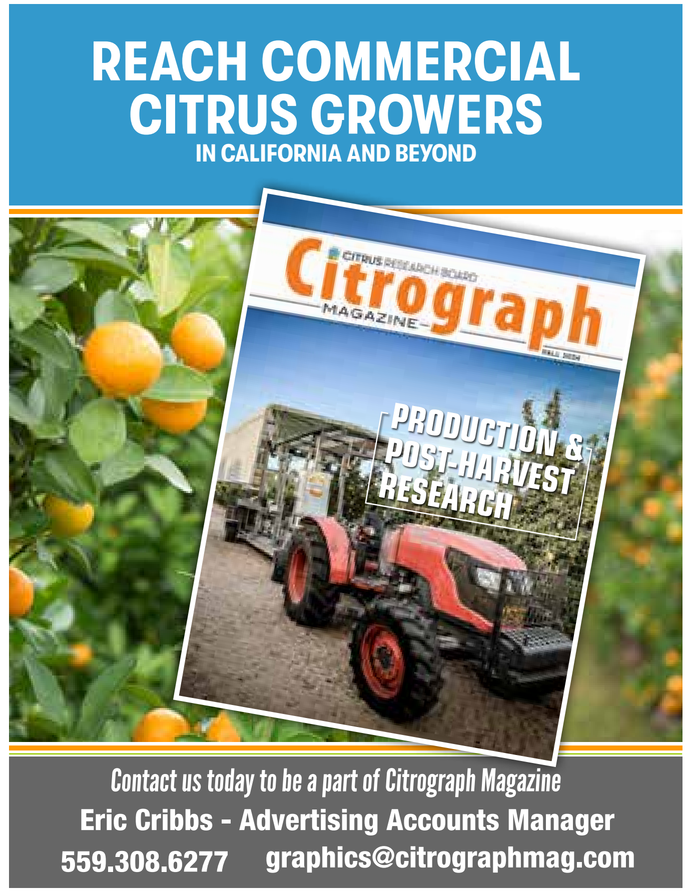
***Ute Albrecht, Ph.D., is an associate professor at the University of Florida/IFAS, Southwest Florida Research and Education Center. For more information, contact* ualbrecht@ufl.edu**



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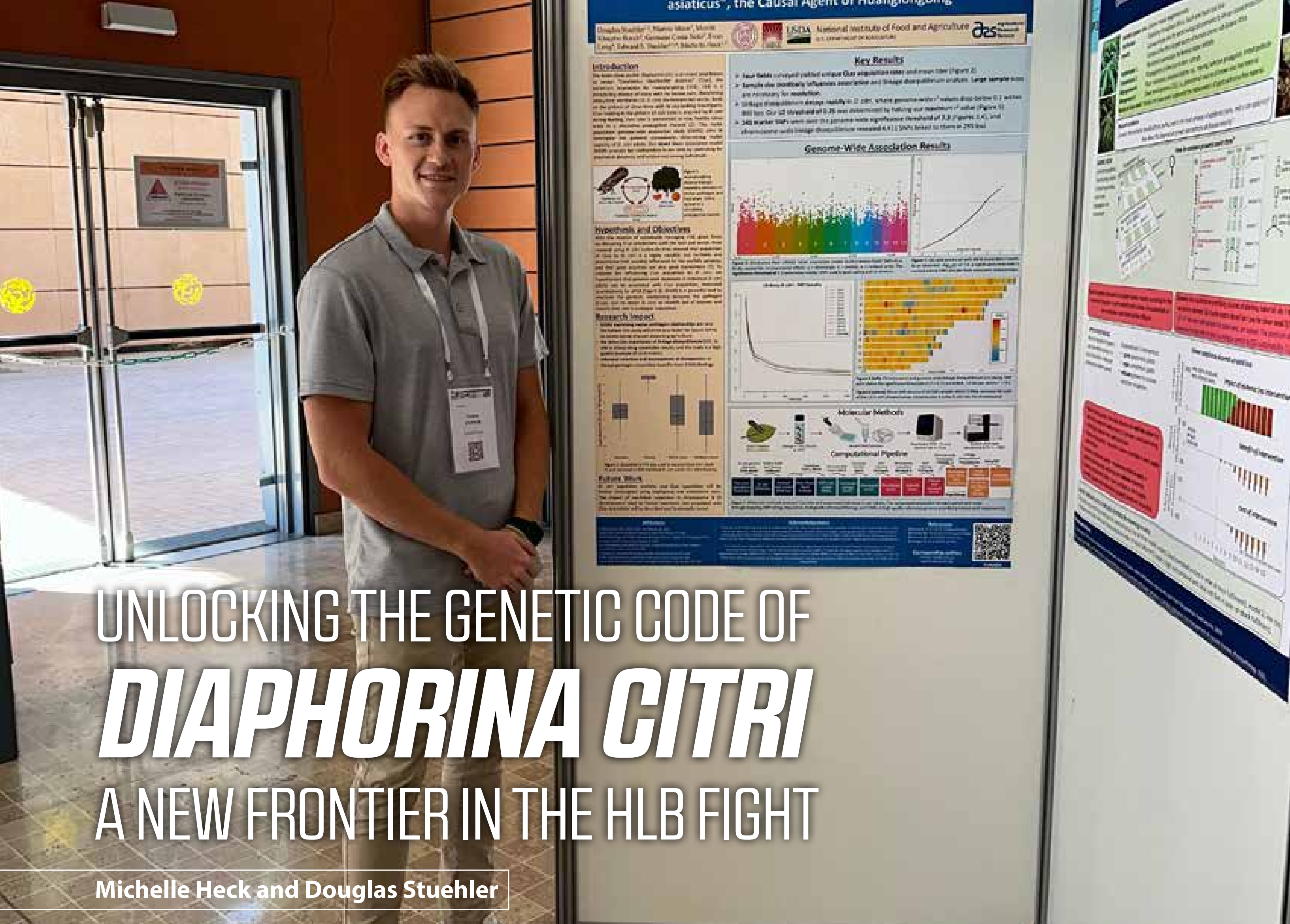

