CITRUS RESEARCH BOARD

Citrograph

MAGAZINE

FALL 2017

CALIFORNIA CITRUS CONFERENCE

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Special Post-Harvest Section Inside

see pg. 32-50

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On the Cover:

The 2017 California Citrus Conference will take place at the Wyndham Visalia on October 11 in Visalia, California. All industry members are invited to attend the free event, which will feature networking opportunities and speakers including California Secretary of Agriculture Karen Ross. See page 8 for additional information.

In This Issue

Fall 2017 | Volume 8 • Number 4 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.

SPECIAL POST-HARVEST SECTION

CORRECTION
In the summer issue, the legend in Figure 5 on page 69 appeared incorrectly. Please find the corrected figure below.

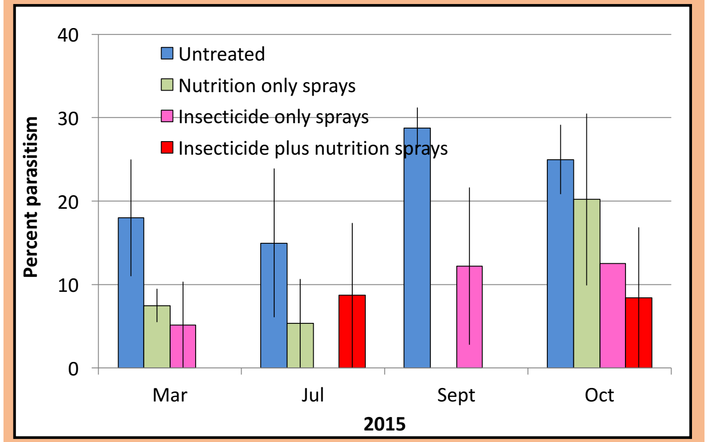

Figure 5. Mean (±SE) percent parasitism by Tamarixia radiata *in nutritional and insecticide spray programs employed to control Asian citrus psyllid and improve tree health. In October, SE is not presented for insecticides only spray program because nymphs were available only from one of the four replicates used to calculate SE.*

The Mission of the Citrus Research Board:

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

Citrus Research Board Member List By District 2016-2017

(Terms Expire September 30)

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Andrew Brown	2019	Vacant	2018
Larry Wilkinson	2019	Jim Gorden	2017
Dan Dreyer	2019	Greg Galloway	2017
Etienne Rabe	2018	Joe Stewart	2017
John Konda	2018	Franco Bernardi	2017
Keith Watkins	2018	Kevin Olsen	2017
Jeff Steen	2018		

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John Gless III	2017		

District 3 – California Desert

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Vacant	2018

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Calendar of Events 2017-18

2017

October 9
Train the Trainer: Best Practices for Citrus Field Crews to Prevent the Spread of ACP/HLB; Bakersfield, California. Pre-registration is required, as space is limited. Register at *www.citrusinsider.org* or by phone at (559) 592-3790.

October 10
Train the Trainer: Best Practices for Citrus Field Crews to Prevent the Spread of ACP/HLB; Parlier, California. Pre-registration is required, as space is limited. Register at *www.citrusinsider.org* or by phone at (559) 592-3790.

October 11
Train the Trainer: Best Practices for Citrus Field Crews to Prevent the Spread of ACP/HLB; Wyndham Hotel (concurrent with California Citrus Conference), Visalia, California. Pre-registration is required, as space is limited. Register at *www.citrusinsider.org* or by phone at (559) 592-3790. See page 10 for details.

October 11
California Citrus Conference; Wyndham Hotel, Visalia, California. For more information, contact the CRB at (559) 738-0246 or visit *www.citrusresearch.org*. See page 8 for details.

October 12
Train the Trainer: Best Practices for Citrus Field Crews to Prevent the Spread of ACP/HLB; Madera. Pre-registration is required, as space is limited. Register at *www.citrusinsider.org* or by phone at (559) 592-3790.

October 15-17
43rd Annual CAPCA Conference and Agri-Expo; Grand Sierra Resort, Reno, Nevada. For more information, contact Dee Strowbridge at (916) 928-1625 X 203 or *dee@capca.com* or visit *www.capca.com*

November 1-3
California Citrus Nursery Society (CCNS) Annual Conference; Embassy Suites, Temecula, California. For more information, contact CCNS at (925) 631-1626 or e-mail *tdelfino@earthlink.net*

2018

February 13-15
World Ag Expo; Tulare, California. For more information, visit *www.worldagexpo.com*

March 8
California Citrus Mutual (CCM) Citrus Showcase; Visalia Convention Center, Visalia, California. For more information, contact CCM at (559) 592-3790 or visit *www.cacitrusmutual.com*

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Citrus industry members listened attentively to speakers at the 2016 conference.

California Citrus Conference to Feature CDFA Secretary Ross

Conference to Present "The Best of the Best" in Citrus Research

Carolina Evangelo

The Citrus Research Board (CRB) is proud to announce that the 2017 California Citrus Conference will be held October 11 at the Wyndham Visalia in Visalia, California. The entire citrus industry is invited to participate in the free one-day conference, which will highlight "the best of the best" in citrus research with a strong emphasis on the work being done to combat Asian citrus psyllids (ACP) and huanglongbing (HLB). This will be the second year in a row for the conference, which returned in 2016 following a four-year hiatus. Last year's gathering drew nearly 400 attendees; and in addition to the speakers and posters, also offered a complimentary breakfast, luncheon and door prizes.

Karen Ross, California Secretary of Agriculture, will address growers at the conference on October 11.

David Roth, Bruce Wileman, Ken Wileman, Jim Phillips and Joel Nelsen were attendees at last year's VIP Reception.

CRB Vice Chairman Dan Dreyer, Chuck Hathaway of Liphatech, CRB Board Member Etienne Rabe, Ph.D., and Fred Gmitter, Ph.D., University of Florida, chatted at the 2016 conference VIP Reception.

The 2017 CCC will once again offer great door prizes. Last year, Justin Qualls won a Yeti ice chest.

This year's featured speakers (confirmed at press time) include:

- Karen Ross, California Secretary of Agriculture
- Georgios Vidalakis, Ph.D., University of California, Riverside
- Beth Grafton-Cardwell, Ph.D., University of California, Riverside
- Carla Thomas, University of California, Davis
- Mkeal Roose, Ph.D., University of California, Riverside
- David Morgan, Ph.D., California Department of Food and Agriculture
- Richard Stouthamer, Ph.D., University of California, Riverside
- Greg Simmons, Ph.D., U.S. Department of Agriculture-Animal and Plant Health Inspection Service
- Spencer Walse, Ph.D., U.S. Department of Agriculture-Agricultural Research Service
- Johan Leveau, Ph.D., University of California, Davis
- Joel Nelsen, California Citrus Mutual
- Nick Hill, Citrus Pest and Disease Prevention Program

Additionally, CRB researchers will display scientific posters, which will provide networking opportunities for growers, industry members and researchers.

"We are greatly looking forward to once again presenting this important and informative event to the growers of California," said CRB President Gary Schulz, "and we also are pleased that Secretary Ross will provide the keynote address to our industry."

Continuing education units have been applied for and will be available to conference attendees, pending approval by the California Department of Pesticide Regulation.

Conference Schedule
(Tentative)

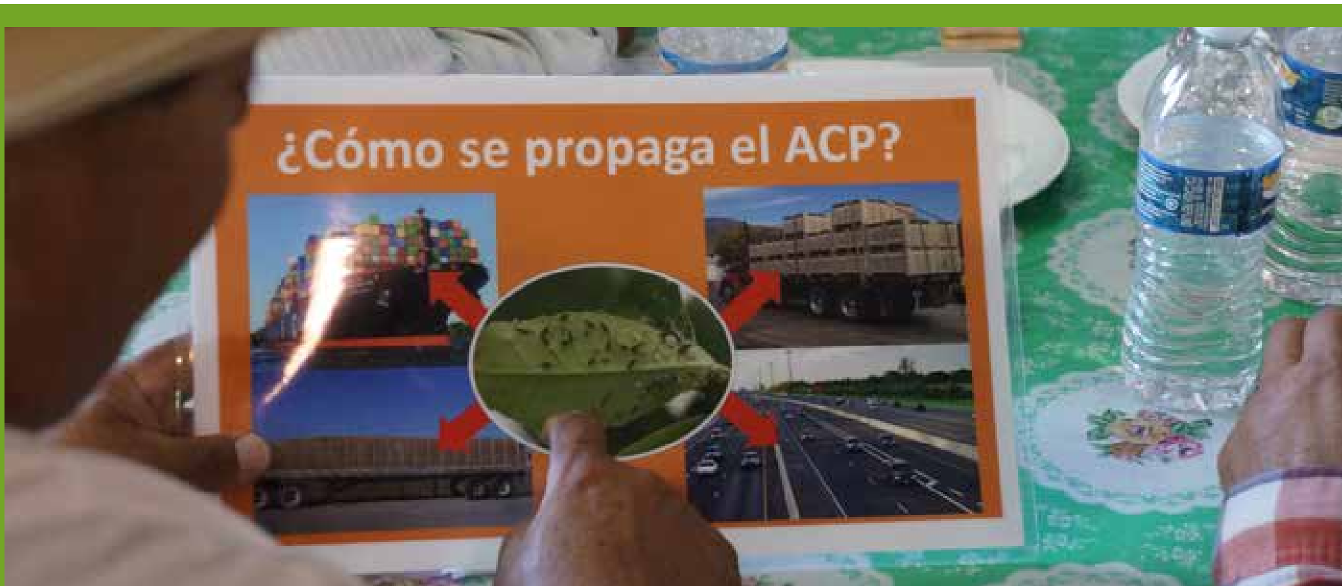
7:00 am	- Conference registration opens
7:30 am	- Complimentary full hot breakfast
9:00 am	- Morning conference session begins
10:00 am	- Morning break
10:15 am	- Morning conference session continues
12:00 pm	- Complimentary lunch
1:30 pm	- Afternoon conference session begins
3:00 pm	- Afternoon break
3:15 pm	- Afternoon conference session continues
5:00 pm	- Conference concludes

Registration and Accommodations

Early registration is requested on-line at *www.citrusresearch.org*, or by calling the CRB at (559) 738-0246. A block of hotel rooms has been set aside at the Wyndham in Visalia. To make hotel reservations at the Wyndham, please call (559) 651-5000 and mention the Citrus Research Board for a special group rate, which will be available until October 4 or until rooms are sold out.

We look forward to seeing you at the the California Citrus Conference!

***Carolina Evangelo is the CRB director of communications and the co-publisher/project manager of* Citrograph. *For more information, contact* Carolina@citrusresearch.org**



CCC will Kick-Off with ACP Workshop for Field Crews

Prior to the start of the California Citrus Conference, at 7:00 am on Wednesday, October 11, the Citrus Pest & Disease Prevention Program and California Citrus Mutual are offering a FREE training workshop designed to teach front-line crews how to mitigate spreading Asian citrus psyllids (ACP) in the field.

Target audiences for this training are crew bosses, foremen, ranch managers and packinghouse representatives. During the workshop, participants will:

- **understand the threat of ACP and huanglongbing to their livelihoods in the citrus industry;**
- **become familiar with best practices for field crews to keep ACP from spreading on plant material, equipment and personal items that leave the field; and**
- **practice using effective communication techniques to help their crews and others understand these best practices and why they are required.**

See the calendar of events on page 6 or visit *www.citrusinsider.org* for a schedule of similar free workshops scheduled throughout the state. Don't miss this opportunity to train your field crews. Everyone in the citrus industry must work together to save California citrus.

Pre-registration is required as space is limited. Register at *www.citrusinsider.org* or by phone at 559-592-3790.

NOTE: TRAINING WILL BE CONDUCTED IN SPANISH ONLY.

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Figure 2. CPDPP representatives sponsored an event in San Gabriel this summer to thank residents for their cooperation and to educate them further about HLB.

Outreach Intensifies in HLB-affected Regions

Gus Gunderson

The final Florida orange crop forecast for the 2016-17 season was 68.7 million 90-pound boxes. This represents a decline of 16 percent from the previous season and a massive drop from the 291 million boxes produced in the 2003-04 harvest before huanglongbing (HLB) ravaged the state (**Figure 1**).

This forecast served as an important reminder that California citrus growers must continue to aggressively defend against HLB. Following multiple hearings and public comment in which the industry voiced support, the California Department of Food and Agriculture (CDFA) has determined to continue the Citrus Pest & Disease Prevention Program (CPDPP)—meaning California citrus growers are promised an additional four years of aggressive disease control.

At press time, more than 100 trees had been confirmed with '*Candidatus* Liberibacter asiaticus' on residential properties. All infected trees have been removed promptly. Given the urban nature of this problem, an aggressive outreach targeting residents in these regions is essential. The CPDPP outreach team is engaged in a variety of efforts to inform residents and encourage them to take action to save California citrus trees, including:

- cooperating with agriculture crews working in the region;
- not moving citrus fruit, plant clippings or trees; and
- removing uncared-for backyard citrus trees.

To spread these messages, the program utilizes a multi-pronged approach, which includes briefing elected officials, using city governments to spread messages, advertising, grassroots community outreach and targeted media relations. Additionally, communications are conducted in multiple languages, including Mandarin, Korean, Vietnamese and Spanish.

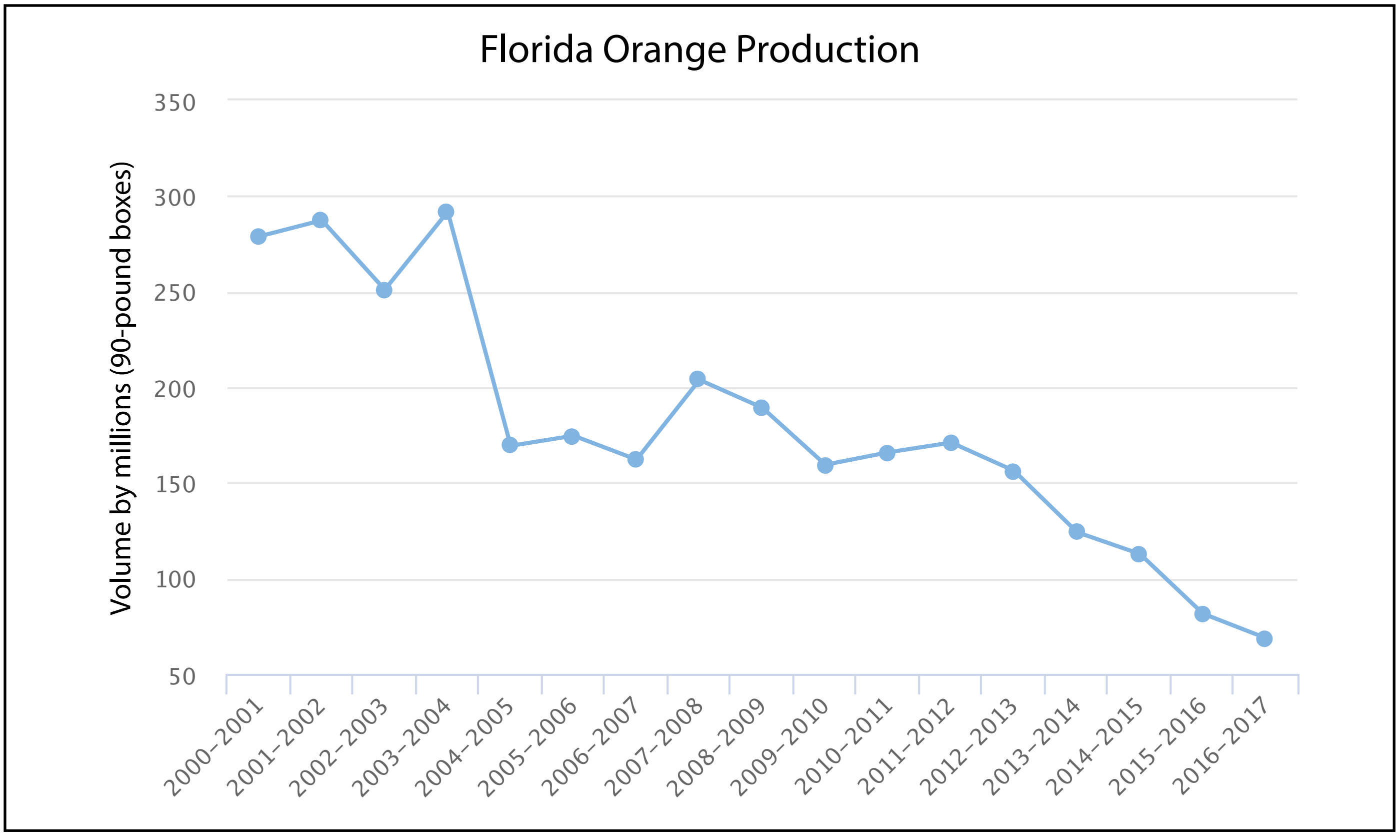

Figure 1. Florida orange production has experienced a precipitous drop since the spread of HLB throughout the citrus-growing regions of the state.

Working with elected officials is helpful since they have direct access to local residents and can also help add a sense of urgency to the threat HLB presents to the community's citrus. The outreach team is meeting with elected officials and presenting at city council meetings throughout Los Angeles, Orange and Riverside counties to secure their help in educating residents. City officials are provided with social media content to post on Facebook and Twitter and with articles to place in local newsletters and community papers. Elected officials also are airing the CPDPP's public service announcement on local public access channels and handing out brochures at city facilities.

The city of Riverside is eager to support HLB eradication in the area and quickly partnered with our program to host grower meetings, distribute yard signs, include information in city mailers and assist with local media outreach.

Additionally, program representatives and local and state agriculture officials are attending community events in the region to speak directly to residents about HLB.

In San Gabriel, where the majority of HLB-positive trees have been found, the program sponsored a summer movie event at a community park to meet with residents, distributing educational materials and encouraging them to protect area trees (**Figure 2**). The event was presented as a "thank you" from California citrus growers for the public's on-going support of efforts to quickly find and eradicate HLB in the community. Free lemonade and snacks were distributed, and children received educational coloring sheets. Hundreds of residents attended the event, which resulted in numerous commitments to check trees, remove citrus trees and cooperate with treatment efforts. The event also helped build a relationship between growers and residents.

On the media relations and advertising front, a series of digital advertisements recently ran on internet-based radio stations through the iHeartRadio app. Mandarin-language advertisements have played on the major Chinese radio station serving the entire Los Angeles region, including Orange, Ventura, Riverside and San Bernardino counties. Facebook advertising also is being run in the region along with on-going social media and traditional media outreach. Additionally, the program's public service announcement is being distributed to local news channels and is airing on both English- and Spanish-speaking stations with an emphasis in the Los Angeles and Riverside markets.

The CDFA staff also has a massive effort underway to detect and remove infected trees and Asian citrus psyllids (**Figure 3**). CDFA crews have been diligently implementing an intensive survey in the area that includes sectioning trees into four to eight sections and evaluating the leaves from each section independently. This thorough approach has proven successful in identifying diseased trees. The CDFA also treats host plants in the surrounding 800-meter area of a find site. State and local crews are building relationships with residents in the

Figure 3. CDFA inspectors closely monitor trees to detect any signs of HLB.

region, which not only helps gain access to properties, but also results in swift removal of diseased trees. Although removal is mandatory, positive homeowner relationships encourage quick cooperation. In fact, many residents have expressed interest in having seemingly healthy citrus trees removed as a proactive means of eliminating host plants in the area. California Citrus Mutual operates a tree removal program for residents in the HLB quarantine, and the CDFA is supporting this effort by sharing the tree removal web site and phone number with residents interested in having their citrus trees removed. This collaborative effort ensures no stone is left unturned. If residents want their trees out, the program explores every means of removal.

While the recent wave of HLB finds in Southern California is disheartening, citrus industry members must remain focused on the pro-active actions they can take to protect their groves, which includes participating in area-wide treatment programs. Please contact your grower liaison through *CitrusInsider.org* to get engaged with coordinated treatment efforts.

Gus Gunderson is chairman of the outreach subcommittee, Citrus Pest & Disease Prevention Program.

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- Tony Lorenzi, Sun Pacific

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Larry Black (second from left) conducted a tour of a Ft. Meade, Florida, citrus grove for (left to right) Etienne Rabe, Ph.D., of Wonderful Citrus in Delano, California; Texas Citrus Mutual President Dale Murden; Tom Aguilar of Mandarin Hill Orchards in Penryn, California; Raul Garcia of Gless Ranch in Riverside, California; California Citrus Mutual Vice-chairman Matt Fisher; and California Citrus Mutual Vice-president Bob Blakely.

Scientific and Observational Information Key to Battling HLB

Michael W. Sparks

As the Florida citrus industry enters its 13th year living with huanglongbing (HLB), one thing has become clear – if we are going to manage the disease effectively, we need to continue to have a free flow of information among growers, scientists and chemical companies.

We've done a pretty good job in Florida putting data out there for the citrus community to absorb. The annual Citrus Growers' Institute, the Citrus Expo and countless field days and other meetings provide growers with an opportunity to see what is working and what isn't.

Of course, the industry's massive scientific research push is the linchpin in finding short- and long-term solutions to HLB. More than $250 million has been spent or appropriated on citrus research by growers and the state and federal governments since its domestic onset in 2005. We are seeing results with new HLB-resistant rootstocks, grove architecture improvements, better nutritional programs and psyllid management.

But field observations are just as important, and they are playing a critical role as growers work together to save our industry. The days of secret production techniques are long gone.

