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On the Cover:
On October 1, Justin Brown officially became chairman of the Citrus Research Board (CRB) and Marcy Martin began work as the new CRB president. For more information, see "CRB Ushers in New Board Leadership" on page 10 and "Chairman's View" on page 12.
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Winter 2020 | Volume 11 • Number 1 The Official Publication of The Citrus Research Board

Citrograph’s mission is to inform citrus producers and other industry members of research progress and results that will help ensure the sustainability of California citrus.

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The Mission of the Citrus Research Board:
Ensure a sustainable California citrus industry for the benefit of growers by prioritizing, investing in and promoting sound science.

Citrus Research Board Member List
By District 2019-2020  (Terms Expire September 30)

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CALENDAR OF EVENTS 2020

January 13-17
Citrus Research Board (CRB) Committee Meetings
Visalia, California. For more information, contact (559) 738-0246, or visit www.citrusresearch.org

January 15
Citrus Pest and Disease Prevention Committee (CPDPC) Meeting
Visalia, California. For more information, visit www.cdfa.ca.gov/citruscommittee

January 28
Citrus Research Board (CRB) Joint Agency Biological Control Task Force Meetings
Riverside, California. For more information, contact (559) 738-0246, or visit www.citrusresearch.org

January 29
Citrus Day at University of California, Riverside
Riverside, California. For more information, visit www.citrusvariety.ucr.edu

January 30
Citrus Research Board (CRB) Meeting
Riverside, California. For more information, contact (559) 738-0246, or visit www.citrusresearch.org

February 11-13
World Ag Expo
Tulare, California. For more information, visit www.worldagexpo.com

February 12
California Citrus Quality Council (CCQC) Board and Annual Meeting
Doubletree Hotel, Bakersfield, California. For more information, visit http://ccqc.org

March 5
California Citrus Mutual (CCM) Citrus Showcase
Visalia Convention Center, Visalia, California. For more information, contact (559) 592-3790 or visit www.cacitrusmutual.com

March 11
Citrus Pest and Disease Prevention Committee (CPDPC) Meeting
Riverside/San Bernardino, California. For more information, visit www.cdfa.ca.gov/citruscommittee

April 16
California Citrus Quality Council (CCQC) Meeting
Doubletree Hotel, Bakersfield, California. For more information, visit http://ccqc.org
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Justin Brown was unanimously elected to a one-year term as Chairman of the Citrus Research Board (CRB) at the organization’s annual meeting on September 24, 2019, at the Lindcove Research and Extension Center in Exeter, California.

Brown farms in the area of Orange Cove, California, where he has worked for more than a decade. He also has served with the CRB since 2009 as chair or vice-chair of numerous committees and as a member of the Executive Board.
The life-long Orange Cove resident holds a B.S. in Ag Business Management from California Polytechnic State University, San Luis Obispo, a J.D. from San Joaquin College of Law and is a California state bar-accredited attorney. Brown has said his priorities are the financial stability of the CRB and ensuring the most efficient and effective use of grower funds to ensure a sustainable and prosperous future for the California citrus industry.

John Konda was elected to serve as Vice Chairman. A Tulare County citrus grower from District 1 who owns and operates Konda Farms, he produces citrus, pistachios and row crops. As vice chairman, one of Konda’s key responsibilities is to chair the Research Priority Screening Committee. Since first being elected to the Board in 2012, he has sat on nearly every CRB committee and is the immediate past chair of the finance committee. The Terra Bella native’s primary areas of interest are new varieties, finance, and research, development and implementation.

Mark McBroom was elected to serve as Secretary/Treasurer. For 15 years, he has farmed and managed more than 3,000 acres of citrus in Imperial, San Diego and Riverside counties for Bloom to Box Crop Care, Inc. The Brawley native has stated that since his farming location is only 50 miles from Mexico, finding solutions to ACP and HLB are his number one priorities.

The CRB also welcomes new District 1 Northern California Board Member Zac Green. A Kansas State University alumnus, Green is the general manager of Johnston Farms and WN Citrus in Bakersfield, California, where he resides. A native of Wichita, Kansas, the new Board Member’s primary interests are new varieties, pest management and the Citrus Pest and Disease Prevention Program. Green will serve a three-year term.

Fresno County Agricultural Commissioner Melissa Cregan was nominated to fill the CRB Public Member seat by unanimous vote. Cregan holds a B.S. in Animal Science from the University of California, Davis (UCD) and brings more than 18 years of regulatory experience and 12 years in civil service leadership. She began her career as an inspector in Madera County in 2001 and was promoted to deputy agricultural commissioner overseeing the pesticide regulatory program in 2007. Cregan joined the Fresno County Department of Agriculture in 2012 as deputy agricultural commissioner overseeing the pest detection and exclusion division until 2018 when she was promoted to assistant agricultural commissioner. Cregan was named agricultural commissioner in March 2019.

“I am excited to join the Board,” Cregan said, “and I look forward to being able to contribute my knowledge and experience in exotic pest detection, exclusion and management.”

Please join us in congratulating our executive board leadership and welcoming our newly elected board members.

Carolina Evangelo is the Citrus Research Board’s director of communications and the co-publisher/project manager of Citrograph. For more information, contact Carolina@citrusresearch.org
October 1 marked the beginning for a new president at the Citrus Research Board (CRB), Marcy Martin. An almost yearlong journey led by a search committee of the Board was required to find this supremely qualified and very well matched candidate.

Marcy joins the CRB with more than 25 years of experience with California commodity organizations. Most recently, she served for 14 years as director of trade for the California Fresh Fruit Association, where she advocated on behalf of the state’s fresh grape, blueberry, pomegranate and deciduous tree fruit production on governmental, legislative and policy issues. Prior to that, she had been controller of the California Apple Commission for a decade.

In 2015, then U.S. Department of Agriculture Secretary Tom Vilsack appointed Marcy to the Agricultural Technical Advisory Committee for Trade in Fruits and Vegetables. Secretary Vilsack said of the appointees, “They are invaluable assets as we work to enact trade agreements and trade policies that deliver the greatest economic benefit for U.S. agriculture and for our nation as a whole.”

October 1 also started my first year as Chairman of the Board. I would like to thank my fellow Board members for this opportunity and for having the confidence in me to serve at this level. I have accepted this honor with great humility and a strong will to see the mission of the CRB carried out successfully this fiscal year.
As the citrus industry moves ahead, we face many challenges. The same is true for the CRB. I have entered my 11th year of serving on the Board of Directors. During the course of this service, I have taken a keen interest in the efficiency and effectiveness of governance by the Board. Moving forward, I have identified major goals to help direct this fiscal year toward a successful path.

**Strategic Planning**

The Board has reached a critical point in time, whereby a comprehensive strategic plan is not only needed, but necessary to effectively move forward over the next two to four years. The Board frequently is required to make funding decisions on very complex research projects. The requested funding commonly is in the six-figure range. The proposed research projects often present high-stakes solutions should their research projects be successful. Understanding that this organization operates under a finite budget that is borne on the backs of the California citrus growers, unlimited mistakes in research project funding decisions are not an option. Under the ever-present cloud of huanglongbing (HLB), our stakes have never been higher and will continue to be at the highest level in history.

My goal with strategic planning is an organized focus from the Board on the most important objectives of the California citrus industry and how best to achieve them. Ultimately, establishing a framework for cohesive, coherent and results-based decision-making at the Board level will best serve the industry.

**Restructuring Committees**

The Board, in large part, operates via committees to handle the detailed and sometimes tedious issues surrounding grant funding, research project selection and follow-up and follow-through of funded project administration. It is inherently germane to the CRB process that committees operate at the most effective level to generate quality recommendations for the entire Board to deliberate where necessary and approve where prudent. To date, this goal has been achieved. A restructured list of committees has been submitted to the California Department of Food and Agriculture, and approval appears imminent. As the year progresses, if it becomes apparent that further restructuring, modification, creation, and/or elimination of committees is needed, it shall be done. Nothing is quite perfect.

**Maintain the Highest Fiduciary Responsibility to the Industry**

As stated above, this organization is funded by you, the California citrus growers. As a sixth-generation California native and proud citrus grower myself, the importance of financial responsibility is a value I hold close – especially when it includes other people’s money. The industry collectively has agreed to be monetarily assessed to fund the CRB and has elected Board members to represent those assessed dollars. Therefore, it is the Board’s first and foremost responsibility to be judicious in how the industry’s money is spent every year.

HLB, cumbersome regulations, labor, water and market access are ever-present struggles facing the industry. Successful research, funded by the industry through the CRB, can be the remedy to these struggles that will result in a more vibrant and successful California citrus industry. I’m looking forward to serving the industry as CRB chairman this fiscal year. Although the challenges are difficult, I believe we are on the right path to a successful CRB.

Justin Brown is a citrus grower in Orange Cove, California, and serves as chairman of the Citrus Research Board. For more information, contact citrusfarmer@aol.com
Five California citrus producers, including four Citrus Research Board (CRB) members, had important seats at the table this December in Washington, D.C. to help identify the research areas that should be prioritized in the allocation of $125 million from the 2018 Farm Bill in the critical fight against huanglongbing (HLB). The five representatives from California who were appointed to the U.S. Department of Agriculture (USDA) National Institute of Food and Agriculture (NIFA)-managed Citrus Disease Subcommittee (CDS) within the National Agricultural Research, Extension, Education and Economics (NAREEE) Advisory Board by USDA Secretary Sonny Perdue included CRB Chairman Justin Brown, CRB Board members Gregory Galloway, John Gless III and Justin Golding, and California citrus producer Julia Inestroza.

CDS members consult and collaborate with NIFA’s Specialty Crop Research Initiative’s Emergency Citrus Disease Research and Extension (ECDRE) Program on citrus research, extension and development needs and also provide research and extension recommendations on key areas of citrus disease that should be funded. This marks the first time that California has filled five of the 11 seats on the CDS, thus resulting in the greatest voice that California citrus has had in the say of these crucial federal government expenditures.

Brown, who has served on the CDS since 2017, said, “Our involvement in this subcommittee is vitally necessary to California citrus growers. It enables us to supplement the research funding that growers provide with substantial federal funds that are targeted to our priorities.”
CRB President Marcy Martin noted, “Adding the NIFA dollars to California grower assessments equals enhanced returns that would not have been possible through grower dollars alone.”

A new addition this year was the Foundation for Food and Agriculture Research (FFAR) leading a Citrus Greening Convening workshop with researchers, funders, growers, CDS members and other interested parties prior to the CDS meeting. Progress to date was reviewed, and bottlenecks to progress were identified, followed by proposals to address those bottlenecks and recommendations for areas of research focus for the next five years. The FFAR workshop recommendations then were provided to the CDS to assist in setting priorities.