UNLOCKING THE GENETIC CODE OF *DIAPHORINA CITRI* A NEW FRONTIER IN THE HLB FIGHT

Michelle Heck and Douglas Stuehler

Douglas Stuehler, a USDA ARS computational biology scholar in the Department of Energy Oak Ridge Institute for Science and Education program, presents his research to identify psyllid genes that regulate the transmission of the citrus greening bacterium at a scientific conference.

Summary

Diaphorina citri*, commonly referred to as the Asian citrus psyllid, is the insect species that spreads the huanglongbing (HLB) bacterium,* 'Candidatus *Liberibacter asiaticus' (CLas), in the United States. Individual D. citri psyllids naturally differ in how easily they pick up and transmit CLas. In this project, we used modern DNA-based tools to explore those natural differences and to understand why, at the DNA level, some psyllids are more effective CLas carriers. The same approach could be used in future work to learn whether psyllids vary in how well they are controlled by the beneficial wasp,* Tamarixia radiata*, which is used in California as an important biological control tool to manage psyllid populations. By understanding differences in psyllid DNA, we can develop precision pest management strategies that protect California's citrus industry from long-term risk.*

Huanglongbing (HLB) is the biggest threat to citrus production in the United States. While California has so far managed to prevent widespread infection through aggressive management of *Diaphorina citri* (*D. citri*), Florida's experience demonstrates HLB's catastrophic effects.

Across California, growers and regulators are maintaining a multi-pronged strategy to keep *D. citri* populations under control. Tactics include area-wide management such as "spray and move" programs at harvest. In the San Joaquin Valley, rapid chemical treatments are aimed to eradicate psyllids in response to trap detections. Biological control tactics are also being used in California, including rearing and widespread release of *Tamarixia radiata*, a parasitoid wasp that lays its eggs in psyllid nymphs (Chen et al. 2014). These tools have helped reduce *D. citri* pressures and keep the California citrus industry largely HLB-free. Interactions between *D. citri* and *T. radiata* are regulated by biotic and abiotic factors