As my friend, agriculture journalist Gary Cooper, said in a recent column, "While very different from the steadfast rules of scientific discovery, the observation method has always had a place in agriculture. Farmers have always been tinkerers, experimenters, researchers, inventors and survivors among other things."

Guided by these thoughts, the 2017 Educational Session at the Florida Citrus Industry Annual Conference featured five growers sharing their thoughts on production in the era of HLB. We usually go science-heavy on the agenda, but believing that growers are having a bit of research fatigue, we went with field observations instead.

The Session drew a packed house of more than 400 people to hear the thoughts of the five growers - Glenn Beck, Larry Black, David Howard, Tom Jerkins and Marty McKenna.

All of the grower presentations can be found at *www.flcitrusmutual.com*, and I highly suggest that readers go and take a look at the knowledge put together by these multi-generational farmers that we were lucky to have at the Conference Educational Session.

I think we can all agree we are only going to find a way out of the current environment through collaboration between everybody – scientists, growers, government officials, regulators – everybody. And that's what events like the Annual Conference are about.

Through collaboration, we have made great headway during the past 13 years, and the success has made Florida growers cautiously optimistic. You can't be any other way in agriculture.

Michael W. Sparks is the CEO and executive vice president of Florida Citrus Mutual, based in Lakeland, Florida.

Peace River Packing Company General Manager Larry Black (far left) provided a tour of the packinghouse in Ft. Meade, Florida, to visiting California and Florida growers.

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CCQC's Jim Cranney (second from left) met with Korean regulatory officials to discuss MRL policy issues while attending an MRL workshop in Seoul, Korea, in February.

Exports: Increasing Importance to California Citrus Growers

Jim Cranney

Summary

In any given year, one-third of the California citrus crop is exported to overseas markets. Furthermore, approximately 40 percent of industry revenue is derived from export, thanks to higher prices in export than in domestic markets. However, the threat from market disruption is a constant concern, because of potential quarantine and Maximum Residue Level (MRL) violations. The California Citrus Quality Council (CCQC), which is largely funded by the Citrus Research Board, works on behalf of the industry to facilitate exports and minimize the threat of export market closures.

California Citrus Acreage 1998-2016

	1998	2016	Change
Navel oranges	136,500	120,784	-12%
Valencia oranges	78,300	29,906	-62%
Lemons	53,700	44,621	-17%
Mandarins	10,100	58,941	484%
Grapefruit	15,100	8,493	-44%
Pummelos	3,700	1,144	-69%
Limes	700	631	-10%
Total	**298,100**	**264,520**	**-11%**

Figure 1. Source: California Department of Food and Agriculture, California Agricultural Statistics Service, California Citrus Acreage Report, 2016.

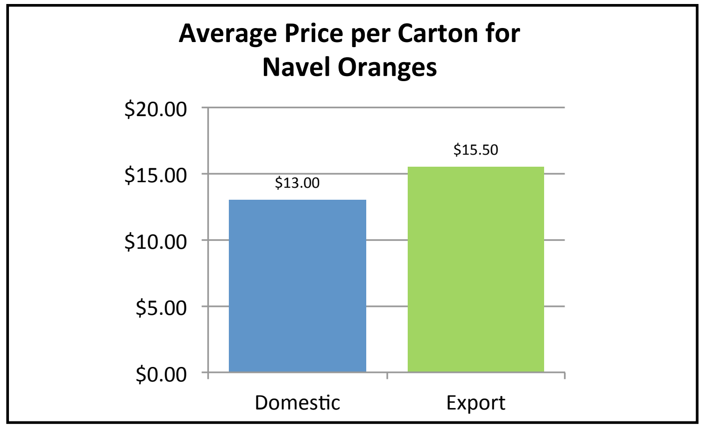


Figure 3. Average Price per Carton for Domestic and Export Navel Oranges.

Rising Mandarin Acreage and Imports Cause Change

Since 1998, California mandarin acreage has increased by nearly 500 percent (**Figure 1**). This remarkable increase has been driven by consumer demand for flavorful, seedless and easy-to-peel fruit. However, as consumers choose to purchase more and more mandarins, it has become apparent that they are buying fewer Valencia and navel oranges. As a result, Valencia orange acreage has decreased 62 percent since 1998, while navel orange acreage has decreased 12 percent (**Figure 2**).

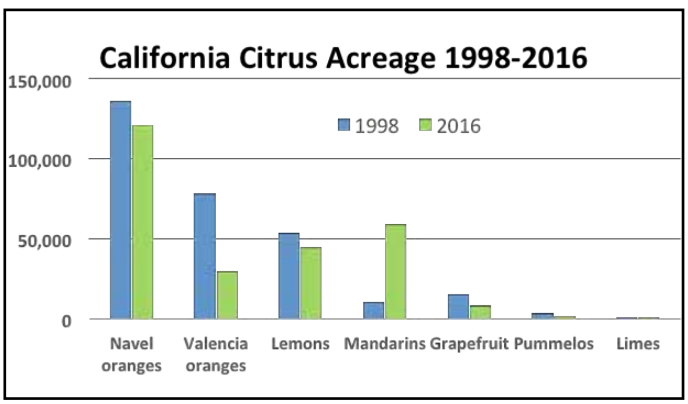


Figure 2. Source: California Department of Food and Agriculture, California Agricultural Statistics Service, California Citrus Acreage Report, 2016.

Export Markets Becoming More Important for Oranges

Domestic marketing conditions are making it increasingly difficult to sell large volumes of oranges at high prices, so packers are looking to export markets as an alternative market for Valencia and navel oranges. While conditions vary from market to market, the average price differential between domestic navel oranges and export navels is approximately $2.50 per carton (**Figure 3**). Higher export prices and difficult domestic markets have led to a greater focus on export markets for navel oranges.

While exports promise greater returns than the domestic market, export markets are also fraught with difficulties. Key export markets such as Korea, China, Australia and a host of others require adherence to expensive phytosanitary protocols, while MRL compliance also can be a difficult issue to navigate. Nevertheless, export markets have become more important for packinghouses that are trying to maximize the value of Valencia and navel oranges.

CCQC Collaborates with University Experts

In export markets, trading partners often require special protocols for growing, harvesting and packing citrus fruit to avoid the establishment of unwanted plant diseases or insects. Exports to Australia, China and Korea require adherence to special requirements before citrus fruit can be approved for export. Some of these protocols include bean thrips for Australia, *Phytophthora* for China and Fuller rose beetle (FRB), California red scale (CRS) and Septoria spot for Korea.

CCQC has partnered with university and U.S. Department of Agriculture experts to explore potential improvements to protocols in the following markets:

Australia

- CCQC is working with the registrant and Australian regulatory authorities to establish an MRL for phosphine, which is highly effective for control of bean thrips. There is a reasonable probability that the phosphine MRL will be established in time for the 2017-18 export season.
- CCQC secured a two-year $510,000 Technical Assistance for Specialty Crops (TASC) grant with the assistance of UC Davis' Beth Mitcham, Ph.D., to research commercial post-harvest fumigations for ethyl formate, which is highly effective against bean thrips and mites.
- CCQC is collaborating with Spencer Walse, Ph.D., of the

USDA-Agricultural Research Service in Parlier, Kearney Ag Center's Sandipa Gautam, Ph.D., and Mark Hoddle, Ph.D., of the University of California, Riverside (UCR) on cold treatments that could be used to control bean thrips.

China

- CCQC is working with the registrant to establish an MRL for potassium phosphite in China. UCR's Jim Adaskaveg, Ph.D., has shown that post-harvest treatments of potassium phosphite are effective in controlling *Phytophthora* infections.
- CCQC and Adaskaveg are collaborating with registrants to facilitate registration of several soil drench and foliar treatments that are highly effective in controlling *Phytophthora*.

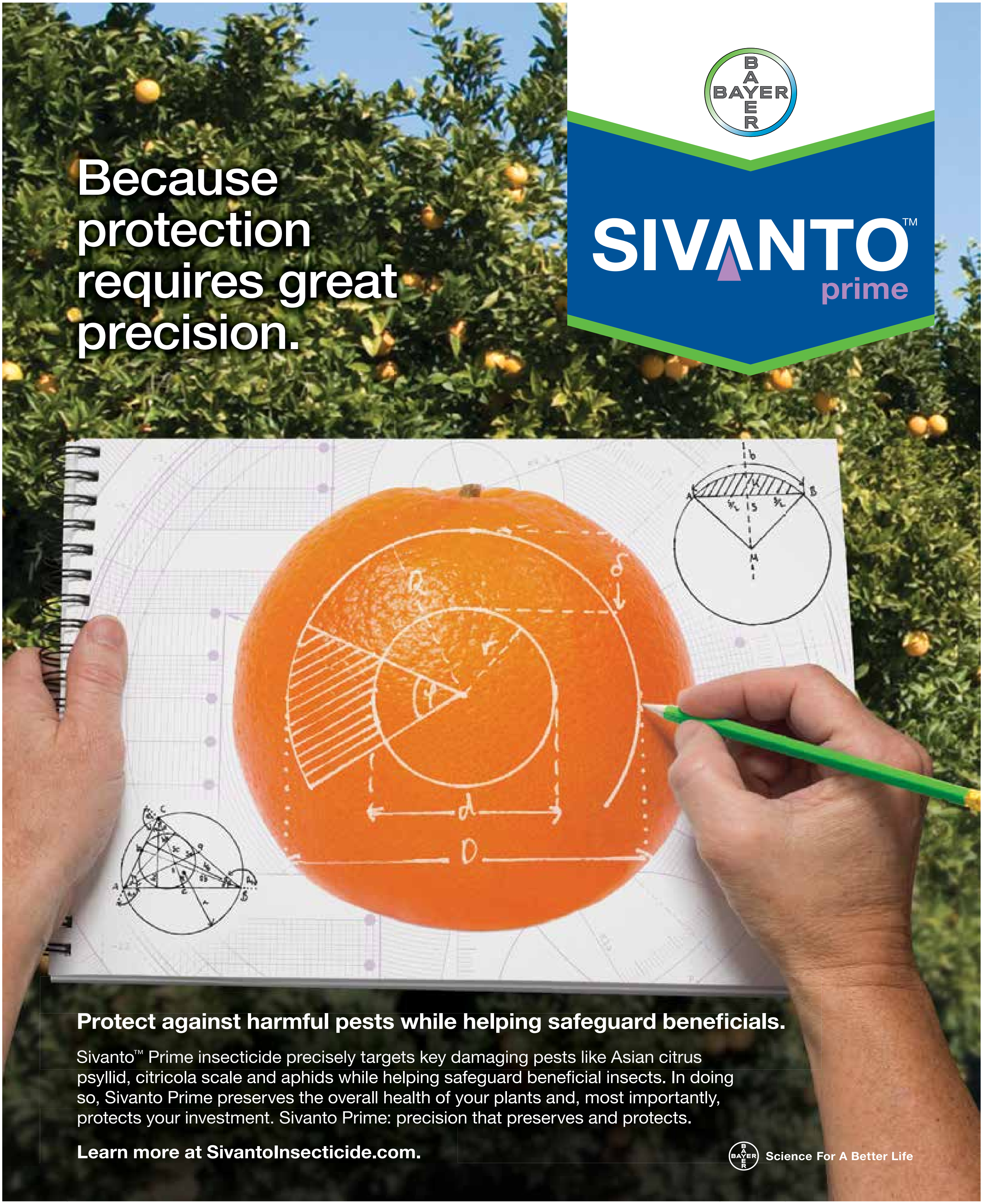
Korea

- Korea is establishing a new MRL setting process, so CCQC is working with several registrants to establish MRLs that will conform to new regulations in Korea.
- CCQC is working with the registrant for Kryocide® to facilitate its use on FRB. Kryocide® is the most effective pesticide treatment for FRB, but it has not been widely used because of the absence of an MRL in Korea.
- Walse is conducting research trials using malic acid and surfactants that show promise in killing FRB eggs in post-harvest packinghouse drenches. CCQC is seeking a registration for malic acid at EPA.
- CCQC is working with the phosphine registrant to establish an MRL for phosphine in Korea.

Progress on all of these fronts ensures that the California citrus industry will continue to have access to important export markets that represent a significant opportunity to maintain grower returns and profitability in the California citrus industry.

The Almond Board of California's Gabriele Ludwig and CCQC's Jim Cranney at the 2017 Codex meeting on pesticide residues in Beijing, China.

Jim Cranney is president of the California Citrus Quality Council (CCQC), which works collaboratively with the California citrus industry, the University of California and domestic and international regulators to manage market access, access to crop protection protocols and food safety issues for the California citrus industry.



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Spotlight on Sunkist

Celebrating 125 Years

Joan Wickham

Sunkist is the largest citrus cooperative in the world, marketing millions of cartons of premium fresh citrus internationally each year to 40 countries. With thousands of grower members in California and Arizona, the Sunkist cooperative reflects the values and legacy of its history: multi-generational family farmers committed to growing the highest quality citrus, being responsible stewards of their land and dedicating themselves to innovation. As a member of a cooperative, each individual grower joins with other growers to participate in a larger market share, creating new opportunities for family-owned farming operations.

A cooperative of growers together can do many things that a grower alone cannot afford to do – access the international market, promote a brand name, leverage a global transportation system, employ comprehensive research capabilities and gain governmental access to overseas markets, to name a few. When buying Sunkist fruit, consumers are not only getting the Sunkist quality that they know and expect, but they're also supporting family farmers – which we believe is really important.

Sunkist will be celebrating its 125th year this upcoming season. Throughout its history, Sunkist has established a reputation as the premier name in citrus offering innovative marketing solutions, a robust on-trend assortment of varieties and a dependable supplier to customers all over the world.

As a sales and marketing cooperative, Sunkist prides itself on the organization's expertise in driving demand. Sunkist's sales and marketing teams have a firm grasp on current and emerging trends that help our partners leverage citrus sales. Sunkist customizes this

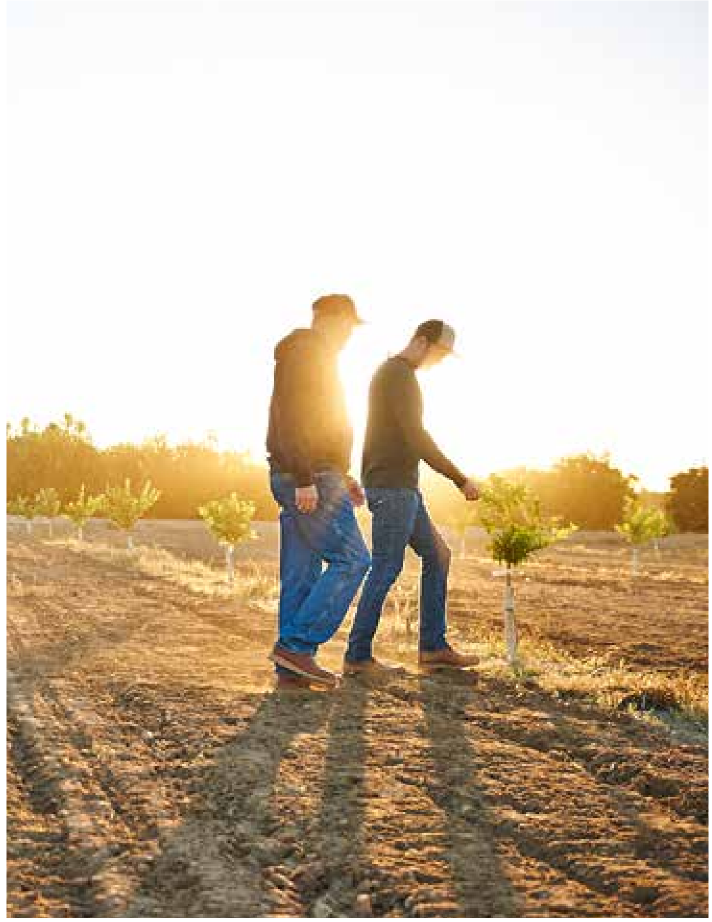
information for our customers to best fit their needs – from point-of-sale display units to social media content. Offering flavor profiles, nutritional information, usage tips and recipe ideas, Sunkist provides customized content and marketing execution to food service and retail customers globally.

California- and Arizona-grown citrus offer a powerful quality proposition to consumers, particularly in export markets, that rewards growers. Sunkist, as a consistent supplier for 125 years, has built a reputation as the premier citrus brand abroad, and our organization continues to uphold that distinction. Sunkist's global reputation is bolstered by its licensing program. One of the largest food and beverage licensors globally, Sunkist's name appears on more than 750 licensed products in 77 countries all over the world, extending the reach of the brand and benefiting our growers.

In this global marketplace, Sunkist is making a strategic and powerful effort to share our story with trade customers and consumers, helping people connect to the heart of our business – our growers. Sunkist Family Stories is a multimedia story-telling project that offers compelling content to our customer base and consumers directly. These stories not only tell Sunkist's story, but offer authentic narrative around industry issues like sustainability, labor, water and weather. Trade customers and consumers can visit *familystories.sunkist.com* to learn more.

The strength of our membership allows Sunkist to offer an impressive portfolio of citrus that affords us a premier reputation in the industry. Sunkist offers more than 40 varieties of premium quality, fresh citrus year-round including oranges, lemons, specialties and organics. This allows us to offer our customers a consistent, broad portfolio of citrus offerings – all from Sunkist.

***Joan Wickham is the director of communications for Sunkist Growers, based in Valencia, California. For more information on Sunkist Growers, visit* www.sunkist.com**



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In Temecula, the retirement of Joe Morse, Ph.D., (third from left) of the University of California, Riverside (UCR), was honored by (left to right) Joe Barcinas, Foothill Ag Resouces; Mary Lu Arpaia, Ph.D., UCR; and CRB Board Members Alan Washburn, Mike Perricone and Chris Boisseranc.

CRB and UCCE Conduct Grower Seminars

Carolina Evangelo

The Citrus Research Board (CRB) and the University of California Cooperative Extension (UCCE) hosted their annual, well-received series of three half-day citrus grower educational seminars in Exeter, Santa Paula and Temecula, California, on June 27, 28 and 29, respectively.

The opportunity for growers to connect with researchers, regional farm advisors and other local growers and to earn continuing education units resulted in a very positive grower turnout of approximately 350 people attending the three seminars.

Each location featured:

- Beth Grafton-Cardwell, Ph.D., University of California, Riverside (UCR) and director of the Lindcove Research and Extension Center, who shared management strategies for the Asian citrus psyllid;
- Joe Morse, Ph.D., UCR, who discussed the future of integrated pest management;
- Ed Stover, Ph.D., U.S. Department of Agriculture-Agricultural Research Service in Fort Pierce, Florida, who talked about huanglongbing tolerance and resistance in breeding citrus; and

Growers packed the Exeter Veterans Memorial Building for the local grower seminar.

- Carol Lovatt, Ph.D., UCR, who presented information on alternate bearing.

Additionally, the three seminars included updates from regional farm advisors:

- Greg Douhan, Ph.D., UCCE Citrus Advisor for Tulare and Fresno counties;
- Ben Faber, Ph.D., UCCE Citrus Advisor for Ventura County; and
- Gary Bender, Ph.D., UCCE Farm Advisor Emeritus for San Diego County.

In Exeter, Douhan moderated and also presented the San Joaquin Valley citrus update, which included a discussion on the excessive moisture this year that occurred due to the break in the drought, along with trends regarding citrus production in the valley. At the Santa Paula seminar, Faber served as the moderator and presented a regional update, where he shared information on research trials involving pest, disease, lemon scion varieties and rootstocks being conducted throughout the coastal region and how they pertain to coastal citrus production. The Temecula seminar was moderated by the UCCE County Director and Area Advisor for Farm Management, Etaferahu Takele, Ph.D. Bender provided the regional update for southern California. Grower attendees asked informed, thoughtful questions and interacted with the speakers at each meeting location.

Morse, who retired in June from his position with UCR after many years of dedicated service *(see Citrograph, Summer 2017, pp. 18-20)*, was recognized at each seminar via a special presentation. The CRB gifted Morse with a painting done by local artist Nadi Spencer entitled "A View from Lemon Cove," along with an award that read, *"Honoring an Outstanding Citrus Researcher. Thank you for your exceptional work and the impact you have made on the California citrus industry."*

Attendee feedback was positive, with many growers mentioning how informative they found the sessions. The CRB is looking forward to holding the series again in summer 2018.

***Carolina Evangelo is the CRB director of communications and the co-publisher/project manager of* Citrograph. *For more information, contact* Carolina@citrusresearch.org**

University of California, Riverside staff gathered together at the Temecula Grower Seminar. (Back row, left to right): Amanda Rawsteran; Ade Gómz; Joe Morse, Ph.D.; Peggy Mauk, Ph.D.; Mike Roose, Ph.D.; Frank Byrne, Ph.D.; Tom Shea, Gary Bender, Ph.D. (Front row, left to right): Eta Tekele, Ph.D.; Beth Grafton-Cardwell, Ph.D.; Tracy Kahn, Ph.D.; Mary Lu Arpaia, Ph.D.; and Carol Lovatt, Ph.D.

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- GEORGIOS VIDALAKIS, PH.D., UC RIVERSIDE
- BETH GRAFTON-CARDWELL, PH.D., LREC/UC RIVERSIDE
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Exeter attendees heard the latest on food safety from panel members (left to right) Donald Schaffer, Ph.D., Rutgers University; Adrian Sbodio, University of California, Davis; Franco Bernardi, Suntreat; Lise Korsten, Ph.D., University of Pretoria, South Africa; and Kevin Severns, Sanger-Orange Cove Citrus Association.