With these recommendations and with the unique perspective and experiences of each citrus growing region well represented (five from California, along with five from Florida and one from Texas), CDS members identified national priorities and needs for the coming year:

 ➢ Develop a delivery system for therapeutics, nutrition and other HLB solutions.
 ➢ Understand the phloem, and improve understanding of the HLB disease triangle (host, pathogen and vector interactions).
 ➢ Standardize screening and testing protocols (for better comparisons across research labs).
 ➢ Prevent ‘Candidatus Liberibacter asiaticus’ (CLas) infection in trees, maintain health/production of CLas-infected trees and cure infected trees.
 ➢ Eradicate Asian citrus psyllid (ACP)/maintain regional control of psyllids where possible (to include resistance management).
 ➢ Optimize ACP/HLB detection and surveillance (including the use of psyllid attractants, and early detection of CLas/HLB).
 ➢ Culture CLas.
 ➢ Develop and evaluate citrus genetic resistance to HLB.
 ➢ Identify ecological aspects and environmental effects that may modify disease activity.

These priorities are expected to be refined prior to inclusion in the upcoming call for priorities for this funding program.

Joining the California representatives on the subcommittee in Washington to develop the priorities listed above were David Howard, Matt McLean, Harold Browning, Ph.D., Gee Roe III and James Snively – all from Florida, and Mani Skaria, Ph.D., from Texas. Representatives from the Citrus Research and Development Foundation, CRB, Huanglongbing Multi-agency Coordination Group and Agricultural Research Service National Programs were in attendance at the NAREEE meeting and contributed to the discussion, both by providing information on past project support and by directly addressing questions from CDS members during the meeting as priorities were set. Following the setting of priorities, the request for applications for the ECDRE Program is expected to go out early in 2020 with an opportunity for further grower involvement as relevancy review panelists. If interested in learning more about the relevancy review process or volunteering as a relevancy reviewer, please reach out to CDS members or the CRB research department.

Melinda Klein, Ph.D., is the chief research scientist for the Citrus Research Board, where she also serves as the science editor of Citrograph. For more information, contact Melinda@citrusresearch.org
It has been two years since I received the congratulatory phone call that I would be heading to California Citrus Mutual (CCM) to eventually assume the position of president and CEO. During that time, I’ve immersed myself in understanding all things citrus – the organizations, history and, most importantly, the people of the industry. We are fortunate to be a part of an industry that is iconic in California, rich in history and with strong organizations to guide its continued success. I knew my biggest task was getting to know many of you since most of us hadn’t crossed paths before. The thought of starting over and establishing new relationships was daunting in the beginning. However, I was comforted by my prior experience...
in the agricultural community. I knew that farmers have a lot in common, regardless of the area and the crop; they are fair, honest, hardworking, humble and caring people. I am blessed to work in an industry that works for them and with them.

As we count our blessings, we must remember that we cannot take them for granted. Farming in California is tough business. We have many challenges, both internal and external, that must be addressed effectively to protect what we have for generations to come. Pests and disease, trade, water, labor and governmental over-regulation are the significant pressures of the day and affect the viability of the industry. Our organization is a vehicle for the industry to collaborate on addressing these challenges. CCM is not alone in this endeavor. To achieve the desired results, we must work in partnership with the Citrus Research Board, California Citrus Quality Council, Citrus Pest and Disease Prevention Program and many other organizations to address specific challenges. We all work for you – the grower, and it must be our mission to coordinate our efforts in order to be sustainable and maximize your return on investment.

Addressing the issues of the day is not our only obligation. Our true ambition is bigger than making a profit. Profit is an important part of the overarching goal of sustaining a way of life and preserving it for the next generation. Yet to do that, we must have a vision for the future that guides our decision-making of the day.

I strongly believe that our challenges are amplified by the disconnect between rural and urban decision-makers. What we do in rural California is essential for urban societies to exist and thrive, yet we are viewed in the eyes of decision-makers as the problem, instead of the solution. This disconnect has grown exponentially because we have been successful in producing more with less, thus enabling an urban voting population to grow and outnumber our voices. This trend will continue and make our problems more catastrophic if we do not begin to address this core issue.

CCM has been a leading agricultural organization in engaging in positive messaging through our Citrus Strong campaign and our Citrus Stride event that is held annually at the State Capitol. We’ve supported food banks, developed scholarship programs and supported many other causes greater than our own. However, negative narratives still drag down the agricultural community. The problem still exists because the true story about the good and hardworking people in agriculture isn’t being promoted enough. Negative stories sell easily; positive ones take a lot of time, resources and persistence. Our positive characteristics, such as humility, make communicating the excellent things we do in the community an uncomfortable experience. While it may be unpleasant for us to “brag” about the positive things we do, we must do so to overcome the inaccurate perception that many decision-makers have about agriculture.

During my career, I’ve experienced the generosity of people in agriculture directly. In order to change the narrative, this generosity does not need to be exaggerated. We just need to make sure to tell the story about the generations of hard-working men and women who have devoted their lives to feeding the world. This will take a sustained and substantiated effort over time, but it is a story that

Times have changed, and we need to change with them. It may feel like our challenges are too big to overcome. Big challenges are just a cumulation of many small ones. If we break them down into smaller segments, they seem more manageable. When we can all roll up our sleeves and work together, I believe we can accomplish a lot more than we may think. I encourage everyone to work on the task in front of us today, with a common vision for tomorrow, knowing that CCM is working right alongside you.

Casey Creamer is president and CEO of California Citrus Mutual, a voluntary non-profit trade association that advocates for commercial citrus growers on matters that affect their economic livelihood. For more information please contact casey@cacitrusmutual.com

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2020 CITRUS SHOWCASE

MARCH 5TH

7:30 A.M. COFFEE AND PASTRIES
Sponsored by Valley Packline Solutions

8:00 - 9:00 A.M. WORKSHOP A
Market Trends: Asking the tough questions
Presented by California Citrus Mutual

9:00 - 10:00 a.m. Tradeshow Open

10:00 - 11:00 A.M. WORKSHOP B
California’s collaborative and multi-layer approach to ward off the threat of Huanglongbing (HLB)
Presented by Citrus Research Board
11:00 - 12:00 p.m. Tradeshow Open

12:00 - 1:30 P.M. LUNCHEON
Luncheon Tickets are $40 and must be purchased in advance by calling California Citrus Mutual.
1:30 - 2:30 p.m. Tradeshow Open

2:30 - 3:30 P.M. WORKSHOP C
Hot Topics in Labor Law and Regulation
Presented by California Citrus Mutual

Event sponsorships are still available!
THIS EVENT IS FREE OF CHARGE.

8:00 - 9:00 A.M. WORKSHOP A

Presented by California Citrus Mutual

Join CCM President and CEO Casey Creamer as he speaks with industry leaders about market conditions, trends and gets their outlook on the 2020-21 season and beyond.

10:00 - 11:00 A.M. WORKSHOP B

Presented by Citrus Research Board

In this presentation, Georgios Vidalakis, Ph.D., will discuss the preventive, concurrent and multi-layered anti-HLB approaches developed and implemented through collaborative efforts of the industry, scientists and governmental agencies that has kept HLB out of commercial citrus. We also will focus on new varieties being processed by the Citrus Clonal Protection Program (CCPP) and plans for their release and evaluation under California growing conditions. The solution to HLB will not be a single silver bullet. It will be based on a systems approach, and all of us will play a critical role in its success.

2:30 - 3:30 P.M. WORKSHOP C

Presented by California Citrus Mutual

Expert panelists will provide an update on hot topics in labor law and regulation including wage and hour requirements, health and safety standards, H2A, and impending state and federal legislation. Panelists include Mike Saqui of the Saqui Law Group and Rudy Mendoza, President of the California Agriculture Labor Association.

FOR MORE INFORMATION ON THE EVENT, PLEASE CONTACT CALIFORNIA CITRUS MUTUAL: (559) 592-3790 OR ALEXIS@CACITRUSMUTUAL.COM

California Citrus Mutual
Charlie Coggins

INDUSTRY REVOLUTIONARY

Carolina Evangelo and Ivy Leventhal | Photos provided by UC Riverside
On August 18, 2019, the California citrus industry, along with numerous family, friends and colleagues, bid farewell to Charles (Charlie) W. Coggins, Jr., Ph.D., a gentleman who was instrumental in revolutionizing the economics, employment and consumer enjoyment of citrus. In addition to being noted for his critical improvements to the industry, he will be missed as a dedicated family man, a devoted, intense and productive scientist and a humble, gentle person.

Best known as the “father of gibbing,” Charlie had been tasked by the University of California, Riverside (UCR) when he joined as a junior plant physiologist in 1957 with developing the use of plant growth regulators to solve production problems and improve the economics of citrus production. He discovered that when gibberellic acid (GA), a plant growth regulator, was sprayed on trees prior to color break, it delayed rind senescence in late-harvested groves. Charlie found that spraying Navel and Valencia orchards with GA allowed fruit to be harvested later in the season while maintaining excellent quality. GA also was found by Charlie to solve a number of citrus fruit quality problems.

“Gibbing” revolutionized the industry. The timeframe for getting fruit to market now could be spread out much longer. This had a number of beneficial effects. The market no longer was flooded with citrus during narrow periods, which had resulted in significant fruit spoilage. The process also ensured that those who earn a living in the industry – pickers, packers, marketers – had work available to them throughout the year; and consumers, too, had a much longer window in which fresh citrus was available for an affordable price. Spreading out the cycle stabilized prices and industry income and allowed for a stable workforce, who could then be trained more effectively, thus improving the economic and production efficiency of packinghouses. Over the ensuing decades, gibbing has become a worldwide standard horticultural practice for not just oranges, but mandarins, lemons and limes, as well.

In a 2012 Citrograph article, Carol J. Lovatt, Ph.D., professor of plant physiology, Department of Botany and Plant Sciences at UCR, wrote, “Gibbing changed everything! Fruit could now be stored on the tree well past the ‘normal’ maturity window for the cultivar, making it possible to harvest, pack and market navels and Valencias over longer periods, supplying consumers with fresh oranges 12 months of the year. Moreover, GA-treated fruit were of superior quality (i.e., the incidence of rind staining, water spot, puff and sticky rind were all reduced by GA.”

Although Charlie may be best remembered for gibbing, he also developed other beneficial cultural practices using different plant growth regulators to increase the size of citrus fruit and to prevent its drop, which increased yield. Additionally, the renowned scientist contributed to improving yield and fruit quality of tomato, Deglet Noor date and avocado.