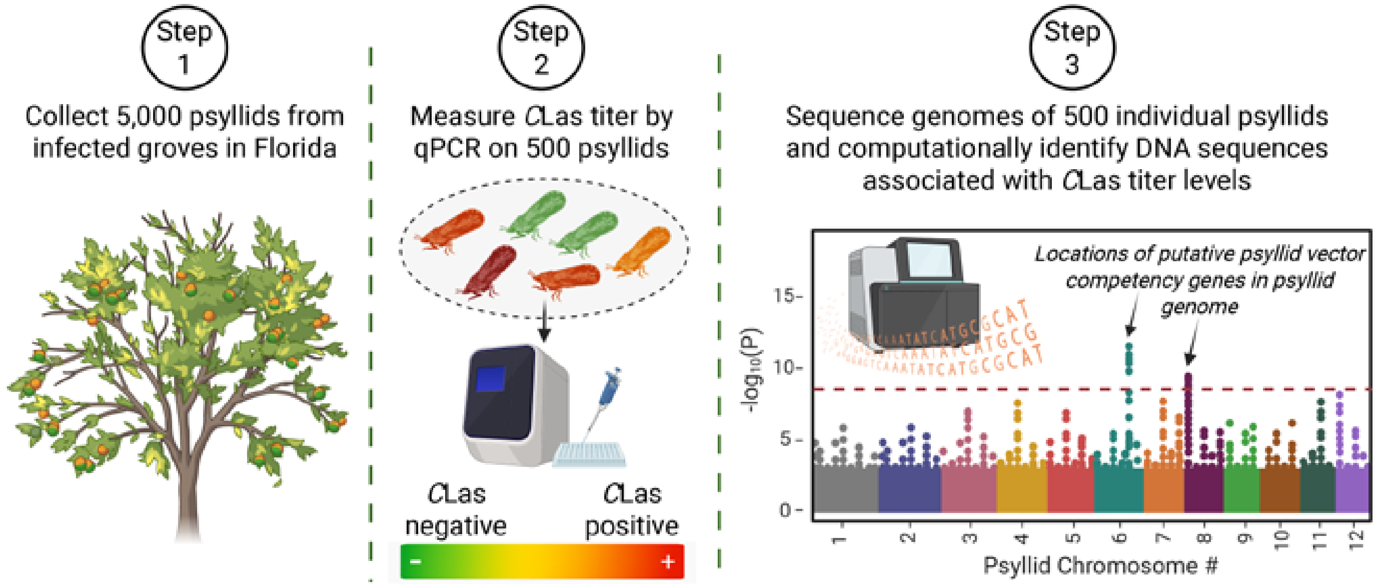

Figure 1. Schematic of the genome wide association workflow we used to identify psyllid genes that regulate *'Candidatus* Liberibacter asiaticus' (*C*Las) transmission. Step 1 involved manually collecting psyllids from Florida groves and transporting them to the lab for analysis. Step 2 involved extracting DNA from the psyllids to measure *C*Las titer using qPCR. Step 3 involved sequencing the psyllid genomes and using computational biology and statistical methods to find DNA sequences associated with vector competency, or the ability of psyllids to transmit *C*Las.

(Milosavljević et al. 2021), yet research questions still remain: Are some *D. citri* individuals more of a threat to transmit CLas from tree to tree when compared to others? Could *D. citri* in California become resistant to parasitism by *T. radiata*, rendering that control tactic less effective?

Our earlier work (Ammar et al. 2018) provided the first definitive evidence that *D. citri* populations vary naturally in their ability to transmit *C*Las. By establishing 15 psyllid populations from grove-collected females in Florida, referred to as isofemale lines, and rearing them under controlled conditions, we discovered that some psyllid isofemale lines were highly efficient at *C*Las transmission while others were not. In fact, some psyllid isofemale lines showed nearly zero capacity to transmit *C*Las to healthy citrus, despite repeated exposure to infected citrus plants. These differences in *C*Las transmission were stable over many generations of rearing the psyllids in the lab, indicating a heritable[1] basis of *C*Las transmission ability, referred to as vector competence.

To understand what makes some *D. citri* individuals better transmitters than others, we turned to a modern genetic tool called Genome-Wide Association Studies, or GWAS. GWAS allows researchers to scan the entire genome[2] of an organism and look for small differences in DNA that are consistently associated with a particular trait. In our case, that trait is vector competence. GWAS also is commonly used to find genetic causes of human disease and to accelerate plant breeding.

To start our GWAS project, we collected over 5,000 adult psyllids from groves in Florida with high HLB and psyllid pressure. From this group, 500 individual psyllids were selected for whole-genome sequencing. Each insect was tested for *C*Las titer[3] using quantitative PCR[4], providing a way to estimate the vector competence trait (Mann et al. 2024). The result was a rich dataset linking the genetic information of each psyllid to its vector competence. The workflow is explained in **Figure 1**.