Post-harvest Conference Focuses on Food Safety

Mary Lu Arpaia and Carolina Evangelo

Post-harvest research expert and meeting chair Mary Lu Arpaia, Ph.D., welcomed more than 60 attendees to the 38th Annual Post-Harvest Pest Control Conference, which was held in mid-April at the Embassy Suites Mandalay Beach Hotel and Resort in Oxnard, California, hosted by the Citrus Research Board (CRB).

This annual, highly technical conference featured 17 guest speakers who provided updates on recent developments in post-harvest disease control and technologies for researchers, industry personnel and service company representatives. An international perspective was offered this year by Lise Korsten, Ph.D., who is an expert on food safety and biocontrol of post-harvest diseases at the University of Pretoria, South Africa. At the beginning of the conference, CRB Chief Research Scientist Melinda Klein, Ph.D., provided an overview of the Board's programs and highlighted current research program priorities.

Citrus Post-harvest Disease Control and Quality

Chang-Lin Xiao, Ph.D., from the U.S. Department of Agriculture-Agricultural Research Service (USDA-ARS) in Parlier, California, shared his on-going research on post-harvest decay of mandarins, which focuses on the management of the emerging diseases caused by *Mucor piriformis* (Mucor rot) and *Botrytis cinerea* (gray mold). He discussed factors involved in disease development and possible control strategies.

Mark Ritenour, Ph.D., of the University of Florida reviewed work he has conducted looking at the potential of essential oils as a possible control strategy for post-harvest diseases. Several groups have been researching this strategy in recent years. Ritenour presented data showing that the essential oils, carvacrol and thymol showed significant antifungal control against Diplodia stem end rot and also reduced fruit weight loss and chilling injury during storage when incorporated into a shellac wax.

Attendees listened intently to speakers at the 2017 Post-Harvest Conference in Oxnard, California.

Arno Erasmus, Ph.D., of Wonderful Citrus in Delano, California, summarized research he conducted while in South Africa on the worldwide challenge of *Penicillium* resistance to imazalil, a key post-harvest fungicide used internationally.

Korsten provided an overview of biocontrol used to manage post-harvest disease. She reviewed past efforts and why they were not ultimately successful and discussed what it would take to make biocontrol a successful strategy in the future. Most importantly, she discussed the relationships and modifications to the microbial biome that can occur across the post-harvest environment, and the challenges that researchers face when developing biocontrol strategies for both post-harvest diseases, as well as food-borne pathogens.

The final presentation on this topic was by David Obenland, Ph.D., of USDA-ARS in Parlier, on the impact of coatings on mandarin fruit quality. Obenland discussed his collaborative work with Arpaia on off-flavor development in mandarins and the importance of temperature management, varietal differences and the interaction of these factors with fruit coatings.

Registration and Regulatory Updates

Matt Lantz, vice-president of global access for Bryant Christie Inc. in Seattle, Washington, discussed the standard Maximum Residue Limits (MRLs) for post-harvest chemicals and the unique challenges for citrus export. Geert de Wever, Ph.D., associate director of global marketing for Janssen PMP (a division of Jannsen Pharmaceutica) in Belgium, covered the topic of imazalil and future challenges for keeping this important fungicide registered and effective. The third presentation was by Paul Kuhn, Ph.D., the technical product lead on fungicides for Syngenta in Greensboro, North Carolina, who provided an update on the fungicide Mentor®, which is effective against sour rot (*Geotrichum citri-quarantii*) and green mold (*Penicillium digitatum*).

Post-harvest Quarantine Issues

Spencer Walse, Ph.D., (USDA-ARS, Parlier) gave an overview about quarantine issues and his research group's work to develop system-based approaches to address insect quarantine issues that challenge citrus exports from California. This presentation was followed by an update from Sandipa Guatam, Ph.D. (University of California, Riverside [UCR]) on the potential use of propylene dioxide as an alternative fumigant for various pests. Lastly, Daniel Kuzmich, Ph.D., (USDA-ARS, Parlier) reviewed current work within the USDA to develop system-based approaches for the control of Tephritid fruit flies.

Food Safety

Korsten opened this topic with a global perspective on food safety. Of great interest to the group was her differentiation of food safety concerns between the European Union, which focuses primarily on chemical usage and residues, and the US, which focuses on the potential of food-borne pathogens. She was followed by Donald Schaffner, Ph.D., of Rutgers University in New Jersey, who provided an update of Food Safety Modernization Act regulations and implementation. Trevor Suslow, Ph.D., of the University of California, Davis reviewed his work on *Listeria* testing in packinghouses, how *Listeria* could potentially become a problem and ways to mitigate this issue.

Paul Kuhn, Ph.D., Syngenta; Richard Kim, Ph.D., Pace International; and Lise Korsten, Ph.D., University of Pretoria, South Africa, at the event reception.

An overview of the history of sanitizers and recent developments was discussed by Joe Smilanick, Ph.D., (USDA-ARS, retired). He was followed by Ritenour, who spoke on the packline sanitation research he has conducted in Florida to control possible *Escherichia coli* contamination. The final presentation was from UCR's Jim Adaskaveg, Ph.D., who highlighted possible new sanitizers that are more effective against both post-harvest diseases and food-borne pathogens.

Other Topics

Additionally, the group heard presentations on sweet cherry post-harvest disease control from Helga Forster, Ph.D., (UCR) and the control of blueberry diseases from Seiya Saito, Ph.D., (USDA-ARS, Parlier).

The 38th Annual Post-Harvest Pest Control Conference once again was a huge success and a valuable research-based program for all who attended.

***Mary Lu Arpaia, Ph.D., is an extension subtropical horticulturist with the University of California, Riverside in the Department of Botany and Plant Sciences. Carolina Evangelo is the CRB director of communications and the co-publisher/project manager of* Citrograph. *For additional information, contact* mlarpaia@ucanr.edu *or* carolina@citrusresearch.org.**

Interior of the in-field fogging for ACP demonstration by Spencer Walse, Ph.D., at the Post-Harvest Seminar.

Spencer Walse, Ph.D. (left), explains the ACP fumigation process to CRB Associate Research Scientist Mojtaba Mohammadi, Ph.D.

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Large controlled atmosphere rooms open (left) and closed (right) for cold storage phosphine fumigations.

Post-harvest Fumigation: Opportunities and Challenges

Breaking trade barriers for California citrus exports

Spencer Walse

Project Summary

The over-arching goal of this research is to develop post-harvest treatments to facilitate movement of California citrus through international trade and marketing channels. Particular attention is paid to the expansion and retention of export markets. This research critically supports compliance with domestic and international regulations related to the protection and distribution of fresh citrus, as well as human and environmental health concerns associated with the use of agrochemicals. Efforts to develop post-harvest fumigation as a tool to maintain the global prominence of California citrus are briefly outlined in this report.

With the ever-increasing demand in domestic and international markets for food quality, safety and security, there also is the critical need to control horticultural crop pests in the safest and most economical ways possible. Whenever horticultural crops traverse political boundaries, pest-related trade barriers can ensue. Frequently, but not always, these barriers can be lowered or removed when sound scientific evidence is provided. The Crop Protection and Quality Research Unit (CPQRU) of the US Department of Agriculture – Agricultural Research Service (USDA-ARS) San Joaquin Valley Agricultural Sciences Center in Parlier, California, has a team of scientists dedicated to addressing horticultural crop export issues, with particular attention given to post-harvest pest control strategies that enhance the competitiveness of American agriculture.

The CPQRU research team comprises three principle investigators who conduct post-harvest citrus research: Spencer Walse, Ph.D., Chang-Lin Xiao, Ph.D., and David Obenland, Ph.D. The team focuses on trade barrier issues, with the major goals of (1) retaining and expanding domestic and/or international markets for US growers and (2) protecting US growers from the agricultural, ecological and economic threat posed by horticultural crop pests. With a research scope that encompasses a variety of key technical and regulatory issues, including Maximum Residue Levels (MRL)[1], pesticide registrations, food safety and IPM strategies, arguably the greatest effort goes to the development of novel theories and treatments for post-harvest insect pest control.

Relative to treatments applied during production, post-harvest opportunities allow for greater synchronization of the treatment with the logistical and infrastructural constraints that funnel into the marketing channel. While efforts continue across the gamut of post-harvest possibilities (e.g., cold-treatments, heat-treatments, irradiation, controlled-atmosphere, fogging, etc.), fumigation is an invaluable treatment option.

Post-harvest fumigation is a critical element of the $3 billion per year California citrus industry. Post-harvest fumigation provides a biological safeguard against pests and, in many cases, is the only available tool for governments, regulators and industry to guarantee pest-free security and food safety. One fumigant, methyl bromide (MeBr), has dominated post-harvest applications in California and beyond. It quickly penetrates commodity loads and has, in general, non-discriminating efficacy against insect and microbiological pests (Bond 1984). As such, MeBr has been used successfully for quarantine[2] and pre-shipment[3] (QPS) disinfestations over the last four decades. Its routine use has left the California citrus industry, producers and port facilities alike, with infrastructural capabilities that are almost exclusively geared toward post-harvest chambers designed specifically for MeBr use.

Many dynamics are involved with the continued use of MeBr by the specialty crop industry as a whole, several of which are currently relevant to the California citrus industry. Methyl bromide use is regulated via international legislation under the Montreal Protocol[4]. The Protocol (Article 2H) *recognizes that QPS methyl bromide is an important remaining use of this ozone-depleting substance that is not controlled*, a clear acknowledgment by the international community that MeBr is critically important and will continue as the "tool of first choice" due to its internationally accepted efficacy and regulatory status. However, (decision XI/13) urges *Parties to implement procedures to monitor the QPS uses of methyl bromide by commodity, and* (decision VII/5) urges *Parties to refrain from using methyl bromide and to use non-ozone-depleting technologies*. This "urging of the Parties" away from QPS MeBr use creates myriad challenges for regulatory, agricultural and industry entities with a stake in post-harvest chamber fumigation, a technology that literally evolved around QPS MeBr use. The situation is further complicated by the fact that continued QPS uses are at the discretion of the importing nation per Food and Agriculture Organization (FAO) standards (ISPM, 2007).

One example is South Korea, one of the key export markets for California citrus, valued at approximately $200 million annually. A true testament to successful negotiations by citrus industry leaders and the US government in the 1980s, the on-arrival QPS MeBr fumigation in South Korea caused minimal disruption to export marketing, resulted in optimal fruit quality and negated the expense of building chambers in California packinghouses. In this light, it was a nearly ideal post-harvest treatment for the California industry. All indications suggest, however, that the use of QPS MeBr for this export is on borrowed time, as it appears South Korea is poised to remove this treatment option, likely due to logistical, regulatory and political pressure on their end.

The following summarizes efforts made by Walse of the CPQRU research team, as well as key University of California, citrus organizations (Citrus Research Board [CRB], California Citrus Quality Council and California Citrus Mutual) and industry collaborators, toward the development of post-harvest MeBr alternatives for the California citrus industry.

Since the US government became a signatory of the Montreal Protocol in 1988, researchers and industry near and far have been working diligently to develop technically and economically viable post-harvest alternatives to MeBr. Foremost, any alternatives must have a domestic food tolerance[5], which can cost a registrant more than $1 million and take five years for the US Environmental Protection Agency (EPA) review, with no guaranteed approval. Secondarily, the alternatives must have MRLs in the target market, which essentially means the process must repeat itself in the foreign regulatory realm.

Table 1. Treatment durations required to control key citrus insect pests with phosphine fumigation conducted on packed, palletized fruit at 40°F.

species	*target life stage*	*market*	*duration (hour)*	*efficacy (% kill)*
bean thrips	adults	Australia	12	> 99
California red scale	all	Korea	24	> 99
Asian citrus psyllid	adults	domestic	36	> 99
Fullers rose beetle	eggs	Korea	48	>95

Only a single post-harvest MeBr alternative, phosphine, can be used to treat citrus in the US. Owing to the pioneering work of Fransiskus Horn, Ph.D., in the late 1990s, phosphine is now used across the globe to treat fresh fruit at cold-storage temperature, and MRLs of 10 parts per billion (ppb) are established in nearly all key exports markets for California citrus, with the notable exception of Australia and New Zealand. MRLs of 10 ppb, consistent with "no detection" per international food standards of Codex Alimentarius, are essentially a regulatory formality because they "acknowledge" a treatment that would otherwise be undetected during residue analysis. The rapid off-gassing of phosphine from fresh fruit, including citrus, during the EPA mandated 48-hour lag (under cold-storage) between fumigation aeration and consumption, enables compliance with such a low food tolerance and ensures that chances of non-compliance in a foreign market are essentially nil. Moreover, the rapid off-gassing of phosphine during aeration minimizes worker exposure concerns, relative to MeBr, which takes more than 20 times longer to off-gas from a given type of fruit. Another advantage of phosphine is that it generally enhances the quality of waxed, packed, palletized fruit. Recently, a CRB-sponsored project began to thoroughly benchmark the quality of phosphine-treated citrus relative to that treated with MeBr, in order to better ascertain the potential use of phosphine for export to Korea.

Figure 1. Due to treatment durations that are long, relative to those for methyl bromide, cold-storage phosphine fumigations can be conducted in large controlled-atmosphere rooms, maintained under cold-storage conditions, as a means to minimize disruptions to export throughput.

There are key features, however, that differentiate phosphine and MeBr. Whereas MeBr works on the timescale of two to four hours at treatment temperatures above 40°F, phosphine is

SPECIAL POST-HARVEST SECTION

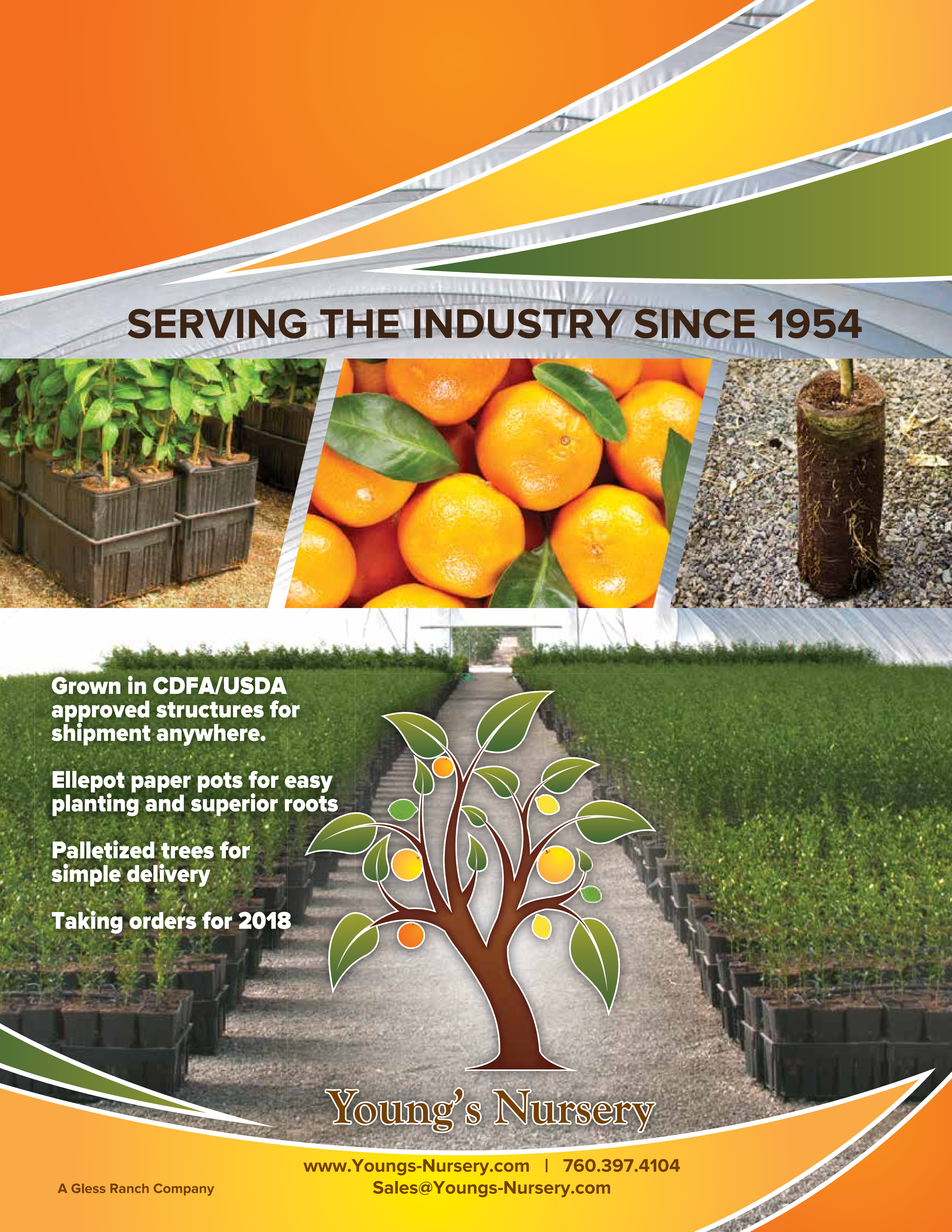
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Figure 2. Phosphine fumigations can also be conducted in methyl bromide chambers (top left), modified reefer containers (bottom left), or even under tarpaulins (top right).

typically used for 12 to 72 hours at "cold-storage" temperatures spanning about 31 to 45°F (**Table 1**). For extremely difficult-to-control insect pests, such as internal feeding Tephritid fruit fly larvae (e.g. Mexican fruit fly, Medfly), treatment temperature can be increased up to nearly 70°F for 48 hours (Williams et al. 2000). The need to treat for such a long period of time, relative to MeBr treatments, requires compensatory scaling of fumigation structures to accommodate packinghouse throughput requirements, particularly for several of the larger export markets. Chile fresh fruit packers and shippers have overcome this logistical complication, primarily by conducting phosphine fumigation in large controlled-atmosphere rooms (**Figure 1**) or banks of modified reefer containers (**Figure 2**). The CRB has funded commercial-scale research with phosphine as a proof of concept for industry and USDA-Animal and Plant Health Inspection Service (APHIS) regulators. A 48-hour treatment with a dose of 1.5 milligrams per liter phosphine at 40°F controlled 95 percent of more than 7,000 Fuller rose beetle eggs buried throughout 88 pallets of navels packed for export to Korea. Whether used as a stand-alone post-harvest fumigation or as a final mitigation step as part of a systems-approach, phosphine is a viable tool for the California citrus industry, and its use is expected to increase.

Two other fumigants, ethyl formate and propylene oxide, are currently being investigated for insecticidal efficacy, residue characteristics and impact on fruit quality. While it is unlikely that two-hour fumigations with ethyl formate and propylene oxide will provide control of Fuller rose beetle and Tephritid fruit flies at doses that do not harm fruit, both ethyl formate and propylene oxide are highly effective against external feeding insects, including mites, psyllids and thrips.

If any citrus packers wish to learn more about post-harvest treatments, including fumigation, please reach out to Spencer Walse of the CPQRU research team. We are always willing to conduct fumigations in Parlier, California, so that packers have an opportunity to observe the treatment and evaluate fruit quality on their own terms/criterion – drop the fruit off, we will treat it and then return it for evaluation.

CRB Research Project #5400-149

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Glossary

[1]**Maximum Residue Levels (MRL):** The highest level of a pesticide residue that is legally tolerated in or on food or feed when pesticides are applied correctly per label instructions.

[2]**Quarantine disinfestation:** Treatments to prevent the introduction, establishment and/or spread of quarantine pests (including plant pathogens).

[3]**Pre-shipment disinfestation:** Treatments applied directly preceding and in relation to export to meet the phytosanitary or sanitary requirements of the importing country.

[4]**Montreal Protocol:** International treaty designed to protect the ozone layer by phasing out the production of numerous substances responsible for ozone depletion. It has been ratified by 197 parties, including 196 states and the European Union, making it the first universally ratified treaty in United Nations history.

[5]**Food tolerance:** Limits on the amount of pesticides that may remain in or on foods marketed in the US (referred to as MRLs in many other countries); set by the EPA and enforced by USDA (meat and poultry) and FDA (other foods).

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Images of Mucor rot (left) and gray mold (right).
(Photos courtesy of J. Smilanick).

Emerging Post-harvest Fruit Rot Diseases of Mandarins

Seiya Saito and Chang-Lin Xiao

Project Summary

Storing mandarins in cold facilities recently has become a common practice to retain fruit quality and expand their marketing window. However, extended storage may increase the risk for development of storage rot. In recent years, some mandarin packinghouses have experienced an unusual post-harvest rot problem affecting stored mandarin fruit. In 2015-16, we collected decayed mandarins from packinghouses to identify the major post-harvest diseases affecting mandarin fruit. We found that Mucor rot caused by the fungus Mucor piriformis *and gray mold caused by the fungus* Botrytis cinerea *are two emerging post-harvest diseases affecting cold-stored mandarins. Mucor rot is a newly recognized mandarin post-harvest disease. The survey results suggest that to reduce post-harvest mandarin losses, decay-control programs should target these emerging diseases in addition to other common post-harvest diseases. Research is currently underway to develop such programs.*

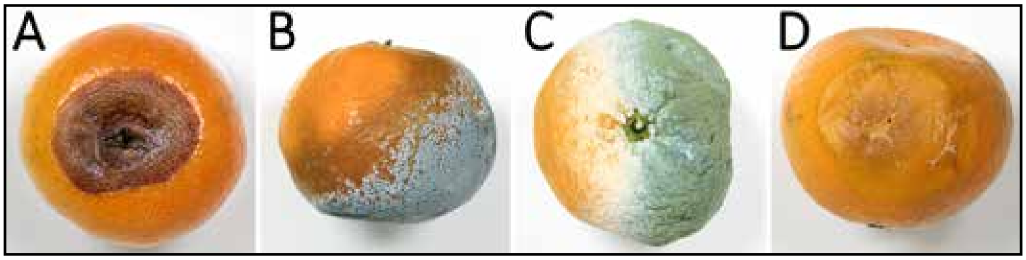

Figure 1. Symptoms of Alternaria rot (A), blue mold (B), green mold (C) and sour rot (D) on mandarin fruit.

