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Fosphite® is a systemic fungicide that has been proven to effectively control pesky crop diseases. Fosphite® has a four-hour re-entry interval and a zero-day pre-harvest interval, making it an excellent component to any IPM program. Protect your crops all season long through harvest and post-harvest applications with Fosphite®.

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Contact Leonard Massey at 661.369.2035 or LeonardM@wncitrus.com.

“Fall in love with the process and the results will come!” - Eric Thomas
On the Cover: The theme of this summer’s issue of Citrograph is new varieties. Pictured on the cover is a bloom from the New Varieties Collection housed at the University of California, Riverside. The photo is courtesy of Mariano Friginal.
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Summer 2021 | Volume 12 • Number 3  The Official Publication of The Citrus Research Board

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## DISTRICT 1 – NORTHERN CALIFORNIA

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<thead>
<tr>
<th>MEMBER</th>
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<tr>
<td>Justin Brown, Chairman</td>
<td>2021</td>
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<td>Scott Carlisle</td>
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<td>Greg Galloway</td>
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<td>John Konda, Vice Chairman</td>
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<td>Etienne Rabe</td>
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<td>Keith Watkins</td>
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<td>Andrew Brown</td>
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<td>Justin Golding</td>
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<td>Zac Green</td>
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<td>Megan Morreale</td>
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<td>Jason Orlopp</td>
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<td>Joe Stewart</td>
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## DISTRICT 2 – SOUTHERN CALIFORNIA – COASTAL

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<tr>
<td>Alan Washburn</td>
<td>2021</td>
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<td>Chris Boisseranc</td>
<td>2023</td>
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<td>John Gless III</td>
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## DISTRICT 3 – CALIFORNIA DESERT

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<tr>
<th>MEMBER</th>
<th>EXPIRES</th>
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<tr>
<td>Mark McBroom, Secretary-Treasurer</td>
<td>2022</td>
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<tr>
<td>Craig Armstrong</td>
<td>2023</td>
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## PUBLIC MEMBER

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<th>MEMBER</th>
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<tr>
<td>Melissa Cregan</td>
<td>2021</td>
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NEW GENERATION K-PHITE 7LP
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DISEASE CONTROL FOR CALIFORNIA CITRUS GROWERS

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✦ Botryosphaeria dothidea
✦ Fusarium
✦ Hyphoderma sambuci

✦ Phytophthora (soil borne and aerial phases including brown rot)
✦ Pseudomonas syringae
✦ Pythium
✦ Rhizoctonia
✦ Xanthomonas ssp. (including citri)

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For more information including research results and scientific publications, contact;
Mark Brady, Western Marketing Manager, Plant Food Systems, Inc. (559) 731-1267
CALENDAR OF EVENTS

AUGUST
10
Citrus Research Board (CRB) Meeting.
For more information, contact the CRB at (559) 738-0246 or visit www.citrusresearch.org

SEPTEMBER
16
Citrus Research Board (CRB) Nomination Meetings.
For more information, contact the CRB at (559) 738-0246 or visit www.citrusresearch.org

OCTOBER
6
California Citrus Conference.
For more information, contact the CRB at (559) 738-0246 or visit www.citrusresearch.org

*Date subject to change.

AUGUST
11
Citrus Pest and Disease Prevention Committee (CPDPC) Meeting.
For more information, visit www.cdfa.ca.gov/citruscommittee

NOVEMBER
10
Citrus Pest and Disease Prevention Committee (CPDPC) Meeting.
For more information, visit www.cdfa.ca.gov/citruscommittee

SEPTEMBER
21
Citrus Research Board (CRB) Annual Meeting.
For more information, contact the CRB at (559) 738-0246 or visit www.citrusresearch.org
Because protection requires great precision.

Protect against harmful pests while helping safeguard beneficials.
Sivanto® Prime insecticide precisely targets key damaging pests like Asian citrus psyllid, citricola scale and aphids while helping safeguard beneficial insects. In doing so, Sivanto Prime preserves the overall health of your plants and, most importantly, protects your investment. Sivanto Prime: precision that preserves and protects.

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From the PRESIDENT'S DESK

Marcy L. Martin
Welcome to the summer edition of Citrograph. The focus of this issue is on new varieties. In these pages, you’ll read about some of the latest research projects into the next generation of citrus that have been funded by the Citrus Research Board (CRB). We’ll also introduce you to the work of the CRB’s New Varieties Research Committee and talk to several industry members about what they believe is important in the development of future citrus.

