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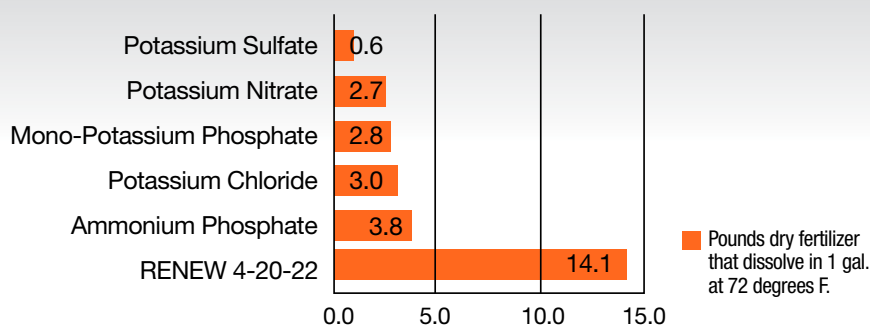
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**On the Cover:** Welcome to the winter 2024 issue of *Citrograph*, which focuses on integrated pest management (IPM). Some of the suspects on IPM's "most wanted list" include Asian citrus psyllids, citrus mealybugs, mites, Argentine ants, California red scale, thrips and Citricola scale. We invite you to delve into these pages to learn more about the valuable research being conducted to control and/or eradicate these pests through IPM.





# In This Issue

Winter 2024 | Volume 15 • Number 1 The Official Publication of The Citrus Research Board  
*Citrograph's* mission is to inform citrus producers and other industry members of research progress and results that will help ensure the sustainability of California citrus.

**10** **From the President's Desk**  
Marcy L. Martin

**12** **2023-24 Research Agenda Approved at CRB Annual Board Meeting**  
Caitlin Stanton

**16** **Meet the CRB's Newest Board Members**  
McCall Machado

**18** **Proactive Education is the Best Defense Against HLB**  
Kevin Ball

**22** **IPM Views Across the Industry**  
Ivan Milosavljević, Ph.D.

**28** **Crushing Citrus Woes**  
Ivan Milosavljević, Ph.D.

**32** **Make Plans to Attend the IRCHLB in March**  
Caitlin Stanton

**36** **Recap of the Citrus Post-harvest Pest Control Conference**  
Caitlin Stanton, et al.

**40** **Advancing Commercialization of Hydrogel Baits for Sugar-feeding Ants**  
David Haviland, et al.

**44** **The CA-CRaFT Program**  
Ariana Gehrig

**48** **California-adapted *Tamarixia radiata* for ACP Biological Control**  
Raju Pandey, Ph.D., et al.

**54** **Core IPM Updates**  
Sandipa Gautam, Ph.D.

**60** **The Phytosanitary Efficiency of California's Post-harvest "System"**  
Sandipa Gautam, Ph.D., et al.

**66** **Precise Genome Editing of the Asian Citrus Psyllid**  
Michelle Bui, Ph.D., et al.

**70** **A Taste of Citrus History**  
Benjamin Jenkins, Ph.D.

Editor's note: In the fall 2023 issue of *Citrograph*, there was an error in the Adaskaveg article "An Increasing Array of citrus Pre- and Post-harvest Fungicides." In Table 2, the trade name of the fungicide mefen-trifluconazole/fluxapyroxad is Elisys, not Mibelya. In addition, it should be noted that both mefen-trifluconazole/fluxapyroxad and pydiflumetofen/fludioxonil have had registration requested in California. An updated version of the table can be found in the digital version of the article at [citrusresearch.org](https://citrusresearch.org)





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# 2024

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# From the **PRESIDENT'S DESK**

Marcy L. Martin

**A**s the new season recently began for many California citrus growers, we are reminded of the joys of a harvest and the trials that come along with agriculture. Pests have been a major issue for many growers during the past year, and we are seeing those effects now as fruit is harvested. Integrated pest management (IPM) long has been one of the Citrus Research Board's (CRB) main research priorities and that continues with the guidance of our Pest Management Research Committee. They are tasked with reviewing and guiding IPM researchers to deliver valuable results to California's citrus growers.

The winter edition of *Citrograph* focuses on IPM and features several of the ongoing IPM projects in California and beyond. I would like to highlight two of the projects included in this issue:

#### **Raju Pandey, Ph.D. (*Tamarixia* Rearing)**

Raju Pandey, Ph.D., of the CRB oversees the high-quality production of *Tamarixia radiata* to support Asian citrus psyllid biocontrol programs in California. Originally overseen by University of California, Riverside researchers, Pandey took over the program in 2021 and is responsible for the collection, maintenance and production of genetically diverse *T. radiata*. He has shifted from the program's original maintenance of Pakistani material to the collection and maintenance of locally adapted *T. radiata* from inland, coastal and desert regions of California. The shift to locally

Marcy L. Martin



sourced *T. radiata* is expected to enhance the biocontrol efforts in state by providing programs with *T. radiata* that are better adapted to California conditions. More information from this research project can be found on page 48.

### David Haviland (Ant Control)

David Haviland, of the University of California Agriculture and Natural Resources, is conducting research to identify improved methods of ant control in citrus orchards. The Haviland lab is employing the use of polyacrylamide hydrogels (water absorbing polymers) containing low amounts of insecticide and sugar that can be readily applied in orchards. Early results from commercial scale field trials are showing promise as populations of Argentine ants are being reduced more than 50 percent following one application and more than 90 percent following a second application. Beyond these trials, the Haviland lab is working in tandem with other UC researchers who are employing similar hydrogel technology. More information from these research projects can be found on page 40.

### Looking Ahead in 2024

The beginning of the 2023-24 fiscal year brought a new executive officer team. We were thrilled to welcome Mark McBroom of Bloom to Box Crop Care, Inc. in District 3 to serve as chairman. John Gless III of Bagdasarian Farms in District 2 will begin his term as vice chairman, and Scott Carlisle of Villa Park Orchards Association in District 1 will serve as secretary/treasurer. We are confident that this slate of officers will expertly guide the CRB through 2024.

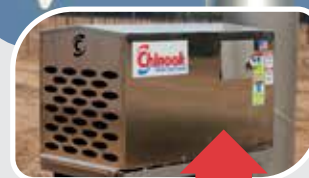
This year is shaping up to be busy for the CRB. Our year will begin with our research committees implementing the slate of industry priorities adopted during the 2022 strategic workshop and developing the 2024-25 request for proposals (RFP) to support these objectives. These committees meet several times per year to assess projects and their alignment with the CRB's priorities. Come spring, the CRB will host the International Research Conference on Huanglongbing VII (IRCHLB VII) in Riverside, California. This event will bring together researchers from around the world to discuss the effects of HLB and determine paths forward to keep this brutal disease from further impacting the world's citrus industry. Additional information about the conference may be found on page 32.

The CRB remains committed to supporting needed research projects in the citrus industry. We hope you enjoy this IPM-focused issue of *Citrograph*, and we look forward to sharing research updates throughout the next year. 🌞

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



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A photograph of five men standing in a row outdoors. From left to right: a man in a white button-down shirt and a baseball cap; a man in a blue and white plaid shirt and glasses; a man in a grey short-sleeved button-down shirt; a man in a green zip-up jacket and a baseball cap; and a man in a blue and white plaid shirt. They are all smiling and standing on a paved area with trees in the background.

Retiring Board members (middle, left to right) Chris Boisseranc, Joe Stewart and Jason Orlopp were commended for their dedicated service by the 2022-23 Chairman Justin Brown (far left) and incoming 2023-24 Chairman Mark McBroom (far right).

2023-24 RESEARCH AGENDA APPROVED AT

# CRB ANNUAL BOARD MEETING

Caitlin Stanton

The Citrus Research Board (CRB) met on September 19, 2023, to approve the annual budget and confirm the 2023-24 research agenda. Board members and guests traveled from around the state to the Visalia Convention Center in Visalia, California, to attend the meeting. Joining the Board members were representatives from California Citrus Mutual (CCM), the California Citrus Quality Council (CCQC), EcoData Technology and the California Department of Food and Agriculture (CDFA).

"The Annual Board Meeting allows our members to reaffirm their support for the valuable research projects funded by the Citrus Research Board," said CRB President Marcy L. Martin.

During the meeting, each committee chair provided an update on their group's activities. They shared an impressive

slate of research projects and informed attendees about current developments at the BSL-3P Laboratory, the California-focused Citrus Research and Field Trials (CA-CRaFT) Program and ongoing work by CRB Integrated Pest Management (IPM) Entomologist Ivan Milosavljević, Ph.D.

Both new and continuing projects were approved in the New Varieties Research, Vectored Diseases Research, Production and Post-harvest Technology Research, Pest Management Research and Citrus Clonal Protection Program committees. A complete list of FY2023-24 research projects will be shared in the upcoming summer issue of *Citrograph*.

The Finance Committee provided an update on the CRB's financial position. The Board remains committed to ensuring that fiscal responsibility is one of the organization's key principles. An overview of the CRB's financial position also will be shared in the summer issue of the magazine.



The Board voted to maintain the current assessment rate of \$0.032/carton, with an estimated total of \$5,800,000 in fiscal year funding. More than 66 percent of this funding will be spent on core research programs that will benefit growers with field-level applications. This rate has been approved by the CDFA Secretary.

Additionally, members of the Board held elections to determine the slate of officers for 2023-24. Mark McBroom of Bloom to Box Crop Care, Inc. in District 3 assumed the role of chairman. John Gless III of Bagdasarian Farms in District 2 was elected vice chairman, while Scott Carlisle of Villa Park Orchards Association in District 1 joined the officers as secretary/treasurer.

Justin Brown of Citricove Orchards in District 1 concluded his four years as chairman of the CRB, where he made a lasting impact on the organization. During his tenure, the Board's committee structure was updated to maximize efficiency and focus on strategic research priorities that will yield field-based solutions for California's citrus growers. As a grower-funded organization, financial responsibility was one of Brown's priorities and during his time as chairman, the CRB has maintained the lowest assessment rate in recent years. Brown has served on the Board since 2009 and will continue to do so while participating in and leading various committees. We are thankful for his remarkable guidance over the past four years and look forward to his continued impact on the CRB.

Retiring Board members Chris Boisseranc, Jason Orlopp and Joe Stewart were recognized for their service as they concluded their terms. Chris Boisseranc of Southwest Ag Consulting, Inc., served on the Board from 2016-23 and chaired the Pest Management Research Committee in addition to being a member of many additional committees. Jason Orlopp of Foothill Ag Services, Inc., was a member of the Board from 2017-23 and served as the vice chairman of the Production and Post-harvest Technology Research Committee. Joe Stewart of Stewart Farms dedicated 19 years to the CRB as a member of the Board from 2004-23. He has served as the chair of both the Citrus Clonal Protection Program Committee and the Production and Post-harvest Technology Research Committee and has been a member of various other committees. We would like to thank these retiring Board members for their dedicated service to the California citrus industry.

Administering crucial research projects, maintaining an appropriate budget, and communicating these activities to the growers remain the primary objectives as the CRB mission is carried out. The CRB staff looks forward to working with the Board and its various committees to continue the mission of the organization as we strive to maintain a sustainable citrus industry established on sound science. 🌱

***Caitlin Stanton is the director of communications with the Citrus Research Board and also serves as the editorial assistant on Citrograph. For more information, please contact [caitlin@citrusresearch.org](mailto:caitlin@citrusresearch.org)***



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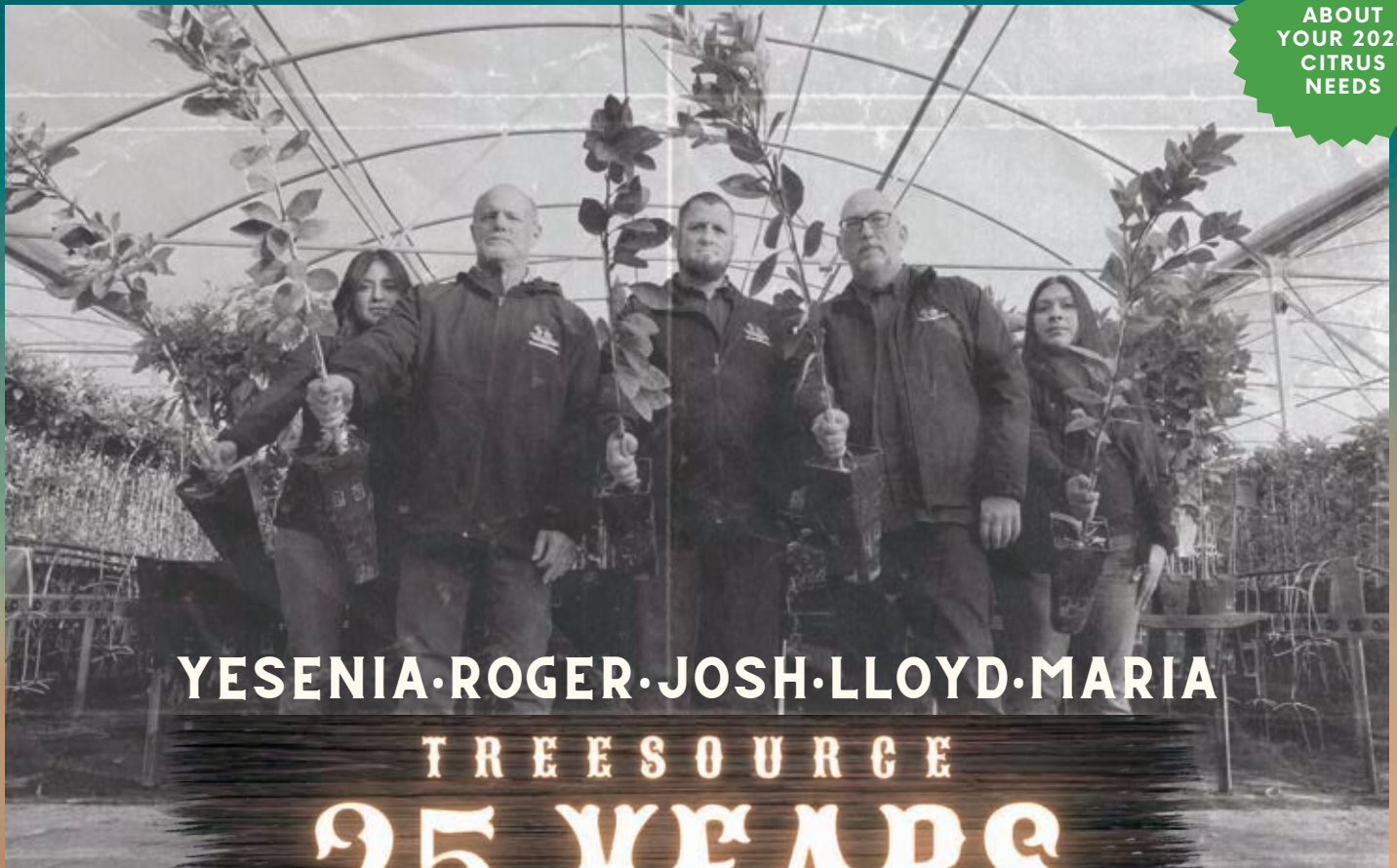


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**KEVIN  
BALL**

## **McCall Machado**

**T**he Citrus Research Board (CRB) welcomed three new members to the Board at nomination meetings held September 12-13, 2023. Jose Lima of Citrolima Citrus Nursery US and Nicholas Theis of AC Foods were nominated to represent District 1, while Kevin Ball of Ag Land Services was nominated in District 2.

Five current Board members were re-elected to serve additional three-year terms – Craig Armstrong of Thermiculture Farm Management, Inc.; John Gless III of Bagdasarian Farms; Henk Griffin of San Joaquin River Ranch, LLC; Megan Morreale of Hogwallow Farms; and Ram Uckoo of Wonderful Citrus.



"This group of newly elected and re-elected members brings a diverse set of industry experiences that will lend themselves well to the mission of the CRB," said CRB President Marcy L. Martin. "We are pleased to welcome them to the Board and look forward to working with each of them."

Lima is a second-generation citrus industry professional, following in the footsteps of his father and uncle, who farmed more than 700 acres of citrus and ran a large citrus nursery in Brazil. He holds a Bachelor of Science degree from Florida Southern College and a Master of Science degree from the Universidad Politècnica de València in Spain, with both degrees focused on citrus production. Lima moved to the Central Valley with his family in 2007 and has worked as a manager and director at several citrus nurseries. In 2018, he planted 16 acres of citrus and then started Citrolima Citrus Nursery US, in Dinuba in 2023. Lima takes pride in being a part of the California citrus industry, where he believes the world's best fresh citrus is grown.

"As a member of the CRB, I am excited about accomplishing several goals for the California citrus industry. Some of these goals include joining various committees that aim to produce practical outcomes for the benefit of California citrus growers. I believe that the research funded by the Board should be relevant and applicable to the challenges and opportunities faced by the growers," said Lima. "I am aware of the threats posed by pests, diseases, climate change and competition, and I want to help the Board find effective solutions and strategies to address them."