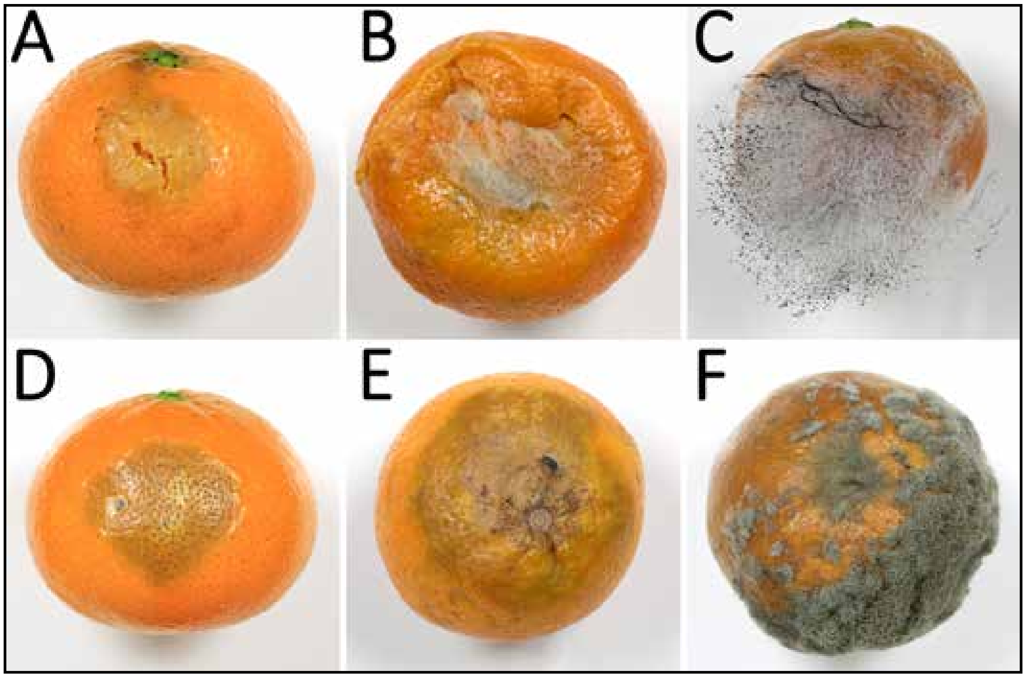

Figure 2. Symptoms of Mucor rot (A-C) and gray mold (D-F) on mandarin fruit. Pictures from left to right represent disease symptom development from an early stage to an advanced stage.

During the 2013-14 mandarin harvest and packing season, some packinghouses reported that in recent years they had experienced an unknown decay on mandarin fruit that had been stored in cold facilities for an extended period of time after pre-sorting, resulting in significant economic losses. To address this problem, we initiated a survey to identify what major post-harvest diseases affect mandarins, particularly previously unknown diseases on fruit that had been kept in extended cold storage. Such information would help us develop and implement relevant programs to control mandarin decay.

Introduction

The biggest change in California's citrus industry in the past decade has been the increasing production of mandarins (Webber et al. 2014). This change has been largely attributed to the increasing consumption of (and consumer demand for) "easy-to-peel" citrus fruit such as mandarins (Ladaniya 2008). Between 2004 and 2016, California tangerine and mandarin acreage increased from 18,512 to 58,941 acres with a U.S. production value in 2015-16 exceeding $600 million (California Agricultural Statistics Service, 2004; USDA-National Agricultural Statistics Service 2016a, 2016b). Mandarins are now the second most important California citrus variety behind navel oranges.

After harvest, mandarin fruit are commonly packed and shipped directly to market. However, as mandarin acreage and volume continue to increase, storing the fruit in cold facilities has become a common practice to retain quality and expand the marketing window. After initial pre-sorting, mandarins may be stored at cold temperatures for up to several weeks prior to packing and shipping. However, extended storage increases the risk for development of storage rot.

Packinghouse Survey

The mandarin post-harvest disease survey was conducted between January and April in 2015 and 2016. Decayed mandarins were sampled from three California Central Valley packinghouses in 2015 and two in 2016. Decayed fruit were collected either from a pre-sorting line ("non-stored" fruit) or from storage bins that had been stored after harvest for two to seven weeks at around 45°F (7.2°C) ("stored" fruit). In 2015, 533 decayed non-stored fruit and 733 decayed stored fruit were sampled from 11 and 21 grower lots, respectively. In 2016, 390 decayed non-stored fruit and 660 decayed stored fruit were collected from nine and 25 grower lots, respectively. Decay on each fruit initially was identified by disease symptoms, if applicable. All decayed fruit were subjected to isolation of pathogens on agar media, and identification of the pathogens (fungi) was based on the morphological characters. Fungi that were not identified based on morphological characteristics were identified by DNA sequence-based molecular approach. No tests were done to fulfill Koch's postulates for fungi with unknown pathogenicity on mandarin fruit. Pathogenic fungi were determined based on the literature.

Results

During the two-year survey, we observed six major post-harvest diseases affecting mandarins – Alternaria rot caused by *Alternaria* spp., blue mold caused by *Penicillium italicum*, green

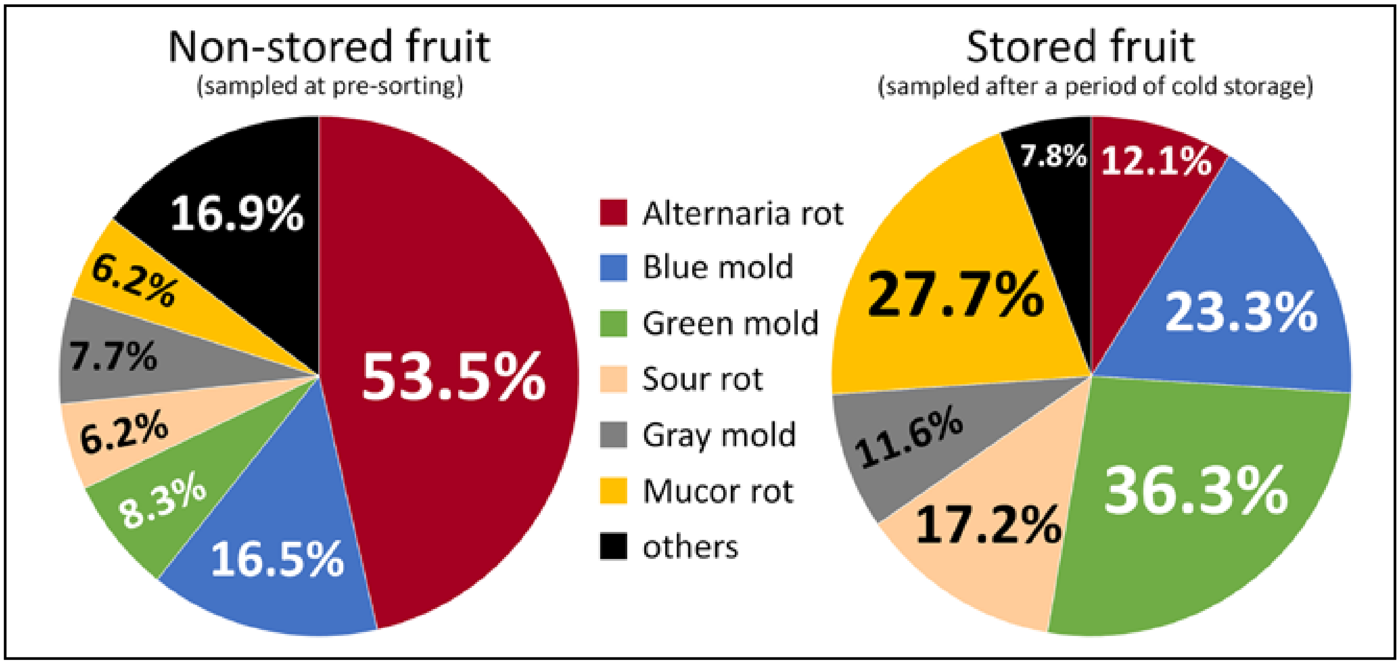

Figure 3. Percentages of post-harvest rot caused by various pathogens in the total decayed mandarin fruit sampled in 2015. Non-stored fruit and stored fruit were sampled from 11 and 21 grower lots, respectively. The values are the mean percentages of each disease in the total decayed fruit within a grower lot. Total percentage may exceed 100 percent due to multiple infections on the same fruit.

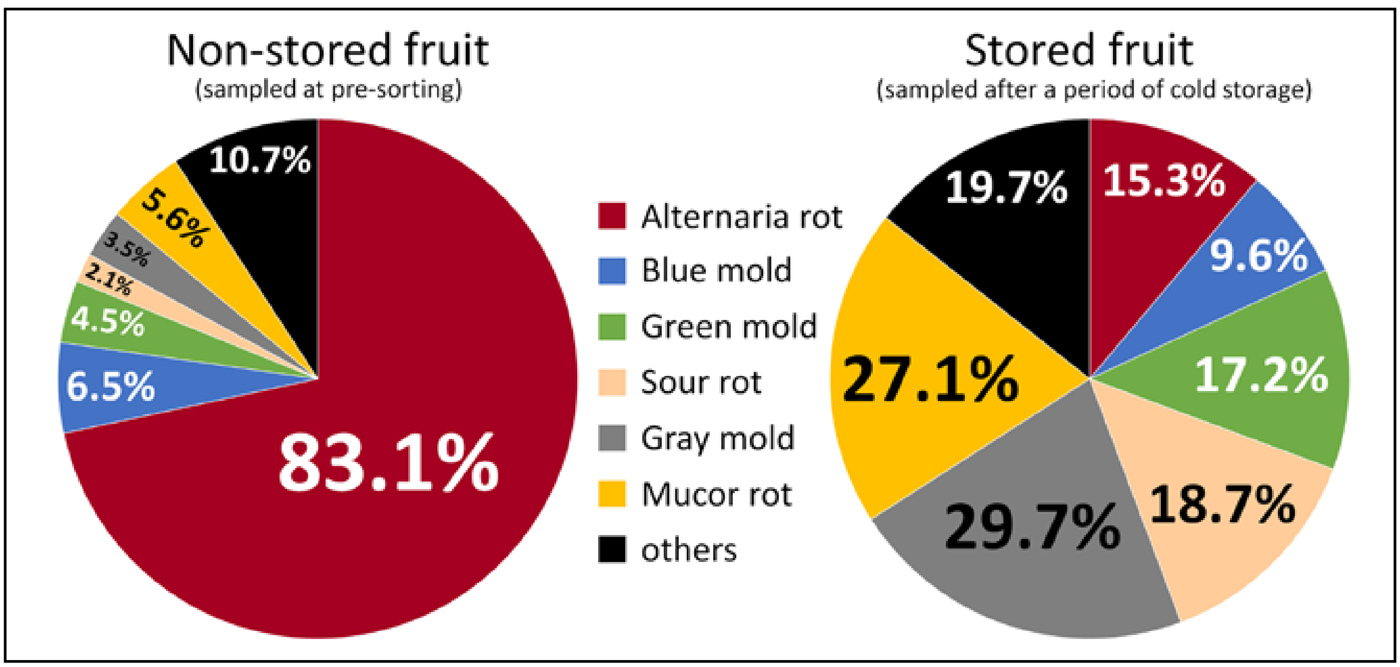

Figure 4. Percentages of post-harvest diseases caused by various pathogens in the total decayed mandarin fruit sampled in 2016. Non-stored fruit and stored fruit were sampled from nine and 25 grower lots, respectively. The values are the mean percentages of each disease in the total decayed fruit within a grower lot. Total percentage may exceed 100 percent due to multiple infections on the same fruit.

mold caused by *P. digitatum*, sour rot caused by *Geotrichum* spp., Mucor rot caused by *Mucor* spp., and gray mold caused by *Botrytis cinerea* (**Figures 1** and **2**). Of the six diseases, Alternaria rot, blue and green molds and sour rot are the common California citrus post-harvest diseases. Mucor rot and gray mold are viewed as emerging since they previously had not been targeted as post-harvest California citrus decay control diseases.

Among the decayed fruit sampled, the percentage of these individual diseases varied by type. On non-stored fruit (i.e., decayed fruit sampled at pre-sorting), Alternaria rot was the most prevalent disease, accounting for 53.5 percent and 83.1 percent in 2015 and 2016, respectively. All other diseases accounted for only small percentages of rot (**Figures 3** and **4**).

On cold-stored fruit (i.e., fruit that had been stored for an extended period of time), green mold was the most prevalent disease (36.3 percent), followed by Mucor rot (27.7 percent) and blue mold (23.3 percent) in 2015, while gray mold was the most prevalent (29.7 percent), followed by Mucor rot (27.1 percent) and sour rot (18.7 percent) in 2016 (**Figures 3** and **4**). The percentage of those post-harvest rots differed greatly from one grower lot to another in each year.

Mucor rot is a newly recognized post-harvest California citrus rot disease (Saito et al. 2016). Our study showed that Mucor rot of California mandarins is caused by five *Mucor* species, primarily *Mucor piriformis*, which is a soil-borne fungus present in the field. Inoculum of *M. piriformis* is likely brought to packing and storage facilities via contaminated harvest bins and fruit. It can actively grow at temperatures ranging from 32-81°F (0-27°C), a range that includes the cold temperatures used to store mandarins, leading to Mucor rot after a period of time in cold storage. This may explain why more of it was seen on mandarins after extended storage. Mucor rot also has been observed on lemons and oranges.

The causal agent of gray mold, *Botrytis cinerea*, is a widespread fungal pathogen. This pathogen infects a wide range of crops grown in the Central Valley of California, including grapes, blueberries, stone fruits, pistachios, etc. Gray mold can be a significant problem on stored mandarins and lemons. Infection of fruit by *B. cinerea* occurs in the field, leading to gray mold during storage. Control is largely dependent on pre- and post-harvest fungicide applications. However, *B. cinerea* is a high-risk pathogen for the development of fungicide resistance. Fungicide-resistant strains of *B. cinerea* are common in blueberry and table grape fields in the San Joaquin Valley, and these resistant strains can be a source of inoculum for citrus grown in adjacent fields, posing a challenge for effective control of gray mold in mandarins.

In summary, our survey results indicated that Mucor rot and gray mold are two emerging post-harvest diseases on mandarins, particularly on those fruit that had been stored at 45°F (7.2°C) for an extended period of time after pre-sorting. As storing mandarins in cold facilities has become a common practice to retain fruit quality and expand the marketing window, control of post-harvest diseases including gray mold and Mucor rot has become particularly important to mandarin storage. Research is currently underway to develop post-harvest programs that are effective not only for control of common post-harvest diseases such as green mold and sour rot, but also for control of emerging mandarin diseases such as Mucor rot and gray mold.

CRB Research Project #5400-150

Acknowledgements

This research was supported in part by the Citrus Research Board. We acknowledge the valuable contributions of Tarcisio Ruiz, who first recognized the importance of the unknown disease, later identified as Mucor rot, to the citrus industry. We thank K. Fjeld, D.A. Margosan and J.L. Smilanick, Ph.D., for technical assistance and the personnel of the three citrus packinghouses for assistance in sample collection.

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Figure 1. Post-harvest treatment of Valencia oranges using a recycling fungicide flooder application system containing an organic acid sanitizer to meet Food Safety Modernization Act (FSMA) regulations on sanitizing water systems in contact with food.

New Food Safety Rules Challenge Recycling Post-harvest Fungicide Systems

James Adaskaveg, Daniel Chen, Kevin Nguyen and Helga Förster

Project Summary

Under the Food Safety Modernization Act (FSMA), fruit packinghouses must sanitize recirculating fungicide solutions to ensure that food-borne human bacterial pathogens do not accumulate to cause illnesses in consumers. Typically, packinghouses use oxidizers such as sodium hypochlorite or peroxyacetic acid for sanitation. These compounds may be incompatible with certain fungicides and produce odors that may irritate workers. To identify alternative, highly effective and odorless sanitizers, we tested two organic acids: lactic acid (LA) and citric acid (CA) that are designated Generally Recognized as Safe or exempt from tolerance in the United States. These organic acids and their commercial formulations are registered for food use and have high toxicity against most food-borne human bacterial pathogens. Using surrogates for bacterial pathogens, we demonstrated that they can be effectively used to sanitize fungicide solutions without loss of fungicide activity for human bacterial pathogens and no phytotoxic effects to fruit.

As part of a post-harvest research project to develop new post-harvest fungicides and effective application strategies, we are addressing challenges for the citrus industry to meet new regulations under the FSMA, whose overall goal is to protect public health and reduce food-borne bacterial pathogens by strengthening the food safety system. The rules provide a preventive framework where safety standards are established by the United States Food and Drug Administration (FDA) for produce safety and preventive controls for human food. The food industries themselves in the United States are responsible for verifying that standards are met through accreditation by third-party auditors for domestic food production and for foreign suppliers through a separate verification program.

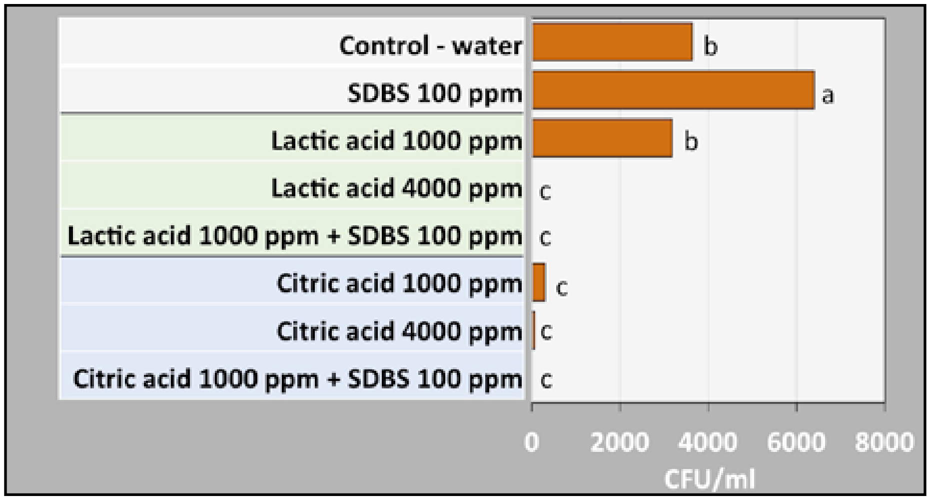

Figure 2. Laboratory toxicity of lactic acid or citric acid alone and in combination with a food-grade surfactant (SDBS) to **Escherichia coli** ***(surrogate strain K-12) after one hour of exposure. Toxicity was determined by plating suspensions onto agar media and enumerating colony-forming units (CFU). Bars followed by the same letter are not significantly different statistically (P<0.05). 1 µg/ml = 1 ppm***

Post-harvest fungicide recycling, in-line drench application systems have become a standard method for achieving the best citrus decay control for the last 15 years. Low-volume systems have an advantage with no disposal issues; however, high-volume systems (**Figure 1**) have out-performed low-volume applications systems because they provide better fungicide coverage. Low-volume systems rely on brushes to distribute high concentrations of a post-harvest fungicide over the fruit surface; whereas, in high volume systems, lower concentrations of fungicides are carried to fruit injuries using aqueous suspensions and evenly coat the entire fruit surface. The result is high efficacy, lower use rates, reduced fungicide residues on fruit and economic use of costly fungicides. For some fungicides, waste fungicide solutions from recycling systems can be disposed of easily through the sewer system or they can be used for dust control on orchard roads. We demonstrated these advantages in numerous studies over many years. Furthermore, the foundation for fungicide resistance management of post-harvest decay organisms such as *Penicillium* species is to obtain the highest level of control to limit the number of survivors that have the potential to develop resistance.

Any aqueous solution in contact with environmental samples and kept for a prolonged period of time at ambient temperatures is subject to microbial contamination. Contaminants may include innocuous environmental bacteria and fungi, as well as pathogenic fungi of citrus, but of most concern may be human bacterial pathogens contaminating these aqueous solutions. Although fruit always are washed with a sanitizing agent prior to exposure to re-cycling fungicides solutions, contamination can still occur because 100 percent of the fruit surfaces cannot be cleaned free of microbial contamination. Additionally, there may be other sources of contamination if solutions are kept for extended periods. To meet safety standards, these solutions can be effectively sanitized using oxidizers such as sodium or calcium hypochlorite or peroxyacetic acid (PAA). Two problems, however, immediately occurred:

1. some fungicides of different modes of action[1] are incompatible with these sanitizers causing a breakdown of the active ingredient and subsequently the performance of the fungicide; and
2. adding these sanitizers at concentrations that are effective for killing human bacterial pathogens may result in offensive odors that may irritate packinghouse workers if adequate ventilation is not available.