Charlie was the recipient of many honors. He had been instrumental in the organization of the International Society of Citriculture and served as its executive secretary/treasurer from 1985 through 2001. In appreciation, the Society elected him as an honorary member and a fellow member (the Society’s most prestigious award). The American Society for Horticultural Science also named him as a fellow, which is their highest form of recognition. Charlie received the National Agri-Marketing Association Award for Agricultural Excellence in Science and the Albert G. Salter Award from the California Citrus Quality Council for his distinguished services and outstanding contributions to the citrus industry.

In Lovatt’s article, she said, “I couldn’t have asked for a better role model and colleague in those early years. To this day, I remain inspired by the magnitude of his accomplishments.”

Charlie hailed from Cherryville, North Carolina, where he was born in 1930. He received his Bachelor of Science with honors and Master of Science in Agronomy degrees from North Carolina State University in 1952 and 1954, respectively. With support from a National Science Foundation Fellowship, Charlie earned his Ph.D. in Plant Physiology from the University of California, Davis. Capping a successful career at UCR, he was named chairman of the university’s Department of Plant Sciences in 1975, which he then led in evolving into the highly regarded Department of Botany and Plant Sciences. Charlie chaired the department through 1982.

The noted scientist was predeceased by two of his sons (Steve and Bruce) due to cystic fibrosis and is survived by his beloved wife of 68 years (Irene), son (Roger) and four grandchildren (Grace, Jemma, Elizabeth and Nicholas).

Contributions in memory of Charlie Coggins can be made to the Cystic Fibrosis Foundation cff.org, The Parkinson’s Disease Foundation Parkinson.org or "The Coggins’ Endowed Scholarship Fund" through the University of California, Riverside.

A special thank you is extended to Carol Lovatt, Ph.D., for contributing to this tribute.
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Outreach Was “In” This Summer

Mark McBroom

For many Californians, the summer months are cherished as a time for relaxation and fun pastimes; but the Asian citrus psyllid (ACP) and huanglongbing (HLB) don’t take summer breaks. The Citrus Pest & Disease Prevention Program (CPDPP) uses the summer months to amplify outreach that naturally aligns with summer activities to continue educating residents on the issue and encourage them to act to protect California’s citrus trees.

Reaching Residents Where They Are

To stay relevant during summer, the CPDPP’s outreach strategies were based on where residents were, what they were doing and how they were enjoying their time off. In particular, three specific activities – summer travel, entertainment and community events – were identified as popular summer activities.

According to the American Automobile Association, more than 68 million families were expected to travel this past summer, thus posing an increased risk of travelers moving citrus material and unknowingly spreading ACP throughout the state. Beginning on Memorial Day weekend, billboards were placed on Interstate 5 between Los Angeles and Orange counties, at the international border with Mexico and on State Route 99 in Kern County advising travelers to refrain from moving citrus in order to protect local agriculture and backyard citrus trees. These billboards were seen an estimated 4.5 million times by travelers.

The CPDPP outreach team also developed social media content using the message of “don’t move citrus” to reach residents with a consistent call-to-action in multiple settings. The content was shared with the program’s contacts at local elected officials’ offices and was made available for city and county governments to use on their own social media accounts and web sites. This information was shared on government and local elected official social media accounts more than 65 times, with a majority of the shares coming from cities in the HLB quarantine area. Not only did this strategy help the program reach more people, but it also leveraged its relationships to add third-party credibility to the issue of HLB.

Additionally, the CPDPP recognized movie theaters and community events as popular activities for residents to enjoy...
during summer. The program's public service announcement ran during movie theater previews in Anaheim, Garden Grove and La Habra, and was targeted to run during months with highly anticipated movie premieres. The program also staffed information booths at local community events in six cities, including towns in southern California and the San Joaquin Valley, where outreach team members educated residents on the pest and disease, inspection tips and what to expect when California Department of Food and Agriculture crew members visit their homes. The program partnered with the Citrus Clonal Protection Program to educate attendees at the California Rare Fruit Growers annual conference about proper citrus tree care.

While all of these activities were planned well in advance, the CPDPP worked quickly to address and respond to HLB detections as they occurred – such as the additional residential HLB detections in Riverside.

**Acting Quickly to Protect the Birthplace of Citrus**

This past July, HLB was detected for the first time in Riverside since 2017. While these detections were all on residential properties, the risk to nearby commercial citrus was heightened and required the CPDPP to quickly educate Riverside residents, growers and elected officials.

Working in partnership with the Riverside County Agricultural Commissioner’s office, the program notified local media of the detections and coordinated interviews with state and local agriculture officials. Several stories ran in local media, including on the front page of Riverside's daily newspaper *The Press-Enterprise*, and in *Excélsior*, a weekly Spanish newspaper serving southern California. Additionally, a post on *CitrusInsider.org* was sent to citrus industry members. Agriculture media outlets, including AgNet West, also published stories about the detections.

Lastly, radio ads were running on local stations KCAL-FM and KOLA-FM in the weeks following the detections, encouraging residents to cooperate with agriculture officials inspecting trees and to refrain from moving citrus tree material off their properties.

**We All Play a Role**

The CPDPP will continue working diligently on behalf of California’s citrus industry to encourage residents and members of the industry to act and protect California citrus trees from the death sentence of HLB. As a citrus grower, I encourage you to use your voice, as well. Educate your neighbors about the issue and/or urge your local commercial citrus community to follow best practices and get involved with pest and disease control. Our livelihoods are on the line, and that is a powerful story to tell others. By working together to all do our part, we can save California citrus for generations to come.

*Mark McBroom is the outreach subcommittee chair for the Citrus Pest & Disease Prevention Program. For additional information, contact desertcitrus@aol.com*
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Opening of Biosafety Level-3 Laboratory for HLB and ACP Research

Carolina Evangelo, Melinda Klein and Joey S. Mayorquin | Photos provided by UC Riverside

On September 26, leaders from the California citrus industry and the University of California, Riverside (UCR) gathered to celebrate the highly anticipated grand opening of a Biosafety Level-3 (BSL-3) Laboratory for huanglongbing (HLB) and Asian citrus psyllid (ACP) research.

The state-of-the-art lab is the product of a partnership between the Golden State’s citrus growers and UCR aimed to protect California citrus trees from HLB.

Located just two miles from the UCR campus on Marlborough Avenue, the lab will allow researchers to conduct work with state and federally regulated plant pathogens, which previously couldn’t be done in southern California.

Construction of the $8 million lab began in 2016 and was entirely funded by the California Citrus Research Foundation (CCRF) through donations by citrus growers and packers via a fundraising campaign that was diligently and successfully led by CCRF Executive Director Joel Nelsen.
The grand opening was marked by a ribbon-cutting ceremony and citrus industry appreciation luncheon at which several university, government and citrus industry leaders spoke about the lab's significance to the future of the California citrus industry.

The collaboration between citrus growers and UCR drew praise from a number of local elected officials, as well as State and Federal representatives in attendance.

### Inaugural Research Projects

BSL-3 laboratory operations are being overseen by Lab Director Le’Kneitah Smith. Six research projects have been approved to begin work in the fight against HLB in the Biosafety Level-3 Lab. The initial projects will focus on several areas of HLB mitigation, including:

- development of HLB tolerant/resistant rootstocks and scions through non-traditional (i.e., CRISPR) breeding,
- identification of HLB-resistance genes from citrus hybrids for the development of HLB-resistant commercial citrus varieties,
- standardization of greenhouse methods to evaluate relative HLB tolerance/resistance of scions and rootstocks,
- identification of prophylactic and potentially curative antimicrobials to suppress or kill the presumptive HLB pathogen ‘Candidatus Liberibacter asiaticus’ (CLas) and testing of a class of CLas proteins (effectors) for their role in the citrus infection process and as a potential early systemic indicator of infection.

### Tolerance/Resistance Breeding and Evaluation

Three of the six research projects focus on the development and evaluation of HLB tolerant/resistant rootstocks and scions. The first project, “Development of non transgenic HLB-resistant citrus varieties using CRISPR-Cas9,” funded by the U.S. Department of Agriculture-National Institute of Food and Agriculture (USDA-NIFA) and led by Nian Wang, Ph.D., from the University of Florida, focuses on developing non-transgenic HLB-resistant citrus varieties using CRISPR technology to modify citrus gene products/pathways that are manipulated by CLas to promote infection and disease. Citrus Research Board (CRB)-funded researcher Mikeal Roose, Ph.D., of UCR is managing the California-based experiments, testing resistant citrus varieties (‘Washington Navel,’ ‘Tango’ and ‘Lisbon’ lemon) generated from this project with California CLas strains. Modification of these CLas-targeted citrus gene products/pathways could prevent CLas from infecting citrus altogether or could lessen the disease severity in CLas-infected citrus containing these modified genes.
The second project “Development of huanglongbing resistant/tolerant citrus through genomic approaches” is funded by USDA-NIFA and led by CRB-funded researcher Chandrika Ramadugu, Ph.D., from UCR. It focuses on determining HLB-resistance genes and validating their activity from citrus hybrids derived from crosses between commercial citrus varieties and HLB-resistant citrus relatives, a project originally funded by CRB project #5200-154 “Development of HLB resistance through inarching, novel disease tolerant hybrids and through breeding.” Identification of HLB-resistance genes from these citrus hybrids could be used in later breeding efforts to introduce HLB-resistance traits into commercial citrus varieties, a process that will be sped up with the opening of the BSL-3 laboratory, as inoculations and disease tolerance assessments of citrus varieties can now be evaluated locally.

The third project, “Refinement and application of greenhouse methods to evaluate scion and rootstock tolerance to CLas,” is funded by the CRB and led by Kim Bowman, Ph.D., from the USDA-Agricultural Research Service, Fort Pierce, Florida, with Roose running the west coast portion of this project. The goal is to evaluate potential differences in HLB tolerance/resistance across different scion and rootstock cultivars when tested against either Florida CLas strains in Fort Pierce or California CLas strains in Riverside under standardized greenhouse protocols. HLB tolerance/resistance evaluation is complicated by several interrelated factors (e.g., uneven distribution of CLas, genetic variability of CLas isolates and citrus varieties, time of disease progression); thus, there is a critical need to establish methods that determine relative HLB tolerance/resistance of rootstocks and scions to ensure the U.S. citrus industry is aware of the options available to them and how best tolerant/resistant varieties can be utilized.

Antimicrobial Therapies for CLas

Two of the six research projects focus on the identification and development of antimicrobial compounds to prevent infection altogether or reduce the bacterial levels of CLas-infected citrus.