This summer, we’re also proud to introduce two new features to the CRB family. The cover story of the magazine is an inaugural look at our new logo and website. The logo is a colorful, modern update that ties directly into the primary purpose of the CRB. Our new website debuted about a month ago. It is designed to be comprehensive, informative and easy to navigate. We hope you’ll bookmark www.citrusresearch.org and refer to it whenever you’re looking for industry news and references. Additionally, the current and past issues of Citrograph will be archived on the site. For more information on the website and logo, please see "CRB Debuts Revamped Website and Logo" on page 12.

Lastly, please look for the CRB’s annual financial report on page 22. Each year, we provide a snapshot of our current status and a review of finances for the previous two years. One of our most important mandates is to be conscientious stewards of the budget and to report our activities back to you. The results of how these funds are being put to use may be found regularly in our e-newsletters, teleconferences, (soon to resume) in-person meetings and Citrograph.

Please enjoy this issue and let us know your thoughts.

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

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Best applied before temperatures reach triple digits.

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800.678.7377 • www.polymerag.com • customerservice@polymerag.com
Helping Growers for Over 30 Years
CRB debuts revamped WEBSITE & LOGO

Tamara Tollison and Ivy Leventhal
The Citrus Research Board (CRB) recently introduced two new family members – a revamped, expanded and accessible interface website and an updated logo.

CRB President Marcy L. Martin said that the organization’s leadership believed it was time to more effectively share the CRB’s mission and work through an updated website. “The previous site did not necessarily reflect the Board’s mission or the marketing order,” she explained, “and we were not reaching our audience. We needed to provide more of what the California industry was seeking – information on our core programs and other projects in our portfolio, access to research reports and results, committee selections and up to the minute news.”

MJR Creative Group was selected last summer to develop the website and logo following a formal request for proposals sent out by the Board. “MJR is established within the agriculture and food sectors,” said Martin. “They were chosen due to their solid foundation in terms of content design and level of creativity.”

Website

Three primary objectives for the website were to:

1. provide a valuable and practical online resource for all information related to California citrus crop research;
2. provide access to news and events affecting the citrus research community; and
3. serve as the primary destination for CRB-funded researchers to submit citrus research proposals and reports easily and for researchers and California citrus growers to review past and current research.

“MJR’s design team created a powerful and contemporary design that reflects and communicates the important contributions the organization makes to the industry,” said Martin.

The new website, www.citrusresearch.org, went live in May. It is much more user-friendly than its predecessor and features a contemporary look. A revamp of the graphics and the site’s aesthetics resulted in a responsive design that fits all device formats and platforms and may be accessed via any browser type.

Primary features and highlights include:

- intuitive and pleasing user experience;
- responsive site design;
- searchable database of research and other downloadable content and a user-friendly process to submit proposals (for current and prospective researchers);
- access to the assessment portal and downloadable handler files;
- improved CRB calendar function with events that easily can be added to a user’s calendar;
- integration with the MailChimp email marketing platform for electronic newsletter sign-ups, etc.; and
- integration with social media platforms – Facebook, LinkedIn and Instagram. Users may click on the links in each page’s header and footer to be redirected to these sites.

Updated tabs offer a great way to find past research projects and supporting documents. There are many more attractive graphics and detailed information on the CRB’s core programs. Citrograph will continue to have its own tab on the website; but now, the magazine’s past issues will be available for easy downloading. In addition to Citrograph, other tabs are About, Our Research, News and Events, For Researchers and Handler Resources.

MJR will be providing ongoing support that includes:

- web hosting;
- web security;
- script and program updates as needed and on time;
- regular back-ups of the website;
- monitoring of server usage and ensuring that appropriate bandwidth is allocated for the site and its associated components;
- updates made to online forms as needed; and
- training for staff to properly update and manage the content and products and creation of tutorials for CRB use.
The logo is only the third iteration of the Board’s identity signature in the past 53 years. It is a stylized update of the CRB’s original logo and features a drawing of a microscope within a blue circle surrounded by the Citrus Research Board’s name in an outer white circle. Contemporary artwork of a citrus slice and leaves are displayed in orange and green on the bottom quarter of the logo. The change resulted from a Board mandate to return to the original logo’s meaning, which was a symbol of the CRB’s primary function – research.

“We created a modern and sophisticated mark that bears a connection to the previous logo with its circular shape,” said MJR Creative Group Account Director Jason Bukilica. “It’s a brand identity that works well as a modern stand-alone icon and also will work well visually across a multitude of digital and print mediums.”