To identify alternative, highly effective and odorless sanitizers, two food-grade organic acids were tested – lactic acid (LA) and citric acid (CA). CA is designated 'Generally Recognized as Safe'[2], whereas LA is 'exempt from tolerance'[3] in the United States. Commercial formulations of organic acids registered for food use have shown high toxicity against most food-borne human bacterial pathogens including *E. coli* O157:H7 and pathogenic strains of *Listeria, Salmonella, Pseudomonas, Shigella*, *Vibrio* and *Yersinia*. LA and CA were tested *in vitro* and in fruit assays against a non-pathogenic surrogate strain of *Escherichia coli* (substitute for pathogenic *E. coli* O157:H7) and against *Pseudomonas syringae* (substitute for *P. aeruginosa*).

In direct exposure assays, bacteria-contaminated solutions of selected post-harvest fungicides with different modes of action including azoxystrobin, fludioxonil, imazalil or natamycin (newly registered post-harvest biofungicide for citrus) were exposed to 1,000-4,000 micrograms per milliliter (µg/ml) LA or CA for selected times and then plated onto agar media. Viability of bacteria was assessed based on colony counts. At ambient temperature (24°C), all bacterial cells were killed within 24 hours. *P. syringae* cells also were killed using lower concentrations of acids and shorter exposure times. The addition of 100 µg/ml of the food-grade surfactant sodium dodecylbenzenesulfonate (SDBS) improved the efficacy of LA and CA against *E. coli*, and all bacterial cells were killed within one hour (**Figure 2**). In comparison, PAA at registered rates also was highly toxic to both bacterial species. Initial studies demonstrated that exposure to heated (48°C) fungicide solutions amended with 1,100 µg/ml CA and 60 µg/ml SDBS inactivated *E. coli* cells within 60 seconds, resulting in 4.3- to 3.6-log population reductions as compared to the heated water control. Subsequent studies adjusting CA and SDBS concentration showed nearly 100% reduction (**Figure 3**). Furthermore, the bactericidal activity was maintained when solutions were re-contaminated with bacteria after 24 hours. These results demonstrate that heated, acidified fungicide solutions are highly effective in killing bacteria within short time periods.

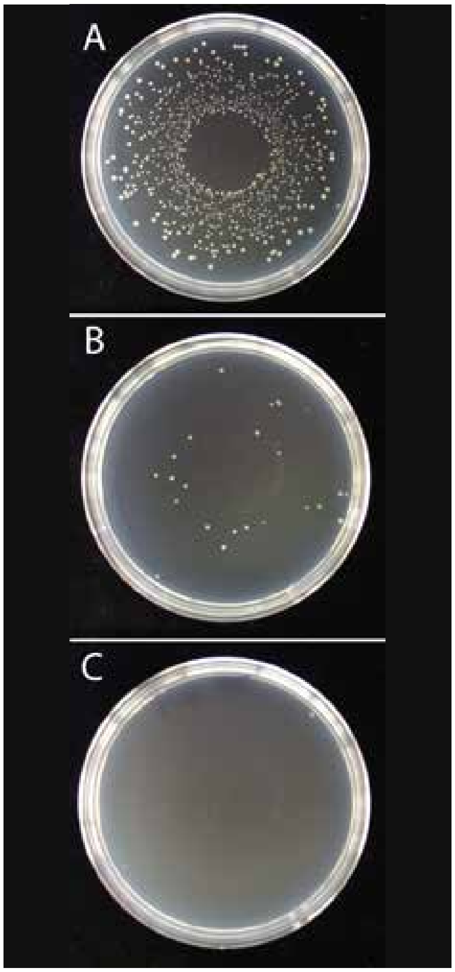

Figure 3. Effect of citric acid (2,000 µg/ml acidification of fludioxonil (300 µg/ml) fungicide solutions on viability of Escherichia coli *strain K12 after two minutes of exposure. A. Control (fludioxonil only); B. Fludioxonil + citric acid; and C. Fludioxonil + citric acid + SDBS (120 µg/ml). 1 µg/ml = 1 ppm*

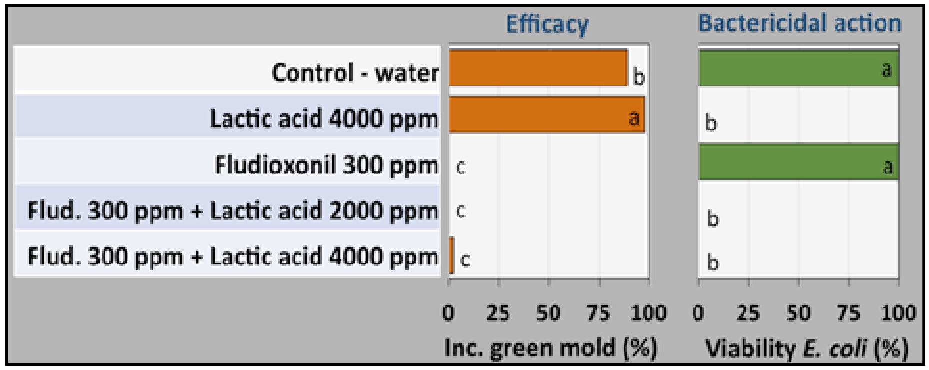

Figure 4. Toxicity and performance of lactic acid, fludioxonil (Graduate®), and combinations of the two compounds. Efficacy was determined by treating lemon fruit 14 hours after inoculation with Penicillium digitatum *and evaluating the incidence of green mold. No phytotoxicity was observed with any of the treatments. Bactericidal action or toxicity was based on the viability of* Escherichia coli *K12 after one hour of exposure to a 24 hour-old solution. Viability was determined by plating suspensions onto agar media and is expressed as a percentage of colonies present relative to the water control. Bars followed by the same letter are not significantly different statistically (P<0.05). 1 µg/ml = 1 ppm*

In fruit studies, fungicide–lactic or -citric acid solutions were prepared 24 hours before dip-treating lemon fruit inoculated with *Penicillium digitatum*. Azoxystrobin, fludioxonil, imazalil and natamycin were similarly effective against *P. digitatum* when used in combination with acids as when used by themselves at ambient temperature (24°C) (fludioxonil and lactic acid results shown in **Figure 4**). Additionally, flooder treatments with heated (48°C) solutions of azoxystrobin, fludioxonil or natamycin containing 2,000 µg/ml CA and 110 µg/ml SDBS were as effective against *P. digitatum* as the fungicide-only treatments. *E. coli* cells added to the heated fungicide solutions before fruit treatment were inactivated within one hour. No phytotoxicity was observed on fruit with any of the fruit treatments containing either acid at rates evaluated when used in combination with the SDBS surfactant.

In conclusion, LA and CA have the potential to be effective sanitizers that are compatible with important post-harvest fungicides of citrus fruit. Our results provide strong evidence that acidification is an alternative, highly effective sanitation method to oxidation for recirculating fungicide solutions. Commercial formulations of citric acid and SDBS (e.g., Pro-San®, Microcide, Inc.) and a mixture of citric and lactic acid (e.g., Veggiexide™, Birko Co.) are available. These products may be used as "drop-in" replacements for chlorine or peroxyacetic acid to sanitize post-harvest fungicide solutions in recycling systems without causing odors in the packinghouse that may be irritants for workers. Additionally, treatments with these products will allow compliance with third-party audits as required by FSMA regulations.

CRB Research Project #5400-103

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Glossary

[1]**Mode of action:** Fungicides inhibit fungal growth by interfering with critical cellular processes. Mode of action (MOA) refers to the specific cellular process inhibited by a particular fungicide.

[2]**Generally recognized as safe (GRAS):** A property of a common substance that is considered by the FDA as not harmful when found or used on food.

[3]**Exempt from tolerance (EFT):** Expression used by the US Environmental Protection Agency for materials that are considered safe for the environment and consumers and that are not required to have a tolerance or maximum residue limit (MRL) on food.

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Tamarixia are released onto a residential tree. (Photo courtesy of CPDPP)

Identification and Characterization of Two Candidate Predator Species for ACP Biocontrol

Aviva Goldmann and Richard Stouthamer

Project Summary

High densities of Asian citrus psyllid (ACP) in residential neighborhoods in southern California pose a greater threat than ever now that additional trees have tested positive for the bacterium associated with huanglongbing (HLB) and the quarantine zone has expanded. Effective biological control of ACP is urgently needed because residential trees are difficult to access. Reports of heavy psyllid mortality in southern California initially suggested the presence of effective local predators. We conducted a three-year project to identify these predators, first quantifying ACP mortality caused by predation, then designing a molecular gut test for ACP predation which we used to test predators collected in a year-long survey. We identified several spiders and two insects that frequently ate ACP and further characterized the predation habits of the two insects, ladybeetle Diomus pumilio *and lacewing* Chrysoperla comanche.

Figure 1a: Adult* Chrysoperla comanche*. ***Figure 1b:* Chrysoperla comanche *larva***

Role of Local Natural Enemies

The complexity of the urban landscape has resisted effective treatment of residential citrus since southern California was invaded by ACP in 2008, undermining area-wide management and leading to the build-up of large ACP populations in residential neighborhoods (USDA-APHIS 2010). Now that HLB has begun to spread among neighborhood trees (Citrus Pest & Disease Prevention Program 2017), treating residential citrus for ACP is more important than ever. Reducing ACP populations is necessary to slow the spread of the HLB-associated bacterium '*Candidatus* Liberibacter asiaticus' from residential areas to commercial citrus, because transmission rate increases with the number of infected insects feeding on a tree (Pelz-Stelinski et al. 2010). Unfortunately, treating residential trees remains difficult and expensive. New control options, including improved biological control—the use of beneficial arthropods to reduce a pest population—are urgently needed to address these gaps in management (USDA-APHIS 2009, Hall et al. 2012).

Effective biological control depends on effective natural enemies. Biological control programs often use specialist co-evolved predators and parasitoids that successfully control a pest in its native region. Unfortunately, this is not an option for ACP, which is not adequately controlled by co-evolved natural enemies, neither in its area of origin (Shivankar et al. 2000, Khan et al. 2014) nor in California, where two of these specialists have been imported (Kistner et al. 2016a, Vankosky and Hoddle 2017). However, local predators kill a large number of ACP in southern California (Goldmann et al. 2014, Kistner et al. 2016b). It follows that the resident predator ACP community in southern California has the potential to yield new species for biological control of ACP, and these species may be capable of causing even more mortality if identified and properly managed.

Project Goals

The goal of this project was to identify effective ACP predators among the citrus predator community in southern California. To this end, we first confirmed that local predators reduce ACP in southern California (Goldmann et al. 2014). We then conducted a year-long survey of predators collected before dawn and during late afternoon in an organic Valencia orange orchard in Orange County and a conventionally managed, but unsprayed, navel orange orchard in San Bernardino County. While conducting this survey, we designed a molecular method to identify the remains of ACP in predators' gut contents and tested approximately half the collected specimens (up to 100 per type) of each of the most abundant predator species for ACP predation. We further evaluated the most promising insect species identified by this procedure: we learned which life stages of ACP they prefer as prey and obtained estimates of how fast each one digests ACP, allowing us to understand how accurately gut content analysis represents how often each species eats ACP.

Survey Results

We collected more than 70 apparent species of predatory insects and more than 40 apparent species of spiders during the year-long survey. Of these, five predatory insects and five spiders were abundant enough to test for ACP predation using a DNA test we designed to detect the remains of ACP in predator guts.

The two insects with the highest rate of positive tests for ACP predation were the green lacewing *Chrysoperla comanche* and the lady beetle *Diomus pumilio*. Three spiders also frequently tested positive for ACP predation, indicating that spiders are likely contributors to conservation biological control[1] of ACP. Spiders cannot be mass-reared and, therefore, are not candidates for augmentative biological control. The two promising insect species have potential for use in both conservation and augmentative biological control. We studied the ACP predation habits of these two species further.

Chrysoperla comanche

The green lacewing *C. comanche* (**Figure 1**), or Comanche lacewing, is native to the American southwest and represented more than 99 percent of lacewings collected in our survey. It previously has been mass-reared and sold commercially,

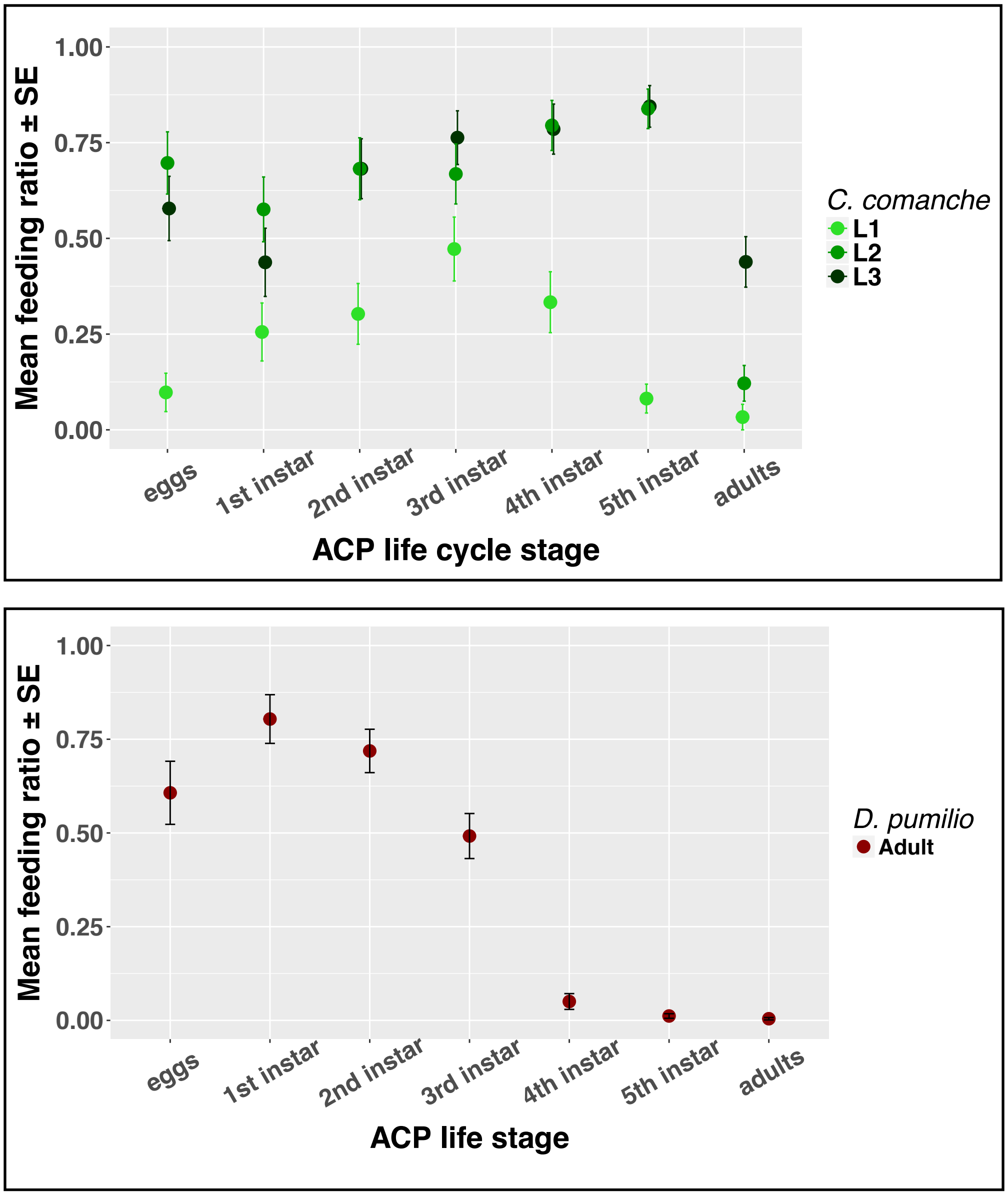

Figure 2: Ratios of feeding events to attacks (probing with mouthparts) by **Chrysoperla comanche** ***larval instars (top) and*** **Diomus pumilio** ***adults (bottom) for all life stages.***

but is no longer on the market. Adults, which are difficult to identify, are completely herbivorous. Larvae are easily distinguished by their head markings (Tauber 1974) and are voracious fluid-feeding predators of ACP. Over the course of development, a *C. comanche* larva can eat every ACP life stage, with younger *C. comanche* larvae preferring younger ACP nymphs (**Figure 2**). At the two study sites, *C. comanche* was present year-round, with a population peak in late winter/early spring at both sites. Digestion time analyses showed that a meal consisting of one second instar ACP nymph becomes undetectable by our molecular gut test in 50 percent of *C. comanche* larvae after only 0.7 hours, indicating that our gut test may underestimate how often or how many ACP are eaten.

Diomus pumilio

The lady beetle *D. pumilio* (**Figure 3**) is not native to the Americas, and its establishment in California citrus is a new and surprising development in a century-long story of citrus biocontrol. Between 1892 and 1970, it was repeatedly brought to California from Australia and released in a string of failed introductions aimed at various citrus pests (Essig 1931, p. 366-367). None became established until the early 1970s, when *D. pumilio* was released in California for biological control of acacia psyllid (Ferris and Klyver 1932). By 1977, *D. pumilio* was established and contributes substantially to the successful biological control of acacia psyllid (Pinnock et al. 1978). Acacia psyllid was brought under control, and the beetle has not been monitored since. Interestingly, the one lacewing that was found preying on acacia psyllid alongside *D. pumilio* was the above-mentioned *C. comanche*. This was the first case of successful classical biological control against a psyllid (Dreistadt and Hagen 1994). Prior to our study, *D. pumilio* had not been found in citrus, and acacia psyllid was its only recorded natural prey (Leeper and Beardsley 1976).

The adult beetle is less than two millimeters long and sexually dimorphic[2] (**Figure 3**). Adults are chewing predators, with both sexes preferring younger ACP nymphs (**Figure 2**). *Diomus pumilio* larvae are fluid-feeders and can be reared to maturity on a diet of ACP. Older larvae can consume up to fifth instar ACP nymphs by seizing a leg and fluid-feeding through it until the psyllid is drained. Digestion time analyses showed that a meal consisting of one second instar ACP nymph becomes undetectable to our molecular gut test in 50 percent of *D. pumilio* adults after only 2.8 hours, indicating that our gut test may underestimate how often or how many ACP are eaten, but to a lesser extent than with *C. comanche*.

Potential Use in Biological Control

Field testing is needed to determine whether mass releases of these insect predators would reduce ACP abundance.

Figure 3: **Diomus pumilio** ***adults, female (top) and male (bottom).***

If so, they could be used to help manage ACP on trees that are not or cannot be treated chemically, such as residential citrus, abandoned orchards and organic groves. Both species previously have been mass-reared, and rearing protocols for *D. pumilio* and *Chrysoperla* species are found in the literature.

Argentine ant control is a major issue in ACP biocontrol, as ants reduce the effectiveness and diversity of predators feeding on ACP. Our study sites were heavily infested with Argentine ants. Good Argentine ant control may improve the performance of *C. comanche* and *D. pumilio* while allowing less ant-tolerant predator species to increase. As residential and other hard-to-reach trees likely will remain ant-infested, some degree of ant tolerance is valuable for ACP biocontrol agents.

These species are the most promising new candidates for ACP biocontrol that could be located within the study's test area – the only area available that contained both untreated groves and sufficient ACP when this study began. However, other regions may harbor additional undiscovered predators. The molecular methods we developed to detect ACP predation are available to other researchers to speed the process of identifying potential ACP predators in other regions.

Although spiders cannot be mass-reared, we have confirmed several as ACP predators. This suggests a potential role for spiders in conservation biological control. Field testing is needed to determine the role of these spiders in reducing ACP.

Conclusions

Chysoperla comanche and *D. pumilio* have potential for future use in biological control of ACP. Both species feed on ACP frequently in the field, are able to consume most or all ACP life stages over the course of their development, and each individual predator has the potential to consume many ACP over the course of its lifespan. The next recommended step in utilizing these species is to field test their ability to reduce ACP on the types of citrus plantings where improved biological control is desired, whether residential trees, abandoned groves or organic orchards, with and without Argentine ant control. Conservation biological control programs may benefit from consideration of pesticide sensitivities of these species, as well as those of spiders.

CRB Research Project #5500-197

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Glossary

[1]**Conservation Biological Control:** Providing support, such as habitat, for natural enemies in order to reduce a pest insect population.

[2]**Sexual Dimorphism:** Differences in appearance between males and females of the same species.

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Female Diaphorencyrtus aligarhensis *searching for suitable Asian citrus psyllid hosts to parasitize*
(Photo: Mike Lewis, Center of Invasive Species Research, UCR).