Theis, a native of Visalia, California, discovered his passion for agriculture during his teenage years while harvesting corn and driving swathers. He pursued a Bachelor of Science degree in Crop Science at California Polytechnic State University, San Luis Obispo. His career began at Wonderful Citrus as a farm supervisor, and he later obtained his pest control adviser (PCA) license before transitioning to Helena Agri-Enterprises as a retail PCA. After four years, he returned to his passion of farm management and joined AC Foods as a Director of Farming.

"I look forward to addressing the challenges we face as growers and presenting modern solutions at the Board level. Throughout my young career, I've learned from people who have guided and mentored me in the citrus industry, and my hope is that I can give back my time and represent them proudly on this Board," said Theis.

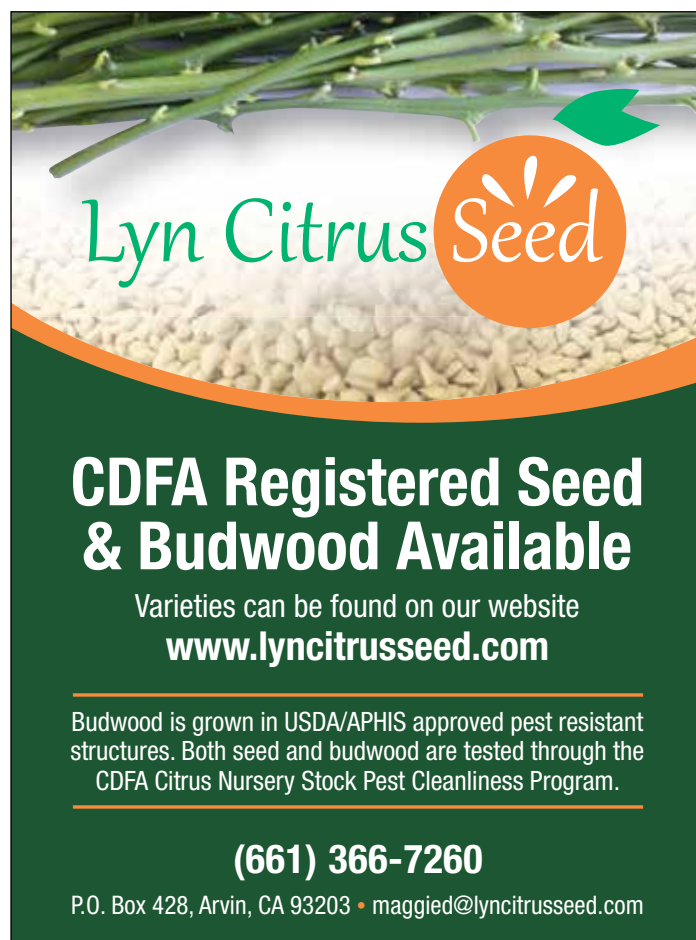
Ball comes from a strong agricultural background, as he grew up on a Valencia and lemon ranch in Fillmore, California. It became clear early on that his career path would be in agriculture, and he earned a Bachelor of Science degree in Agricultural Systems Management from California Polytechnic State University, San Luis Obispo. For more than two decades, Ball has worked at Ag Land

Services where he currently serves as the Vice President of Operations and oversees 1,100 acres of lemons and 1,200 acres of avocados in Ventura and Santa Barbara counties. Along with his family, he farms 120 acres of lemons and 150 acres of avocados. Ball also serves on the California Avocado Society and the Citrus Pest & Disease Prevention Committee. In his latter capacity, he authors quarterly articles about the Committee's activities for *Citrograph* (see page 18).

Ball added, "I love working in agriculture and it's the only career I wanted to have. I hope to bring my growing experience from the coastal regions to the CRB to ensure the citrus industry remains viable for future generations."

The CRB looks forward to a productive year with the 2023-24 Board. Please join us in welcoming our newly elected Board members and congratulating those who were re-appointed. 🍊

**McCall Machado is the communications coordinator for the Citrus Research Board. For more information, please contact [mccall@citrusresearch.org](mailto:mccall@citrusresearch.org)**



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# PROACTIVE EDUCATION IS THE **BEST DEFENSE AGAINST HLB**

Kevin Ball





The California citrus industry has done a remarkable job of delivering a strong counterpunch to the pests and diseases that threaten our livelihoods. For more than 15 years, our industry has rallied to outmaneuver the threat of the Asian citrus psyllid (ACP) and huanglongbing (HLB) and has developed proactive and reactive strategies that have allowed our state to continue to thrive. This commitment to getting ahead of the problem is also a key part of what drives our outreach strategies. We recognize that we can't wait for issues to arise to begin communicating best practices, regulatory requirements or response plans. Rather, we work to identify proactive opportunities for the industry and residents to continue learning about the pest and disease and feel prepared for when HLB may be in their area.

## Leveraging Anticipated Seasonal Spikes to Proactively Increase Awareness

While the Citrus Pest and Disease Prevention Program's (CPDPP) outreach team often leverages grim milestones – such as quarantine expansions or detections in new areas – to build awareness and maintain urgency for this important issue, proactive outreach efforts also are an important part of the communication mix. Recognizing that the Kern County area historically has seen a spike in ACP detections in residential areas around the later parts of summer and early autumn each year, the outreach team developed a comprehensive campaign to help educate local industry members and residents on the importance of keeping ACP populations low and also how to take action before any expected spike may occur. To bring awareness to the dangers that an increase in ACP populations can bring, an opinion-editorial (Op-Ed) piece bylined by citrus grower and Citrus Pest and Disease Prevention Committee (CPDPC)

member Keith Watkins was secured with the *Bakersfield Californian* in September 2023. In addition, the CPDPP team secured an ad placement and advertorial mention in *Valley Ag Voice*, a local publication based in Bakersfield with a strong agriculture focus. Through the Op-Ed and placements in *Valley Ag Voice*, the program worked to illustrate what could happen if the area is unable to control a potential ACP population surge, with each element providing a unique perspective on the important role residents play in remaining vigilant in the fight against ACP.

In addition, English and Spanish advertisements were placed on two local radio stations, KIWI-FM and KUZZ-FM, with messaging focused on encouraging residents to learn more about what the ACP is, how it can spread HLB and what they can do to protect not only their own trees, but Kern County's robust commercial citrus production. Lastly, an eye-catching billboard was placed along State Route 58 in south Bakersfield directing residents to learn more at the CPDPP's website. Overall, the program and local Bakersfield residents were successful in keeping the historically high ACP populations to a minimum last fall.





**In late summer 2023, citrus industry members in Santa Paula and Exeter attended Train-the-Trainer sessions to learn more about Asian citrus psyllid and huanglongbing best practices.**

## Hands-on Education with Regional Field Crews

Protecting commercial citrus groves from the devastation of HLB starts with those on the front line of citrus groves and packinghouses, as these industry members are truly at the forefront of stopping ACP in its tracks. Prior to the fall harvest season, the CPDPP hosted two "Train-the-Trainer" workshops in Ventura and Tulare counties to directly connect with these key stakeholders. These workshops were conducted in Spanish and informed key leaders in the field about what they can do to reduce the risk of spreading ACP populations from grove to grove and – ultimately – how they can protect California's commercial production from HLB. The training workshops taught effective communication techniques and included hands-on exercises to help crews and other industry members understand these best practices. The training workshops also fulfilled the requirement listed in the California Department of Food and Agriculture (CDFA) harvesters/farm labor contractors' compliance agreement,



**Video crews worked with California Department of Food and Agriculture staff to showcase what growers can expect if they are working with an agriculture official in their groves.**



established in early 2023, for these industry members to stay abreast of ACP and HLB prevention best practices by providing learning opportunities prior to harvest. These two meetings secured attendees from more than 25 different companies or organizations in the area, which included field crew staff, foremen and packinghouse representatives.

## Preparing for a Potential Commercial HLB Detection

While citrus growers may not want to think about a day when our commercial operations may experience a detection of deadly plant diseases such as HLB, it's best that we prepare now and be well equipped to act swiftly to handle the unthinkable. To ensure all of our industry partners are aware of what's coming down the pipeline, the CPDPP created an educational video to accompany its Response Guide for a Confirmed HLB-positive Detection in a Commercial Grove, which details the mandatory response taken by the CDFA and actions required of the property or grove owner should a confirmed citrus plant sample test positive for the HLB-associated '*Candidatus Liberibacter asiaticus*' (CLas).

The video highlights the key actions in the response guide that represent the most effective tools known to the citrus industry at this time and are meant to protect California's citrus groves and support the CDFA's current required regulatory response. The video is accessible on [CitrusInsider.org](http://CitrusInsider.org).

While we can't predict what the future will hold, we can plan ahead for scenarios that may come to fruition. By working together, we can all do our part in protecting the future of California citrus from ACP and HLB. 🍊

**Kevin Ball is the outreach subcommittee chair for the Citrus Pest and Disease Prevention Committee. For additional information, please contact [kevin.ball@aglandca.com](mailto:kevin.ball@aglandca.com)**

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# IPM VIEWS

## ACROSS THE INDUSTRY

Ivan Milosavljević



Given *Citrograph's* focus on integrated pest management (IPM) in this edition, we reached out to multiple citrus pest control advisers (PCAs) to gather their insights on current IPM challenges in California, spanning from California's southernmost points through the coastal areas of Ventura and Santa Barbara counties to the Central Valley.





A syrphid fly larva consuming an Asian citrus psyllid (ACP) nymph.  
(Photo by Mike Lewis, UC Riverside)



**SAN DIEGO COUNTY  
ENRICO FERRO**

Farm Consulting Services, PCA  
San Diego County Citrus Pest  
Control District Manager

**What notable pest challenges did your region face in 2023, and what are your expectations for 2024? Were there any unexpected issues in 2023 compared to previous years?**

Notable pest challenges in 2023 included citrus mealybug (CM) damage to the crop, the buildup of black scale and rust/silver mites and anthracnose damage to the fruit rind. Looking ahead in 2024, the prediction of increased precipitation raises concerns for the same pests and the potential for additional issues like brown rot, septoria and

rind stippling. In an unexpected turn of events in 2023, despite applying copper as a fungicide and bactericide, substantial anthracnose rind damage occurred, leading to significant losses due to downgrading at the packinghouse.

**How do you envision IPM evolving to provide stronger support to the industry? Which particular advancements or tools do you think should be a focus to effectively address a range of pests?**

Pest management is always evolving as conditions change from year to year and new chemistries and products become available. The industry needs more ongoing field research to determine what works and what doesn't work (or doesn't work as well as it used to). Prioritizing ant control is crucial, not only for reducing Asian citrus psyllid (ACP), CM, mite and aphid populations, but also for reducing overall pesticide use. Baiting with thiamethoxam and boric acid is critical



in managing various pests, and the development of new products for ACP and other pests holds great importance.

**What strategies are organic growers in your region employing to manage pests? Which pests pose the most significant challenges for them, and what specific tools or advancements do they lack and need for effective pest control?**

Organic growers have very limited tools. Most of them are struggling to keep up with six ACP treatments per year to reduce ACP populations. Some continue to release beneficial insects; but with poor market returns, that has been challenging, as well. Their main hurdles are ACP and ants, as there are few effective products available and they come with high costs.



**VENTURA AND SANTA  
BARBARA COUNTIES**  
**TOM ROBERTS**  
Integrated Consulting  
Entomology, Entomologist, PCA

**What notable pest challenges did your region face in 2023, and what are your expectations for 2024? Were there any unexpected issues in 2023 compared to previous years?**

In coastal lemon groves, our most challenging pests are broad mites, ACP and pest-tending ants. In 2023, reduced populations of broad mites and ACP were observed due to increased winter rainfall and an extended, cool, foggy spring and summer, which led to delayed leaf flush and fruit set. Spring also saw the presence of aphids and an abundance of hoverfly larvae. The combination of hoverflies and the ACP parasitoid *Tamarixia radiata*, along with cooler temperatures, likely kept ACP populations very low. Similar pest levels are expected in 2024 with an approaching El Niño event.

**How do you envision IPM evolving to provide stronger support to the industry? Which particular advancements or tools do you think should be a focus to effectively address a range of pests?**

I envision IPM continuing as it has in the past. When new pests like ACP invade, the citrus industry responds with initial quarantines and eradication. If an invasive pest establishes itself, they use strategies like area-wide management, incorporating pheromones, cultural controls, coordinated treatments and introducing natural enemies. In my opinion, the citrus industry should focus on long-term alternative strategies to address ACP and other pest resistance, especially if replacement pesticides are eliminated due to environmental or regulatory issues. Coastal regions have managed ACP successfully for more than a decade, with no

reported huanglongbing (HLB) cases in commercial orchards, thanks to collective efforts in reducing ACP populations, the resilience of lemon varieties against HLB in contrast to oranges and other citrus varieties, regular pruning and increased treatment frequency due to historical pest problems like broad mites and bud mites.

**What strategies are organic growers in your region employing to manage pests? Which pests pose the most significant challenges for them, and what specific tools or advancements do they lack and need for effective pest control?**

Organic citrus growers employ various strategies like cover crops, emitter relocation away from tree trunks and tree skirt maintenance to disrupt ant movement and promote beneficial insects. Coastal lemon growers face challenges with the persistent broad mite, often needing sulfur products that disrupt mealybugs and attract Argentine ants, leading to increased pest issues such as California red scale (CRS) and black scale, among others. Inland organic groves have fewer mite issues, requiring minimal pest control except for ACP treatments. Currently, organic growers in Ventura apply two treatments under the ACP management plan. The key requirement for both conventional and organic citrus orchards is an effective, economical liquid bait to manage Argentine ant populations.



**VENTURA AND SANTA  
BARBARA COUNTIES**  
**JANE DELAHOYDE**  
Oxnard Pest Control  
Association, PCA

**What notable pest challenges did your region face in 2023, and what are your expectations for 2024? Were there any unexpected issues in 2023 compared to previous years?**

2023 was celebrated because it broke years of severe drought, yet it has been one of the worst lemon markets our growers have experienced due to oversupply. Ventura and Santa Barbara counties experienced a very cool spring and gloomy June that reduced common pests like bud mites, broad mites, citrus thrips, ACP, CRS, CM and ants. Beneficial predator populations were high until warmer weather returned in August. Excessive rain led to a rise in brown snails, while low lemon prices required cost-cutting on cultural practices, affecting fruit quality. The discovery of HLB in residential citrus trees prompted a five-mile quarantine zone around our orchards. In the last month, we started finding trace blotchy mottling inside some orchards and have been working with the growers, the local University of California (UC) Farm Advisors and UC Riverside researchers to get them tested. We completed our fall spraying operations, and we're gearing up for potential challenges in 2024 guided by the "Hope for the best, plan for the worst" principle.



**How do you envision IPM evolving to provide stronger support to the industry? Which particular advancements or tools do you think should be a focus to effectively address a range of pests?**

IPM only works when there's close monitoring in the fields by experienced PCAs, growers and trained workers; not reliance on calendar sprays, as microclimates play a huge part in timing. Ventura County's area-wide ACP sprays reduced ACP populations but virtually destroyed IPM, causing participation decline and efficacy issues. This past cool, rainy season allowed IPM programs to reduce field trips to one or two sprays yearly, targeting specific pests when they reach economic thresholds. Ant gel bait development is promising, but should address potential bait-shyness in ants. Wind rows and barriers slow pest spread, but we need better barrier crops with less plastic use. An efficient tree removal method for HLB, using a tractor with a scissor attachment and tailored systemic herbicide, shows promise in Florida. Ensuring access to quality and affordable sources of beneficial insects, such as *Neoseiulus californicus*, *Tamarixia radiata* and *Cryptolaemus* is vital. Increasingly stringent pesticide regulations have resulted in the loss of being able to knock back several pests at once. This results in more field visits, broad-spectrum pesticide reliance, drift risks, higher costs and resistance. Pesticide-spraying drones may work where there are large, flat ranches, but they are unaffordable for Ventura County.

**What strategies are organic growers in your region employing to manage pests? Which pests pose the most significant challenges for them, and what specific tools or advancements do they lack and need for effective pest control?**

Successful organic citrus growers are found in microclimates like Ojai, Fillmore and Santa Paula, where they mostly grow mandarins and oranges. They use coarse woody mulch for weed control, build up microbes in the soil and attract beneficial insects with pollinizer patches. They monitor closely for CRS, broad mite, black scale, ACP, aphids and citrus thrips and apply appropriate treatments as needed. Beneficial organisms are introduced, and spray treatments encompass diluted oils, a Pyrethrins/oil combination and Spinosad/oil mixtures for pest control. Snail management includes skirt maintenance and copper-painted trunks. Some growers experiment with ducks for snail control and goats for weed management if selling at farmers' markets. They are looking forward to seaweed hydrogel registration for ant control.

Conversely, coastal organic citrus farmers facing milder temperatures could not grow sellable lemons as they were too heavily damaged by pests. They used all tactics listed above, but pesticides were never enough to knock the pest pressure low enough for clean fruit. Ant infestations are a significant issue, but ironically, ACP is not a concern.



**CENTRAL VALLEY  
MARCO RINALDI**

Rinaldi Ag. Services, Inc,  
President, PCA, CCA and QAL

**What notable pest challenges did your region face in 2023, and what are your expectations for 2024? Were there any unexpected issues in 2023 compared to previous years?**

In 2023, we encountered significant pest challenges, including a surprising surge in citrus thrips and a notable rise in citrus mealybug populations. The elevated citrus thrips pressure caught us off guard, especially considering the wetter-than-usual winter and spring conditions leading up to it.