The dataset showed that vector competency is, primarily, encoded in the psyllid's DNA. Our results highlighted that the trait is complex and influenced by many genes, each with a small effect. We identified several regions of the psyllid genome associated with high or low *C*Las titer, providing a roadmap for future research into the molecular basis behind vector competence. First-degree relatives (e.g., parents and siblings) had *C*Las titers that were more similar to each other as compared to non-relatives. The similarity in titer between parents and siblings may be due to a combination of genetics as well as the feeding biology of sibling nymphs on individual citrus flush points. These are key findings for California because under the right environmental conditions, a female psyllid with the right combination of high vector competency genes can produce hundreds or thousands of offspring with similar genes and increase the chance for an HLB outbreak.

Just as the ability to transmit *C*Las is heritable, studies with other insect systems have shown that the likelihood of an insect to be attacked by a parasitoid, known as host susceptibility, is also heritable. In the fruit fly *Drosophila*, for example, genetic studies have shown that some populations lose immune genes that normally defend against parasitism, possibly due to tradeoffs that favor other survival traits (Arunkumar et al. 2023). In pea aphids, scientists have found heritable resistance to parasitic wasps that is tied to specific aphid genes (McLean and Parker 2020) and even protective symbionts[5] (Gwynn et al. 2005). This raises an important research question: Could California's psyllid population evolve resistance to biological control with *T. radiata* over time? This question could be addressed with the same GWAS tools we used to study the genes that regulate *C*Las transmission. If certain psyllid genotypes are less susceptible to parasitism by *T. radiata*, we could detect this early by developing a simple assay to determine the psyllid genotype and adopt our biological control strategies before they lose effectiveness. California's biological control program has already taken steps to conserve genetic diversity in *T. radiata* by maintaining parasitoids as separate isoline colonies rather than unrestricted mass-reared populations (Hoddle et al. 2022).

We hypothesize that with future research, California growers and regulators will be able to identify whether a detected psyllid carries high-risk genes for *C*Las transmission, improving decision-making around treatments, quarantine, and eradication zones. The work could also facilitate the development of precision biopesticides, targeting genes to block *C*Las transmission rather than kill the insect. This project aligns with our priority to provide HLB management tools that are safe, affordable and available.

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Glossary

[1]**Heritable:** A genetic trait that can be passed from parents to children.

[2]**Genome:** The complete set of genetic information an organism carries, made from DNA.

[3]**CLas titer:** The amount of CLas bacterium within an individual. A high titer means more bacteria.

[4]**Quantitative PCR:** A lab-based method that measures the amount of a unique DNA sequence.

[5]**Symbionts:** Organisms that live in a close, cooperative long-term relationship with another dissimilar organism.

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Michelle Heck, Ph.D., is a research molecular biologist in the United States Department of Agriculture Agricultural Research Service Emerging Pests and Pathogens Research Unit. Douglas Stuehler is a computational biologist scholar in the Department of Energy Oak Ridge Institute for Science and Education in Dr. Heck's lab. For further information, please contact Michelle Heck at **Michelle.Heck@usda.gov**

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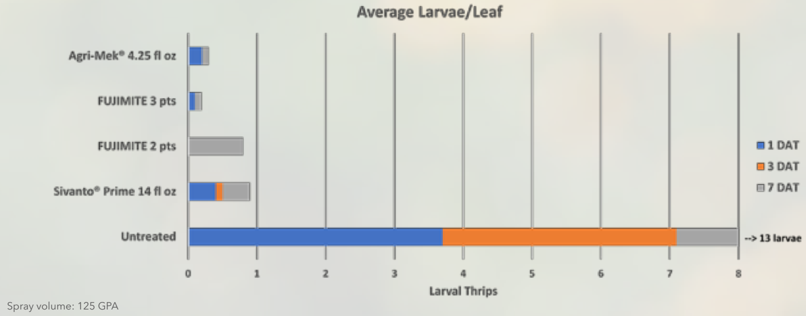

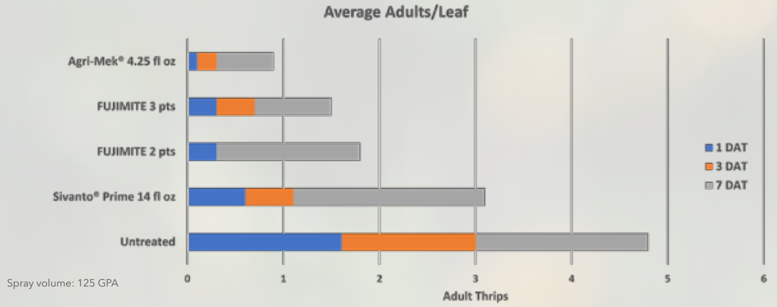

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