Building a Coalition

Biocontrol of ACP by Tamarixia *and* Diaphorencyrtus *in California*

Ivan Milosavljević, David J.W. Morgan and Mark S. Hoddle

Project Summary

*The Asian citrus psyllid (ACP) –'*Candidatus *Liberibacter asiaticus' (CLas) complex associated with huanglongbing (HLB) has become an increasingly prevalent and serious threat to California citrus producers. ACP-CLas threatens both backyard, and more importantly, commercial citrus groves. Decisive actions were made in 2011 and 2014 with the release of two Pakistani biocontrol agents,* Tamarixia radiata *and* Diaphorencyrtus aligarhensis *to combat ACP, and by extension, the spread of CLas in southern California. One priority for California's citrus industry has been to slow the spread of CLas by establishing these two natural enemies across infested regions to suppress ACP populations in areas where management with insecticides is either minimal or not feasible, such as urban environments or organic citrus groves. It is anticipated that biological control of ACP in California will complement other management efforts targeting ACP-CLas because lower density ACP populations will reduce the rate of spread of CLas.*

Figure 1. Asian citrus psyllid life stages: (A) adult psyllid feeding on a citrus leaf; (B) ACP eggs (yellow spots in flush crevices), dried curly white ACP honeydew, large fifth instar nymphs (some exhibiting wing pads) and a winged adult psyllid on citrus flush **(Photos: Mike Lewis, Center of Invasive Species Research, UCR).**

Classical biological control of ACP-CLas in California

California's $3 billion-per-year citrus industry faces an unprecedented threat from the ACP-CLas complex[1] (Milosavljević et al. 2017), where it is still largely an urban problem. ACP (**Figure 1**) is widely established in southern California's residential settings where homeowners provide little control and eradication efforts have been impractical. However, migration of CLas-infected ACP from urban infestation foci into nearby commercial citrus groves could greatly exacerbate this problem. Thus, a priority for the industry has been to reduce CLas spread through population reduction of its vector, ACP. In the absence of a sustained and effective pesticide treatment campaign in urban areas, classical biological control[2] was viewed by the industry as a viable management option for ACP control in areas devoid of pesticide-based management (Schall and Hoddle 2017). While very high levels of vector control are needed to stop the spread of CLas, reduction of ACP densities in urban areas may help diminish rapid CLas spread from infected backyard trees into commercial citrus production areas.

The main objective of the ACP biocontrol program developed cooperatively by researchers from the University of California, Riverside (UCR), the Citrus Research Board, the California Department of Food and Agriculture (CDFA), and the U.S. Department of Agriculture–Animal and Plant Health Inspection Service (USDA-APHIS) is to mass-rear and make widespread releases of two ACP parasitoids[3] imported from Punjab, Pakistan, *Tamarixia radiata* and *Diaphorencyrtus aligarhensis*. The goal is to establish these two parasitoid species throughout ACP-infested areas of California.

Why release both parasitoids in California?

Tamarixia radiata and *D. aligarhensis* have been released in southern California because it is expected that they may complement each other if both species establish, and together they will provide superior ACP pest population reduction than either parasitoid species alone. Both parasitoids have been credited with significantly decreasing the ACP densities in Taiwan and Réunion Island. In California, *T. radiata* was first released in December 2011. Considerable program success has been achieved with *T. radiata* over the last five years including the mass production and release of approximately six million parasitoids throughout the infested areas of eight counties in southern California (Imperial, San Diego, Orange, Riverside, San Bernardino, Los Angeles, Ventura and Santa Barbara) (**Table 1**). Evidence of widespread establishment at more than 90 percent of release sites in southern California, including recoveries in areas distant from release sites indicating extensive natural dispersal (Hoddle et al. 2016), and parasitism by *Tamarixia* as high as 63 percent have been observed in some areas at certain times of the year (Kistner et al. 2016a). Post-release monitoring in southern California indicates that average year-round parasitism by *T. radiata* is moderate at about 21 percent, varying greatly across locations

Table 1. Releases of *Tamarixia radiata* and *Diaphorencyrtus aligarhensis* parasitoids across eight counties in southern California as of June 2017 (data provided by CDFA).

County	Urban grid releases				Grove releases			
	# Grids / County	# Release events	*T. radiata*	*D. aligarhensis*	*T. radiata*		*D. aligarhensis*	
			# Released	# Released	# Groves	# Released	# Groves	# Released
Imperial	80	658	171,737	10,295	5	14,800	0	0
Los Angeles	188	3,630	1,502,975	79,518	9	6,440	6	1,240
Orange	70	1,093	438,690	46,023	3	1,285	0	0
Riverside	197	3,472	1,260,649	86,997	91	61,606	3	2,961
San Bernardino	72	1,259	463,758	35,644	52	298,181	19	1,939
San Diego	206	2,441	1,043,122	59,158	144	114,217	12	13,173
Ventura	69	1,023	558,008	16,830	17	24,700	0	0
Santa Barbara	58	92	35,212	9,931	2	2,000	0	0
TOTAL	**940**	**13,668**	**5,474,151**	**344,396**	**323**	**523,229**	**40**	**19,313**
Total *T. radiata* released: 5,997,380			**Total *D. aligarhensis* released: 363,709**			**Total parasitoids released: 6,361,089**		

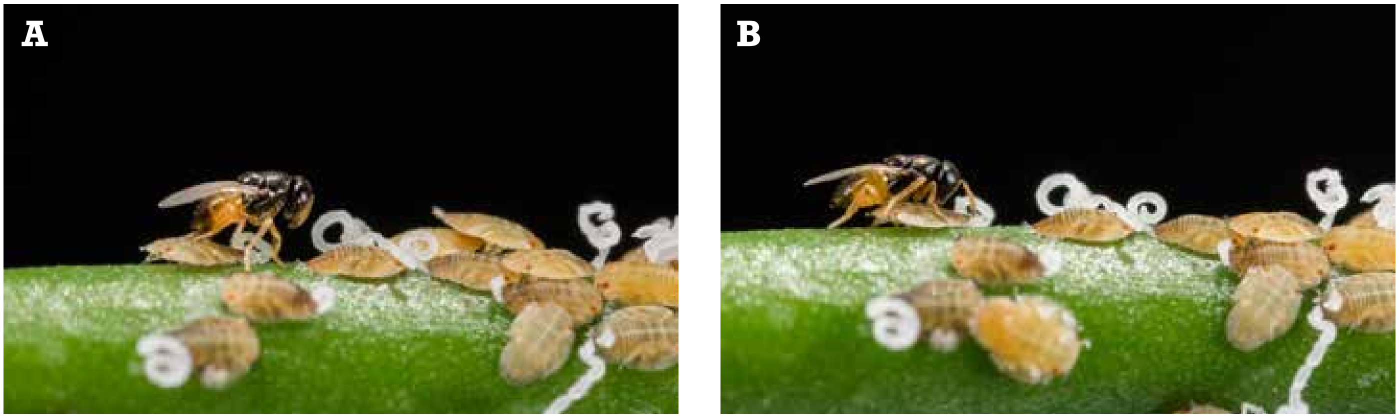

Figure 2. A female **Diaphorencyrtus aligarhensis** *host feeding on ACP nymphs. (A) Female* **D. aligarhensis** *obtain nutrients from their hosts by "stabbing" them with the ovipositor*[9] *and (B) consuming the leaking body fluids. The combined trauma of "stabbing" and "drinking" is often sufficient to kill ACP nymphs and host feeding by parasitoids is likely an under-appreciated ACP mortality factor* **(Photos: Mike Lewis, Center of Invasive Species Research, UCR).**

and over time. However, it is likely that *Tamarixia*-inflicted mortality on ACP is under-estimated because mortality from host feeding[4] and loss of parasitized ACP nymphs through intraguild predation[5] are very hard to quantify in the field (Kistner et al. 2016b).

In December 2014, *D. aligarhensis* joined the urban crusade against ACP. *Diaphorencyrtus aligarhensis* kills ACP nymphs, as does *T. radiata*, through a combination of parasitism and host feeding (**Figures 2** and **3**). To date, more than 360,000 *D. aligarhensis* have been released in southern California by UCR and CDFA (**Table 1**). Releases are made based on a standardized grid release system (i.e., within a one square mile grid overlaid on the greater release area). *Diaphorencyrtus aligarhensis* releases encompass 285 sites spanning more than 285 square miles of residential areas in eight counties (**Figure 4**). Attempts were made initially to release *D. aligarhensis* in ACP-infested areas where competition from the widely established *T. radiata* may be low or non-existent, thereby easing at least one establishment barrier, interspecific competition. The rationale for attempting to establish *D. aligarhensis* in California was to complement parasitism by *T. radiata*, as the two natural enemies[6] successfully co-exist in citrus orchards in the native ACP range (i.e., Punjab, Pakistan), and they both additively contribute to ACP control. The two parasitoids attack different host stages and densities. *Diaphorencyrtus aligarhensis* lays eggs within the second through fourth stage nymphs and performs better under lower pest densities. *Tamarixia radiata* lays eggs on fourth and fifth stage nymphs and prefers higher ACP densities (reviewed by Milosavljević et al. 2017). Furthermore, *D. aligarhensis* and *Tamarixia radiata* may have different climate and habitat preferences, and heterogeneous climates and variations in citrus production habitat across major citrus production areas may favor establishment of *D. aligarhensis* in parts of southern California that could be unfavorable for *T. radiata*. Although speculative at this stage, both parasitoids may have potential to simultaneously contribute to ACP control in California, perhaps through resource, geographic or habitat partitioning.

Figure 3. The developmental biology of **Diaphorencyrtus aligarhensis***: (A) gravid female lays an egg within (parasitizing) an ACP nymph, (B)* **D. aligarhensis** *is a specific endoparasitoid of immature ACP (attacks second through fourth stage nymphs) and* **D. aligarhensis** *larvae feed and develop within a host and (C) adult* **D. aligarhensis** *emerge through a circular hole cut with the mandibles in the posterior of the host's abdomen* **(Photos: Mike Lewis, Center of Invasive Species Research, UCR).**

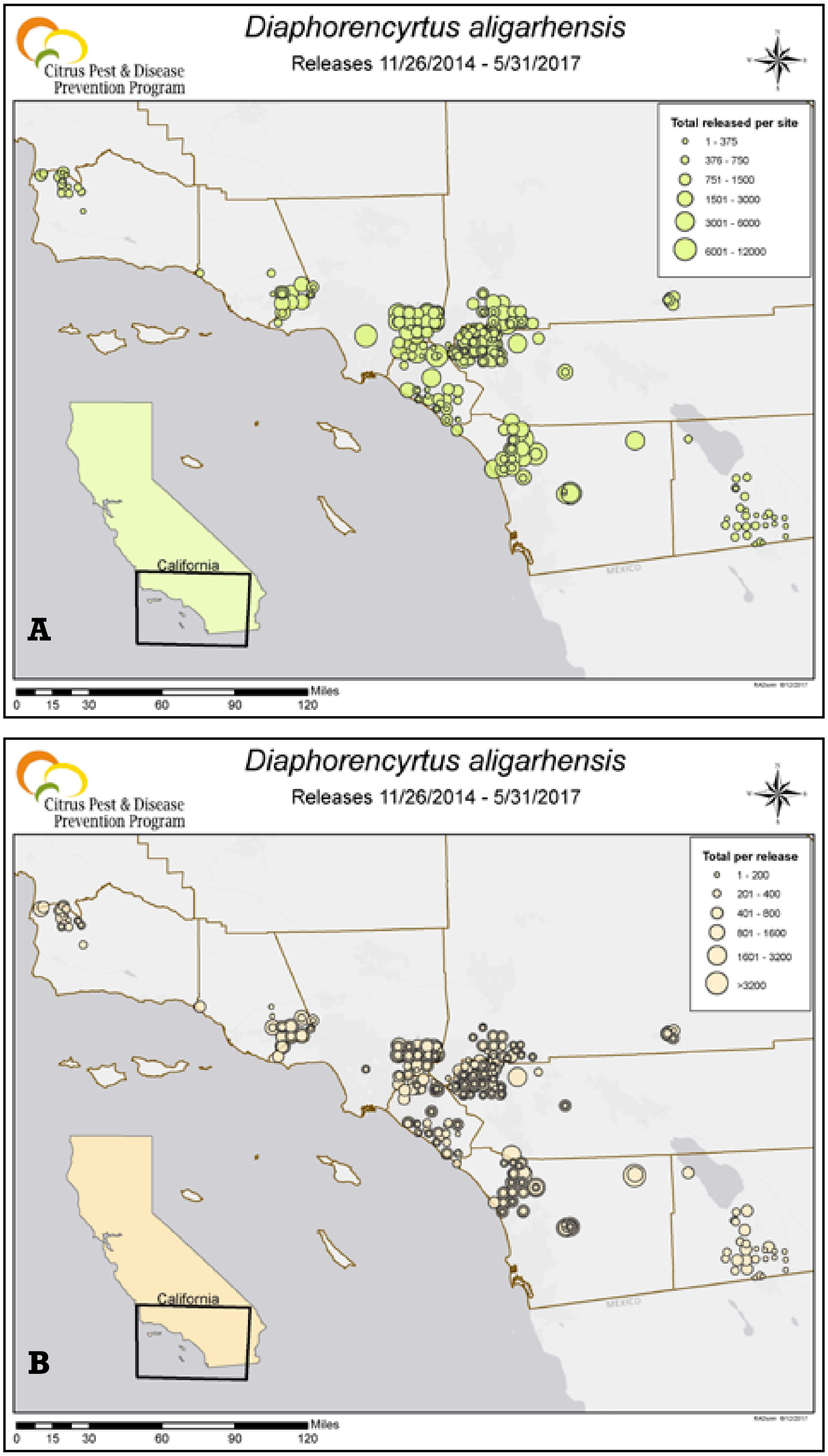

Figure 4. Release locations (as of June 2017) of **Diaphorencyrtus aligarhensis** *in southern California. The total number of parasitoids released per location (A) and release event (B)* **(Maps courtesy of Rick Dunn, Citrus Research Board director of data and information management, Visalia, California).**

Can *D. aligarhensis* establish and contribute in California ACP biocontrol?

A major research question that has arisen from the *D. aligarhensis* release program is "Can *D. aligarhensis* establish and have an impact on ACP in California?" As part of the classical biological control program targeting ACP, *D. aligarhensis* is being released and monitored at 15 residential gardens in Los Angeles, Orange, Riverside, San Bernardino and San Diego counties (**Table 2**; **Figure 5**). The release-monitoring program for *D. aligarhensis* began in California in October 2015. As of June 2017, 76,917 parasitoids (male and females) have been released in total at these 15 study sites. At each site, parasitoid releases and numbers released are contingent upon the presence of ACP life stages susceptible to *D. aligarhensis* attack and available flush on trees susceptible to ACP infestation. One orange and one lemon tree at each location will be monitored for a minimum of 24-26 months to determine (a) the capacity of *D. aligarhensis* to establish permanent populations in southern California and (b) the effect of *D. aligarhensis* activity (parasitism and host feeding) on ACP populations. To date, evidence of *D. aligarhensis* parasitism of ACP has been found at 13 surveyed sites, and multiple recoveries of D. aligarhensis have been made at about 60 percent of sites where this species had been released (**Table 2**). Average year-round parasitism of ACP nymphs by *D. aligarhensis* varies greatly across study sites and time of the year (up to 37.5 percent at certain locations), and higher rates of parasitism by *D. aligarhensis* have been observed on orange trees rather than lemons, which may be possible evidence of habitat or host plant preference (Milosavljević et al. unpublished data).

D. aligarhensis has failed to establish permanent populations in Florida, which may be attributable to several factors:

Table 2. Characteristics of 15 long-term study sites in urban areas across five Southern California counties as of June 2017. Traits measured include numbers released, numbers recovered, and parasitism rate of *Diaphorencyrtus aligarhensis* and presence or absence of *Tamarixia radiata*.

Site	County	*D. aligarhensis*			*T. radiata* status
		# Released	# Recoveries	Average % parasitism	
Los Angeles	Los Angeles	6602	1	0.23	Invaded
Claremont	Los Angeles	2788	2	3.85	Not Invaded
Pomona	Los Angeles	5750	3	2.59	Invaded
Fullerton	Orange	7086	0	0	Not Invaded
Anaheim	Orange	7670	6	13.83	Invaded
Irvine	Orange	6584	4	8.75	Invaded
Riverside	Riverside	3252	4	0.47	Invaded
Orange Crest	Riverside	4669	10	37.5	Invaded
Hemet	Riverside	3065	3	7.5	Invaded
Warner	San Bernardino	4138	0	0	Invaded
Loma Linda	San Bernardino	3058	2	1.0	Invaded
North Redlands	San Bernardino	2455	1	16.67	Invaded
Fallbrook	San Diego	4389	4	0.43	Invaded
Valley Center	San Diego	8547	2	10.0	Invaded
Ramona	San Diego	6864	11	12.13	Invaded
***D. aligarhensis* released: 76,917**					

***Figure 5. Ivan Milosavljević releasing* Diaphorencyrtus aligarhensis *parasitoids onto urban citrus flush infested with ACP nymphs* (Photo: Mike Lewis, Center of Invasive Species Research, UCR).**

1. feminization[7] effects of *Wolbachia* bacterial infections, which eliminate males from populations and the possibility of sexual reproduction, thus lowering genetic variation, which could negatively affect fitness traits such as survivorship, fecundity[8] and offspring sex ratio;
2. intensive broad-spectrum insecticide use in citrus production areas where *D. aligarhensis* has been primarily released;
3. lower competitiveness of *D. aligarhensis* when compared to the widely established *T. radiata*; and
4. relatively low release numbers and release frequencies, which further reduce the likelihood of *D. aligarhensis* establishment (Vankosky and Hoddle 2016).

It is possible, however, that California provides a better environment for *D. aligarhensis* establishment and proliferation than Florida. First, unlike Florida's ACP biocontrol program, which utilizes *Wolbachia*-infected female-only populations from Taiwan, California's biocontrol program releases sexually reproducing bi-parental populations of *D. aligarhensis* sourced from Punjab, Pakistan, an area with a 70 percent climate match with California. Sexual reproduction increases genetic variation and the possibility for adaptation to varying environmental conditions. Second, the majority of California's releases of *D. aligarhensis* occur exclusively in urban-residential areas where pesticide applications are limited, compared to Florida's releases, which primarily are focused on commercial citrus groves subjected to intensive pesticide treatments. Third, multiple recoveries of *D. aligarhensis* across different release sites over time tentatively suggest that *D. aligarhensis* could establish in southern California, which may further reinforce ACP biocontrol.

Answers to questions pertaining to the efficacy, establishment likelihood and rates of spread of *D. aligarhensis* into non-release areas are being obtained. As the field research phases of the classical biological control program are drawing to a close, analyses of collected data will provide insight as to how effective *D. aligarhensis* is likely to be and whether or not it is complementing population suppression provided by other species of ACP natural enemies in California, notably *T. radiata*.

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Glossary

[1]**ACP-CLas complex:** A pestiferous vector-disease partnership that threatens California's citrus production. ACP successfully vectors the plant pathogenic bacterium, *'Candidatus* Liberibacter asiaticus,' a causative agent of a deadly citrus disease, huanglongbing.

[2]**Classical biological control (biocontrol):** Intentional introduction of exotic natural enemies (i.e., predators, parasitoids and pathogens), usually those that are host specific and have co-evolved with the target pest in the native range, to suppress invasive pest populations in the introduced range to less damaging levels.

[3]**Parasitoid/parasitism:** An insect that feeds upon and develops within or attached to a host, eventually killing it. *Diaphorencyrtus aligarhensis*, an endoparasitoid that lives inside its host, is a specific parasitoid of immature ACP that prefers second through fourth stage nymphs. In contrast, *T. radiata*, an ectoparasitoid that feeds on the outside of its host, is a specific parasitoid of immature ACP that prefers fourth and fifth stage nymphs.

[4]**Host feeding:** Parasitoids obtain nutrients from their hosts by "stabbing" them with the ovipositor (see below) and consuming the emerging body fluids. Host feeding is very traumatic to the host and usually causes death.

[5]**Intraguild predation:** The attacking, killing and eating of potential competitors in the animal (including insect) world.

[6]**Natural enemies:** Parasitoids, predators or pathogens that attack pestiferous organisms.

[7]**Feminization:** Males infected with *Wolbachia* bacterium develop as females or infertile pseudo-females (or they may be killed during the early stages of development).

[8]**Fecundity (fertility):** The number of offspring produced by reproductively active females, which affects population growth rates.

[9]**Ovipositor:** Tubular organ through which female parasitoids deposit eggs on or inside their hosts.

Real-time PCR Co-detection of *'Candidatus* Liberibacter' Species and S. *citri*

Fatima Osman, Deborah Pagliaccia and Georgios Vidalakis

Project Summary

The purpose of this project was to resolve a diagnostic question faced by the California citrus industry. California and Arizona are the only states in the U.S. where 'Candidatus *Liberibacter asiaticus' (CLas) will super-infect trees that already may be infected with* Spiroplasma citri *(citrus stubborn). These two biologically similar microorganisms colonize the same tissues and induce similar symptoms in citrus plants. Therefore, interactions between CLas and* S. citri *in mixed infections are possible. These interactions–synergism or competition–may affect the titer and uneven distribution of* S. citri *and CLas in infected trees, thus making pathogen detection with the regulatory test of quantitative polymerase chain reaction (qPCR) challenging.*

If stubborn is misdiagnosed as huanglongbing (HLB), then unnecessary and costly regulatory actions may be taken. On the other hand, any HLB-positive trees that escape detection because they are misdiagnosed as stubborn-affected trees will serve as inoculum sources for the spread of HLB. Such diagnostic challenges may be catastrophic at this point in the spread of HLB in California. In this study, we developed biological and genomic information for the design and validation of a multiplex qPCR assay[1] for the simultaneous detection of

S. citri and 'Candidatus Liberibacter' species (i.e. asiaticus, africanus and americanus) in a single reaction. The biological results indicated that it was difficult to dually graft-inoculate citrus plants maintained under standard greenhouse conditions with CLas and S. citri. *However, when the dual infection was successful, the symptoms were devastating. The single sweet orange seedling, out of the eight co-inoculated with CLas and* S. citri *in our small-scale greenhouse experiment, developed severe yellowing and die-back symptoms in the first 12 months and died within 18 months post-inoculation, while the non-infected and single-infected plants remained alive. The analysis of available genomic sequences of the two targeted microorganisms indicated that the best region for multiplex detection of* 'Candidatus *Liberibacter' (CL) species and* S. citri *were the 16S rDNA and spiralin genes, respectively. This result also was supported by the validation and evaluation of 11 different qPCR assays designed for CL species and* S. citri *in various genomic regions. The selected multiplex qPCR assay was able to detect CL species and* S. citri *in a wide range of California and non-California samples, including those that tested negative with previously developed CLas detection assays. During this project, it also became obvious that while full genome sequences were available for the design of CLas qPCR assays, full genome sequences were not available for* S. citri, *thus limiting the qPCR assay designing potential. Therefore, we initiated the full genome sequencing of several* S. citri *isolates for future use in diagnostics and other citrus pathology studies.*

California and Arizona are the only states in the U.S. where CLas will infect trees that already may be infected with *S. citri*. The focus of this project was to resolve the unique diagnostic question faced by the California citrus industry to simultaneously detect CLas and *S. citri* with a regulatory-approved test of real-time quantitative polymerase chain reaction (qPCR[2]). This question had two parts – one biological for citrus (*S. citri* vs. CLas) and one technical for qPCR (SYBR green vs. TaqMan®[3]).