**How do you envision IPM evolving to provide stronger support to the industry? Which particular advancements or tools do you think should be a focus to effectively address a range of pests?**

I'm optimistic that in the near future, we will see the development of a mating disruption pheromone specifically designed to combat CM. Additionally, I look forward to the introduction of novel chemical solutions for managing citrus thrips, CRS, citricola scale and CM.

**What strategies are organic growers in your region employing to manage pests? Which pests pose the most significant challenges for them, and what specific tools or advancements do they lack and need for effective pest control?**

Organic growers employ CRS mating disruption, introduce beneficial insects to assist in managing CRS and CM, and utilize weed mats to control weed growth within the tree rows.



**CENTRAL VALLEY  
NICK BRANDT**

Sun Pacific Farming, PCA and  
CCA, Tulare and Kern counties

**What notable pest challenges did your region face in 2023, and what are your expectations for 2024? Were there any unexpected issues in 2023 compared to previous years?**

In 2023, citrus growers faced an unprecedented challenge with a dramatic increase in citrus thrips populations. Unfortunately, commonly used thrips insecticides proved ineffective and lacked persistence. Typically, thrips hatch in waves during spring and early summer; but in 2023, their numbers remained high during the fruit's vulnerable period,





Mature decollate snail, *Rumina decollata*, a natural predator of snail pests. (Photo by Mike Lewis, UC Riverside)

persisting from early spring well into July. Furthermore, specific areas in the valley have grappled with a growing CM issue in recent years. It's crucial to sustain research and investigate CM biology and monitoring to determine optimal treatment times for effective control.

**How do you envision IPM evolving to provide stronger support to the industry? Which particular advancements or tools do you think should be a focus to effectively address a range of pests?**

The citrus industry is facing a critical need for innovative insecticidal solutions, particularly concerning our primary

pest concerns, CRS and citrus thrips. Many of the most effective materials for combating these pests are outdated and have seen diminishing effectiveness through the years due to prolonged use. In California, the development of new, broad-spectrum agrochemicals has reached a standstill, leaving the citrus industry with a shortage of registered insecticidal chemistries. To address this issue, it is imperative to prioritize the continuous development of materials with novel, safer modes of action. Additionally, there should be a strong focus on implementing mating disruption and pheromone-based trapping tools wherever applicable, both for existing and emerging pest problems. The recent resurgence of CM populations highlights the need for a



multifaceted IPM strategy. Mating disruption already has demonstrated its efficacy in managing CRS and reducing the reliance on insecticide applications. A similar approach is required to effectively control CM and other significant citrus pests.

**What strategies are organic growers in your region employing to manage pests? Which pests pose the most significant challenges for them, and what specific tools or advancements do they lack and need for effective pest control?**

Organic citrus farming heavily relies on citrus spray oils for pest management. We regularly test new insecticidal products to address specific pests; but it's widely recognized that organically certified insecticides, while gentler, are generally less effective against citrus pests. Consequently, precise adherence to IPM principles, such as timing, becomes paramount in organic citrus farming. We also consistently introduce and rely on natural predators to combat various pests in our organic citrus groves. While the

most challenging pests are similar to those in conventional farming, honeydew-producing insects like citricola scale and CM prove to be especially difficult to control organically. Additionally, ACP poses a significant threat to citrus farming, with even greater emphasis in organic settings. The San Joaquin Valley's Guide for ACP/HLB Management in Organic Citrus serves as a valuable resource for organic citrus growers to develop effective ACP management strategies. 🌱

**Ivan Milosavljević, Ph.D., works as an integrated pest management (IPM) entomologist for the Citrus Research Board. For additional information, contact [ivan@citrusresearch.org](mailto:ivan@citrusresearch.org)**

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# CRUSHING CITRUS WOES

## BATTLING HLB, ANTS AND LEMON PITTING WITH IPM

Ivan Milosavljević

Aerial drone perspective of a commercial citrus orchard in southern California. (Photo by Mike Lewis, UC Riverside)

Safeguarding California's vital citrus industry relies on integrated pest management (IPM) to confront substantial challenges from invasive and resident insect pests. This article showcases IPM progress in combating persistent citrus issues involving insects such as Asian citrus psyllid (ACP) and huanglongbing (HLB) disease, Argentine ants (AA), and examines uncertain cases like lemon pitting, where insect involvement remains inconclusive.

## Combating ACP and HLB with All Our Resources

Addressing ACP and the '*Candidatus Liberibacter asiaticus*' (CLas) bacterium it carries, which causes the incurable HLB citrus disease, requires a robust IPM program (**Figure 1**). ACP was first detected in San Diego in 2008, sparking comprehensive efforts to protect citrus from HLB, which was subsequently identified in California in 2012. Ongoing efforts, encompassing coordinated insecticide treatments and statewide monitoring (Hoddle et al. 2022), collectively embraced by growers, show promise, particularly in specific regions. Moreover, the use of biological control agents from

Pakistan has demonstrated impressive effectiveness in reducing ACP populations within urban areas of southern California, especially in residential citrus areas recognized as breeding grounds (Milosavljević et al. 2021), thereby mitigating disease transmission in these environments. Additionally, the removal of infested residential trees and the rigorous enforcement of regulatory quarantine boundaries by state authorities have been crucial measures in containing HLB's spread to commercial citrus orchards.

Despite our best efforts, the enduring ACP/HLB challenge in California can be attributed to the state's diverse environmental conditions favoring ACP survival, necessitating a collective community effort, as the presence of a single infected tree has the potential to infect all nearby citrus trees. Addressing this challenge hinges on an adaptable approach that integrates region-specific strategies and embraces best treatment practices. Additionally, continuous research and collaborative initiatives involving growers, their workforce, PCAs, researchers and regulatory bodies are indispensable for effectively combating the ACP and HLB threat within California's citrus industry, with an emphasis on maintaining a constructive approach rather than being overly critical when issues arise.





**Figure 1.** Female *Tamarixia radiata* parasitizes Asian citrus psyllid (ACP) nymphs by attaching eggs to their undersides. Following this, the parasitoid larva emerges, consuming the ACP nymph internally and exiting through the upper back portion of the nymph. (Photo by Mike Lewis, UC Riverside)

## Revolutionizing AA Control with Hydrogels and Automated Monitoring

Combatting sugar-feeding ants like AA and their associated sap-sucking pests (SSPs) such as citrus mealybugs, soft scales and ACP in commercial citrus orchards requires an integrated biological strategy within an IPM framework, utilizing natural enemies. However, AA's protection and nurturing of SSPs in exchange for honeydew can compromise the effectiveness of these natural enemies (**Figure 2**). Consequently, effective ant control is crucial for enabling natural enemies to efficiently regulate SSPs (McCalla et al. 2023).

A promising solution for dealing with AA issues involves employing hydrogels, which are water-absorbing polymeric substances designed for targeted insecticide delivery. This approach entails infusing hydrogels with a small amount of

insecticide and sugar-water to create enticing baits for ants. Thiamethoxam works best for conventional orchards, while spinosad proves effective for organic systems. When applied via hydrogels, both insecticides swiftly reduce ant populations (McCalla et al. 2020; Milosavljević, *unpublished data*). As AA populations decline significantly, the role of natural enemies in pest control becomes crucial, underscoring the approach's importance in promoting sustainable citrus production (McCalla et al. 2023).

Implementing AA control, however, faces a major challenge due to the absence of an efficient, cost-effective sampling method for estimating ant densities and defining action thresholds for treatments. Infra-red (IR) sensors attached to irrigation lines offer potential solutions, enabling near real-time monitoring of ants and precise counts GPS-tagged to specific orchard areas, (Hoddle et al. 2022). Despite being in early stages of development, this technology shows promise in automating AA counts in citrus orchards, providing detailed and accurate insights into their foraging behaviors. However, establishing an action threshold, a critical ant density above which SSPs cause economic damage, is urgently needed to advance AA IPM in citrus orchards and to effectively utilize emerging technologies like hydrogels for ant control.

## The Growing Challenge of Lemon Pitting

When tackling the issue of lemon pitting (**Figure 3**), which was initially noted during the 2015-16 growing season in the San Joaquin Valley (SVJ), the industry faced a puzzling challenge (Mayorquin & Klein 2023). Initially, affected lemons in a few blocks exhibited webbed scarring and depressions on their surfaces. Although suspected to be mite-related, it did not display typical mite feeding damage, and pathogen testing yielded



**Figure 2.** Argentine ants consuming low-concentration insecticide in sugar water within biodegradable hydrogel beads. (Photo by Mike Lewis, UC Riverside)



**Figure 3. Fruit displaying distinctive web-scarred depressions, indicative of lemon pitting observed in the San Joaquin Valley.**  
(Photo by Ashraf El-Kereamy, Ph.D., UCCE)

inconclusive results. At that time, no definitive cause(s) could be identified for the lemon pitting observed.

Most recently during the 2021-22 and 2022-23 growing seasons, lemon pitting was identified in numerous SJV groves. Consequently, in early 2023, the Citrus Research Board (CRB) engaged with SJV producers to devise an action plan to address this issue; and through this, the CRB launched a collaborative research project with UC researchers to investigate the root causes of this issue. Producers within

the SJV were contacted to initiate large-scale grove surveys for researchers to collect comprehensive data on grove characteristics, symptoms, management practices, damage stages and potential biotic factors contributing to the problem.

Given the varied symptoms observed, such as depressions, scarred areas and reddish-brown patches on the fruit, a more rigorous insect sampling approach, covering different times of the day and night, various stages of fruit development and considering

both flying and crawling insects, may be warranted. Maintaining consistent and synchronized insect sampling with the fruit's development is vital to gaining a holistic understanding of potential insect involvement or the absence thereof. By heeding these recommendations and fostering interdisciplinary collaboration, we can enhance our understanding of this issue and collectively work toward effective solutions for the citrus industry. 🌱

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The background of the page is a photograph of a conference. At the top, a banner reads "Joint Conference of the INTERNATIONAL ORGANIZATION OF CITRUS VIROLOGISTS XII". Below this, large white text is overlaid. In the lower portion of the image, a man is seen from the side, speaking at a wooden podium. A microphone and a bottle of Aquafina water are on the podium. The podium has a white sign with a green logo on it.

# MAKE PLANS TO ATTEND THE IRCHLB IN MARCH

Caitlin Stanton

Olufemi Alabi, Ph.D., of Texas A&M University, shared his research with the audience during the IRCHLB VI.





The International Research Conference on Huanglongbing VII (IRCHLB VII) will be held March 26-29, 2024, at the Riverside Convention Center. Researchers from around the world will convene in Riverside, California, to share the latest updates on HLB and Asian citrus psyllid (ACP) research. This event is sponsored by the Citrus Research Board (CRB) with the support of California's citrus industry and organized in collaboration with researchers from many leading institutions, including the University of California, Riverside; University of Florida; US Department of Agriculture-Agricultural Research Service (USDA-ARS) and more.

This year's conference theme is "Transitioning Research to Field Reality," with the goal of sharing solution-based research and practical applications for growers. The opening session will focus on lessons learned since the previous IRCHLB in 2019. More than 160 abstracts have been submitted by researchers from 10 countries who will speak on topics such as:

- » host, pathogen and vector research, as well as the interactions between the three,
- » disease management,
- » infection consequences and
- » epidemiology and cultural control.

These abstracts represent much of the ongoing research aimed at combating the spread of HLB and will be shared in presentations and poster sessions throughout the conference.

Five keynote speakers will provide insight on HLB, ACP and related research:

- » Ute Albrecht, Ph.D., from the University of Florida, will share an update on practical measures underway in the United States to maintain commercial citrus production in HLB-endemic areas.
- » Michelle Heck, Ph.D., of the USDA-ARS, will provide an overview of field-based research that provided new insights into the biology and management of HLB.
- » Michael Knoblauch, Ph.D., of Washington State University, will share his research on phloem biology and the potential effort to counteract bacterial infections.
- » Denise Manker, Ph.D., of Bayer US, will share an overview of the compound development pipeline, highlighting the pathway from discovery through the testing process.
- » Leandro Peña, Ph.D., from the Polytechnic University of Valencia in Spain, will provide insight on international efforts in HLB control, including the degree of success of various management strategies and scientific technologies that have been utilized.



Conference attendees listened intently during talks given at the 2019 IRCHLB VI. A group of keynote speakers will share insights from around the world at next year's conference.



Attendees networked during the IRCHLB VI Conference in 2019.



The IRCHLB VII in 2024 will provide ample opportunities for researchers, regulatory representatives and industry members to discuss the latest HLB research.

"It has been nearly four years since the HLB research community has met to discuss their findings on strategies to manage the devastating effects of citrus greening disease," said MaryLou Polek, Ph.D., Chair of the IRCHLB Steering Committee. "I am looking forward to the International Research Conference on Huanglongbing VII to hear about research progress being made by scientists from around the world."

Conference registration is now open, and the cost per attendee is \$550 through March 15. Limited on-site registration will be available at \$675 per attendee. Special conference rate hotel rooms have been set aside near the convention center. Reservation links for each room block can be found on [irchlb.com](http://irchlb.com).

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Please visit [irchlb.com](http://irchlb.com) for additional details and to secure your place at this important event. For more information on the IRCHLB, please contact Caitlin Stanton at [events@citrusresearch.org](mailto:events@citrusresearch.org) or call +1 (559) 738-0246.

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# Recap of the Citrus Post-harvest Pest Control Conference



Jim Adaskaveg, Ph.D., University of California, Riverside, updated attendees on fungicide resistance management practices of post-harvest pathogens.

Caitlin Stanton, Joey S. Mayorquin and Mary Lu Arpaia

*The Citrus Post-harvest Pest Control Conference is a long-standing meeting for the California citrus industry to share the latest information in post-harvest technologies. This meeting originally was organized as an extension course in 1978 through the University of California, Riverside (UCR) and was overseen by Joseph Eckert, Ph.D., a renowned citrus post-harvest pathologist. In 1993, UCR Cooperative Extension Specialist of Subtropical Horticulture Mary Lu Arpaia, Ph.D., assumed responsibility for producing the conference. While the Citrus Research Board (CRB) became the official co-organizer of the conference in 2017, the CRB began providing input into the program in 2009. Since its inception, the conference remains committed to its original objective of providing technical information to packinghouse personnel and service providers in the areas of post-harvest pest management, quarantine requirements, trade issues and, more recently, food safety issues.*



## Conference Recap

On September 6, 2023, the 41st Citrus post-harvest Pest Control Conference was held in Visalia, California. The conference was well attended by more than 100 participants from both academia and industry. A diverse group of speakers representing academia, government and private industry provided conference attendees with the most up-to-date information in the critical areas of post harvest disease control, chemical residue management and food safety. The conference also provided attendees with the opportunity to network and receive continuing education units.

Eleven speakers were invited to share their expertise with conference attendees in one of four main areas: food safety, disease management, fruit quality maintenance, and trade and pest management issues. This year's speakers represented George Nikolich Consulting, California State University, Fresno, the University of California (Riverside and Davis), JBT Corporation, the United States Department of Agriculture-Agricultural Research Service, Syngenta, Janssen PMP and the California Citrus Quality Council (CCQC).



**Attendees of the Citrus Post-harvest Pest Control Conference gathered during a conference break.**

## Food Safety Session

Food safety presentations were provided by George Nikolich and Steven Pao, Ph.D. Nikolich opened the conference with a look into the importance of a root cause analysis during an illness outbreak in peaches—an informative break from the conference's typical focus on citrus topics. Although the citrus industry has not suffered illness outbreaks like other commodities, attendees learned that prompt response from the industry, including cooperation between producers, greatly assisted the peach industry in responding and working to mitigate potential future outbreaks. Pao's presentation provided results from his previously funded CRB project investigating the control of cross-contamination in fruit washing and fungicide application systems. It remains critically important for each packinghouse to conduct its own food safety evaluations as individual packinghouse systems and practices vary, but proper sanitizer applications are important to prevent the contamination of recirculated water systems.

## Disease Management Session

The Adaskaveg lab, led by James (Jim) Adaskaveg, Ph.D., provided two presentations on post-harvest fungicide use. Albert Nguyen's presentation focused on a promising post-harvest fungicide, cyproconazole, which was shown to be effective against the green mold and sour rot pathogens with moderate to high resistance to imazalil and propiconazole, respectively. Although imazalil remains a critical post-harvest fungicide, cyproconazole would offer the industry an additional fungicide. Cyproconazole also was shown to be compatible with other sanitizers and fruit coatings. The Adaskaveg team is hopeful that this fungicide will be registered in the near future. Jim Adaskaveg's presentation focused on strategies for managing fungicide resistance in green mold, sour rot and brown rot, including additional guidelines for the prolonged storage of lemons.



Speaker and conference co-organizer Mary Lu Arpaia, Ph.D., of the University of California, Riverside, spoke about how decisions made in the field can influence the quality of fruit at the consumer level.