Tamara Tollison is the communications specialist with the Citrus Research Board and also serves as the communications assistant on Citrograph. Ivy Leventhal is the managing editor of Citrograph. For more information, please contact tamara@citrusresearch.org
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Iron baits from Neudorff offer both conventional and organic growers a powerful tool for year-long control because iron baits remain effective under cool and wet conditions. These baits are highly palatable to pests and can be used around pets and wildlife. Plus, they provide a maximum number of baiting points.

Ferroxx AQ or Sluggo Maxx.

Make your choice. Then, Bait Back!
In the fall of 2020, California’s citrus growing regions saw a rise in sporadic Asian citrus psyllid (ACP) detections popping up across the state, including in regions like Kern County with historically low ACP activity. While the citrus industry’s efforts have thus far kept huanglongbing (HLB) – the deadly citrus tree disease that can be carried by ACP – out of commercial groves, the rise in ACP detections prompted concerns from local growers and industry stakeholders. The Citrus Pest & Disease Prevention Program (CPDPP) outreach team sprang into action to quickly institute a fully integrated outreach campaign throughout Kern County and beyond, targeted at residents and industry members alike.
The campaign was designed to put the seemingly sudden detections into context, while reinforcing best practices for keeping HLB at bay through effectively managing and suppressing ACP populations – both in backyards and commercial groves. In addition to the important one-on-one guidance provided by Kern County Grower Liaison Judy Zaninovich and other liaisons throughout the state, the outreach team identified additional strategies for communicating to the broader community. The efforts focused on encouraging growers and homeowners to remain vigilant and stay on top of this elusive pest so that the dangerous disease doesn’t spread to more California citrus.

Connecting with All Audiences

After a relatively quiet year, Kern County saw nearly 75 new ACP detections in residential and commercial areas during the past six months. While HLB has not been detected in Kern County, just one detection of the disease could cause a major shift in the CPDPP’s fight against HLB. To bring awareness to the spike in recent ACP detections, an opinion-editorial (op-ed) piece bylined by citrus grower and Citrus Pest & Disease Prevention Committee (CPDPC) member Keith Watkins was secured with the Bakersfield Californian in November 2020.

By illustrating what could happen if the area is unable to control the ACP population surge, the op-ed provided a peer-to-peer perspective for the local citrus industry on the importance of remaining vigilant in the fight against ACP. It also highlighted how beneficial citrus crops are to the Kern County economy as the third-highest grossing commodity for the county in 2019.

Along with serving as an opportunity to connect directly with citrus growers, the op-ed alerted homeowners of the threat ACP and HLB pose to backyard citrus trees. The article reinforced that whether residents had one lemon tree in their front yard or a grove with hundreds of acres, the threat is on our collective doorstep, and homeowners and growers alike need to work together to stop psyllids in their tracks.

To elevate the important messages shared through the op-ed, an interview also was secured in AgNet West with Victoria Hornbaker, director of the Citrus Pest & Disease Prevention Division, to discuss the division’s response to the recent ACP detections.

Diversifying Media Channels to Maximize Reach

To fully blanket the county with the recent news, the outreach team developed a robust advertising campaign – including Facebook ads, radio ads and billboards – to supplement the earned media outreach and grower liaison efforts and encourage residents’ support in the fight against...
ACP and HLB. To ensure that the campaign resonated with the diverse populations in Kern County, in-language messages were coordinated to reach English- and Spanish-speaking audiences.

Billboards on Interstate 99 in Bakersfield read “Dangerous Citrus Pest Found in Kern. Protect Local Citrus” in both English and Spanish. Combined, these billboards were seen an estimated two million times. In addition, informational ads ran on local radio stations KUZZ-FM and KIWI-FM for two weeks to maximize reach, earning a combined 663,800 audience impressions.

A variety of social media ads were deployed to target specific zip codes in which ACP detections have been made, earning 44,558 audience impressions. The ads focused on what residents can expect should they see an agricultural official in their area and on what would happen if California citrus disappeared.

Emphasizing Best Practices Across the State

As ACP detections continued to appear in the final months of 2020 in areas with typically low ACP populations (Madera, San Luis Obispo, Santa Barbara, Santa Clara, Tulare, Contra Costa and others), the outreach team deployed another peer-to-peer strategy by working with CPDPC Chairman Jim Gorden to draft a letter about the detections directed toward fellow growers. Through close collaboration with grower liaisons and industry members, the team uncovered a sentiment among some growers that the ACP detections were being seen as a sign of program failure. Through entomological guidance from our partners at the University of California, the letter emphasized that “while we should expect to see this type of ‘flare up’ occasionally, we need to remain vigilant – even when things are quiet – to ensure we continue to stay on top of this elusive pest and the dangerous disease it spreads.” The letter highlighted the need to rely on the sound science used to develop the Voluntary Grower Response Plan recommendations. Ultimately, the letter was well received by industry stakeholders, shared by various industry partners and garnered coverage in industry outlets, including Ag Info Radio, Fresh Plaza and California Fresh Fruit Magazine.