The first project, “Deployment of a spectrum of bactericides to cure and prophylactically treat huanglongbing in citrus,” funded by USDA-NIFA and led by Caroline Roper, Ph.D., from UCR, focuses on the development of two classes of antimicrobial compounds to suppress or kill CLas – silver and sulfur nanoparticles and naturally derived antimicrobial compounds from microbes residing in “survivor trees.” The development of antimicrobial nanoparticles and naturally derived compounds ensures these compounds can be
used both in conventional and organic farming practices. Additionally, several application methods will be studied in detail to determine the most effective mean(s) to deliver antimicrobial compounds to the phloem. As CLas is a phloem-limited bacterium, the use of therapies that can be effectively delivered to the site of the bacterium are important to ensure compounds come into direct contact with CLas.

The second project in this area, “Combatting citrus HLB using citrus-derived antimicrobial peptides,” led by Hailing Jin, Ph.D., from UCR and also funded by USDA-NIFA, focuses on using a new group of antimicrobials discovered in HLB-resistant citrus relatives to suppress or kill CLas and potentially affect ACP development. The use of a citrus-derived antimicrobial has the potential to move quickly through the regulatory process with greater acceptance by the general public, two traits that can benefit the California citrus industry’s ability to swiftly implement an antimicrobial product for CLas mitigation while maintaining the public’s interest in environmentally responsible and ecologically sustainable practices.
CLas-secreted Protein Functions and HLB Diagnostics

The final project, “Establishing diagnosis and understanding the virulence mechanisms of Citrus Huanglongbing (HLB),” funded by USDA-NIFA and led by Wenbo Ma, Ph.D., from UCR, focuses on understanding how a class of proteins secreted by CLas helps the bacteria infect citrus trees and whether these proteins provide an early indication of CLas infection. As the number of new CLas-infected trees continues to rise in residential areas of California, there is a need to understand how CLas infects and multiplies in citrus trees, to inform researchers about potential methods to prevent or reduce its spread. Additionally, since these secreted proteins move throughout plants and can be detected significant distances away from the initial site of infection, these proteins may allow for early systemic detection of the bacteria compared to the approved regulatory PCR method.

Prospects for Researchers and the California Citrus Industry

The opening of the BSL-3 near UC Riverside – originally the site of what is now known as the Citrus Research Center and Agricultural Experiment Station – is not only befitting given the area’s rich citrus history, but is also a significant advancement for California researchers feverishly working towards finding a solution for HLB. When asked how the opening of the BSL-3 would benefit HLB research efforts, Ma said, “It will provide the much-needed HLB-diseased material to support our research. We also will be able to monitor the disease progression in a controlled environment. Since HLB is strictly quarantined in California, we have been relying on infected samples shipped from Florida and Texas as
freeze-dried tissues. This has been a major hurdle that significantly slows down our research.”

Considering how research in the BSL-3 would lead to a solution for California citrus growers, Ramadugu said, “Long-term, sustainable cultivation of citrus in the presence of HLB would require availability of disease-resistant and -tolerant citrus. We already have generated hundreds of advanced hybrids with potential resistance. Our work in the BSL-3 will help us to select promising hybrids for cultivar development in California.”

When asked what impact the BSL-3 will have for long-term HLB research, Roper said, “The opening of the BSL-3 facility will allow us to have sustained and ongoing research projects, which is crucial when understanding diseases that impact perennial trees like citrus. Opening this facility to researchers studying CLas pathogenesis will directly tie into the research efforts of the California citrus industry as we all seek to mitigate HLB.”

Carolina Evangelo is the director of communications for the Citrus Research Board in Visalia, California, and the co-publisher/project manager of Citrograph. Melinda Klein, Ph.D., is the chief research scientist for the Citrus Research Board in Visalia, California, and the science editor of Citrograph. Joey S. Mayorquin, Ph.D., is a research associate with the Citrus Research Board in Visalia, California and serves as associate science editor of Citrograph. For more information, contact Carolina@citrusresearch.org
On October 4, Lindcove Research and Extension Center (REC) Director Beth Grafton-Cardwell, Ph.D., hosted a gala at the center and officially named its conference facility the “Ray Copeland Citrus Center” after the late Ray Copeland. It is very fitting that the Center be named after Ray, because as superintendent of the field station from 1965-87, he was instrumental in developing the orchards, facilities and relationships with the first group of scientists who conducted research at Lindcove. Susan Fritz and Karen Bray, Ray Copeland’s daughters, talked about their father’s many achievements and their memories of growing up living at Lindcove. Jim Gorden, chair of the Citrus Pest and Disease Prevention Committee and local grower, also spoke about his many years of partnership with Ray, as well as Ray’s contributions to the citrus industry.

The gala also was an opportunity to honor Georgios Vidalakis, Ph.D., the director of the University of California (UC) Citrus Clonal Protection Program (CCPP) and specialist and professor of plant pathology at UC Riverside. The CCPP is a renowned program that brings in new citrus germplasm from around the world, cleans it free of disease and provides the California nursery industry and homeowners with “clean” budwood. Lindcove REC is the location from which the budwood is distributed. In 2019, the Citrus Research Board (CRB) and the UC Office of the President co-funded a $1 million endowment. Subsequently, Vidalakis was awarded this “Citrus Research Board Presidential Researcher for Sustainable Citrus Clonal Protection Endowment.” At the gala, he spoke about the endowment funds and their importance for supporting the CCPP program at Lindcove, as...
and a modern laboratory with high-tech equipment. The CRB also provides grant funding to the scientists for the majority of the 30 research projects conducted at Lindcove each year. These facilities and projects have given Lindcove REC a world-renowned reputation as a “Center of Excellence” for citrus breeding, horticulture and pest management.

While research at Lindcove REC is cutting edge, outreach programming has been limited due to Lindcove’s small staff, small conference center and the undeveloped roads and parking around the conference center. The current outreach program focuses primarily on tours and field days for growers, pest control advisors and nurserymen. For the general public, outreach has been limited to a yearly master gardener workshop, bringing in local Ag Academy high school students for one-day experiences with agricultural mechanics and agricultural science, small tours of the citrus orchards, and the December fruit display and tasting. The level of outreach and impact on the local communities could be greatly expanded with improvements in infrastructure and staffing.

The UC ANR recently committed to providing funds to redevelop the outreach facilities at Lindcove to better serve the needs of the citrus industry and the local community. Redevelopment plans include constructing a larger conference center to create a hub for citrus industry and university interactions. This building could be used for 300-person industry meetings or subdivided for committee meetings. Plans also include building a “youth experiential laboratory” where students are taught agricultural science using microscopes, biotech equipment and computers, as well as hands-on learning about citiculture in a nearby citrus demonstration orchard and agricultural mechanics in the pack-line. This training in citiculture and science will help prepare the next generation of farmers and scientists who will work together to ensure the future health and success of the citrus industry.

Beyond the facilities improvements funded by UC ANR, additional funds are needed to support staff who specialize in education to develop the outreach programs, and funds are needed to purchase equipment for these new outreach programs.

well as Lindcove’s role in training future scientists.

The gala also was the kick-off of a fundraising program to improve the conference center area and outreach programs at Lindcove REC. During the past 25 years, the University of California Agriculture and Natural Resources (UC ANR) and the CRB have partnered again and again to develop high quality facilities and equipment for research. These facilities include the pack-line and fruit grading system, screenhouses and greenhouses to protect citrus from pests and disease,
facilities. This is why the “Sweetening the Future of Citrus at Lindcove” fund-raising campaign has been initiated. The Ray Copeland and Jim Gorden families together have very generously contributed $150,000 as a match for funds donated by others to the fundraising campaign. The goal is to raise at least $2 million in donations that, combined with the UC ANR funding, will provide the facilities’ improvements, equipment and program support to take Lindcove REC educational outreach into the future. With these changes, Lindcove REC will greatly expand its ability to attract top research programs, provide a hub of interaction between the research community and the citrus industry, inspire local youth to pursue careers in agriculture and science and educate the general public about citrus. For more information about the campaign or to make a donation, please visit the campaign web site at lindcovecitrus.com.

Elizabeth Grafton-Cardwell, Ph.D., is an integrated pest management specialist and research entomologist in the Department of Entomology, UCR and director of the Lindcove Research and Extension Center. For additional information, contact eegraftoncardwell@ucanr.edu
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Foothill Mandarin Orchards
Benefit from Mulch

Cindy Fake, Bob Bonk and Louise Ferguson

Project Summary

For the last three seasons, five Placer County mandarin growers, in collaboration with the University of California Cooperative Extension, have conducted mulching trials in Satsuma mandarin orchards. The purpose is to determine whether mulch can reduce herbicide and water use while improving soil and tree health and orchard productivity. The trials are still in progress. To date, the data show lower and more stable soil temperatures in mulched compared to unmulched plots. Soil moisture under mulch is maintained longer and rarely depleted at 6- to 12-inch depths. Higher fruit drop is observed in unmulched plots, indicating reduced temperature and moisture stress under mulch.

Grower collaborators have hosted 12 on-farm workshops to date, sharing what they have learned with other producers. This participatory research has led to a 97 percent increase in the use of mulch since the start of the project by 44 of the growers who attended the workshops.
Background

The foothill mandarin growing areas above the Sacramento Valley are at the climatic limit for citrus production. The wet, cold winters and hot, dry summers can be stressful for citrus. A changing climate adds stress through unpredictable temperature fluctuations in late spring and early summer.

In the last decade, foothill citrus growers have started using organic mulches to mitigate tree stress. Research shows that organic mulch increases organic matter and reduces erosion. It decreases evaporative losses, maintaining soil moisture in coarse soils (Cerdà et al. 2018, Faber et al. 2001, Lui et al. 2014). Mulch also is reported to modify temperatures (Mesejo et al. 2012) and increase biological activity in the root zone (Homma et al. 2012). This evidence points to increased drought resilience and mitigation of tree stress. However, mulching practices needed to be evaluated to develop grower recommendations.

The goal of this study is to determine if mulching can reduce herbicide and water use while maintaining or improving soil and tree health and orchard productivity. The study documents the effects of mulch on soil properties, including moisture, temperature, organic matter and microbial activity, as well as tree health, nutrient status, yields and fruit quality.

Research Trials

Research trials were established in five commercial Owari Satsuma mandarin orchards in 2017. Each trial plot is composed of three uniform trees, with the center tree as the data tree. Each trial replicate contains a mulch treatment and an unmulched control. Mulch is applied annually, in the spring, at a depth of four to six inches. The mulch is a mix of 50 percent horse manure and 50 percent wood chips placed along the tree row under the canopy. These materials are readily available and would otherwise be sent to the landfill.