## Fruit Quality Maintenance Session

Arpaia opened the afternoon session on fruit quality maintenance with a presentation stressing the importance of in-field practices in ensuring excellent fruit quality. Every aspect of fruit production ultimately affects the quality of fruit delivered to consumers; but how the fruit is handled is essential for producing high quality, saleable fresh fruit. Following Arpaia, Irwin Donis-González, Ph.D., discussed optimal cold storage conditions for citrus fruit and cold treatment schedules for management of various citrus pests. Charlene Jewell closed the session with a presentation focused on the ins and outs of post-harvest fruit coatings, especially best practices for the care and handling of coatings.

## Trade and Pest Management Session

The conference concluded with four presentations focused on various aspects of trade and pest management. Spencer Walse, Ph.D., provided an update on post-harvest fumigants including the special citrus use for ethyl formate as an alternative to spray and move mitigations for the Asian citrus psyllid that can be used on bulk fruit in bins. Heidi Irrig reviewed the process of establishing citrus post-harvest maximum residue levels (MRLs) and the global challenges facing MRL policies. Pauline Voorbraak discussed the challenges and potential opportunities within the global post-harvest industry, highlighting the need for continued collaboration between the scientific community and industry in developing creative solutions to maintain quality fruit at a global level. The final presentation was delivered by James (Jim) Cranney and highlighted the recent activities of the CCQC on market access issues and protocols, as well as registrations and label changes.

## Looking Forward

The Citrus Post-harvest Pest Control Conference continues to provide the California citrus industry with a forum where valuable technical information can be exchanged. The industry is committed to staying at the forefront of post-harvest issues and the research needed to address these issues. The CRB looks forward to continuing its role as a co-organizer of the conference. 🌱

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# ADVANCING COMMERCIALIZATION OF HYDROGEL BAITS FOR **SUGAR-FEEDING ANTS**

David Haviland, Mark Hoddle, Ivan Milosavljević,  
Ben Faber, Sandipa Gautam and Hamutahl Cohen

Figure 1. The Argentine ant protects Asian citrus psyllid nymphs from natural enemies, like *Tamarixia radiata*. Consequently, ants exacerbate infestations of sap-sucking pests in citrus orchards. (Photo credit: Mike Lewis, UC Riverside)





Sustainable production of citrus in California requires the use of integrated pest management practices that maximize reliance on biological control. However, biological control is often disrupted by Argentine and native gray ants, which prefer feeding on sugary liquids (Milosavljević et al. 2021; Hoddle et al. 2022). These ants protect honeydew-producing insects, like citrus mealybug and Asian citrus psyllid (ACP), from natural enemies (**Figure 1**). In return for this protection, ants are rewarded with a nutrient-rich honeydew, a waste product that results from a sap-feeding diet. Reducing the disruptive impacts of sugar-feeding ants from citrus orchards has clearly shown that natural enemies can provide excellent control of sap-sucking pests (McCalla et al. 2023).

University of California (UC) researchers have evaluated different types of hydrogels (polymers that absorb water) for control of sugar-feeding ants in citrus orchards and vineyards (**Figure 2**). Hydrogel chemistry permits the uptake of 25 percent sugar water (i.e., artificial honeydew) infused with an ultra-low amount of chemical insecticide (e.g., 0.0001 percent). Baits can be applied to the ground either by hand or mechanically under trees where ants forage. Ants rapidly recruit to this sugar-rich resource.

Research in the Hoddle lab at UC Riverside (UCR) has focused on the development of biodegradable alginate hydrogel beads, which are made using ingredients readily available online (i.e., sodium alginate, calcium chloride). Additionally, there are multiple online videos demonstrating how to make them. Once the beads are made, they are 'conditioned' for 24 hours by leaving them in sugar water that contains an ultra-low level of insecticide. Following this conditioning period, hydrogel beads are ready to be applied in the orchard. Alginate hydrogel beads are highly biodegradable and have the potential for use in organic production systems. At this early stage of development, alginate hydrogel beads are not produced on a large commercial scale but should be amenable to mass production.

Consequently, research at the Haviland lab (UC Cooperative Extension, Kern County) has focused on the use of commercially available polyacrylamide hydrogels for large scale field applications. Dry polyacrylamide crystals readily can be purchased in bulk, mixed with sugar water and an insecticide and left overnight to condition. When crystals are fully hydrated the next morning, they can be distributed in orchards. A five-gallon pail of pre-crosslinked hydrogel crystals is sufficient to produce about 400 gallons of finished bait, effectively covering a treatment area of 40 to 80 acres. Conversely, biodegradable beads necessitate crosslinking and soaking in a sugar-insecticide water solution to form the bait.

The Hoddle and Haviland labs have looked at a wide range of insecticides for use within sugar water-loaded hydrogels. Both research groups have come to the same conclusion that the most effective insecticide for use in conventional orchards is thiamethoxam, and the most effective for use in organic systems is spinosad. In their studies, the concentrations of insecticide that were used (0.0006 to 0.0015 percent for thiamethoxam; 0.01 percent for spinosad) were effective when applied at five to ten gallons of hydrated bait per acre (McCalla et al. 2020;



**Figure 2. Argentine ants feeding on biodegradable hydrogel beads applied to the soil in a commercial citrus orchard. (Photo credit: Mike Lewis, UC Riverside.)**

Milosavljević, Hoddle, & Haviland, *unpublished data*). These concentrations resulted in per-acre amounts of active ingredients that were 10 to 200 times lower than the amounts that safely can be applied to the foliage on the day of harvest (hydrogels are applied to soil, so they have no contact with foliage or fruit).

Commercial-scale field trials using baits with thiamethoxam in lemons and oranges consistently have shown a greater than 50 percent reduction in the number of Argentine ants during the first month after the first application (Haviland, *unpublished data*). After a second application one month later, Argentine ant

populations were reduced by more than 90 percent for at least two additional months. These results are consistent with those seen in trials conducted against native gray ants in table grape vineyards, against pavement ants in wine grape vineyards and against dark rover and bicolor pyramid ants in citrus (Haviland, *unpublished data*).

Commercial scale field trials using baits containing spinosad have had mixed results that appear to be based on rates and concentration. In research trials by UCR, where either 125 grams or 250 grams of alginate beads were spread evenly around the base of citrus trees by hand at monthly intervals for two to three months, ant control was excellent (Milosavljević & Hoddle *unpublished data*). In the UCCE trials, where the same volume of polyacrylamide baits was applied using a broadcast spreader to large acreages, but with a lower concentration of active ingredients, efficacy was greatly reduced (Haviland, *unpublished data*). More work is needed to fine tune insecticide concentrations in hydrogels and application amounts for effective use of spinosad using broadcast spreaders.

UCR has documented the massive impacts that natural enemies have on sap-sucking pests when Argentine ant densities are reduced significantly (McCalla et al. 2023). When ant densities are reduced by more than 90 percent, populations of citrus mealybugs declined by more than 90 percent on foliage and were eliminated completely from fruit. The amount of flush growth infested by ACP nymphs was reduced by 75 percent. These reductions were the direct result of increased biological control by both generalist and specialist predators, such as syrphid fly larvae and *Tamarixia radiata*, the ACP parasitoid imported from Pakistan and established in California.

Large-scale field evaluations of hydrogels as an insecticide delivery system for ant control in commercial orchards have demonstrated clearly that this novel technology is efficacious, extremely safe and uses ultra-low concentrations of insecticides in a



highly targeted manner to control sugar-feeding ants. Once ant densities are reduced, extremely high levels of pest control are provided by natural enemies. Efforts currently are underway to work this hydrogel bait delivery system through the regulatory process at the California Department of Pesticide Regulation. Significant progress already has been made, and we anticipate that progress will continue into 2024 due to strong support within California for integrated pest management technologies that are environmentally safe and promote sustainability. 🌱

## Acknowledgements

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# THE CA-CRAFT PROGRAM

## HIGHLIGHTS FROM THE FIRST YEAR

Figure 1. Mature oleander windbreak across from a lemon orchard in Brawley, California. (Photo credit: Luis Melgar)

Ariana Gehrig





*The California-focused Citrus Research and Field Trials program (CA-CRaFT) is a multi-year research project similar to programs in Florida and Texas where partnerships are formed with commercial citrus growers to limit the impacts of the citrus disease huanglongbing (HLB). The bacterium that cause the disease are spread when the Asian citrus psyllid (ACP) feeds on young citrus leaves. Trees infected with HLB produce inferior quality fruit, lower yields and may eventually die. The United States Department of Agriculture (USDA) Huanglongbing Multi-Agency Coordination (HLB-MAC) Group has funded programs in three major citrus-growing states to address the needs of the citrus industry and combat the threat of HLB. The CA-CRaFT program is focused on supporting growers in areas already impacted by ACP, notably southern California, who are interested in field testing additional ACP mitigation strategies to protect their groves.*

## Introduction

To slow the spread of ACP and the threat of HLB disease to commercial citrus in California, the Citrus Research Board (CRB) received funding from the USDA to implement a study of ACP mitigation strategies in California





**Figure 2. Kaolin clay application on lemon trees in Brawley, California. (Photo credit: Luis Melgar)**

groves in 2022. The CA-CRaFT program is grower-based and focuses on testing mitigation strategies like compound treatment applications and grove border protection in southern California citrus groves.

## Program Highlights

With funding secured, one of the first steps of program initiation was to hire a project manager in spring 2022. After reviewing program objectives with the CA-CRaFT Technical Advisory Committee (TAC), the CRaFT team developed

the program requirements, grower application materials and grower outreach activities. During summer and fall of 2022, outreach efforts included meetings with pest control districts, farm bureaus, CRB newsletter updates and several grower-specific informational webinars about the program.

Prior to the call for grower applications in autumn 2022, program staff began working with the Partnerships for Data Innovations (PDI) team, an initiative of the USDA-Agricultural Research Service (USDA-ARS), to develop an online grower application. Since that time, staff have continued to work with the PDI team to develop several data collection surveys



and data portals to collect, monitor, visualize and export program sampling data for further analyses.

The CA-CRaFT program received 14 applications for 20 individual grove sites. After sites were evaluated for program suitability, the program selected 13 groves to move forward. Ten groves (1,711 acres) were selected from District 3 (Riverside and Imperial counties), and three groves (55 acres) were selected from District 2 (Santa Barbara and Ventura counties). Lemon is the only variety enrolled in the program from District 2, whereas lemon, grapefruit and mandarin varieties are enrolled in the program from District 3. In total 1,766 acres are enrolled in the program, with lemons comprising the majority of the acreage.

The ACP mitigation strategies being tested in the CA-CRaFT program fall into two categories – preventive and threshold-based. Preventive mitigations are permanent barriers like mesh fencing and living windbreaks (**Figure 1**) that can prevent the movement of psyllids into groves along high-risk borders – next to a major transportation corridor, for example. Threshold-based mitigations are ongoing treatments like additional pesticide applications or psyllid repellent sprays (**Figure 2**), which are applied once ACP counts rise above the University of California Integrated Pest Management (UC IPM) Program threshold (UC IPM Guidelines, 2022). Kaolin clay is an example of a psyllid repellent spray that is applied to the whole grove or grove borders to make the leaves and new flush less attractive to psyllids. Other examples of threshold-based treatments include the release of biological control agents, generalist predators that feed on ACP, and ant control.

ACP monitoring is being conducted in several ways, via tap and visual sampling for psyllid adults and nymphs conducted by a field scout hired and trained to conduct ACP sampling for the program. The ACP tap and visual survey follows UC guidelines - sampling a total of 50 trees per block: 10 trees in each cardinal direction and 10 trees toward the center of the block. At each tree, a tap count is collected for adult ACP plus a visual flush inspection for adult and nymph stages. Monitoring is conducted by installing sticky traps in all program blocks. The adhesive on the traps immobilizes psyllids, and the traps are regularly serviced and inspected for ACP counts. Canine scouting is also being conducted to detect and confirm ACP infestations. Field technicians are using program specific surveys in real time to collect the data on ACP counts, foliar growth flush status, canine alert coordinates where ACP are visually confirmed by the canine handlers and overall grove conditions.

Aerobotics has conducted aerial grove imaging at program locations to map the groves and provide metrics on tree counts, canopy area, and vigor and transpiration values that relate to overall grove health. This information monitors general tree and grove health.

## What's Next

ACP monitoring and data collection are continuing at all experimental field sites throughout the second year of the program. While initial data are analyzed, monitoring is ongoing as permanent barriers are installed and windbreak plantings mature. Threshold-based treatments are continuing into year two and will continue to be based on ACP population fluctuations expected with the seasonal foliar flush cycles that vary between locations, citrus cultivars and standard grove operations. The CRB recently hired Ivan Milosavljević, Ph.D., to serve as the CRB IPM entomologist who, in addition to developing applied citrus research projects for the industry, will lend his expertise to the CRaFT program and provide additional program support.

The CA-CRaFT program looks to expand and add more grower participants this year. While the program will continue to focus on ACP mitigations in commercial citrus, new grower participants are being sought to enhance program efforts by introducing additional citrus varieties and selecting those program-approved mitigations with limited representation in the program's first year.

## Conclusion

The CA-CRaFT program is the first large-scale grower-based project to field test preventive and threshold-based ACP mitigation treatments in California commercial citrus. CRaFT staff are building on the successes of the first year by continuing to monitor ACP populations and mitigation strategies at participating groves in 2023. We expect to have an open application window this winter for growers interested in participating with this research effort. For more information on the program, please contact Ariana Gehrig via e-mail or visit the CRB website. 🌱

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Single female *Tamarixia radiata* wasp is used to start an isofemale line to preserve their unique genetic characters.

# CALIFORNIA-ADAPTED *TAMARIXIA* *RADIATA* FOR ACP BIOLOGICAL CONTROL

Raju Pandey, Gregory Simmons and David Morgan

## Introduction

The Asian citrus psyllid (ACP) is one of the most serious citrus pests due to its ability to vector the presumed huanglongbing (HLB) pathogen, '*Candidatus Liberibacter asiaticus*' (CLas). The ACP-HLB complex had begun to show its impacts in Florida before its detection in California in 2008. Researchers at the University of California, Riverside (UCR), in collaboration with California Department of Food and Agriculture (CDFA) and United States Department of Agriculture (USDA) scientists, initiated the





development of a classical biological control (BC) program targeting ACP populations in urban southern California (Milosavljević et al. 2017). This work was supported by the California citrus industry. The Punjab region of Pakistan, an endemic ACP locale, was selected to search for *Tamarixia radiata* and other BC agents based on a more than 70 percent climatic match with California's major citrus growing area (Hoddle and Pandey 2014). *Tamarixia radiata*, a tiny wasp, is the most widely used and effective biological control agent against ACP worldwide. The female *T. radiata* lays its egg on the underside of the ACP nymph. Upon hatching, its larva feeds on the nymph, thus killing the host. The adult wasp can also kill ACP nymphs by host feeding. A single female can kill up to 500 ACP nymphs in its lifetime (Chien et al. 1995). Though the introduction of *T. radiata* to California began in 2010, its first release was made only in December 2011 (Milosavljević et al. 2017). Imported *T. radiata* were held in a controlled (temperature, humidity, light, etc.) environment within quarantine until the non-target impact studies were completed.

There are risks associated with rearing parasitoids under controlled conditions that include the loss of desired fitness characteristics such as the ability to survive in a range of weather conditions and locate the host at low densities. Artificial rearing environments, longer time in captivity and smaller population size could lead to rapid loss of genetic variability due to inbreeding (Bueno et al. 2017, and references therein). To avoid loss of genetic diversity due to inbreeding, scientists at UCR maintained 16 different inbred lines collected at different times and places in Pakistan (Stouthamer 2015). Individuals from multiple inbred lines were pooled together in a mass-sting cage to produce a heterogenous population that was further multiplied through mass production. More than 28 million mass-produced wasps have been released in the last ten years throughout ACP-infested areas in southern California from these 16 original lines.

Citrus-growing areas in California are climatically disparate, encompassing a mild climate along the Pacific coast to an extremely hot and dry climate in the low Sonoran Desert and an intermediate climate in between. When released, *T. radiata*

must survive the local environmental conditions and locate hosts under a natural low-density distribution (compared to the rearing cages where thousands of nymphs are available within a small, enclosed space). It is expected that *T. radiata* with fitness traits suitable to the environment will be selected and the less fit will be eliminated. Field monitoring has shown that *T. radiata* have been established throughout southern California (Hoddle et al. 2022) from the coast to the desert.

## Establishing Locally Adapted Lines

The California ACP biocontrol program recognized the importance of the isofemale line<sup>1</sup> rearing system to maintain the high quality and genetic diversity of *T. radiata*. The program also realized it would be appropriate to replace the original Pakistan lines (that had been in captivity for more than ten years) with California-adapted *T. radiata*. Collecting field-adapted wasps and initiating new isofemale lines from various climatic environments within California will create genetically successful stock in terms of host searching<sup>2</sup> and surviving in the given climatic conditions.

The main objective of the project is to supply high-quality, genetically diverse *T. radiata* starter material to mass rearing programs to support the ACP biological control program. Producing and releasing wasps that are better adapted to each environment is expected to provide better pest control and ultimately benefit California citrus growers. The Citrus Research Board took over the role of collection, maintenance and production of *T. radiata* isofemale lines in 2021 and the collection of locally adapted *T. radiata* isofemale lines promptly began that fall.