A Reminder to Stay Vigilant

We know that California's citrus growing regions are as diverse as the growers and groves within them. Yet, each share the same challenge of protecting their trees from ACP and HLB. If the industry does not remain vigilant in the fight against this dangerous pest and the deadly disease it carries, these recent spikes can become the norm in areas like Kern County.

While a variety of statewide initiatives educate growers and homeowners on threats to California citrus, as we've seen with Kern County, there is also value in tailoring an approach to fit the features unique to each region. To complement the ongoing outreach activities conducted on a statewide level, the CPDPP outreach team is ready at a moment’s notice to launch fully integrated campaigns tailored for specific areas.

Mark McBroom is the vice chair for the Citrus Pest & Disease Prevention Program and outreach subcommittee chairman. He also serves as secretary-treasurer of the Citrus Research Board. For more information, contact desertcitrus@aol.com
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Watsonville, California

Since we sell primarily to home growers, we are very interested in varieties with extended ripening seasons where the fruit will hang on the tree and still have good flavor. Home growers want to enjoy fruit as long as possible and cannot always use everything that sets on the tree if the ripeness season is short. We also are interested in varieties that can set multiple crops per year – mostly lemons and some hybrids like Calamondin or limequats. Again, this gives home growers more chances to enjoy their fruit throughout the year.

We are anxiously awaiting more selections of colored finger limes. In a finger lime trial located at the University of California, Riverside (UCR), seeds of highly pigmented Australian finger limes are being cooperatively evaluated by the U.S. Department of Agriculture and UCR personnel. During the California Citrus Nursery Society web series this past November, the industry was shown promising selections along with evaluation data and was able to provide input on the development of the evaluation criteria. There were hundreds of selections to choose from, and we are eagerly looking forward to seeing some of the best ones move toward release.

We also are interested in cold hardy selections that can help expand the regions where citrus can be grown. A new rootstock, US-852, is a trifoliate mandarin cross that is reported to be hardy to 0°F. Additionally, the Citrus Clonal Protection Program will be re-releasing a cleaned-up version of the Bidwell orange. According to the UC Citrus Variety Collection, “The original Bidwell’s Bar tree was planted in 1856 in Butte County, California, and is often called the ‘Mother Orange Tree.’ It is still alive and believed to be the oldest living orange tree in California.” Since Butte County is located much further north than the current citrus production region in California, a selection like this could pave the way for the industry to expand its current footprint. Growers likely will be interested in new cold-hardy selections, as well. Finally, we are always on the lookout for dwarfin g rootstocks like US-897. Not only is this selection HLB tolerant, it also produces trees that reportedly max out around ten feet tall, making it a good choice for homeowners and for density production plantings.
I'm sure many folks are keen on water tolerance, given the scarcity issues with ground and surface water. Trees that can be stressed without compromising fruit quality and size would be a great help. However, that being said, new varieties that are developed MUST taste great and be able to be shipped easily. There is no point in growing something that won't sell or won't ship well!

As a citrus nurseryman at TreeSource Citrus Nursery, this question comes up often. The simplest answer is “commercial potential.” A new variety must have the ability to make the grower money in either a market niche capacity or as a new commodity that sits on every supermarket shelf in America. There is not one specific trait that meets these criteria, but seedless with good flavor and mouth feel are essential. After that we need a variety to ship well and be easy to peel. Red colored flesh or rind pigment seems to attract consumers, as well. Probably an overlooked criteria is asking whether this new variety is different than what is already in the stores. We saw the Summer Gold mandarin variety fail because its season was the same as Tango, which dominates the market, but Sumo Citrus® was able to carve out a market niche even though its harvest timing is the same as Summer Gold.

New varieties need to have good flavor, be easy to peel and seedless for consumer acceptance, have good shelf life, be easy to ship for retailers’ acceptance, and hold well on the tree to meet the timing of an extended market.