Data logger stations monitor temperature and relative humidity in the canopy, as well as soil temperature and moisture. Tensiometers also measure soil moisture. Data are collected weekly in each orchard. Soil, mulch and leaf tissue are analyzed annually. Fruit yields and quality are assessed annually at harvest in November and December.

Results to Date

Soil moisture is constantly monitored under mulched and unmulched (control) trees. Moisture levels in the control depleted more rapidly throughout the soil from zero to 12 inches. Most moisture depletion occurs in the top six inches, but mulched trees have substantially less depletion compared to the control. At 6-12 inches, the mulched soil consistently retains moisture with minimal depletion.
Soil temperature is being monitored at a depth of nine inches. Data from control plots show significant fluctuations in temperature in short periods of time. Data from the mulched trees reflect more temperature stability with the highs lower than in the control. The control trees may be under more stress due to the rapid temperature swings. Our trial data have documented canopy temperatures of lows in the 40s to highs above 105°F in the same week. Field observations show that fruit drop (June drop) occurs at a much higher rate and volume in unmulched treatments.

The study is still in progress, but growers are adopting mulching practices. Forty-four growers now use mulch in their citrus orchards, a 97 percent increase since the project started. In a July 2019 grower survey, 68 percent of respondents reported using mulch. The five most important reasons cited for using mulch (in ranked order) are:

1. save water (47 percent of respondents),
2. improve soil health (58 percent),
3. reduce herbicide use (21 percent),
4. improve tree health (47 percent) and
5. improve fruit quality (44 percent).

Thirty-two percent of survey respondents did not mulch. They cited the following reasons (in ranked order):

1. too much labor (32 percent of respondents),
2. cost of materials and/or application (26 percent),
3. too much time (32 percent),
4. increases rodent problems (11 percent) and
5. difficulty hauling or applying (32 percent).

Conclusions and What We Have Learned
Mulch may benefit citrus orchards by mitigating tree stress due to temperature and moisture fluctuations, reducing water use, reducing weeding and/or herbicide use and reducing fruit drop. This may improve orchard productivity and returns. However, there are some caveats. Timing is critical. Mulch should be applied only to wet soil. For mandarins, it is best to apply it in spring when soil is still moist. Spring application provides a six-month interval between application and harvest, which is important if manure is used. It also allows decomposition of mulch before the rainy season, when it can be a detriment in a wet year. Monitoring soil moisture is important. Mulch changes irrigation timing and frequency. In our study, the interval between irrigation cycles in mulched orchards has increased. Mulch reduces moisture loss from the soil. If irrigation was not adjusted, soil under the mulch stayed too wet, increasing tree stress.

Manure provides beneficial microbes and more nutrients than wood chips. However, manure may increase phosphorus
in the soil, so regular soil testing is needed. Wood chips require less labor as they typically last 18 months to two years in a wet winter climate.

Mulching can be a beneficial practice, helping to build healthy soils, reduce water use and reduce tree stress. However, mulching may not fit into every orchard system. Our experience and grower evaluations show that multiple factors influence the effective use of mulch in citrus orchards.

References


Cindy Fake is a farm advisor and Bob Bonk is a research manager with the University of California Cooperative Extension in Placer and Nevada counties. Louise Ferguson, Ph.D., is a pomology specialist with the University of California Cooperative Extension at the University of California, Davis. For additional information, contact cefeake@ucanr.edu
Citrus Archives Move to New Home

Benjamin Jenkins

Recently, the Citrus Roots: Preserving Citrus Heritage Foundation transferred its impressive collection of historical documents to Archives and Special Collections at Wilson Library, the University of La Verne. Now located on La Verne’s main campus in eastern Los Angeles County, this impressive collection includes historical newspapers, photographs, books, old issues of Citrograph, artifacts and much more detailing the citrus industry in California. Materials from the collection date back to the early 19th century and the days of William Wolfskill, an American who planted one of the first orange groves in Los Angeles in the 1870s. The collection touches on a variety of topics relevant to modern citrus agriculture, including frost protection, pest control and transportation. The University of La Verne is excited to make these materials available on-line, in museum exhibits and in the pages of Citrograph. A sample of digitized documents from the collection is available at https://digitalcollections.laverne.edu/, under the “Citrus Roots Collection” link.

A significant part of the collection consists of citrus crate labels. These vivid examples of early graphic art from California were placed on the sides of wooden boxes containing oranges that were shipped across the United States from the 1890s to 1954. Eye-catching and vivid, citrus crate labels such as the one above used by the W.A. Snyder & Sons Company of Upland, California, attracted customers to the orange and lemon bounty of the Golden State. Note the glass of orange juice, which is prominently displayed in the image. The rise of orange juice as a staple of the American breakfast was largely a result of the California Fruit Growers Exchange’s aggressive marketing campaign to boost the consumption of vitamin C. That Exchange is now known as Sunkist Growers, Inc., which maintains its role as a leader in the citrus industry.

Benjamin Jenkins, Ph.D., is the university archivist of the Wilson Library and an assistant professor in the Department of History and Political Science at the University of La Verne in La Verne, California. For more information, contact bjenkins@laverne.edu

New Feature...

Please be sure to check out page 70, where you will find our new regular feature, A Taste of Citrus History. In this same location in each issue of Citrograph, University of La Verne Wilson Library Archivist and Assistant Professor Benjamin Jenkins, Ph.D., will present a historic California citrus industry photo. We look forward to sharing with you these fascinating glimpses into the industry’s past.

Ivy Leventhal, Managing Editor
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On October 4th Lindcove Research and Extension Center officially launched the campaign “Sweetening the Future of Citrus at Lindcove” to enhance our research facilities and grow our educational outreach programs.

Thank you to the many generous donors that have supported our efforts thus far!

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Sometimes the development and naming of a new cultivar follows a complicated route. One example is the newly named US Superna mandarin. Referred to as either Lee x Nova, Supernova or USDA 88-2, this cultivar was created by Jack Hearn, a plant geneticist, formerly of the U.S. Department of Agriculture-Agricultural Research Service (USDA-ARS) U.S. Horticultural Research Laboratory in Orlando, Florida, in 1966. Even after 20 years of low yields and considerable dieback at the USDA-ARS Leesburg Farm, Hearn believed that US Superna was the “best tasting” citrus he produced in 32 years as a breeder. Convinced this seedless selection had commercial value in California, he sent budwood to the Citrus Clonal Protection Program (CCPP) for evaluation in 1988. The CCPP began distribution in California as USDA 88-2 in September 2007. On June 28, 2019, came the USDA announcement of an official name and release of US Superna, thus completing this journey.

Evaluations of US Superna fruit characteristics were conducted as part of our (Kahn and Siebert) CRB-funded research on new citrus introductions from 1996-2002 (https://citrusvariety.ucr.edu/citrus/88-2.html). Fruit of this cultivar was compared with Clementine Fina Sodea fruit on Carrizo and C35 rootstocks at the Lindcove Research and Extension Center in Exeter, California. Fruit of US Superna had slightly higher Brix, yet similar acidity in late-October/early-November (Table 1). By mid-December, US Superna had higher Brix and slightly lower acidity than Fina Sodea, with

This Mandarin By Any Other Name Would Taste As Sweet

USDA Official Name and Release of US Superna

Tracy Kahn, Toni Siebert Wooldridge and Ed Stover
larger fruit and better rind color. Although US Superna trees were under heavy cross-pollination conditions, they had less than one seed per fruit, whereas Fina Sodea seed numbers ranged from 3.7 to 12 per fruit.

The name, Supernova, was coined by LoBue Brothers, who first planted US Superna in 2010 and now have 110 acres grown on Rich 16-6 and Pomeroy trifoliate rootstocks. Robert LoBue described the fruit as seedless with dark orange color and great fragrance and flavor. Compared to Tango, US Superna is a medium producer with some alternate bearing and a large “June drop.” Fruit is typically in the 113 navel orange size category. It can be harvested warm, but it needs to be chilled while packing. Trees respond best when grown on good soil with minimal stress. The LoBue Brothers harvest their fruit beginning in mid-December in Lindsey, California, when the rind is dark orange (sweating the fruit is not effective) and continuing for about six weeks. Emily Ayala from Friend’s Ranches in Ojai, California, harvested their fruit in late January and early February for farmers’ markets in 2018. Ayala feels US Superna will be a good variety for them because of the excellent flavor and consumer acceptance (Emily Ayala, personal communication).

To obtain budwood of US Superna (currently listed as USDA 88-2 VI 501) in California, please visit the Citrus Clonal Protection Program website at http://www.ccpp.ucr.edu for more information.

Acknowledgements

The authors thank David Karp for background information, Robert LoBue from LoBue Brothers and Emily Ayala from Friend’s Ranches for their insights.

Tracy Kahn, Ph.D., is the curator and Givaudan Citrus Variety Collection Endowed Chair at the University of California, Riverside. Toni Siebert Wooldridge is a senior museum scientist associated with the Givaudan Citrus Variety Collection and Citrus Breeding Program at UCR. Ed Stover, Ph.D., is a research horticulturist at the USDA-U.S. Horticultural Research Laboratory in Fort Pierce, Florida. For more information, contact Tracy.kahn@ucr.edu

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The two rootstocks used in this planting were Carrizo (CZO) and C35 Citrange (C-35). Further results for specific sample dates and additional characteristics for US Superna and the standard are posted at the UCR Citrus Variety Collection website under US Superna: https://citrusvariety.ucr.edu/
Data Analysis and Tactical Operations Center (DATOC)

Expert Panel Meets in Denver

Holly Deniston-Sheets and Neil McRoberts

Project Summary

The mission of the Data Analysis and Tactical Operations Center (DATOC) is to provide analyses and science-based guidance to manage huanglongbing (HLB). A diverse team of experts fulfills the need for rapid, flexible and responsive analyses to interpret complex issues surrounding the Asian citrus psyllid (ACP) and HLB control. In September 2019, the team gathered together in Denver, Colorado, for two days of data-driven discussions.

The Citrus Pest and Disease Prevention Program (CPDPP) is funded by grower assessments, and spending grower money wisely and effectively always has been a priority for the CPDPP. Driven by this responsibility to growers, the CPDPP asked DATOC to evaluate the effectiveness of the current huanglongbing (HLB) management program in southern California, and to identify when or how management strategies should change. While this seems to be a straightforward request, it has proven to be a complex task to complete. Luckily, one of DATOC’s strengths is in bringing together different perspectives from around the country to work through problems, and much of the meeting in Denver focused on how to provide an answer to the CPDPP’s request.