### Collection of Parasitized ACP

Field visits were made to select residential sites with *T. radiata* activity that were at least two miles away from recent *T. radiata* releases. Visiting these types of sites was expected to increase the probability of collecting 'established and adapted' wasps. Mummified ACP and/or late instar nymphs were collected and brought to the laboratory to be incubated and observed daily for *T. radiata* emergence. Site locations visited in the past two years are summarized in

**Table 1.**

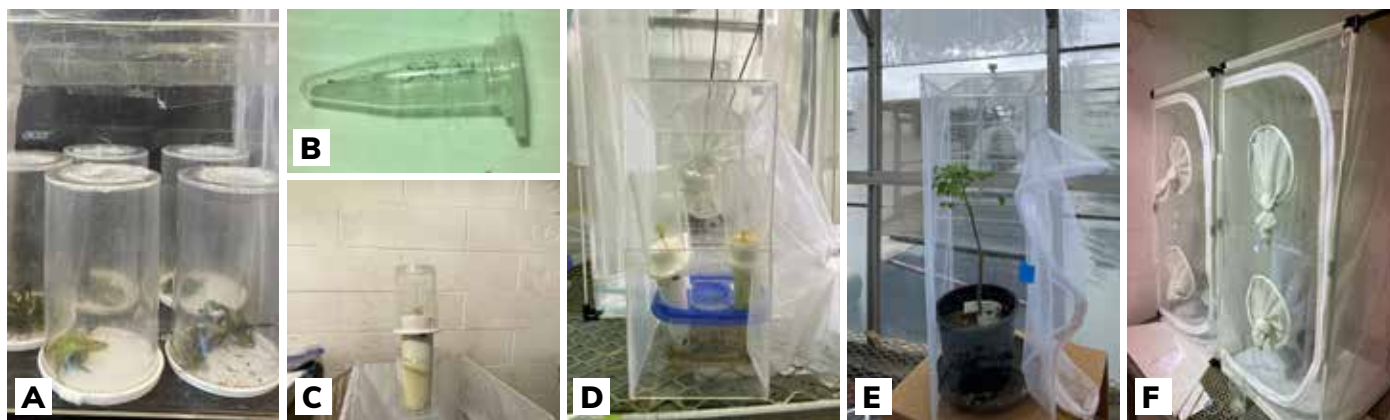
**Table 1. List of sites visited for parasitized Asian citrus psyllid nymphs and mummies.**

| COUNTY         | CITIES  |
|----------------|---|
| San Bernardino | Redlands, Fontana   |
| Riverside      | Riverside, Temecula, Murietta, Hemet, Corona, Thermal, Palm Springs |
| Orange         | Lake Forest, Huntington Beach                                       |
| Los Angeles    | Azusa   |
| San Diego      | Poway, Fallbrook, Oceanside, Vista                                  |
| Imperial       | Brawley and Calipatria  |

### *T. radiata* Nursery

A newly emerged single female wasp was paired with a male from the same collection site. A curry leaf plant seedling with 25 late-instar nymphs was provided to the female wasp every day for four consecutive days (100 nymphs total). In about 12-15 days, the next generation of wasps emerged from parasitized mummies. Progeny from the single female were collected and used for establishing a new isofemale line.





**Steps of iso-female line establishment. A:** Field-collected foliage with mummified Asian citrus psyllid (ACP) placed in a ventilated vial. **B:** Emerged *Tamarixia radiata* collected in micro centrifuge vial. **C and D:** Female provided with late instar nymphs on curry leaf plants. **E:** Newly emerged *T. radiata* transferred to an acrylic cage with ACP infested plant. **F:** The acrylic cages are placed within a BugDorm.

## Isofemale Lines

All the Pakistani lines have now been replaced with 15 California-adapted isofemale lines. Nine of the lines came from the inland region, four lines from the coastal region and two lines from the desert environment. Mitochondrial DNA analysis has revealed a high degree of genetic diversity among the collected lines (**Table 2**). As more California-adapted lines from each climatic region are collected, old locally collected materials also will be replaced.

## Mass Producing Locally Adapted Lines

The program has a dual purpose of establishing and maintaining isofemale lines, while also mass-producing *T. radiata* for use in other release programs. To accomplish both goals, a small number of wasps (usually 10-15) from each line are used for maintaining individual lines while the rest are used for larger scale production of *T. radiata* in mass sting cages. These materials will serve as source material for mass production from each climatic region to streamline ongoing biocontrol efforts and will help rejuvenate the colony vigor, especially as it relates to the host searching ability of the parasitoid.

To date, we have been combining all lines into a single mix in the mass sting cages. Soon we will begin to produce two mixes: (1) a coastal mix by combining the coastal and inland materials and (2) a desert mix by combining the desert and inland materials. Once we have more lines from the desert and coastal regions, three population mixes may be produced to represent coastal, inland and desert regions to provide a more targeted impact.

**Table 2. Results of mitochondrial DNA analysis of various *Tamarixia radiata* (TR) lines collected from within California.**

| TR LINE        | COUNTY      | LOCATION           | HAPLOTYPES <sup>a</sup> |
|----------------|-------------|--------------------|-------------------------|
| Coastal region |             |                    |                         |
| 21C1           | Orange      | Lake Forest        | H20                     |
| 21C2           | Orange      | Lake Forest        | H16                     |
| 22C1           | San Diego   | Fall Brook         | H2b                     |
| 22C2           | San Diego   | Oceanside          | H31                     |
| Inland region  |             |                    |                         |
| 21N3           | Riverside   | Orange Crest       | H3                      |
| 21N4           | Riverside   | Corona             | H2                      |
| 21N6           | Riverside   | Corona             | H2b                     |
| 21N8           | Riverside   | RCRCD <sup>b</sup> | H4                      |
| 22N1           | Riverside   | Murieta            | H2                      |
| 22N2           | Riverside   | Temecula           | H2                      |
| 22N3           | Los Angeles | Azusa              | H16                     |
| 22N4           | San Diego   | Poway              | H20                     |
| 22N5           | Riverside   | Corona             | H2                      |
| Desert region  |             |                    |                         |
| 22D1           | Imperial    | Brawley            | H2C                     |
| 22D2           | Imperial    | Brawley            | H2                      |

<sup>a</sup>Haplotype grouping is determined by common variations at specific locations in the mitochondrial DNA sequence.

<sup>b</sup>RCRCD=Riverside-Corona Resource Conservation District





*Tamarixia radiata* originally imported from Pakistan were maintained as 16 isoline populations, which have now been replaced with California-adapted lines.

## Future Efforts

The program will continue to maintain and produce *T. radiata* collected from southern California. Although the Central Valley is the main citrus production area in California, ACP is not established in this area due to eradication efforts.

Should ACP become established in the residential areas of the Central Valley and biological control is sought, material will be initially combined from all three regions and released. Subsequent efforts will be made to establish isofemale lines adapted to the Central Valley environment should that be needed. Releasing better-adapted wasps in each

**Table 3. Numbers of *Tamarixia radiata* produced from individual lines and mass sting cages.**

| YEAR                      | INDIVIDUAL LINES | MASS STING |
|---------------------------|------------------|------------|
| 2021 (October -December)  | 3,543            | 52,743     |
| 2022 (January – December) | 16,626           | 183,027    |
| 2023 (January – August)   | 10,057           | 92,407     |





Female *Tamarixia radiata* attempting to lay an egg on the underside of Asian citrus psyllid (ACP) nymph.

environment is expected to benefit California citrus growers by providing better vector control and reduce the risk of HLB spreading out of residential areas. 🌱

## Glossary

**<sup>1</sup>Isofemale line:** Small insect populations bred using male and female progenies of a single wild female wasp. Because unfertilized *T. radiata* eggs produce males while fertilized eggs produce female wasps, breeding within the population preserves the genes from the founding female in the entire line.

**<sup>2</sup>Host searching:** Adult female parasitoids often use host-related chemical cues to help them find prey, even when population densities are low.

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**Figure 1. Citrus mealybug field day on May 5, 2023. Field day was organized in a grower field in Ivanhoe, California, that had ongoing pesticide trials. (A) Attendees listening to Sandipa Gautam's lecture. (B) Attendees using microscope stations to observe pests and natural enemies. (C) Georgina Reyes with her poster. (D) Attendees visiting the poster. Citrus mealybug has been an increasingly concerning pest in the San Joaquin Valley.**

# CORE IPM UPDATES

## CITRUS IPM EXTENSION/OUTREACH EFFORTS

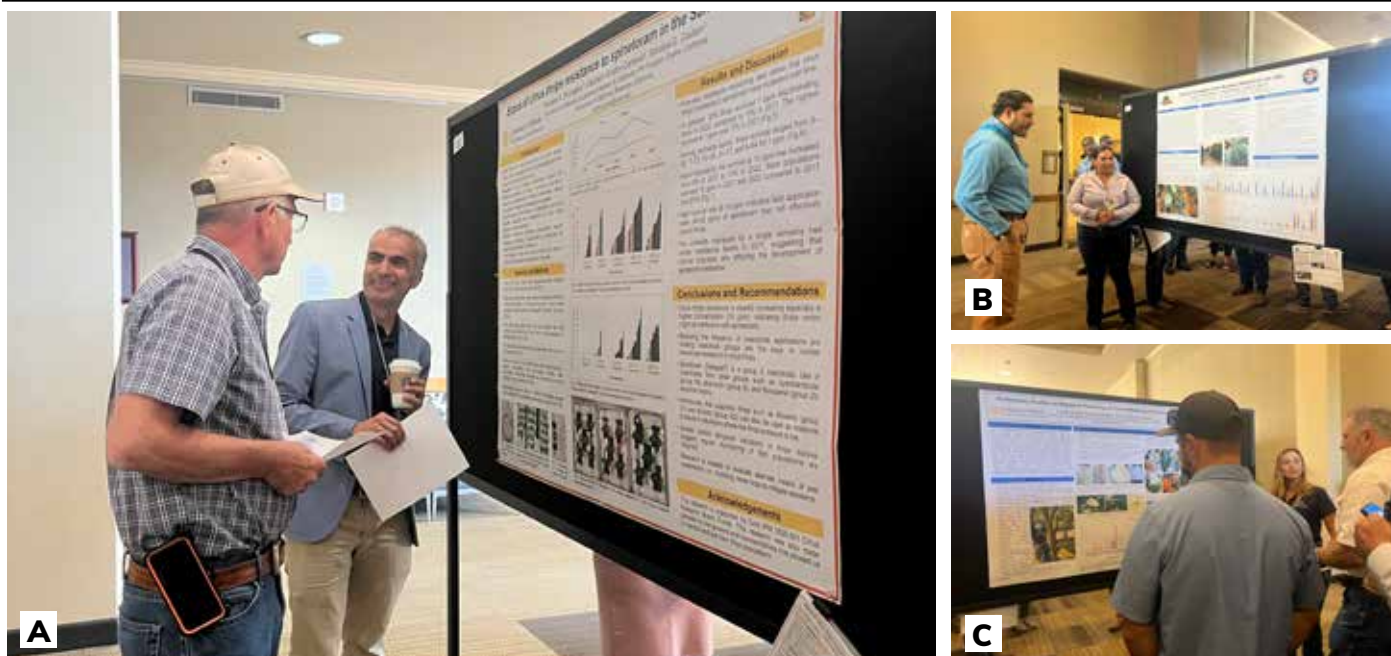
**Sandipa Gautam**

**T**ranslating research outcomes and communicating technical information in a way that is understandable and accessible is key to realizing the full value of the Core Integrated Pest Management (IPM) program. After starting as an area citrus IPM advisor in July 2021, I trained with Beth Grafton-Cardwell, Ph.D., to organize my first extension and outreach event. Since then, outreach efforts have evolved to address the needs of industry stakeholders. Below are some ongoing and future outreach efforts and a list of resources where citrus pest and pest management information can be accessed.

### Field Days and Workshops

Growers and pest control advisers (PCAs) have informed me that hands-on field days and workshops are an effective means to learn about their pest management needs and to get research updates. To address this need, the citrus IPM research and extension program led by my team and me organize two to three events at the Lindcove Research and Extension Center (LREC) each year. These events aim to educate attendees about the biology of a pest, scouting and monitoring techniques and updates on the best management practices highlighting recent work. Since July





**Figure 2. Poster presentations by Citrus IPM researchers at the 2022 California Citrus Conference. (A) Sanjeev Dhungana, Ph.D., discussed citrus thrips insecticide resistance. (B) Georgina Reyes presented pesticide trials on citrus mealybug. (C) Lauren Vuicich shared the seasonal phenology of citrus mealybug.**

2021, we have developed and organized seven in-person outreach events:

- » a citrus thrips field day,
- » citricola scale biology and management,
- » California red scale (CRS) and its natural enemies workshop in 2021,
- » another citrus thrips field day,
- » CRS and parasite life stages workshop in 2022,
- » citrus mealybug field days in 2023 (**Figure 1**) and
- » CRS field days in 2023.

In addition, we also invite other University of California researchers to bring outreach events to the LREC to serve San Joaquin Valley (SVJ) clientele. On September 19, 2023, we hosted a workshop at the LREC on “Management of sap-sucking insects and ants in citrus.”

All the outreach events are developed for growers and pest management professionals with continuing education units (CE) available for PCAs and Qualified Applicator License holders.

## Outreach via Grower and Industry Meeting Presentations

Every year, I present more than 20 invited talks at various meetings organized by the Citrus Research Board, California Citrus Mutual, Association of Applied IPM Ecologists, Crop Consultants Conference, California Association of Pest Control Advisers, California Citrus Quality Council and other

grower and industry meetings organized by agro-chemical companies. These presentations are designed to provide research updates to citrus industry stakeholders, especially growers, PCAs and field scouts. My team members also share findings via talks and posters at these meetings (**Figure 2**). Sanjeev Dhungana, Ph.D., is an assistant specialist working for me (**Figure 2A**). Georgina Reyes is a student at California State University, Fresno, and her graduate research on citrus mealybug seasonal phenology<sup>1</sup> and management is supported through the Core IPM program (**Figures 1C** and **2B**). Lauren Vuicich is a laboratory assistant working for me.

## List of Citrus Pest Management Resources

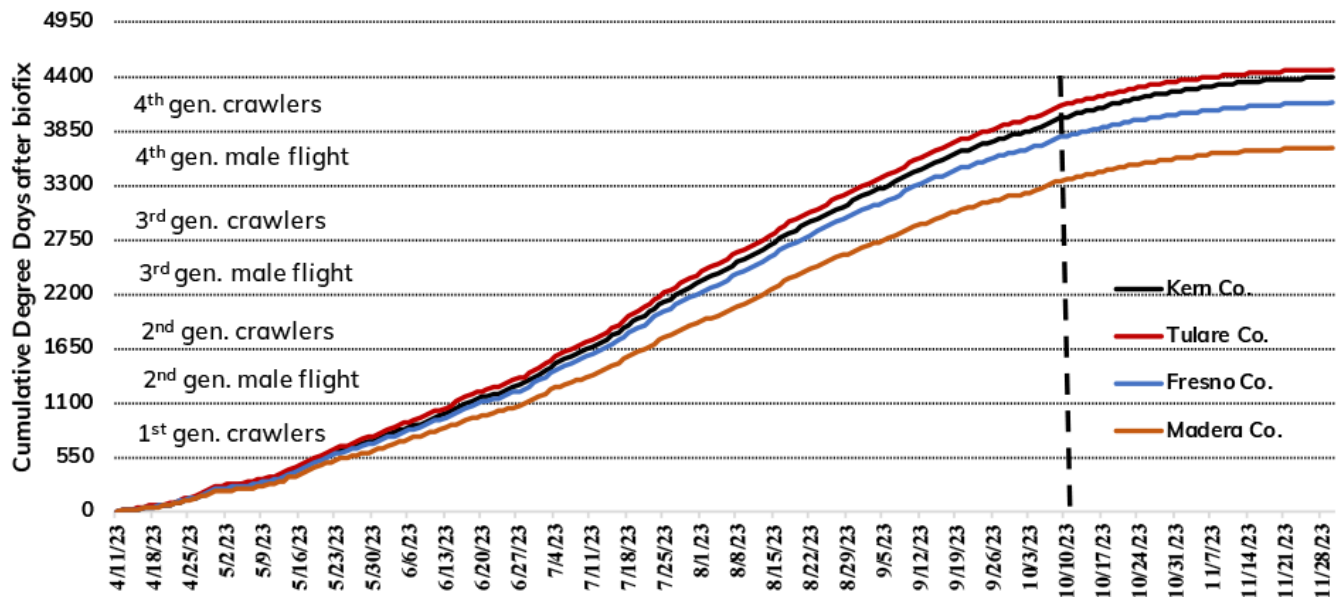
A tremendous amount of learning takes place online. There are several resources that can be tapped into to learn about citrus IPM, including archives of published articles, webinar records and live documents that are constantly updated with new information. Below, I describe the extension and outreach resources my group develops and updates and the list of resources that I update with other researchers and the University of California Integrated Pest Management (UCIPM) team.