In terms of biology, the two pathogens induce remarkably similar citrus symptoms (e.g. blotchy mottle on leaves, off-season blooming and lopsided, unevenly matured fruit with aborted seeds). In addition, they are both low titer, unevenly distributed, phloem-limited and insect-transmitted bacterial pathogens, which means there is a possibility for pathogen interaction in dually-infected citrus trees. Such interactions, synergism or competition, may affect the titer and distribution of *S. citri* and CLas in citrus trees, thus making pathogen detection with qPCR challenging. California is in HLB exclusion and eradication mode; therefore, we cannot afford any misdiagnosis. In terms of qPCR, many researchers have developed *S. citri* and CLas detection methods; however, these methods were not designed to be compatible with each other and were not validated for use in a multiplex reaction for the simultaneous detection of both pathogens. For example, the regulatory-approved TaqMan®-probe qPCR assay targeting the 16S rDNA gene (Li et al. 2006 and 2007) cannot be multiplexed with the *S. citri* SYBR green qPCR assays targeting genes in the *S. citri* genome housekeeping (i.e. spiralin, P89 and P58) or prophage genes[4] (Yokomi et al. 2008 and Wang et al. 2015). When the multiplex qPCR assays developed in this study were compared to the regulatory-approved qPCR assay (Li et al., 2006 and 2007), they produced results with lower Cq valued samples and detected three *'Candidatus* Liberibacter' species (asiaticus, americanus and africanus), some of which were not detected by the approved *'C.* Liberibacter' qPCR assay (**Table 2**).

We developed biological and genomic information for the design and validation of a multiplex qPCR assay for simultaneous detection of *S. citri* and CL species (i.e. asiaticus, africanus and americanus) in a single reaction that can be used in field surveys and as part of a comprehensive high-throughput citrus diagnostic program.

HLB Plus Stubborn Biology on Co-infected Citrus

Citrus plants co-infected with CLas and *S. citri* were generated at the U.S. Department of Agriculture-Agricultural Research Service (USDA-ARS) Exotic Pathogens of Citrus Collection, Beltsville, Maryland, using standard graft-inoculation procedures. CLas isolates from Japan, China and California were co-inoculated with *S. citri* isolates from California on sweet orange and lemon seedlings maintained under standard greenhouse conditions. Plants were tested regularly by qPCR for the detection of the two microorganisms.

Based on the results of this experiment, co-infection of citrus plants via grafting was not easy. Only one out of the eight inoculated seedlings tested positive for both CLas and *S. citri* 13 months post-inoculation. The biological impact of co-infection of CLas and *S. citri* was devastating. The sweet orange plant developed severe yellowing and die-back in the first 12 months and died within 18 months post-inoculation; while plants singly infected with CLas and *S. citri* and non-infected plants remained alive (**Figure 1**). This observation was the result of a single tree in a small-scale greenhouse experiment and suggests that further work is needed to identify the interactions of these two pathogens when co-localized in citrus.

CLas Plus *S. citri* Multiplex qPCR

A significant amount of genomic information (i.e. sequences) for the targeted microorganisms is required for the proper design of multiplex qPCR detection assays. Following design, qPCR assays must be validated (for efficiency, robustness and sensitivity) and evaluated using real-life samples.

Table 1. Samples tested positive with the developed multiplex qPCR assay for '*Candidatus* Liberibacter' species and *Spiroplasma citri*.

Geographic Origin[1]	Number of samples	Citrus Host	Number of samples	Pathogen[2]	Number of samples	qPCR Cq Values Range[4]
Brazil	32	Blood Orange	5	CLaf	3	23.58-37.80
California	77	Calamondin	2	CLam	2	22.46-23.23
China	10	Citron	4	CLas	175[3]	20.02-37.60
Egypt	1	Citrus macrophylla	10	CLas+Sc	2	24.71-24.72+33.97-34.01
Florida	47	Grapefruit	14	Sc	50	25.88-37.73
France	1	Kaffir Lime	2		232	
Japan	1	Kumquat	1			
Pakistan	43	Lemon	4			
Reunion France	1	Mandarin	6			
South Africa	3	Navel Orange	5			
Syria	1	Psyllid	4			
Taiwan	1	Pummelo/Lemon	2			
Texas	9	Sweet Orange	61			
Thailand	1	Tangelo	1			
Turkey	4	Trifoliate Orange	2			
	232	Valencia Orange	11			
		Unknown	98			
			232			

[1]Brazil States: São Paulo and Minas Gerais, California areas: Hacienda Heights, San Gabriel, Kern and Tulare
[2]CLaf: 'Candidatus Liberibacter africanus,' CLam: 'C. L. americanus,' CLas: C. L. asiaticus, Sc: Spiroplasma citri
[3]Five samples tested negative for CLas by regulatory-approved qPCR (Li et al. 2006 and 2007) tested positive with the developed multiplex qPCR assay.
[4]qPCR: Quantitative polymerase chain reaction, Cq: quantification cycle

Table 2. Comparison between qPCR assays including multiplex qPCR to detect three HLB-associated '*Candidatus Liberibacter*' species in infected samples across a range of different geographical distribution.

Isolate	Origin/Genotype	qPCR COX[1]	qPCR 16S[2]	CL/*S. citri* Multiplex qPCR[3] CL	CL/*S. citri* Multiplex qPCR[3] *S. citri*
CM-3	*C. macrophylla* *	14.72	28.22	27.5	-
HH-11	Hamlin/Volk *	16.71	24.24	23.24	-
64	SCS/ Volk *	15.59	25.55	24.03	-
H11D	Psyllid	13.29	25.53	24.14	-
B1	Reunion France *	13.74	26.07	26.80	-
B430	Japan *	13	26.19	26.16	-
B437	Florida *	14.1	24.62	23.72	-
11-2003	South Africa #	13.39	25.98	23.58	-
C.L. afr.	South Africa #	15	28.02	27.54	-
Jose Bomifacio	Brazil @	15.92	29.41	27.3	-
Santa Maria	Brazil @	15.32	29.9	28.8	-
Lisbon Lemon	Hacienda Heights, Calif.*	16.95	27.08	26.88	-
SG CLas 2	San Gabriel, Calif.*	14.32	36.95	37.74	-
Comendado Gomes	Brazil @	17.64	-	34.76	-
Motuca	Brazil @	13.06	26.13	25.62	-
Aparecida D'oeste	Brazil @	16.59	-	37.60	-

** represents 'C. Liberibacter asiaticus', # 'C. Liberibacter africanus', @ 'C. Liberibacter americanus'*
[1]COX – Cytochrome C Oxidase gene – a plant host 'housekeeping' gene included to normalize qPCR expression levels across different samples.
[2]16S – Primers per Li et al. 2006
[3]Gene primers designed in this study, 16S rDNA for CL and spiralin gene for S. citri

Based on the GenBank available sequences of all CL species (191 sequences) and *S. citri* (20 sequences), we designed 11 qPCR assays (i.e. primers and probes) targeting different areas of the CLas and *S. citri* genomes. Preliminary validation and evaluation of these assays indicated poor efficiency and robustness or, most importantly, samples cross-reacted with the wrong set of primers. Such diagnostic assays are, of course, unacceptable and can have severe consequences for the spread of HLB in California.

The best regions for multiplex qPCR detection of CL species and *S. citri* were the 16S rDNA and spiralin genes, respectively. The 'C. Liberibacter' qPCR assay designed for this project has been designed in the same genome location as the approved regulatory diagnostic assay (Li et al. 2006); however, the multiplex qPCR assay designed in this report is universal and detects all

***Figure 1. Disease progression and real-time qPCR detection of* 'Candidatus *Liberibacter asiaticus' plus* Spiroplasma citri *co-infected sweet orange seedling. (*Photos by G. Vidalakis and C. Paul.)**

three CL species (CLas, Clam and CLaf) together with *S. citri* in a single reaction. In addition, the forward qPCR primers in the universal CL multiplex qPCR assay have been designed to detect extra genome isolates in the GenBank – in particular, CLas genomes of Hacienda and San Gabriel isolates, that posed the first threat to the California citrus industry. This multiplex qPCR assay was able to detect CL species in a wide range of California and non-California samples (**Table 1**). For California, the assay effectively detected 16 CLas isolates from Hacienda Heights and San Gabriel, as well as *S. citri* from the Citrus Clonal Protection Program (CCPP) Disease Bank and the Central Valley. In addition, the assay successfully detected CL species and *S. citri* from around the world, including isolates that tested negative with previously developed CLas detection assays (**Table 1**).

In conclusion, our results showed that the developed multiplex qPCR assay is capable of detecting both CLas and *S. citri* in mixtures, as well as in singly-infected samples from various geographic areas and citrus types. When compared to the currently approved CLas regulatory assay (Li et al. 2006 and 2007), it performed equally well or better (**Table 2**). Finally, the developed multiplex qPCR assay can streamline testing for multiple pathogens by replacing separate singleplex assays, thus reducing time and labor while retaining sensitivity and specificity. It can be incorporated into any high throughput qPCR citrus diagnostics laboratory such as those at the California Department of Food and Agriculture, USDA, universities, industry, the CRB or the Citrus Pest Detection Program of the Central California Tristeza Eradication Agency (CPDP-CCTEA).

Next Step: Full Genome Sequencing for *S. citri*

One of the biggest challenges of this project was the limited genomic information for *S. citri*, both in terms of number of sequences in the GenBank (20) and the coverage area of the genome (two genes and a few contigs[5]). As a result, we did not have many options for designing the primers and probes for the *S. citri* qPCR assay. Even though the spiralin gene produced acceptable Cq values, a large number of them were in the upper 30s, which is not preferable, especially in the case of mixed infection with CLas (**Table 1**). Therefore, in this project, we laid the foundation for the full genome sequencing of several *S. citri* isolates for future use in improved diagnostics, as well as other citrus pathology studies.

So far, pure cultures have been generated from nine *S. citri* isolates, using the protocol of Lee and Davis (1984). *Spiroplasma citri* DNA was purified (Li et al. 1990) and evaluated (UC Riverside Genomic Core Facility), and DNA libraries were constructed according to Pacific Biosciences (PacBio[6]) protocols. We currently are performing bioinformatics analysis[7] of the sequence data produced by the PacBio SMRT sequencing technology[8].

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Glossary

[1]**Multiplex qPCR assay:** A polymerase chain reaction-based technique that enables the simultaneous amplification of more than one target (pathogen) in a single reaction using qPCR probes carrying different reporters (dyes) with distinct fluorescent spectra.

[2]**Real-time qPCR:** Real-time polymerase chain reaction also known as quantitative polymerase chain reaction (qPCR), is a molecular biology technique based on the polymerase chain reaction (PCR). It monitors the amplification of a targeted DNA sequence during the PCR – i.e., in real-time, and not at its end.

[3]**SYBR green and TaqMan®:** Two specific probes used in qPCR assays to detect and differentiate microorganisms. In this study, both probes were compared for specificity, sensitivity and efficiency to distinguish CLas and *S. citri* from one another.

[4]**Prophage genes:** Essentially bacterial virus (bacteriophage or phage) genes incorporated into the genome of (or exists as an extrachromosomal element in) its bacterial host.

[5]**Contig:** Contiguous set of overlapping DNA segments that represent a consensus region of DNA.

[6]**PacBio:** A gene sequencer instrument that produces longer high-throughput sequence reads.

[7]**Bioinformatics analysis:** An interdisciplinary field that develops methods and software tools for understanding biological data. It combines computer science, statistics, mathematics and engineering to analyze and interpret raw sequencing data, annotate genomes of unknown pathogens and identify genes using computer software.

[8]**SMRT sequencing technology:** Single molecule real-time sequencing (SMRT) is a parallelized single molecule DNA sequencing method by which the precise order of nucleotides within a DNA molecule is determined.

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Aerial pesticide application to a commercial grove in the Pauma Valley.

Fall 2016 Pauma Grower Control of ACP

Nastaran Tofangsazi, Beth Grafton-Cardwell and Enrico Ferro

Project Summary

Eleven commercial orchards in San Diego County that were treated with various insecticides by ground or by air were sampled to determine their impact on fall 2016 populations of Asian citrus psyllid (ACP). Both air and ground applications of Actara®, ground applications of Leverage® 360 or Exirel®, and two ground applications of Surround® WP significantly reduced ACP densities for six to seven weeks. In contrast, Delegate® WG, Entrust® SC and Agri-Flex® were not as successful in decreasing ACP populations. This study is part of an ongoing project to develop an area-wide management approach to facilitate management decisions to control ACP and huanglongbing (HLB) in California commercial citrus orchards.

Until a cure for HLB is found, suppression of ACP, the insect vector[1] of the HLB-associated bacterium '*Candidatus* Liberibacter asiaticus' (CLas), is critical to reduce spread of the disease (Grafton-Cardwell et al. 2016). Foliar applications of broad-spectrum insecticides and soft insecticides[2] along with soil applications of systemic insecticides are becoming common practices for managing immature and adult ACP. The research described in this article provides information on the efficacy and residual control provided by Entrust SC (spinosad), Delegate WG (spinetoram), Actara (thiamethoxam), Agri-Flex (abamectin + thiamethoxam), Leverage 360 (imidacloprid + beta cyfluthrin), Exirel (cyantraniliprole), and Surround WP (kaolin clay), used to suppress ACP during fall 2016 under southern California inland conditions.

Eleven commercial citrus orchards were sampled for all developmental stages of ACP during September to November 2016 in Pauma Valley, San Diego County. Changes in population densities of ACP (adults were counted by tap sampling; nymphs and eggs were counted by flush examinations) were estimated prior to the treatment date and were monitored at approximately weekly intervals for up to 75 days after treatment application to determine the level and persistence of control. The sampled orchards varied in plant variety and age (sites 1-3 were young orchards less than five years, and all other orchards sampled were mature trees) with an acreage range of 1.32 to 6.99 acres (**Table 1**). The numbers of flushes from three randomly chosen ¼-square-meter areas of each sampled tree were recorded each week. Twenty trees

Table 1. Description of the sample site varieties, acreage and treatments.

Sites	Variety	Acres treated	Insecticide rate/acre	Adjuvant	Water volume (gal/acre)	Method of application	Treatment dates 2016
1	Lemon	1.75	Entrust SC 10 fl oz	1% 440 Supreme spray oil	400	Ground	22 Sep 10 Oct
2	Ruby Red grapefruit	3.4	Entrust SC 10 fl oz	1% 440 Supreme spray oil	400	Ground	14 Sep 6 Oct
3	Ruby Red grapefruit	3.4	Entrust SC 10 fl oz	1% 440 Supreme spray oil	50	Air	22 Sep Oct 6
4	Valencia orange	1.7	Delegate WG 6 oz	1% 440 Supreme spray oil	400	Ground	19 Sep
5	Valencia orange	2.7	Delegate WG 6 oz	1% 440 Supreme spray oil	50	Air	22 Sep
6	Valencia orange	2.3	Actara 5.5 oz	1% 440 Supreme spray oil	400	Ground	22 Sep
7	Ruby Red grapefruit	1.3	Actara 5.5 oz	1% 440 Supreme spray oil	50	Air	22 Sep
8	Valencia orange	1.8	Agri-Flex 8.5 fl oz	1% 440 Supreme spray oil	400	Ground	22 Sep
9	Valencia orange	1.9	Leverage 360 6.4 fl oz	1% 440 Supreme spray oil	400	Ground	22 Sep
10	Valencia orange	2.5	Exirel 20.5 fl oz	1% 440 Supreme spray oil	400	Ground	22 Sep
11	Valencia orange	1.3	Surround WP 50 lbs	None	400	Ground	22 Sep 10 Oct

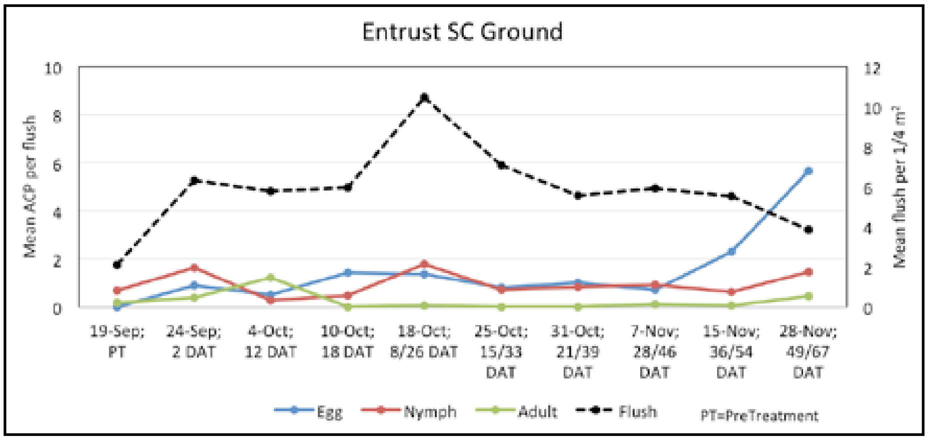

***Figure 1. Site 1 – the impact of Entrust SC + Oil applied by ground to a lemon orchard on September 22 and October 10.* NOTE: in all Figures, impacts are on the densities of ACP eggs, nymphs and adults and the number of flush per ¼-meter-squared.**

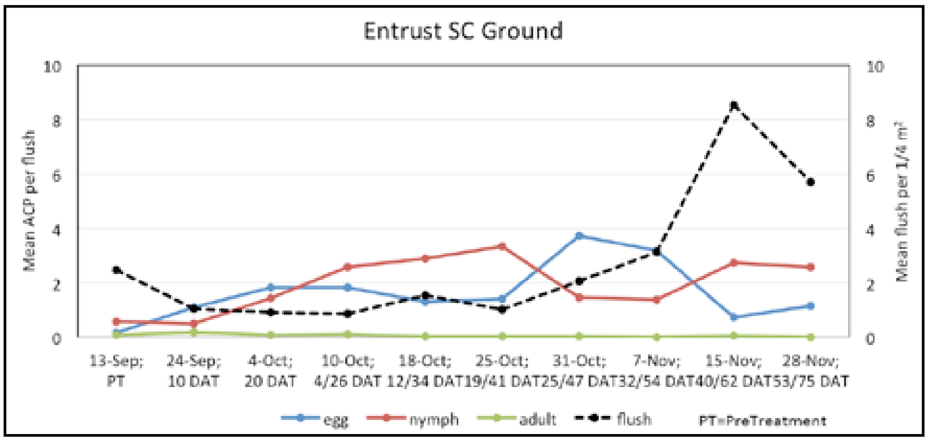

Figure 2. Site 2 – the impact of Entrust SC + Oil applied by ground to a Ruby Red grapefruit orchard on September 14 and October 6.

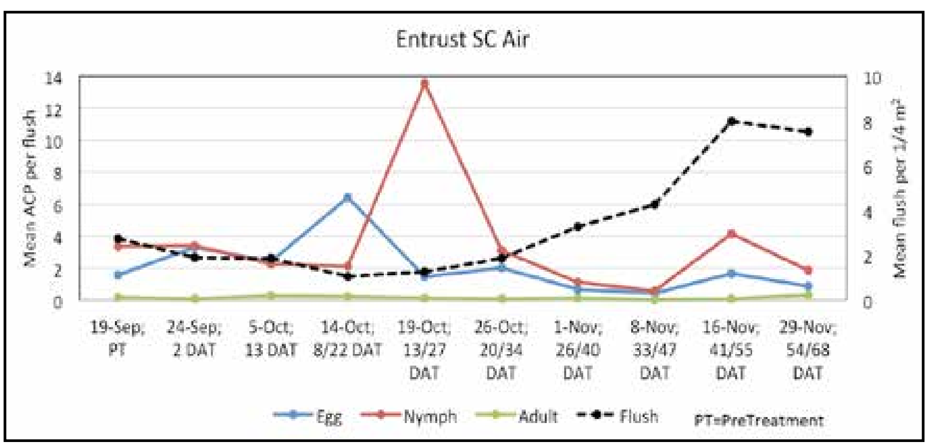

Figure 3. Site 3 – the impact of Entrust SC + Oil applied by air to a Ruby Red grapefruit orchard on September 22 and October 6.

were sampled in each orchard, with four trees sampled from each of four border rows and one central row (12 flushes were collected from each row, totaling 60 flushes per orchard). Adult population densities were estimated by tapping foliage into a sweep net three times on different sides of the same trees in which the above flushe counts were collected.