While the total number of diseased trees detected and destroyed in southern California has continued to rise, most diseased HLB-affected trees likely go undetected due to methodological restrictions, as well as to other biotic
and abiotic factors. For that reason, the presence or absence of diseased trees cannot be used to definitively evaluate program success or disease spread. DATOC panelists reviewed research from the U.S. Department of Agriculture-Agricultural Research Service U.S. Horticultural Research Laboratory in Fort Pierce, Florida, which suggests disease spread may depend more on psyllid populations than infected trees: infected psyllids move the HLB-associated bacterium, ‘Candidatus Liberibacter asiaticus’ (CLas), around the environment independent of whether diseased trees are present before the psyllids feed. This insight adds strength to the idea that detectably infected trees may not be an effective way to judge where HLB is spreading. However, diseased trees that are not destroyed eventually lead to increasing CLas levels in the ACP population, and psyllids with higher levels of CLas tend to spread the bacterium more efficiently, resulting in a rapid increase of disease occurrence and distribution. Tree and psyllid management are, therefore, likely to remain an important part of the CPDPP’s overall strategy. How resources should be allocated for the greatest effectiveness remains to be determined.

The problem facing the CPDPP, and the one posed to DATOC, is how to best allocate program resources to improve disease detection, limit the negative effects of infected trees and manage the spread of CLas by ACP. The Denver meeting provided an opportunity for the expert panel to meet and make significant advancements on this issue. The team determined that a Citrus Research Board-funded model developed by a team in Fort Pierce (CRB project 5300-154) will be used to simulate how disease progress would have been expected to develop in one area of southern California that has undergone intensive pest and disease management by the program. By comparing the results of the model to what has actually occurred, the team can estimate the effects of the management strategies that were applied by the program in that area. We will explore altering models currently available.
used to guide CPDPP program activities, to switch the focus from diseased trees to psyllids and undertake a thorough examination of ACP data across southern California. Lastly, the team will try to estimate the risk to commercial citrus from the HLB quarantine zone and the risk from ACP in the urban landscape closest to commercial citrus. Depending on the results of these analyses, the team may recommend changes to the program’s activities. These activities will be completed by spring 2020, which will allow time for discussion by the CPDPP Committee ahead of budgetary decisions for the next fiscal year. Any suggested major changes to program activities will require approval of the Committee and would be incorporated into operations during the next budget cycle.

**CRB Research Project #5300-182**

_Holly Deniston-Sheets is the DATOC coordinator. Neil McRoberts, Ph.D., is an associate professor of plant pathology at the University of California, Davis, a panelist in the University of California Agriculture and Natural Resources Sustainable Food Systems Strategic Initiative, and the western region director for the National Plant Diagnostic Network. For additional information, contact holly@citrusresearch.org or visit www.DATOC.us_

---

**Figure 5. Tom Delfino, former executive director of the California Citrus Nursery Society.**
Huanglongbing (HLB) is the most devastating citrus disease worldwide and threatens all commercial citrus production. Since 2005, Florida has lost 72% of its citrus production.1 According to California Citrus Mutual, infected trees have been found in Southern California and quarantine areas are growing.2

The Asian citrus psyllid (ACP) feeds on new leaf growth and is a vector of the bacterium that causes HLB. Once a citrus tree is infected, the disease is fatal. At this time there is no known cure for the disease. Symptoms of the disease include yellowing leaves and bitter fruit followed by the death of the tree.

Best management practices center around controlling ACP through use of insecticides and removing infected trees.

ACP and Insect Management Options from Bayer

Bayer has a proven portfolio of insecticides that provides the foundation for season-long ACP control and controls other important California citrus pests. The Bayer portfolio of insecticides encompasses multiple modes of action to limit insecticide resistance and is flexible relative to application timing and method to optimize crop quality and to help growers stay ahead of HLB.

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*Suppression only. **Insecticide Resistance Action Committee’s mode of action groups.

How ACP Affects Citrus Plants³

The psyllid damages citrus directly by feeding on new leaf growth (flush).

More importantly, the psyllid is a vector of the bacterium, Candidatus Liberibacter asiaticus (CLas), that causes HLB and transmits the bacteria into the phloem when it feeds on flush.

HLB disease spreads from tree to tree when a bacteria-carrying psyllid flies to a healthy plant and transmits the bacteria as it feeds on the leaves and stems.

The bacteria multiply in the tree’s phloem tissue, blocking the flow of nutrients through the plant. If not well managed, trees will eventually die within three to five years.

Effective control of Asian citrus psyllid reduces the chance that a citrus tree will become infected by the bacteria and helps ensure a healthy, productive tree.


²https://www.farmprogress.com/fruit/california-citrus-mutual-marks-four-decades-important-industry-watchdog-group

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NMR as a Promising Screening Tool for Identifying HLB

Emily Padhi, Darya Mishchuk and Carolyn Slupsky

Project Summary
A tiny bacterium wreaking havoc on the Florida citrus industry is making its way to California’s golden groves. Many have tried and failed to come up with a cure that will kill ‘Candidatus Liberibacter asiaticus’ (CLas), the bacterium that is associated with the disease huanglongbing (HLB). Without a cure, our only hope of saving the California citrus industry is by detecting the pathogen before the plant shows symptoms using an early detection technology (EDT), as this tool will allow growers to quickly identify infected trees in their groves. Teams of scientists across the country assembled to tackle this problem, with our lab joining the mix to develop an EDT. Based on the principles of Nuclear Magnetic Resonance¹ (NMR) spectroscopy and metabolomics, our screening tool measures the systemic responses of infected trees to CLas; and using greenhouse and field data, we showed that our EDT successfully distinguishes infected trees from those that are disease-free.

What Was Achieved
Our approach was based on the simple idea that when a living thing becomes sick, its metabolism changes. Instead of blood, plants have something called phloem² sap, which carries nutrients and other small molecules throughout the plant. These small molecules are called metabolites. Like the blood that courses through our veins, phloem sap is
transported to all parts of the plant – from the leaf veins all the way down to its roots. Metabolomics is a cutting-edge field of study that provides snapshot information about the metabolism of living things.

We hypothesized that the metabolism of a sick plant differs from that of a healthy one, that the differences would be detectable, and that our tool would be able to tell diseased plants apart from healthy ones. To test these hypotheses, we used NMR to assess the metabolism of citrus leaf samples collected from greenhouse and field experiments and created a model for diagnosing HLB. NMR is a powerful tool because it allows us to identify and accurately measure several components of metabolism in a single sample (Chin et al. 2015; Slupsky et al. 2013), which saves time and money.

Our previous research examined changes in citrus metabolism solely due to psyllid feeding and showed that higher insect loads dampen the plant defense response (Chin et al. 2017; Chin et al. 2018). In other words, NMR metabolomics successfully captures the stress responses initiated by psyllid feeding alone, allowing us to distinguish plants infested with ACP from those that are insect-free. The next step was to see if we could isolate the effects of psyllid feeding from those caused by CLas. This would tell us if NMR metabolomics can detect a specialized systemic response unique to CLas infection. To do this, the Slupsky lab participated in greenhouse experiments at the University of California, Davis (UC Davis) Contained Research Facility (CRF) and the U.S. Department of Agriculture (USDA)-Agricultural Research Service (ARS) U.S. Horticultural Research Laboratory in Fort Pierce, Florida.

Greenhouse Experiments

In the first experiment at the CRF, lemon or navel orange scions were grafted onto Carrizo rootstock, and additional grafts were made with material that either were infected with CLas or were CLas-free. Citrus metabolism was tracked by measuring leaf metabolites in samples from the canopy for about one year using NMR spectroscopy (Chin et al. 2014; Chin et al. submitted). To paint a detailed picture of the progression of HLB, we used a systems biology approach that combined multiple data sets from transcriptomics, proteomics and metabolomics. We found that CLas initiated a response in navels, significantly changing how plant cell walls are structured and how the plant makes energy during photosynthesis (Chin et al. submitted). As expected, these changes are more pronounced as plants become more diseased. Importantly, changes at the metabolite, protein and transcript level were detectable during the pre-symptomatic stage of disease.

The effects of CLas on citrus metabolism were also captured in the root system (Padhi et al. 2019), with NMR metabolomics distinguishing diseased plants from healthy plants (Figure 1). Overlap in the varietal response to CLas included a reduction in sugars and some amino acids and increased trigonelline, a metabolite that maintains water balance in plants during stress. Unique features in lemon root metabolism included reductions in two metabolites important for the production of defense related compounds – malic acid and quinic acid. By contrast, these metabolites increased in the roots of navel plants.

Figure 1. Nuclear magnetic resonance (NMR)-based metabolomics of root samples from healthy and huanglongbing-positive (HLB+) navel and lemon plants. Each of the small circles represent the root metabolite profile of a single plant. The large circles represent the average position of each group. Samples were collected at the same time point (46 weeks post-psyllid exposure). NMDS stands for non-metric dimensional scaling. It is a statistical technique that shows the similarity of individual samples to one another.
In collaboration with Johan Leveau, Ph.D., at UC Davis, we examined bacterial and fungal communities in and around the root system (Padhi et al. 2019). Interestingly, although the same rootstock (Carrizo) was employed, the microbial community structure in and around the roots varied by the scion showing that there is a direct effect of the tree canopy on microbial structure of the roots. In addition, the presence of CLas altered the microbial structure of the root system, with some changes that were shared between the two varieties.

In a second experiment at the UC Davis CRF, Lisbon lemon trees grafted onto Carrizo rootstock were exposed to CLas-positive psyllids or CLas-free psyllids. As in the first greenhouse experiment, NMR-based analysis tracked the metabolism of plants through leaf samples for one year. Similar to the graft study, the metabolism of infected trees changed during the two months following exposure to CLas-positive psyllids, and strong metabolic differences emerged four months post-exposure (Figure 2).

A few trends emerge when comparing Lisbon lemon plants in the graft- and psyllid-inoculation experiments. Leaf data show that the same amino acids decrease very early during infection, followed by an increase as the disease develops. The impact of CLas-infection on lemon roots was similar, regardless of whether the plants were inoculated with CLas by grafting or psyllid feeding. Roots in both experiments lost a substantial amount of sugar and amino acids and saw an increase in trigonelline. This suggests that the metabolic response in the root system is the same, regardless of how CLas is introduced.

In a longitudinal greenhouse study (performed in collaboration with Mark Hilf, Ph.D., and Greg McCollum, Ph.D.) using Valencia scions grafted onto US-812 rootstock at the USDA-ARS U.S. Horticultural Research Laboratory, we observed differences in metabolite concentrations of samples obtained from trees continuously exposed to CLas-positive psyllids compared to trees not exposed to psyllids over several months. These differences were similar to those observed in graft and insect inoculated plants in the CRF experiments.