### CRS Degree Day Updates

CRS is a key pest of citrus. Monitoring males and using degree day accumulations above the lower threshold of 53°F to predict life events such as crawler emergence and male flights for successive generations helps PCAs and growers make management decisions. Weather events can



### California Red Scale - cumulative degree days for four counties in the SJV



**Figure 3. Cumulative degree days (DD) in four San Joaquin Valley counties after California red scale biofix. Accumulation of 550 DD is required between flight to crawler emergence, 1,100 DD for next generation male flight. Data after October 8, 2023, are the predicted values based on long-term averages.**

affect degree day accumulation, thereby affecting seasonal phenology. For instance, the cooler spring in 2023 delayed first generation male flight by four to five weeks in all counties, which is something that hasn't happened in more than 15 years. Fourth generation crawler emergence was also delayed by three to four weeks (**Figure 3**). This may mean low population pressure going into the 2024 season. Moreover, 2024 also is expected to have a cool and wet climate. Biofix<sup>2</sup> and successive crawler emergence may be delayed again as it was in 2023.

Degree day updates for CRS were identified as a critical need by PCAs for monitoring this pest. I worked with Grafton-Cardwell on those and have been updating degree days since 2022. My group monitors and updates degree days weekly from May through November and biweekly in March through April using California Irrigation Management Information System (CIMIS) weather station data for Kern, Tulare, Fresno and Madera counties. These updates are available at [lrec.ucanr.edu/citrus\\_IPM/Degree\\_Days/](https://lrec.ucanr.edu/citrus_IPM/Degree_Days/) (**Figure 4**).

#### Citrus IPM Pest Memos

Another outreach need identified was access to regular and updated information in the form of pest memos for key citrus pests. To address this need, my group develops two- to three-page informal worksheets on citrus pests to provide information on pest monitoring, scouting and best management practices. For example, a memo on CRS sent in May 2023 included information on biofix and degree days in four counties, available options for management and links to the pesticide trial reports published in *Arthropod Management Tests* since 2012. Additional pest memos for citrus thrips and Citricola scale were developed in 2023 and

shared via mass email and made accessible on the LREC website, [https://lrec.ucanr.edu/Citrus\\_IPM/](https://lrec.ucanr.edu/Citrus_IPM/). We will continue to develop and share pest memos and update the industry.

#### UCIPM Pest Management Guidelines

Through decades, many researchers have contributed to developing the UCIPM pest management guidelines (PMGs) that are constantly revised as new data are gathered (Grafton-Cardwell et al. 2022). Since my appointment, I have worked with Grafton-Cardwell and the UCIPM team to update citrus PMGs. The most recent updates are pest monitoring forms for several key citrus pests, mating disruption updates for CRS, a document on organic pest management guidelines for San Joaquin Valley for Asian Citrus Psyllid/Huanglongbing Management and post-harvest treatment updates for bean thrips. Citrus PMGs can be accessed at <https://ipm.ucanr.edu/agriculture/citrus/>

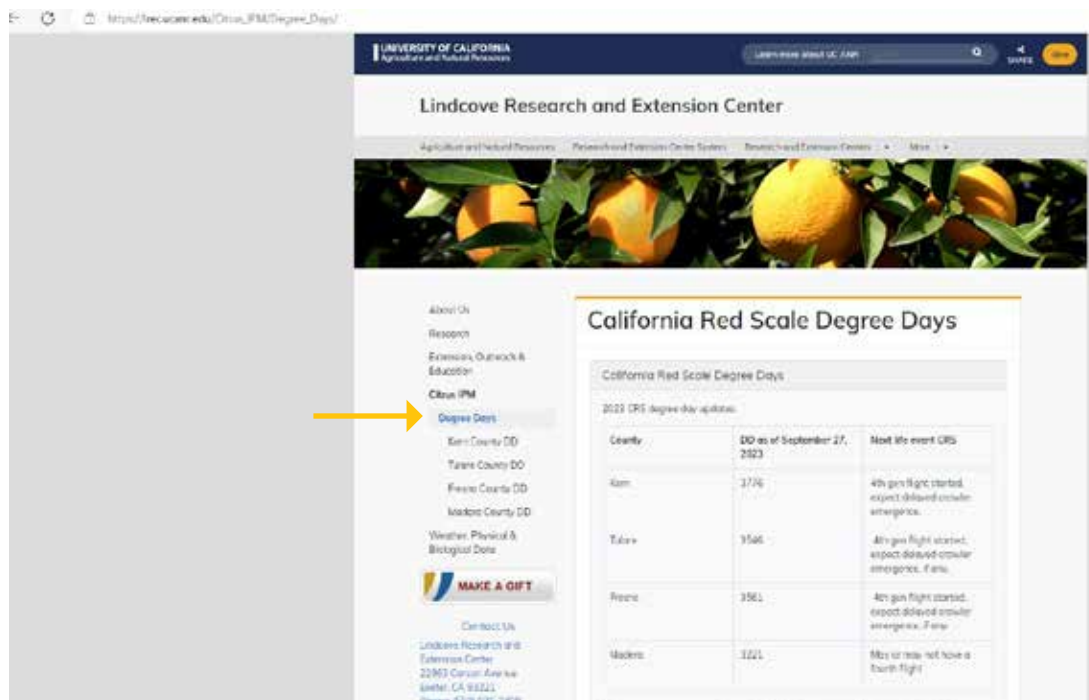
#### Journal Publications and Newsletter Articles

Core IPM pesticide trial results from field, greenhouse and lab studies are published as journal articles in *Arthropod Management Tests* and can be accessed for free online at <https://academic.oup.com/amt>. Citrus IPM research results also are published as newsletter articles in industry magazines like *Citrograph*, *Progressive Crop Consultant*, *The Adviser*, *Topics in Subtropics* newsletters and on blog posts at Citrus IPM News (<https://ucanr.edu/blogs/IPMBlog/>) and *Topics in Subtropics* (<https://ucanr.edu/blogs/Topics/>).

The project's educational materials are and will continue to be available in various formats, as journal articles, newsletter articles, fact sheets, briefs, pest memos and recordings.



A



B

| DD As of | 2021 | 2023 | 30 year avg |
|----------|------|------|-------------|
| 15-Mar   | 0    |      | 81          |
| 22-Mar   | 46   |      | 134         |
| 29-Mar   | 104  |      | 182         |
| 5-Apr    | 161  |      | 238         |
| 12-Apr   | 224  | 0    | 298         |
| 19-Apr   | 295  | 72   | 365         |
| 26-Apr   | 382  | 144  | 439         |
| 3-May    | 478  | 257  | 532         |
| 10-May   | 584  | 326  | 629         |
| 17-May   | 699  | 425  | 738         |
| 24-May   | 823  | 541  | 853         |
| 31-May   | 957  | 664  | 978         |
| 7-Jun    | 1094 | 798  | 1120        |
| 14-Jun   | 1248 | 936  | 1269        |
| 21-Jun   | 1413 | 1089 | 1430        |
| 28-Jun   | 1589 | 1254 | 1603        |
| 5-Jul    | 1774 | 1430 | 1792        |
| 12-Jul   | 1966 | 1616 | 1967        |
| 19-Jul   | 2156 | 1808 | 2186        |
| 26-Jul   | 2350 | 1997 | 2388        |
| 2-Aug    | 2541 | 2191 | 2591        |
| 9-Aug    | 2734 | 2382 | 2782        |
| 16-Aug   | 2912 | 2575 | 2977        |
| 23-Aug   | 3092 | 2754 | 3157        |
| 30-Aug   | 3267 | 2933 | 3339        |
| 6-Sep    | 3421 | 3108 | 3515        |
| 13-Sep   | 3562 | 3262 | 3675        |
| 20-Sep   | 3705 | 3403 | 3820        |
| 27-Sep   | 3832 | 3546 | 3963        |
| 4-Oct    | 3929 |      | 4089        |
| 11-Oct   | 4029 |      | 4191        |
| 18-Oct   | 4098 |      | 4287        |
| 25-Oct   | 4154 |      | 4369        |
| 1-Nov    | 4203 |      | 4431        |
| 8-Nov    | 4236 |      | 4481        |
| 15-Nov   | 4263 |      | 4519        |
| 22-Nov   | 4280 |      | 4551        |

Degree days as of September 27<sup>th</sup>, 2023. Tulare County: 3546. Fourth generation flight has started. It is likely that there will not be enough degree days for 4<sup>th</sup> generation crawler emergence. Expect delayed crawler emergence, if any.

**Biofix:** 1<sup>st</sup> male flight: April 11 (nearly 4 weeks later than previous years)

**Lower developmental threshold = 53°F**

**Degree days from first male flight to crawler emergence: 550 DD**

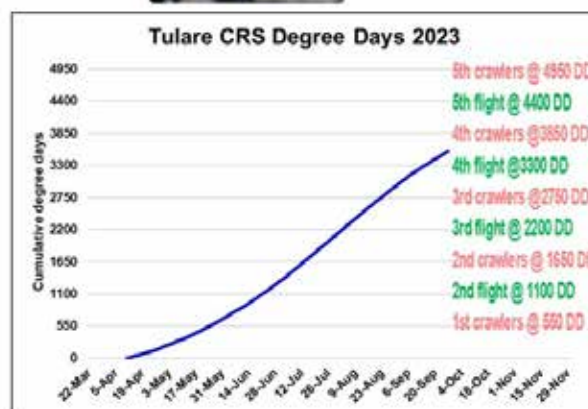
**2<sup>nd</sup> male flight: 1100 DD**

**2<sup>nd</sup> generation crawlers: 1650 DD**

Individual field conditions may vary. Monitor individual fields. For treatment recommendations [UCIPM Guidelines for citrus](https://ucanr.edu/citrus_ipm)



The crawler stage can be monitored using double sticky tape wrapped around a branch with females on it. Initiate after male flight and examine the tapes using a hand lens/microscope.



**Figure 4. (A) California red scale degree day (DD) updates, screenshot of the webpage, DD at the arrowhead. [irec.ucanr.edu/citrus\\_ipm/Degree\\_Days/](https://irec.ucanr.edu/citrus_ipm/Degree_Days/) (B) PDF file summarizing degree day update for Tulare County for the week of September 27, 2023.**

All materials are written targeting both professional and community audiences. We are working on creating an archive of recent publications that are accessible on the LREC website.

**YouTube Recordings: UC Ag Expert Talks, CRB Webinar Series, Science for Citrus Health Webinars**

Recordings of Citrus IPM presentations on YouTube are another useful resource for pest management. Our group has contributed to UC Ag Experts Talk, which are hour-long

webinars designed for growers and pest management professionals. Some citrus-related UC Ag Expert Talks include “Citrus Mealybugs in the San Joaquin Valley” by Sandipa Gautam (2021) and “Mites in California Citrus” by David Haviland. For a full list of UC Ag Experts Talk on citrus, visit <https://ucanr.edu/sites/ucexpertstalk/> and select “Citrus Pests” from “Past Webinars.”

Similarly, YouTube recordings are available for various topics covered during the CRB Grower Webinar Series, <https://citrusresearch.org/news-events/videos>, and by the Science for Citrus Health Group, <https://ucanr.edu/sites/scienceforcitrushealth/>.

To receive news and updates on Citrus IPM research, outreach events and resources, please email [sangautam@ucanr.edu](mailto:sangautam@ucanr.edu). You also can keep up with outreach events and stories by visiting [https://lrec.ucanr.edu/Citrus\\_IPM/](https://lrec.ucanr.edu/Citrus_IPM/). We will continue to develop outreach programs to offer the most valuable information regarding pest management needs. Announcements for every event and update will be shared via mass email, on the LREC website, via the monthly CRB newsletter and on social media. 🌱

**CRB Research Project #5500-501**

## Glossary

**<sup>1</sup>Phenology:** The study of the field ecology of insects in relation to biotic and abiotic factors.

**<sup>2</sup>Biofix:** An indicator of a developmental event that initiates the beginning of degree day calculations. For example, the California red scale biofix is the first male flight in the season.

## References

Grafton-Cardwell, E.E.; Morse, J.E.; Haviland, D.R; et al. 2022. *UC IPM Pest Management Guidelines Citrus*. UC ANR Publication 3441. Oakland, California.

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# THE PHYTOSANITARY EFFICIENCY OF CALIFORNIA'S POST-HARVEST “SYSTEM”

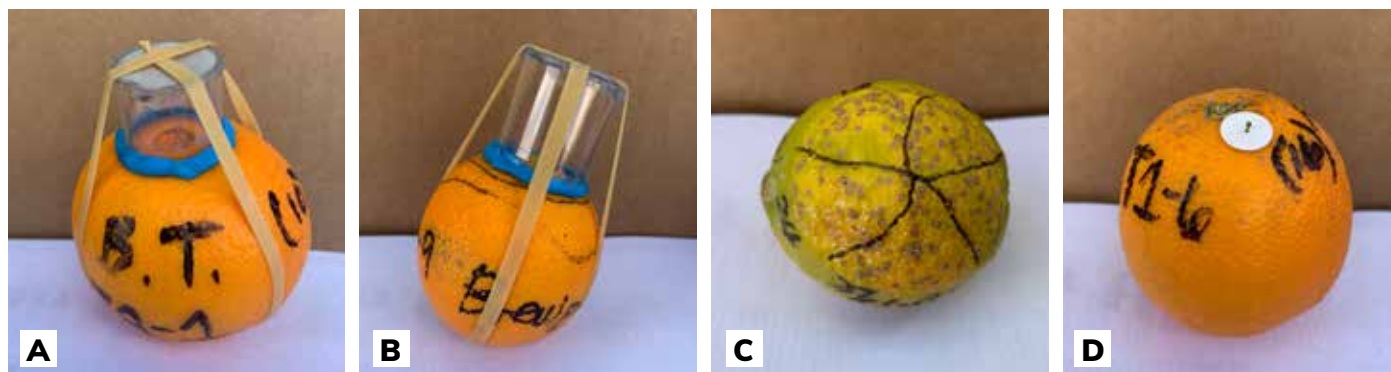


Sandipa Gautam, Jim Adaskaveg and Spencer Walse

## Project Summary

*The overall goal of this project is to develop treatments and strategies to control insect and microbiological pests so that pest-free and high-quality California fresh citrus enters key export marketing channels. Three key export markets for California fresh citrus recently have been critically impacted by pest-related trade barriers: Korea, China and Australia/New Zealand. To identify critical events in the citrus production and marketing chain and, importantly, the efficacy of each event toward the suite of pests important to the respective markets, researchers tracked the control of key insects (flat mites, thrips and scale) and decays (green mold, sour rot, brown rot and Septoria spot) on oranges and lemons subject to standard packing operations with fungicide treatment, followed by a 21-day cold storage to simulate oceanic transit to export markets. These treatments suppressed decay throughout the cold storage as expected and yielded complete control of the insect pests. Research also quantified ethyl formate fumigation of bulk citrus prior to packing, as well as a 12-hour phosphine fumigation of packed, palletized citrus prior to shipping.*





**Figure 1. Specimens of: bean thrips (A), *Brevipalpus californicus* flat mites (B), California red scale (C) and Fuller rose beetle eggs (D) were contained on fruit during Events (treatments) 1 (ethyl formate), 3 (phosphine) and 4 (cold shipping). The restraints were removed for Event 2 in which the effect of the pack line on insect removal and mortality was evaluated.**

## Introduction

The last 70 years saw an exponential increase in the trade of horticultural goods to keep pace with a growing population. Maintaining this critical course in light of reduced or negligible access to key pesticides is a grand challenge that is particularly daunting for quarantine scenarios where pest-free security must be “guaranteed” to the satisfaction of the importer (International Standards for Phytosanitary Measure [ISPM] 28 - Phytosanitary treatments for regulated pests). Accordingly, the tools needed to ensure the safety and security of horticultural goods must evolve. Only through understanding how sequential practices, procedures and processes collectively serve to eliminate pests, can industry maintain the marketing of quality citrus fruit.

Retained export market access for fresh California citrus to Korea, New Zealand, Australia and China are all impacted by pest-related trade barriers. Korea has indicated that methyl bromide fumigation of imported lemons and oranges no longer may be conducted upon arrival at port facilities for control of Fuller rose beetle (FRB) and California red scale (CRS). Moreover, Korea has been concerned with *Septoria citri* for years. New Zealand and Australia have highlighted Asian citrus psyllid (ACP), bean thrips (BT) and three flat mite species endemic to California on the Biosecurity New Zealand Pest Risk Assessment (PRA): *Tarsonemus bakeri* (TBM), *Brevipalpus californicus* (BCM) and *Brevipalpus lewisi* (BLM). Lastly, China ceases imports of navel oranges due to interceptions of brown rot caused by *Phytophthora* species. Suspension is based on *P. syringae* (PS); however, other *Phytophthora* species such as *P. hibernalis* also are on the Chinese quarantine list.