Ground applications were made using an Aerofan sprayer and applied at 400 gallons per acre (gpa), and the air treatments were made by helicopter and applied at 50 gpa. Sites 2 and 3 and sites 4 and 5 were two orchards that were divided so that half was treated by ground and half treated by air. Each insecticide treatment was applied to one of 11 different sites using the label rate per acre (without untreated areas) during fall 2016 (**Table 1**).

Orchards were flushing during most dates in the sampling period. The adult population was low (less than two adults per tap) in all sites (**Figures 1-11**). Nymph populations increased after treatments with Entrust SC + Oil in Site 1, although populations remained below two nymph/flush throughout the sampling period (**Figure 1**). The mean egg population increased from 0.9 per flush two days after Entrust SC + Oil application to 5.7 at the end of the sampling period in site 1 (**Figure 1**). Ground applications of Entrust SC + Oil at Site 2 only slightly reduced nymphs after the first application, and nymphs continued to increase in number after the second application to more than two nymphs/flush (**Figure 2**). Air applications of Entrust SC + Oil had little impact on eggs, which increased from 1.5 to 6.4 eggs per flush eight days after the second treatment, and little impact on nymphs, which increased from two to 13.5 nymphs per flush 13 days after the second treatment (**Figure 3**).

Sites 4 and 5, which received Delegate WG by ground or by air, had two to four nymphs per leaf at the time of application (**Figures 4** and **5**). Neither Delegate WG application suppressed ACP nymphs and eggs for more than 21 days after treatment (DAT), in spite of the fact that the mean flush per ¼-square-meters was low in both sites.

In contrast with the Entrust and Delegate treatments, the ground application of Actara suppressed all stages of ACP to undetectable levels on five dates through the 46 DAT sampling period (**Figure 6**). Air application of Actara suppressed the egg, nymph and adult populations below one per flush or sweep for 33 DAT (**Figure 7**). Surprisingly, the Agri-Flex ground treatment (**Figure 8**) only suppressed the nymphs for 12 days, even though one of the active ingredients, thiamethoxam, is the same as for Actara.

Single applications of Leverage 360 (**Figure 9**) or Exirel (**Figure 10**) and two applications of Surround WP (**Figure 11**) provided very good ACP control, reducing their densities below one per flush or sweep net for more than 54 DAT. In fact, there were many weeks when nymphs were undetectable in these sites. The efficacy of Surround WP should be investigated further, as it is an organically approved product. If it is demonstrated to be consistently successful, it would improve ACP control in organic situations. Additional grower orchard treatments are planned for Ventura County to confirm the insecticidal activity of Surround WP against ACP.

The 11 sampled sites were not replicated trials, but simply grower orchards with different varieties, different flushing patterns and different initial densities of psyllids. However, our data suggest that both methods of application (ground vs. air) can be beneficial in suppressing ACP populations, and four of the insecticides (Actara, Exirel, Leverage 360 and Surround WP) were clearly more efficacious than Entrust and Delegate, suppressing the nymphs below one per flush or even to non-detectable levels for many weeks.

Previous studies conducted in fall 2015 and spring 2016 found that Actara

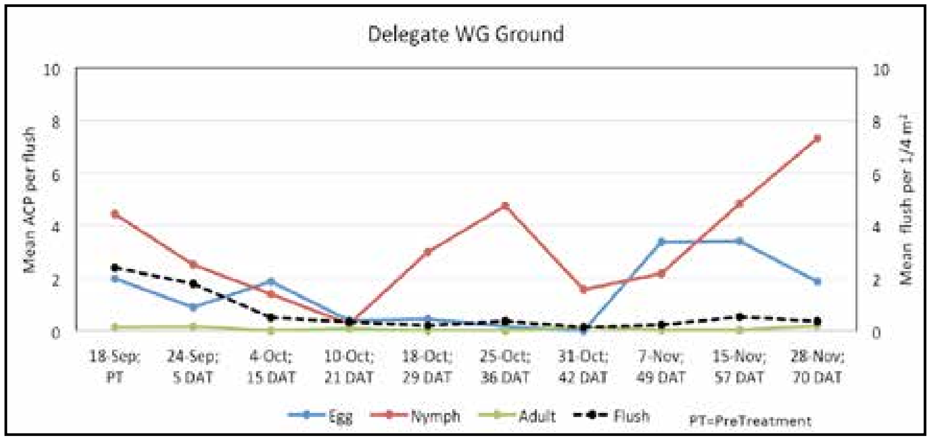

Figure 4. Site 4 – the impact of Delegate WG applied by ground to a Valencia orange orchard on September 19.

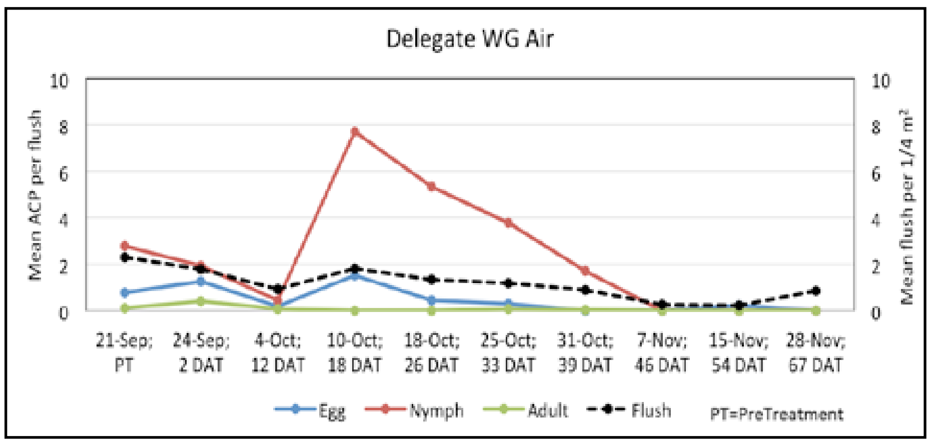

Figure 5. Site 5 – the impact of Delegate WG applied by air to a Valencia orange orchard on September 22.

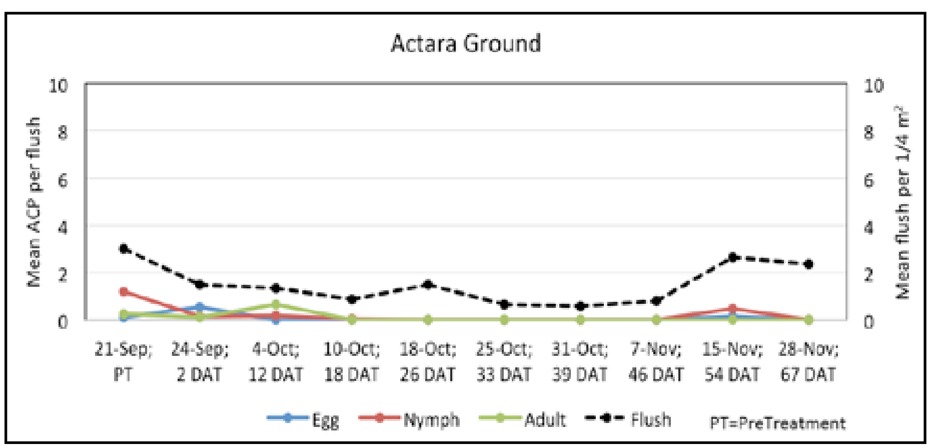

Figure 6. Site 6 – the impact of Actara applied by ground to a Valencia orange orchard on September 22.

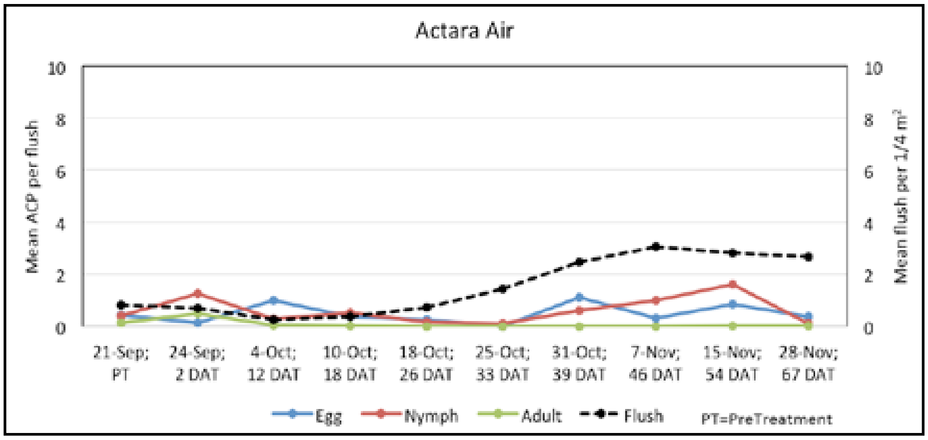

Figure 7. Site 7 – the impact of Actara applied by air to a Ruby Red grapefruit orchard on September 22.

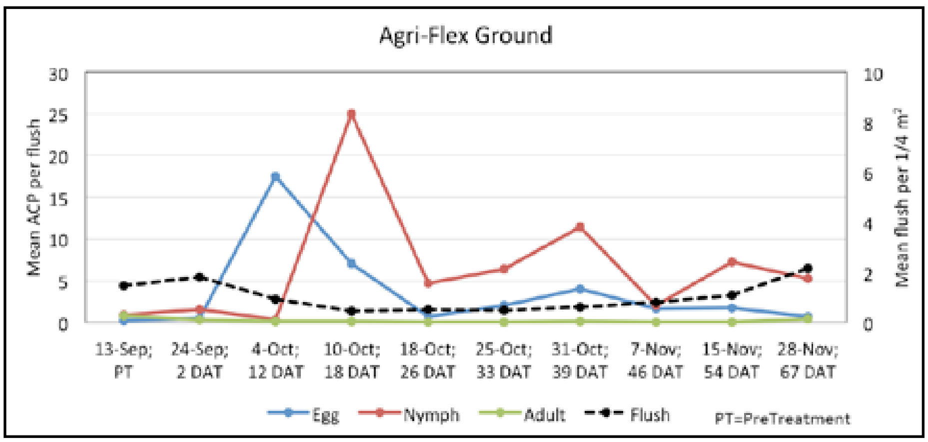

Figure 8. Site 8 – the impact of Agri-Flex applied by ground to a Valencia orange orchard on September 22.

was highly effective in reducing ACP populations to a very low level for at least five weeks after treatments in three of four sites (Tofangsazi et al. 2016, 2017). These studies also demonstrated that Entrust SC had little ability to suppress ACP nymphs when the population was moderate to high and Delegate WG failed to suppress nymphal population beyond 18 days in one of two sampling sites. Thus, two seasons and two years of sampling confirm that broad-spectrum insecticides provide greater, more persistent and more consistent control of psyllids than soft insecticides.

CRB Research Project # 5500-189

Field and laboratory assistance was provided by Brandon Rogers and Imon Riaby.

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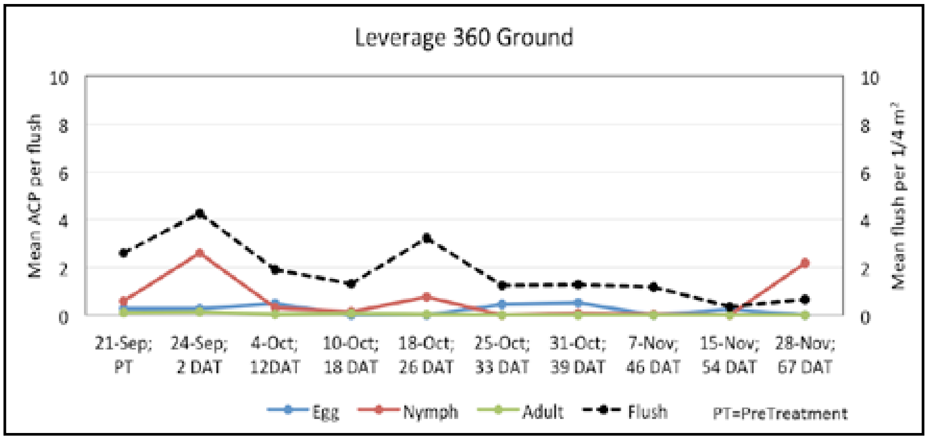

Figure 9. Site 9 – the impact of Leverage 360 applied by ground to a Valencia orange orchard on September 22.

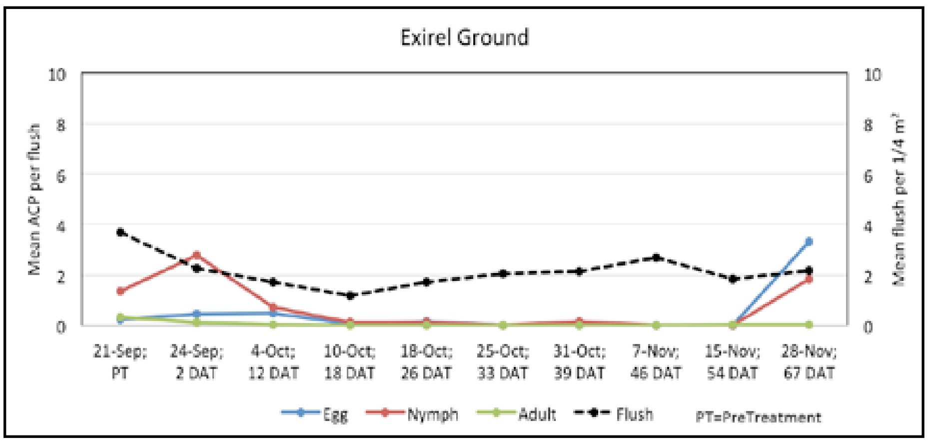

Figure 10. Site 10 – the impact of Exirel applied by ground to a Valencia orange orchard on September 22.

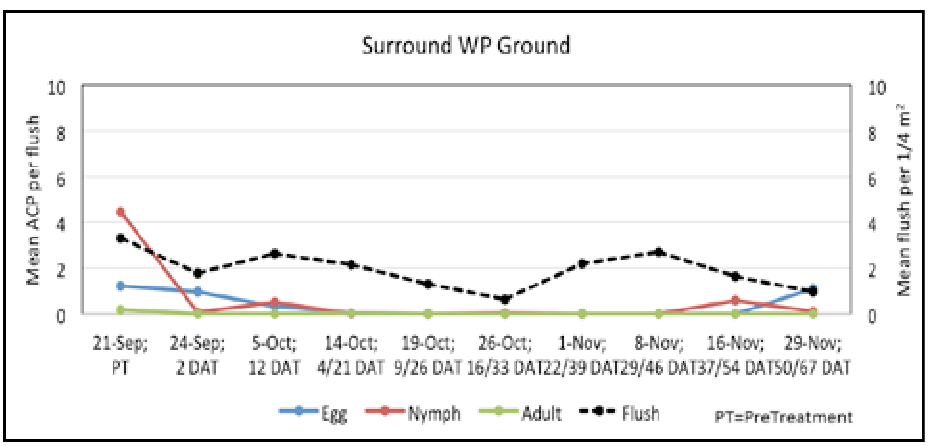

Figure 11. Site 11 – the impact of Surround WP applied by ground to a Valencia orange orchard on September 22 and October 10.

Tofangsazi, N.; Grafton-Cardwell, B.; Ferro, E. 2017. Asian citrus psyllid control in Southern California: Whole orchard studies of the efficacy of grower applied spring insecticides. *Citrograph* 8(3): 58-61.

Glossary

[1]**Insect vector:** Insects that carry and transmit a plant pathogen such as a bacterium or virus.

[2]**Soft insecticides:** Insecticides that pose minimal negative impact on non-target organisms.

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A section of a grove with properly installed frost protection bags.

Correctly installed frost bag.

Incorrectly installed frost bag. Note that the microsprinkler is outside of the bag.

General Guidelines for Using Frost Fabric Bags in Citrus

Roger Smith

Since 2008 when Trigloos were first introduced, the use of frost fabric in citrus orchards has steadily been on the rise. Wider use and acceptance has been seen in the past few years when the TreeGlove™ became widely available at a low retail price and Yeti Bags became available. Unfortunately, the experiences of growers since 2008 hasn't been widely reported, so this guideline came about.

Frost fabric is spun polyester, similar to the material used inside disposable diapers. Its translucent qualities allows the trees to photosynthesize, yet tiny holes in the fabric allows air exchange so the plant can "breathe." The fabric is fairly durable, but high winds can cause some damage. If installed incorrectly, they provide almost no protection. If used as discussed below, the bags can provide a baby tree with up to 8°F of frost protection.

1. Buy in advance and store them out of the sun and protected from rodents. Suppliers run out of inventory during the frost season, so purchase before November 15.
2. Don't install the bags too early! The white bags act like a greenhouse and will keep the tree active, making it more vulnerable to a freeze. Unless an early hard freeze is forecast (as in 2013), delay putting on the bags until early December or when dormant.
3. Some growers wait until a hard freeze is forecast before they install. If stored as in #1, the bags can last for many years if not used. Life is about three years if used annually.
4. Make sure the bag is long enough to allow 12 inches to touch the ground so dirt can be placed around the base. The bags are ineffective if tied to the tree trunk or are not sealed to the ground. The bag should be "floating" on the tree to reduce leaf burn that occurs when a leaf is tight against the fabric. Be sure to patch holes.
5. A microsprinkler inside the bag is best. If installed correctly, when water is turned on, the bag saturates from the "steam" produced when the air temperature is colder than the water temperature. The bag freezes at 32°F, which stops the air exchange of the fabric and traps more heat.
6. Without water inside the bag, a grower can expect about 2°F of protection.
7. If installed properly with at least four gallons per hour of water running inside, a frost bag actually works better the colder it becomes. When the temperature inside the bag drops below 32°F, the change of state reaction from water to ice releases a lot more heat which is trapped inside the bag. The more water you apply, the better the protection.
8. Low gallons per sprinkler of frost water is better than no sprinkler. Even drip will help, but is not as effective as higher flow microsprinklers.
9. Wind machines and frost fabric bags are incompatible. The Wind machines tend to thrash the bags, tear them open, or blow them off. Bagging the boarder rows and corners works well.
10. If water supplies are limited, pick which plants to protect and stick with it. Alternating rows on alternate nights will guarantee 100 percent failure to protect.
11. If buying used bags, make sure they are long enough for your trees and have been used no more than twice.

***Roger Smith is general manager of TreeSource Citrus Nursery and has worked with frost fabric for frost protection in outdoor nurseries since 1992 and in orchards since 2008. For more information, contact* rsmith@citrustreesource.com**


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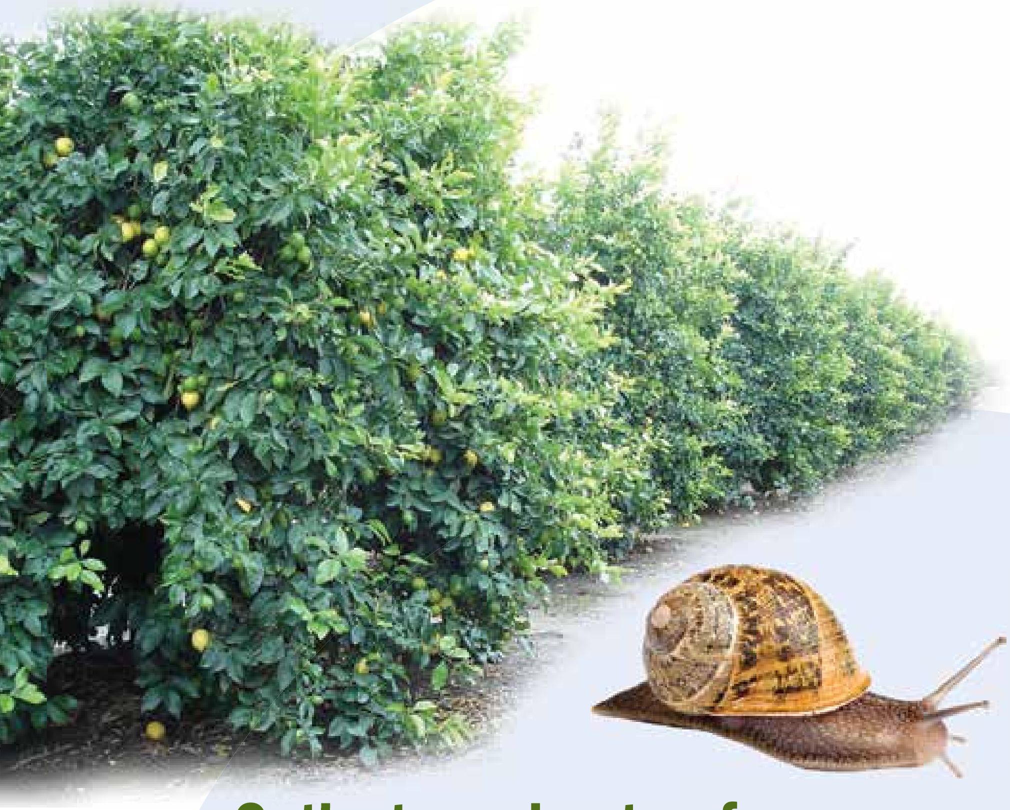

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