The trees used in all greenhouse experiments were very young (one-year-old at the beginning of the experiment) and different plants experienced growth spurts at different times, making it challenging to compare one group of trees to another. However, the greenhouse setting is a tightly controlled environment, and the fact that we reproducibly observed similar changes in metabolism with CLas infection, regardless of mode of transmission, rootstock or scion, allows us to confidently say the observed changes in metabolism are due to CLas infection and not some unknown factor. The next step of our research program involved validating our findings in the greenhouse with data collected from the field.

Field Studies

In collaboration with John da Graça, Ph.D., from Texas A&M-Kingsville Citrus Center, Weslaco, Texas, and the California Department of Food and Agriculture, we analyzed samples from field trees in Texas and California, some of which were confirmed CLas-positive trees. We compared the results from these field samples with results from greenhouse studies to
help develop a biomarker profile, or set of metabolites, for our EDT (Mishchuk et al. 2017). Using these data, we also participated in field trials in Texas and California including the TX-2 EDT study from 2015-16 and the CA-1/CA-1b EDT study from 2017-18 to test how our screening tool performed with field samples (LeVesque and McRoberts 2017).

We were able to successfully identify CLas infected trees 100 percent of the time (LeVesque and McRoberts 2017). In those instances where it was absolutely known that trees could not be infected, our test performed well, successfully identifying trees that were not infected 100 percent of the time. In other studies, such as the CA-1b field validation study, we identified several trees that may be CLas-positive; but regulatory PCR testing did not identify the trees as CLas-positive, and we are unaware of any additional PCR testing that may have occurred since the project was completed in 2018. Nonetheless, these experiments highlight the strong predictive value of our EDT, which is not impacted when other diseases such as tristeza or citrus stubborn are present.

What These Results Mean for Growers

These projects looked at the impact of CLas on plant metabolism in greenhouse and field settings, with CLas exposure via graft or insect inoculation and in multiple varieties of citrus.

We found that when CLas enters a citrus plant, it initiates a metabolic response that is detectable by NMR-based metabolomics with a high degree of accuracy. This was confirmed experimentally in the greenhouse and tested with field trees. Collectively, these studies tell us some important things about HLB and the approaches growers can take to prevent the devastation of their groves. If our EDT method were to be automated, it could be used by growers to monitor new or existing infections and control disease progression through identification of infected trees.

It can take years for a mature tree to show any symptoms of HLB, all the while harboring the bacterium, which replicates inside the tree and spreads to other parts of the plant. The longer CLas is in a plant, the more likely the Asian citrus psyllid, the tiny insect that feeds on citrus leaves, will pick it up. Psyllids transmit the bacterium to other trees as they make their way through an orchard, and the results are devastating. Just look at the impact of HLB on the Florida citrus industry. 😞

CRB Research Project #5300-150

References


**Emily Padhi, Ph.D.,** is a post-doctoral researcher and **Darya Mishchuk, Ph.D.,** is a staff research associate in the Slupsky lab at the University of California, Davis. **Carolyn Slupsky, Ph.D.,** is a professor in the Department of Food Science & Technology and in the Department of Nutrition at the University of California, Davis. For more information, contact cslupsky@ucdavis.edu

**Glossary**

1. **Nuclear Magnetic Resonance:** A technique based on electromagnetic radiation used to determine the content(s) of a sample.

2. **Phloem:** The plant vascular tissue through which sap containing sugars and other products or metabolites from the leaves are transported to all other parts of the plant. Movement generally occurs from source tissues (photosynthetically active leaves) to sinks (developing fruit, new leaves and roots).

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*For Brochure Contact: Roy Pennebaker #0845764 (559)302-1906 or Matt McEwen #01246750 (559)280-0015*
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Resistance of ‘Tango’ Mandarins to Fork-tailed Bush Katydid

Bodil Cass, Elizabeth Grafton-Cardwell and Jay Rosenheim

Project Summary
The current integrated pest management (IPM) guidelines for key California citrus insect pests are based on research done on oranges. We are using a combination of “big data” analyses and field experiments to help create specific recommendations for the mandarin cultivars now being grown. We previously reported results from analyses of a database of pest control advisor (PCA) and grower records of citrus grove management in the San Joaquin Valley showing very low fruit scarring by fork-tailed bush katydids in ‘Tango’ and ‘W. Murcott’ mandarins, despite similar densities of these insects in mandarin and orange groves after petal fall. Presented in the following article are results from field experiments to test hypotheses regarding the mechanisms underlying this observation. We found that katydids reject opportunities to feed on ‘Tango’ fruit. Instead of chewing deep holes in the fruit, as was commonly observed for oranges, the katydids only scratched the surface of the ‘Tango’ fruit. At harvest, the superficial scratches on the ‘Tangos’ were not easily distinguishable from other minor damage. This indicates that in contrast to sweet oranges, ‘Tango’ mandarins have natural resistance to fork-tailed bush katydids, making them a non-pest in this crop. Preliminary analyses of ongoing experiments indicate that these results extend to ‘W. Murcott’ mandarins, but not to Clementines.
Katydids are Early-season Pests in Oranges. What about Mandarins?

Fork-tailed bush katydids (Scudderia furcata) are known to be early season pests of California sweet oranges. The nymphs feed directly on young fruit, creating scars that persist to harvest and often cause the fruit to be downgraded from “fancy” or “choice” to “juice” quality, resulting in a nearly complete loss of value (Grafton-Cardwell et al. 2003). Katydid nymphs are mobile and can damage many fruit in a short amount of time. Therefore, one katydid find in a three-minute search triggers an insecticide application (Grafton-Cardwell et al. 2017). Grower understanding of this serious pest is based on years of experience and knowledge gathered in sweet oranges. However, California citrus production has changed substantially in recent years, with a sharp increase in mandarin acreage (CDFA-CASS 2018). Research is needed to understand the biology of insect pests in mandarins and to optimize guidelines for effective pest management.

We previously reported results emerging from a large “Citrusformatics” database of management records from growers and PCAs in Tulare and Fresno counties, indicating that similar densities of katydid nymphs are found in commercial ‘Tango’ and ‘W. Murcott’ mandarin and sweet orange groves after petal fall. Despite these similar densities, however, almost no katydid scarring is recorded in ‘Tango’ and ‘W. Murcott’ mandarins in surveys of harvest bins (Cass et al. 2018; Cass et al. 2019b). These observations raised the question of whether fork-tailed bush katydids should be considered economic pests in these mandarin cultivars.

Testing Hypotheses Arising from Grower and PCA Data

We conducted a series of field experiments to test four possible explanations for why katydid scarring is lower in mandarins than in sweet oranges, despite similar katydid field densities:

1. a feeding aversion by the katydids to mandarin fruit,
2. preferential shedding of damaged fruit by mandarins,
3. healing of the feeding-damaged mandarins and
4. different scar appearance on mandarins, causing misclassification of the damage at harvest.

It was important to test these hypotheses in replicated field experiments, because they have different management implications (Cass et al. 2019a).

We collected katydid nymphs (approximately 2nd instar; E.E. Grafton-Cardwell Extension Online Campus Course) and caged them onto terminal branches of ‘Parent Washington Navel’ sweet navel orange trees and ‘Tango’ mandarin trees at the Lindcove Research and Extension Center at two different times soon after petal fall. After five days, the katydids were removed and the feeding damage on fruit and leaves was examined. Each fruit was tagged and monitored through harvest for fruit shedding and scar development (Figure 1).
Katydids Don’t Feed on ‘Tangos’

We found strong support for the first hypothesis of feeding aversion. The katydids fed readily on the sweet oranges, chewing deep holes in the fruit; but on ‘Tango’ mandarins, they almost exclusively caused only superficial cuts and occasionally some superficial chewing in the later experiment when they were larger (Figure 2). We interpret the chevron-shaped cuts seen on some fruit as the marks left when the katydid’s paired mouthparts sliced into the fruit, but the katydid then rejected the fruit upon making an initial tasting bite. In contrast, the deep holes in oranges appear to be the result of many repeated bites and ingestion of the fruit. This provides an explanation for the perplexing observation from grower and PCA-generated data: fork-tailed bush katydids are present in ‘Tango’ trees, but are not eating the fruit.

The only part of the ‘Tango’ plant on which the katydids fed were the floral tissues: stigmas, styles and floral discs, all of which become unavailable as the fruit grows. This feeding on flower parts appears to have no economic significance. Leaf feeding observed in the cages was low, even in the oranges (data not shown).

The second hypothesis of preferential shedding of damaged fruit does not appear to contribute substantially to the difference in final scarring rates between oranges and mandarins. The third hypothesis of damage recovery also was not supported by our experiments. For example, when we used a sharp metal punch to create small holes in the rind of young fruit that mimic the holes katydids chew into oranges, the ‘Tango’ fruit did not heal, and the resulting scars were deep and scabby (Figure 3a), similar in appearance to the scars seen from the same simulated damage in navel oranges (Figure 3b). Thus, it appears that the absence of such scars

Figure 2. The katydids fed readily on sweet oranges, chewing deep holes in the fruit; but on ‘Tango’ mandarins, they almost exclusively caused superficial cuts or surface scratching. Graph indicates the mean percentage of fruit per cage with each damage type upon removal of the katydids. A and B: examples of deep holes chewed in oranges. C and D: examples of superficial cuts in mandarins.

Figure 3. Experimental fruit that had been scraped with a sharp metal punch after petal fall to simulate katydid feeding (a) ‘Tango’ mandarin, (b) navel orange, showed scarring at harvest (indicated with arrows).
on harvested 'Tangos' reflects the refusal of katydids to chew deep holes into the young 'Tango' fruit.

We did observe some small, shallow, irregular-shaped scars in the 'Tango' rind (Figure 4a), but could not trace them back to the original katydid damage. In contrast to the large scars seen in navel oranges (Figure 4b), these marks were indistinguishable from the other minor damage seen on the initially undamaged control fruit, likely caused by wind or rubbing.

Conclusions

Heavy feeding damage by fork-tailed bush katydids is extremely rare in 'Tango' mandarins, and the rare, heavily damaged fruit often promptly shed. Fork-tailed bush katydids do cause superficial feeding damage, but this damage is unlikely to be of economic consequence at harvest. With the combined support of these experimental results and the previous observational database results covering hundreds of commercial groves and growing conditions, we suggest that 'Tango' and, based on the database analyses, 'W. Murcott' mandarins have innate resistance to fork-tailed bush katydids. Applications of insecticides for katydid control in these cultivars can, therefore, be eliminated in favor of relying on the plant's natural defenses. Similar experiments have been conducted to test these same hypotheses in Clementine cultivars. This ongoing work will be reported separately; but from preliminary analyses, a very different picture is emerging again for fork-tailed bush katydids in Clementines compared to 'Tango' mandarins and sweet oranges.