The goal of a phytosanitary “systems approach” is to harness the pest control afforded by specified treatments during production, packing, transportation and marketing. The citrus industry can draw on two successful examples of “systems approach” from the recent past where sequential treatments were combined into a quantitative metric of pest control. In approximately 2010, Walse evaluated Asian citrus psyllid (ACP) removal during “standard” packing line events (Walse et al. 2014) to defuse concerns from Australia

and New Zealand about the security of California exports. Packing line processes effectively removed ACP, a finding that ensured continued export from packinghouses. In approximately 2013, China threatened California imports due to the potential incidence of brown rot. Walse teamed with Adaskaveg to quantify the cumulative effect of a pre-harvest cultural practice (skirting), a pre-harvest copper spray and a post-harvest phosphite treatment (Adaskaveg et al. 2013). The “systemic” joint probabilities of these management treatments demonstrated control/management efficacies of greater than 99 percent, which was a risk reduction sufficient for the continuation of trade.

For the last ten years, the Citrus Research Board (CRB) has partnered with the California Citrus Quality Council (CCQC), the United States Department of Agriculture-Agricultural Research Service-San Joaquin Valley Agricultural Science Center (USDA-ARS-SJVASC) and the University of California (Riverside, Davis and Agriculture and Natural Resources) to target federal funding under the USDA-Foreign Agricultural Service (FAS)-Technical Assistance for Specialty Crops (TASC) program. TASC funding totaling more than \$5 million was used to:

1. Evaluate the removal of ACP and other insects during “standard” pack line treatments (Walse et al. 2014);
2. quantify the insecticidal efficacy of post-harvest fumigation with ethyl formate (Bikoba et al. 2019; Gautam et al. 2019; Gautam et al. 2023; Pupin et al. 2013), phosphine (Walse 2017; Walse 2018) and propylene oxide (Gautam et al. 2021); and
3. most recently, couple all this research together to quantify the collective impact of these treatments when conducted in succession, as part of the “system.”

## Present Work

In the present work, four post-harvest practices were tested for efficacy toward BCM, BT, CRS and FRB eggs (Figure 1). The first two years of the study examined each practice



**Figure 2. Events (treatments) of the systems approach: Treatment 1 – (A) ethyl formate fumigation using eFUME™ applied to bulk citrus in bins under tarpaulin; Treatment 2 – (B) pack line processes including brushing, pressure washing, waxing and drying; Treatment 3 – (C) phosphine fumigation at 41°F lasting 12 hours per the Australia export protocol; and Treatment 4 – (D) cold storage at 41°F lasting 21 days to simulate oceanic shipping.**

individually, while the last three years evaluated the post-harvest practices in succession. Post-harvest applications offer several key advantages when incorporated with production treatments. Importantly, post-harvest treatments are extremely reproducible and, accordingly, the variance associated with their efficacy differed minimally across treated locations of an individual application, as well as from one application to the next. Numerous studies reinforce the prominent role of post-harvest chemical treatments and, notably, fumigation in quarantine entomology where the demonstration of reproducible efficacy is of paramount importance. Moreover, post-harvest treatments typically allow for greater synchronization of the treatment with the logistical, infrastructural and regulatory constraints of marketing.

The first experimental treatment (Event 1) evaluated was an ethyl formate fumigation of bulk citrus in bins with 250 grams per cubic meter (g/m<sup>3</sup>) eFUME™ applied at ambient field temperature, which has been proposed to the California Department of Food and Agriculture as an alternative to field packing and “spray and move” for ACP control (**Figure 2**).

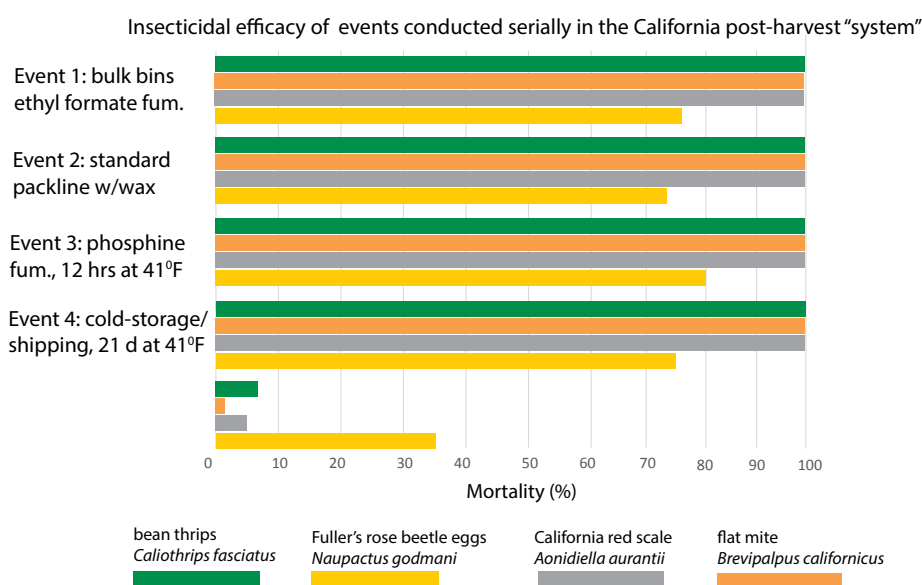
Treatment 2 (Event 2) was a standard packing procedure conducted at Lindcove, which consisted of a dry dump, brushing with pressure washing, sorting, a wax application and drying (no fungicide was applied), followed by packing palletizing and storing the fruit at 41°F.

Treatment 3 (Event 3) was a 12-hour phosphine fumigation per the protocol for export to Australia (BICON 2023).

Treatment 4 (Event 4) was a 21-day cold storage, which simulated oceanic shipping transport to key export markets, including Australia, China, Japan and Korea.

The total numbers of insect specimens tested across all trials involving two varieties each of oranges and lemons were 2,800 BT, 9,202 BCM, 10,241 CRS and 5,537 FRB eggs. Approximately 10 percent of these totals were used as non-treated controls. Importantly, suppression of decay (green mold, sour rot, brown rot and Septoria spot) was evaluated during standard packing in treatment 2, as well as in treatments 1, 3 and 4 to determine if additional benefits in decay management can be obtained.

When treatments 2 (packline) and 4 (cold shipping) were conducted sequentially, the only insect specimens that survived were approximately 25 percent of the FRB eggs (**Figure 3**). Fortunately, the skirt pruning and spraying conducted under the Korea shipping protocol (CCQC 2023) have been decimating this pest in the field. The effect of treatment 2 (packing line) alone can be gleaned by the



**Figure 3. Percent mortality of specimens (mean +/- standard deviation) exposed to different events (treatments) across three replicated trials for each of two lemon varieties, Lisbon and Eureka.**



percentage of specimens washed away during by the pressure wash: 50 to 90 percent for bean thrips, 16 to 54 percent for BCM, 40 to 98 percent for CRS and 0 to 32 percent for FRB eggs. These results indicate that the incorporation of treatment 1 (ethyl formate) before packing or alternatively treatment 3 (phosphine) after packing provide an added level of security to further satisfy the phytosanitary standards of the key export markets. It follows that treatments 1 and 3 need not be conducted on the same fruit.

Management of post-harvest decay was achieved with the fungicide pre-application prior to the four treatment events. Importantly, as expected, neither ethyl formate nor phosphine consistently decreased incidence of the four decays evaluated. In general, treatments 1, 2, 3, and 4 did not affect the performance of pre-applications with the post-harvest fungicides fludioxonil/azoxystrobin (Graduate A+®), propiconazole (Mentor®) or phosphite (ProPhyt®) for green mold and Septoria spot, sour rot and brown rot, respectively. Data were analyzed as split-plot trials.

**Table 1** shows the main plot effects as pairwise comparisons for fungicide pre-treatments as compared to no pre-treatment for each decay. Clearly, the fungicide pre-treatments had the greatest effect on decay management and were not consistently affected by the fumigation and handling procedures of the four treatment events. Some sub-plot treatment sequences separate statistically in the columns; however, they do not differ from Sequence IV, which did not receive any fumigation. Thus, fruit handling procedures in Sequence IV alone resulted in a similar reduction of green mold and brown rot decay as in Sequence II for both fungicide pre-treated and non-pre-treated fruit. This possibly is due to desiccation or temperature effects during fruit handling between treatment events that required several hours to 24 hours for processing each of the treatments prior to cold storage (the fourth treatment event).

Historically, fumigation treatments with methyl bromide never have provided decay control and were used for insect

pest control. Similar to methyl bromide, ethyl formate and phosphine are for insect management. Fumigation with any of the fumigants for decay control would have to be at much higher rates that would cause phytotoxicity. Still, these new fumigants provided the much-needed alternatives to methyl bromide for insect pest management to meet national and international quarantine requirements while providing the highest quality fruit in these markets.

## Conclusion

The overall goal of this project is the long-term export retention of California-grown fresh citrus to foreign markets. These research efforts quantitatively demonstrate that a “system” comprising standard packing operations, followed by cold storage during oceanic transit significantly reduces the probability that decay and viable insects make it to the export destinations. For the first time, industry will be able to address claims of pest interceptions by providing estimates of control and risk reduction associated with well-executed post-harvest procedures, including the added security afforded by an ethyl formate fumigation of bulk citrus prior to packing, as well as a “clean-up” phosphine fumigation of packed, palletized citrus. Although it is difficult to project the impact of this work, the team believes that if these post-harvest procedures are adequately documented, trade partners will recognize and appreciate the phytosanitary “system” used to maintain the global prominence of California citrus.

This research provides growers and packers with greater flexibility in marketing export fruit, and these results are an important first step in harmonizing all export treatments. The team has begun the process of transferring results to the USDA-Animal and Plant Health Inspection Service trade directors for presentation to counterparts in Australia, New Zealand and Korea in an effort to gain phytosanitary acceptance of a standard marketing “system.” Hopefully, a success story can be shared with the industry soon. 🍊

**Table 1. Studies on the efficacy of post-harvest fumigation treatments against decays of citrus fruit pre-treated or not treated with post-harvest fungicides.**

| No.               | Treatments/Events                  |                            |                                |                        | Green Mold<br>Valencia orange |          | Sour rot<br>Limoneira lemon |          | Septoria spot<br>Navel orange |          | Brown rot<br>Limoneira lemon |          |
|-------------------|------------------------------------|----------------------------|--------------------------------|------------------------|-------------------------------|----------|-----------------------------|----------|-------------------------------|----------|------------------------------|----------|
|                   | 1st<br>Ethyl formate<br>fumigation | 2nd<br>Standard<br>packing | 3rd<br>Phosphine<br>fumigation | 4th<br>Cold<br>storage | Pre-treatment                 |          | Pre-treatment               |          | Pre-treatment                 |          | Pre-treatment                |          |
|                   |                                    |                            |                                |                        | No                            |          | No                          |          | No                            |          | No                           |          |
|                   |                                    |                            |                                |                        | Inc. (%)                      | Inc. (%) | Inc. (%)                    | Inc. (%) | Inc. (%)                      | Inc. (%) | Inc. (%)                     | Inc. (%) |
| V.                | -                                  | -                          | -                              | -                      | 45.8 b                        | 4.2 bc   | 93.2 ab                     | 51.4 a   | 87.5 a                        | 13.3 c   | 92.0 a                       | 2.1 a    |
| I.                | +                                  | +                          | +                              | +                      | 79.9 a                        | 11.9 a   | 98.9 a                      | 27.6 b   | 87.5 a                        | 23.6 b   | 55.9 c                       | 5.1 a    |
| II.               | -                                  | +                          | +                              | +                      | 14.8 c                        | 2.1 c    | 90.0 b                      | 23.4 b   | 86.8 a                        | 31.4 a   | 41.7 d                       | 5.3 a    |
| III.              | +                                  | +                          | -                              | +                      | 84.7 a                        | 7.5 ab   | 94.0 ab                     | 21.0 b   | 87.3 a                        | 35.8 a   | 63.0 b                       | 2.7 a    |
| IV.               | -                                  | +                          | -                              | +                      | 20.5 c                        | 2.8 bc   | 89.0 b                      | 18.3 b   | 82.8 a                        | 28.9 ab  | 51.5 cd                      | 6.2 a    |
| Main plot effects |                                    |                            |                                |                        | 49.1 A                        | 4.2 B    | 93.0 A                      | 28.3 B   | 86.4 A                        | 26.6 B   | 60.8 A                       | 4.3 B    |

Fruit were wound-inoculated with *Penicillium digitatum* (1,000,000 spores/ml), *Geotrichum citri-aurantii* (500,000 spores/ml in lemon juice/10 ppm cycloheximide), or *Septoria citri* (5,000,000 spores/ml), or were non-wound inoculated with *Phytophthora citrophthora* (25,000 zoospores/ml). Fruit were dip-treated with fungicides after about 15 hours for the first three decays and after 24 hours for brown rot. In each trial, the ethyl formate fumigation was done for three hours approximately two hours after fungicide treatment, and the phosphine fumigation was done the following day. After treatments, fruit were stored at 4.4C for 21 days and then incubated at KARE at 20C. Control (No. 1) fruit were stored immediately at 20C. Numbers are the average of three experiments for green mold, sour rot and brown rot. For Septoria spot, two studies were done. Analysis of variance and LSD mean separation was done using SAS (ver. 9.4). Treatment effects are by column and main plot effects are pairwise by row.

## Acknowledgements

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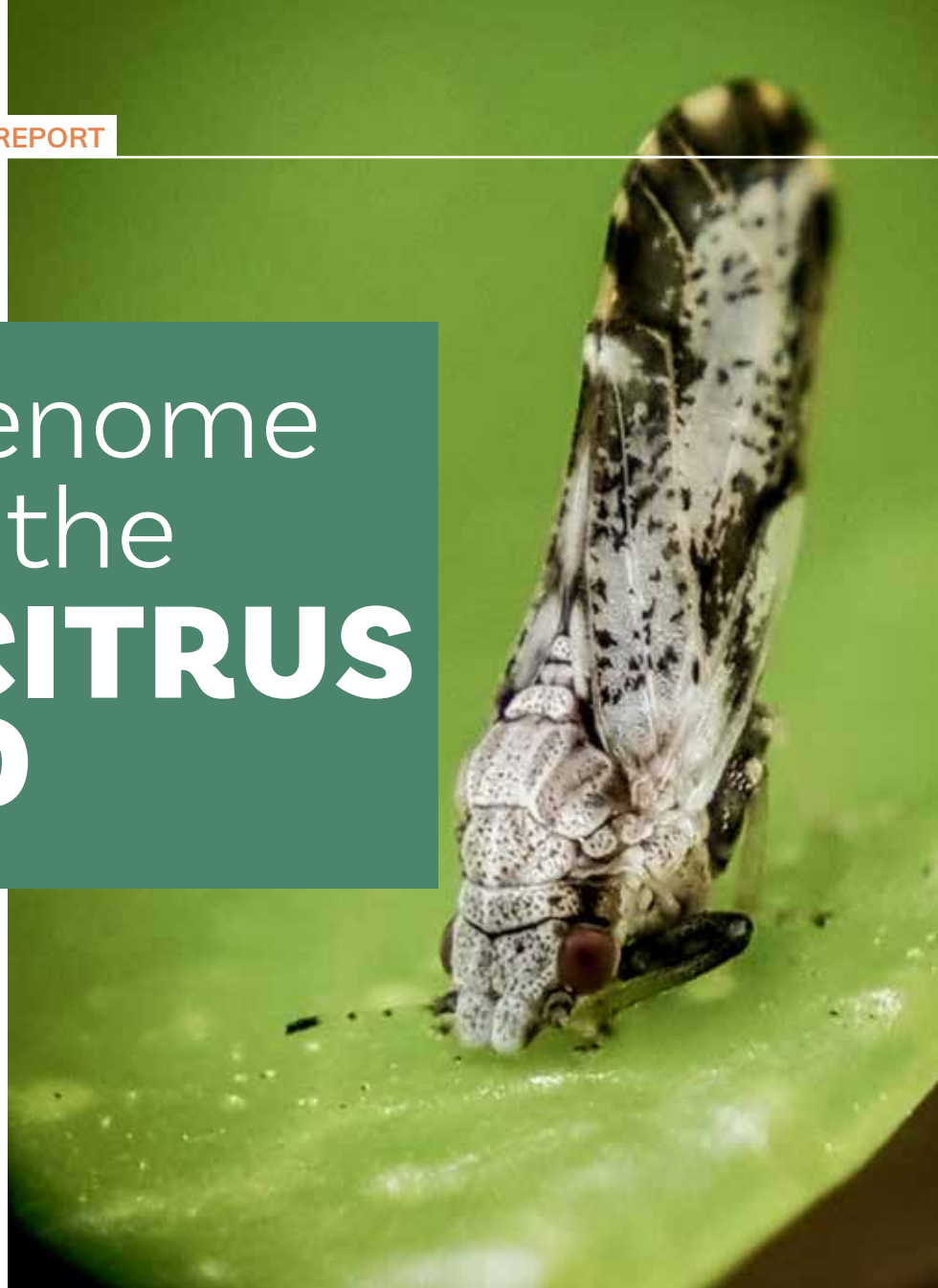