CRB Research Project #5500-214

References


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Scientists Screen for New Plant-based Antibacterials

Expanding growers’ options for HLB natural product therapies

Michelle Heck, Stacy DeBlasio, John Ramsey, Laura Fleites, Robert Shatters and Mark Trimmer

PROJECT SUMMARY

Natural products made by plants, bacteria and fungi have been the source of numerous antimicrobial compounds used in both human medicine and crop protection. Advances in genome sequencing have enabled the development of molecular-based control strategies focused on the identification of antibacterial peptides naturally produced by plants to combat bacterial infection. In this project, we are screening plant-derived...
antibacterial peptides to identify novel, natural products effective at killing the Huanglongbing (HLB)-associated bacterium. The goal of our strategy is to minimize the development of resistance and to provide citrus growers with a range of naturally occurring antibacterial compounds with different killing modes of action. Our tools could contribute to the long-term sustainability of the citrus industry in California and elsewhere.

The presumptive causal agent of HLB, ‘Candidatus Liberibacter asiaticus’ (CLas), is genetically related to the soil bacterium Sinorhizobium meliloti, an important nitrogen-fixing plant symbiont². S. meliloti infects the roots of plants in the pea family, including Medicago truncatula (alfalfa), triggering the formation of specialized plant nodules, which are colonized by the bacteria. In these organs, the plant produces hundreds of nodule-specific peptides which halt multiplication of the bacteria and transform the cells into the symbiotic structure called the bacteroid. This symbiotic relationship is an abundant source of biologically available nitrogen in agriculture and is beneficial for plant growth.

Several of these plant-derived nodule-specific peptides disrupt the growth of plant and animal bacterial pathogens, including Salmonella and Listeria, by disturbing their membrane systems. These peptides contain distinctive structural features and an enrichment of the amino acid³ cysteine that make them easy to find in plant genome sequences using bioinformatics⁴. To date, more than 600 of these nodule-specific peptides have been predicted from the M. truncatula genome (Durgo et al. 2015). The large functional diversity of these peptides, their high stability in nature, their ability to kill genetically diverse bacterial species and their underlying genetic features make them a promising, untapped resource for use in the control of HLB.

We have developed an antimicrobial peptide screening strategy to identify natural product CLas therapeutics that hold promise for commercial development (Figure 1). Our team used bioinformatics to identify regions of these plant-derived peptides with antibacterial properties. We synthesized a subset of these peptides to evaluate their performance in killing CLas and the culturable CLas relative, Liberibacter crescens. We have implemented two bioassays to test for antibacterial activity, one that gauges bacterial membrane disruption and one that indicates the minimum peptide concentration needed to inhibit bacterial growth.

In year two of our project, we will be completing the screen, and the top candidate antibacterial peptides will be tested for their ability to block CLas transmission by its insect vector, the Asian citrus psyllid. Our team has partnered with AgroSource, Inc. for future work involving field trials and commercialization of a cocktail peptide product. The peptides identified in our screen could be the foundation of a field-deployable therapeutic strategy for HLB based on natural product antibacterials, which is vitally needed for the maintenance of citrus production in California and throughout other citrus growing regions of the world.

CRB Research Project #5300-196

References


Glossary

1Antibacterial peptides: Small proteins composed of five to 100 amino acids, produced by all living organisms that have the ability to kill bacteria.

2Symbiont: An organism living in close physical association with another organism in a mutually beneficial relationship.

3Amino acid: An organic compound that is a building block of proteins.

4Bioinformatics: A computer-based field of study that analyzes complex biological data.

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Project Summary

Control of the Asian citrus psyllid (ACP) is a priority for the viability of the California citrus industry, and it must be addressed immediately. Genetic engineering to control wild populations has been developed in other insects, including mosquitoes and fruit flies. While these technologies also may prove promising for ACP, currently genetic engineering of the psyllid remains elusive. To overcome this limitation, which could enable the engineering of potent genetic control systems for ACP, we aim to first develop a “toolkit” to reliably engineer ACP that can be exploited to develop population control technologies to combat this pest in California.
‘Candidatus Liberibacter asiaticus’ (CLas) is a bacterium associated with huanglongbing (HLB) disease. It is transmitted by the ACP (Diaphorina citri) (Figure 1). Current HLB management practices are insufficient to completely control this disease. Thus, there is a significant need for novel tools and techniques to control HLB. Genetic methods can be used to eliminate the ACP, which will affect this species only and not require insecticides. For instance, our goal is to produce sterile males through genetic engineering so, if released into the environment, it will reduce wild populations. As more of these genetically engineered sterile males are released, the population will decline until it is eliminated. These technologies work exclusively against ACP populations, so beneficial insects, like pollinators, will not be affected. Hence, this non-insecticidal, targeted method to control ACP could mitigate costs, technical obstacles and other negatives associated with insecticide use, making it a more sustainable approach for pest control.

Certainly, the advancement of genetic control technologies for ACP will provide new options to halt the spread of HLB. However, the genetic transformation of ACP has been thus far challenging and elusive. Currently, few laboratories have been able to achieve reliable genetic changes in this insect successfully; and none has been able to introduce foreign DNA (Hunter et al. 2018) into the ACP, a required step to generate genetic control technologies.

Our research focuses on developing the tools required to create genetic control technologies for ACP that can be applied as HLB control strategies in California. Specifically, we are designing CRISPR-Cas9-mediated genetic transformation tools for ACP. We have successfully created CRISPR-Cas9 engineered lines for multiple mosquito species (Li et al. 2017a; 2017c; 2019), wasps (Li et al. 2017b) and pests such as Drosophila suzukii (Buchman et al. 2018b).

The biggest challenge for a real-world application of these strategies for pest control is to guarantee the spreading of the engineered gene in the field population. For instance, to suppress a wild ACP population, it would be possible to release genetically-engineered ACP (geACP) that are able to produce sterile female offspring and fertile males. Thus the number of ACP in the field would be reduced, and geACP males would be available to transmit engineered genes. However, to spread their engineered genes, these males will have to compete for wild females with wild-type ACP (wACP) males. In the field, the encounters of geACP males with wACP females would be very low compared to encounters between wACP females and males. Also, each time that a geACP male mates with a wACP female, only 50 percent of his offspring will have the engineered genes. Consequently, in each generation, the proportion of geACP is reduced (Figure 2A).
One way to circumvent this problem is to incorporate a genetic element that increases the inheritance of the engineered gene. Such elements are present in nature and are called gene drives. An ideal gene drive element introduced in a geACP mated to a wACP would increase the transmission of the engineered gene to roughly 100 percent of the offspring instead of 50 percent. As a consequence, over time the wACP will be more likely to encounter geACP to mate, and the frequency of the gene increases with each generation (Figure 2B). CRISPR-Cas9 can be used as a tool to create the geACP and can also be used as a gene drive, facilitating the spread of modified genes into the wild population.

Our goals are to:

1. generate a protocol to create genetic modifications in the ACP, and then
2. generate a protocol to use CRISPR-Cas9 as a gene drive and evaluate its potential application to drive such genetic changes in the field and reduce the ACP population (Figure 2C).

To achieve the first goal, we must surpass the major obstacle in ACP genetic transformation, which is the successful delivery of the Cas9-guideRNA (gRNA) molecules into the eggs and the survival of the eggs injected with these...
molecules. Eggs must be collected early after they have been laid, then aligned and injected with a Cas9-gRNA mixture. After injections, the eggs are placed on host plants or other favorable environments, allowing them to hatch into nymphs that are screened to identify the modified individuals. To optimize ACP transformation, we have to carefully choose genes that can be screened to identify the best conditions for injections.

We identified several potential target genes for which mutations can be observed in nymphs or adults (e.g. eye color, body color or short wings). From these, we selected four genes related to eye color and designed gRNAs that we co-injected with Cas9 into the eggs of ACP to test their effect on egg survival. These preliminary egg injections with Cas9-gRNA produced between 10-14 percent survival of embryos. We are now aiming to increase the rate of survival by testing different conditions for egg collection, injection and hatching. Injection protocols may be difficult, but we have successfully adapted this technology to many insect species (Buchman et al. 2018a; Li et al. 2017a; 2017b; 2017c; 2019).

As an alternative to egg injections, we also have evaluated the injection of the Cas9-gRNA into adult females using the technology termed Receptor-mediated Ovary Transduction of Cargo "ReMOT Control" (Chaverra-Rodriguez et al. 2018). This system uses a peptide, called P2C, to deliver the molecules specifically into the developing ovaries of insects. Researchers have not evaluated this system in ACP; hence, we tested the efficiency of P2C to introduce a red fluorescent protein in the ovaries of ACP females. We detected the fluorescent protein in the developing eggs (Figure 3). This is encouraging and suggests that P2C can be used to deliver Cas9-single gRNA (sgRNA) into the ovaries. The next step is to inject females with P2C-Cas9-sgRNA to investigate the efficiency of this method.

### Conclusions

Genetic transformation traditionally has been difficult for many insect species, including ACP. However, with the advent of CRISPR-based gene editing technologies, genetic engineering is becoming a reality for many organisms. Our preliminary results to generate targeted genetic changes in ACP are encouraging. Successful achievement of this goal will allow us to produce technologies based on genetically-modified ACP that can be used to control CLas transmission. These technologies also will increase research capabilities, facilitating discovery of new methods for ACP control that could benefit California citrus growers. For example, in other pest species, genetic technologies allow researchers to study insecticide resistance mechanisms, pathogen transmission patterns and other functions with direct application to ACP and HLB control.

### CRB Research Project #5500-217

### References


**Glossary**

1**CRISPR-Cas9**: Clustered Interspaced Short Palindromic Repeats refers to DNA sequences present in bacteria that are used to target and degrade foreign DNA. The system has been adapted to cut and paste DNA into other organisms such as bacteria, fungi, plants and animals, in order to change specific sequences within their genomes.

2**Gene drive**: A system that biases the frequency that a gene is inherited from a parent by its offspring.

3**Cas9-guideRNA**: An RNA molecule that guides the Cas9 protein to a specific region of a gene. The gRNA molecule can be designed to target virtually any gene.

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The interior of a packinghouse in Upland, California, as it appeared around 1898. Men and women worked here to clean, size, sort and prepare oranges for transit by rail to markets across the United States.

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