# Precise Genome Editing of the **ASIAN CITRUS PSYLLID**

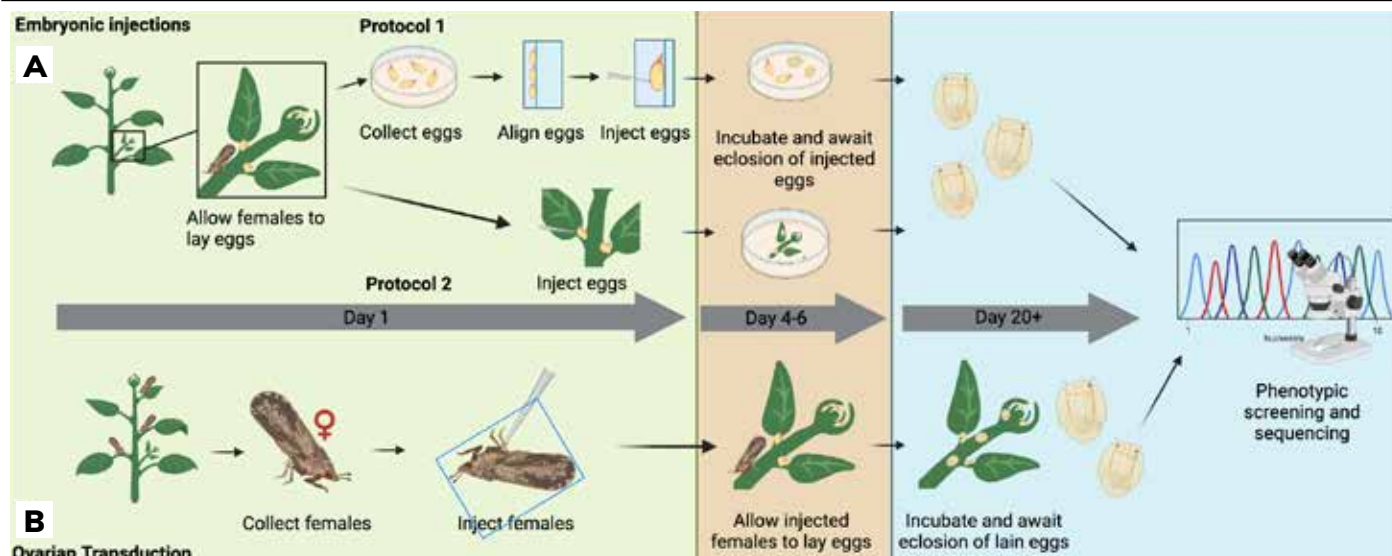
Michelle Bui, Robyn Raban and  
Omar S. Akbari

## Project Summary

'*Candidatus Liberibacter asiaticus*' (CLas), the presumptive causal agent of huanglongbing (HLB), through its insect vector<sup>1</sup>, the Asian citrus psyllid (ACP), has negatively impacted the citrus industry. Chemical insecticides, biocontrol agent releases, the removal of infected trees and establishment of quarantine zones with HLB-affected trees are the current control methods for this disease in California. Although these methods have thus far prevented the spread of HLB into commercial citrus in California, it remains uncertain how long they may be effective. Other insect pests and pathogen vectors, such as mosquitoes and fruit flies, have had their available control tools expanded through the development of gene-based vector control tools. These include a wide range of tools from the use of sterile insects to the development of population suppression or modification-based gene drives<sup>2</sup>. The goal of our work is to lay the foundation for building such tools for effective ACP control. Until now, genetic engineering has remained elusive and difficult in ACP. We are the first to demonstrate CRISPR/Cas9<sup>3</sup> mutagenesis of the ACP genome through embryo microinjections and have developed ACP-specific genome editing methods that can support the expansion of gene-based technologies in ACP. This advancement could help stimulate the future development of gene-based methods to control the spread of ACP and increased incidence of HLB.







**Figure 1. Methods developed for delivering Cas9 protein and guide (gRNAs) to Asian citrus psyllid eggs.** For embryonic injections, freshly laid eggs were collected by either complete removal from curry leaf flush or collected still attached to the plant. Eggs then were injected and allowed to grow and emerge to the nymphal stage (known as eclosion). The resulting individuals were observed for potential phenotypes and collected for genome sequencing. Adult female injection methods were performed by injecting Cas9 protein and gRNAs directly into the female abdomen near the ovaries. These females then were mated to males and allowed to lay their eggs on curry leaf flush. The resulting offspring were observed, and their genomes were sequenced for potential mutations. Credit: Chaverra-Rodriguez, D.; Bui, M.; Gilleland, C.L.; et al. 2023. CRISPR-Cas9-mediated mutagenesis of the Asian citrus psyllid, *Diaphorina citri*. *GEN Biotechnology*. 3(4):317-329, by permission of Mary Ann Liebert, Inc. Publishers. Created with BioRender.com.

Huanglongbing is a major disease with a tremendous cost to the citrus industry. HLB is spread through the transmission of the bacterium CLas through ACP. Current methods for HLB management in California, including chemical insecticides, biocontrol agent releases, removal of infected plants and establishment of quarantine zones, have been moderately successful. However, these tactics are insufficient at preventing the increased incidence of the disease, while also being costly to growers. Thus, the aim of this project was to develop additional genetic methods and tools for ACP control.

In recent years, there have been significant advancements in gene-based control tools in many insect species, such as mosquitoes and fruit flies. Gene drives, for example, which can rapidly spread desired genes throughout wild insect populations at higher than normal rates of inheritance, have been at the forefront of current research due to their potential to rapidly control vectored diseases (Wang et al. 2021) and pest species (Legros et al. 2021). Other genetic tools also are being developed to produce sterile males, such as precision guided sterile insect technique (pgSIT). The pgSIT and other genetic-modification technologies for sterility can be released in large numbers in the field to reduce pest populations (Kandul et al. 2019; Li et al. 2021). To produce similar genetic modification-based control tools in ACP, robust genetic modification methods are required. However, stable germline (heritable) genetic modification of the ACP has remained elusive.

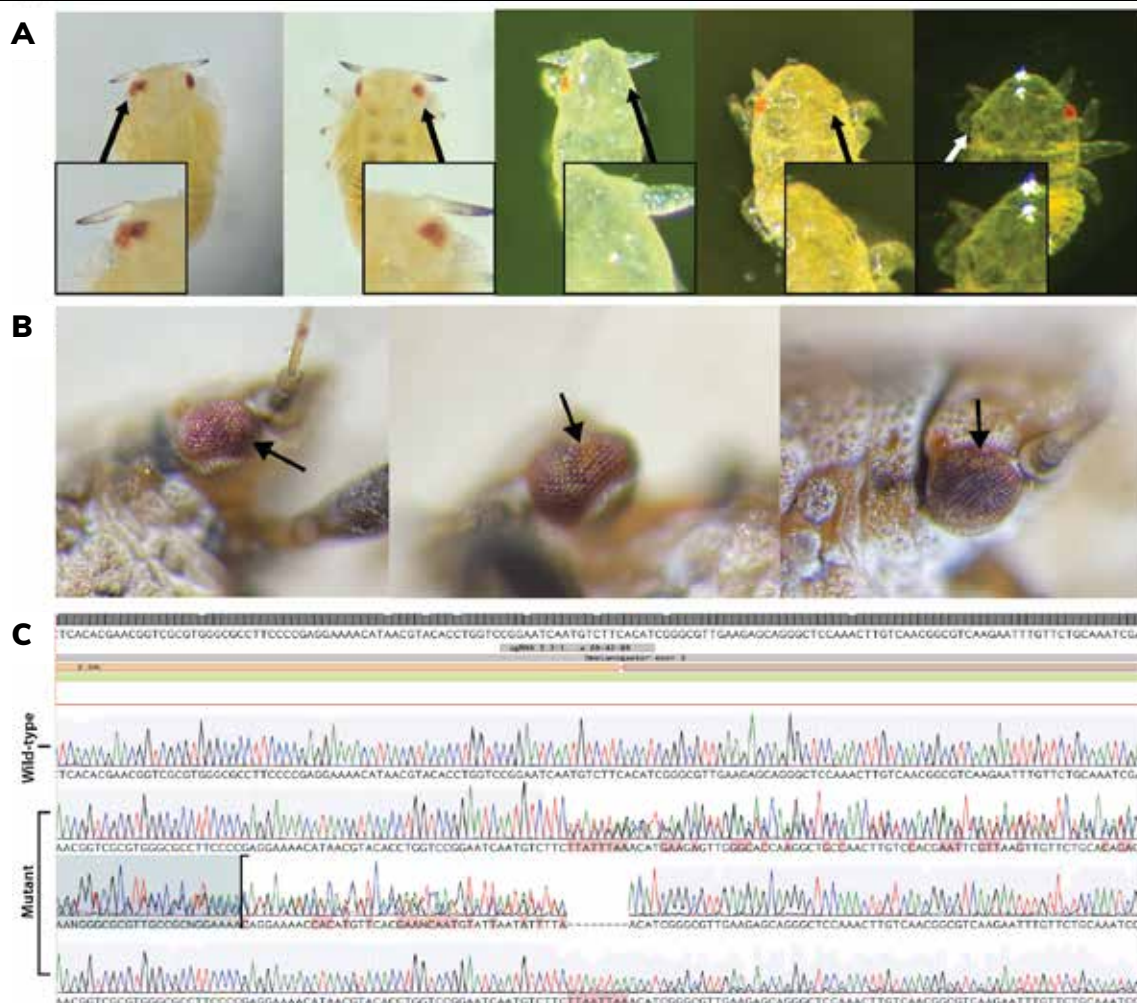
Below, we describe the first ACP genomic modification methods. These methods set the groundwork for further development of gene-based control tools in ACP.

## Embryonic Injections

Precise genetic modification in insects is commonly achieved by injection of molecular gene editing components, such as CRISPR/Cas9 and guide RNAs (gRNAs), directly into freshly laid insect eggs. Cas9 protein and gRNAs are key components of the CRISPR/Cas9 gene editing system. Cas9 protein functions as “molecular scissors” to cut DNA at a target location in the genome. To inform Cas9 protein where to cut, specific target gRNAs, designed to match the target DNA sequence, are injected along with Cas9 protein.

Our first goal was to develop a method for embryonic injections of CRISPR/Cas9 to mutate the genes that can give rise to an easily visible phenotype, including *white(w)* and *kynurenine monooxygenase(kh)*, that affect insect eye color in many insects. Normal insect eyes are black or dark red, depending on the species; but when these genes are mutated, the pigmentation is lost, resulting in eye shades lighter than normal or even completely white. Selecting these as target genes, therefore, can serve as an easy marker for successful mutation of the genome.

To perform embryonic injections, ACP eggs were collected within 30 minutes to three hours after they were laid.



**Figure 2. Non-heritable mutagenesis of the Asian citrus psyllid (ACP). A) ACP nymphs with non-heritable mosaic mutations were commonly identified by loss of pigmentation in one eye. These mutants were generated through both embryo and adult female injections. B) ACP adults also demonstrated mosaic mutant phenotypes. Shown are some eye cells that show reduced pigment in some regions adjacent to fully pigmented regions. C) Genomic sequencing data confirmed mutations within individuals with mutant phenotypes.**

Because ACP lay their eggs attached to the flush of their host plant, we cut the flush from the plant and, to minimize egg handling, separated the egg-containing fragments (**Figure 1**). After collection and sterilization, we used a customized automated embryonic microinjection system to simultaneously deliver Cas9 protein and gRNAs into the eggs.

In our experiments, we have designed our gRNAs to target either the *w* or *kh* gene. Eggs injected with Cas9 protein and gRNAs, along with fragments of plant tissue the eggs were laid on, were kept on 5.6 percent gelatin plates containing sugar and nutritional yeast until they hatched into nymphs. Based on our experiments, we generated ACP with some of their cells mutated, but these mutations were only in somatic cells (nonreproductive cells) and could not be transmitted to the next generation (**Figure 2**). However, this is a very important step in the development of genetic technologies and the first example of mutagenesis in the ACP via embryonic injections. These exciting results merit future research to continue to develop methods so that genome edits are heritable.

## Adult Female Injections

In addition to direct embryo injections, we also injected Cas9 protein and gRNAs into adult female abdomens, whereby the Cas9 protein and gRNAs could be absorbed into the ovaries and developing oocytes<sup>4</sup>. We evaluated three methods of Cas9 protein and gRNA delivery into oocytes without the need for direct injections, including Receptor-Mediated Ovary Transduction of Cargo (ReMOT Control), which consists of a modified Cas9-protein with a bound peptide, Branched Amphiphilic Peptide Capsules (BAPC) and Direct Paternal (DIPA)-CRISPR that use large concentrations of commercially available Cas9 protein to encourage oocyte uptake (Chaverra-Rodriguez et al. 2018; Huang et al. 2021; Hunter et al. 2021; Shirai et al. 2022). Adult female injections are easier to perform than embryo injections since adult females are significantly larger and less delicate than embryos.

In addition, the equipment required for adult injections is more affordable and accessible than what is needed for embryo injections. Using this injection method along with



the Cas9 delivery methods outlined above, we were able to partially change eye color in some of the psyllid offspring (Figure 2).

## ACP Transgenesis

To develop tools for pest control, foreign DNA needs to be inserted into the ACP genome<sup>5</sup>. This foreign DNA could encode genes that make ACP incapable of transmitting CLAs or encode Cas9 protein and gRNAs that can kill ACP by mutating genes required for their survival or fertility. We used CRISPR/Cas9 and foreign DNA injections to insert a foreign genetic sequence for a red fluorescent protein (RFP) and the components needed to express this protein. ACP do not naturally express RFP; but when properly mutated to contain our foreign RFP sequence in their genome and excited at the correct wavelength, they will glow red and allow us to identify genetically modified individuals.

## Conclusions

Gene modification-based technologies could revolutionize the control of ACP. Basic genetic tools are limited in the ACP; however, we have developed multiple methods that improve genome engineering capabilities in the ACP. More notably, we were the first to generate visible, non-heritable mutations in the ACP using embryonic and adult female injections of CRISPR/Cas9 (Chaverra-Rodriguez et al. 2023). These accomplishments demonstrate that we are close to developing the necessary tools for genetic control technologies. Our next steps in this work are to confirm the ability to generate heritable mutations in the ACP genome as well as the integration of foreign DNA. The same methods used to perform these tasks can then be used to generate a functional genetic-based control tool for ACP such as gene drives and pgSIT. 🌱

### CRB Research Project #5500-217

## Glossary

<sup>1</sup>**Vector:** A living organism that can harbor and transmit infectious agents from one infected organism to another.

<sup>2</sup>**Gene Drive:** A system that biases the frequency of a gene above rates derived from normal inheritance. Gene drives may be used, for example, to spread genes detrimental to survival, reproduction or disease agent transmission into insect populations.

<sup>3</sup>**CRISPR/Cas9:** Clustered Regularly Interspaced Short Palindromic Repeats is a genomic editing system with the capability of making precise genomic modifications by removing, adding or altering sections of DNA.

<sup>4</sup>**Oocyte:** Egg cell.

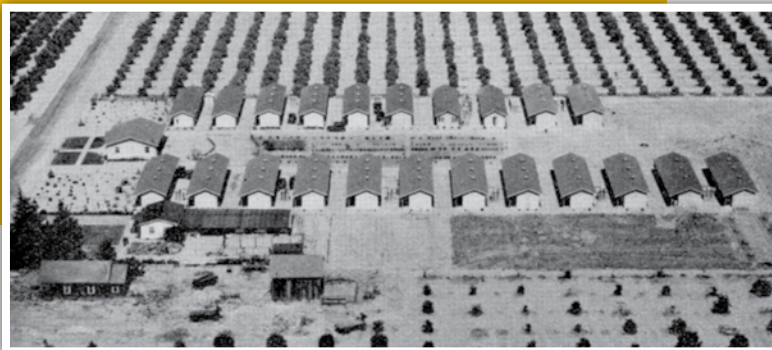
<sup>5</sup>**Genome:** A cell or organism's complete set of genes.

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# EARLY INTEGRATED PEST MANAGEMENT IN CALIFORNIA'S CITRUS INDUSTRY



Citrus growers in the Golden State have a long history of using integrated pest management to control insects. As early as the 1880s, they released ladybugs into their groves to curtail the spread of cottony-cushion scales. While fumigation became increasingly common in orange and lemon groves in subsequent decades, scientists continued to advocate for biological solutions to insect infestations.

One excellent early study supporting this approach appeared in 1931. Written by Harry S. Smith and H.M. Armitage of the Citrus Experiment Station operated by the University of California at Riverside, "The Biological Control of Mealybugs Attacking Citrus" was published by the University of California College of Agriculture. This bulletin provided an early form of integrated pest management. The piece focused on the common and citrophilus mealybugs, *Pseudococcus citri* and *Pseudococcus gahani*, respectively, which first infested groves in San Diego County in approximately 1904 and spread quickly across the citrus empire of southern California.

Rather than relying on fumigation to deal with the pests, Smith and Armitage suggested that growers enlist "insect enemies of mealybugs" to combat mealybugs. The authors noted that insectaries such as the one in Orange County (pictured here) had success controlling pests with black ladybirds, *Cryptolaemus monstousieri*, a natural predator of the mealybug. This form of what Smith and Armitage called "biological control" not only had proven effective in protecting oranges from damaging insects but presented a drastically cheaper solution than fumigation.

The roots of integrated pest management in the California citrus industry are deep.

**For more information, contact [bjenkins@laverne.edu](mailto:bjenkins@laverne.edu)**

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