Tamara Tollison is the communications specialist with the Citrus Research Board and also serves as the communications assistant on Citrograph. For more information, contact tamara@citrusresearch.org
By the Numbers: Financial Report

2018 Income
$12,474,361

- Assessment Income: $9,521,283 (76.3%)
- CPDPP Grant Income: $2,050,403 (16.4%)
- Federal Grant Income: $651,454 (5.2%)
- Investment Income: $51,927 (0.4%)
- Other Income: $199,294 (1.7%)

2018 Expenses
$11,194,767

- Research Expenses: $8,199,085 (73.3%)
- Operations Expenses: $1,185,883 (10.6%)
- Communications Expenses: $391,212 (3.5%)
- Administrative Expenses: $1,373,258 (12.2%)
- Other Expenses: $45,329 (0.4%)

2018 Increase (Decrease) in Net Assets
$1,279,594

2019 Income
$14,663,216

- Assessment Income: $10,254,849 (69.9%)
- CPDPP Grant Income: $2,428,549 (16.6%)
- Federal Grant Income: $1,179,290 (8%)
- Investment Income: $58,970 (0.4%)
- Other Income: $741,549 (5.1%)

2019 Expenses
$12,273,677

- Research Expenses: $7,935,871 (64.7%)
- Operations Expenses: $1,217,793 (9.9%)
- Communications Expenses: $1,244,245 (10.1%)
- Administrative Expenses: $1,554,728 (12.7%)
- Other Expenses: $321,040 (2.6%)

2019 Increase (Decrease) in Net Assets
$2,389,539

These data represent the audited financial statement of the Citrus Research Board for the years ended September 30, 2018, 2019, and 2020. You are welcome to visit us at any time to discuss any elements of the program and explore our portfolio of work. This program is paid through your grower assessment dollars, and the board welcomes your feedback.

-Marcy L. Martin, CRB President
These data represent the audited financial statement of the Citrus Research Board for the years ended September 30, 2018, 2019 and 2020. You are welcome to visit us at any time to discuss any elements of the program and explore our portfolio of work. This program is paid through your grower assessment dollars, and the board welcomes your feedback.

-Marcy L. Martin, CRB President
California citrus producers in Districts 1 (Northern California) and 2 (Southern California-Coastal) should make plans to attend the appropriate Citrus Research Board (CRB) nomination meetings. Six positions in District 1 expire on September 30, 2021, and there is one vacancy to be filled that subsequently will expire in 2022. One position in District 2 expires on September 30, 2021. The public nomination meetings will be conducted by officials of the California Department of Food and Agriculture (CDFA) and the CRB.

In addition to the elected positions mentioned above, the Board will have the opportunity to consider the extended appointment of the public member at its annual meeting on September 21, 2021.

The detailed list of seats expiring this September may be found on page 6, where the current Board member roster appears by name, district and year of term expiration. Member terms are for three years.

**Board Member Responsibilities**

The bulk of the Board’s time is spent considering a broad portfolio of citrus research proposals and projects totaling approximately $10 million. Members are involved in:

- developing research priorities and requests for proposals,
- prioritizing responses, awarding funds,
- devising successful implementation strategies,
- assessing progress and
- providing critiques of project results.

The 21-member CRB is served by 16 staff with headquarters in Visalia and two laboratories in Riverside. There is a relatively high time commitment compared to many other volunteer commodity boards, but those involved with the CRB are integral in directing the response to critical citrus research needs in California. Members are expected to attend Board meetings and to serve on research and/or administrative committees. A typical fiscal year has five Board meetings in various geographic locations, occasional committee meetings and the opportunity to attend a number of citrus-related conferences and events.

**Voter Qualifications**

(As provided by the CDFA Marketing Branch)

- Any owner, officer or employee of an entity in California in the business of producing, or causing to be produced for market, 750 or more standard field boxes (or the equivalent) of any variety of citrus is qualified to participate in the nomination proceedings.
- If you wish to nominate a person at a nomination meeting to serve on the Board, you should determine the candidate’s eligibility and willingness to serve prior to the nomination meeting.
- An individual person is entitled to represent only one legal entity at a nomination meeting.
- In the case of a partnership, only one of the partners may vote.
- In the case of a corporation, a person affiliated with the corporation, preferably an officer, may represent the corporation.
- A married couple operating a production entity is entitled to just one vote, unless each spouse owns and operates separate and distinct entities.
- To participate in a district’s nomination meeting, a business entity must have citrus production within that district. Any entity with production in more than one district must choose a single district in which to participate to vote. If a separate production entity can be proven as the operating entity in another district, the person qualified to act as the representative of that entity may vote in that district, even if he/she has voted as a representative of another entity in another district. Essentially, each separate citrus-producing business entity is entitled to one vote in the district in which it operates.
- Voting by proxy is not permitted.

For more information, the California Citrus Research Program Marketing Order may be viewed on-line at: [http://www.cdfa.ca.gov/mkt/mkt/pdf/Laws/CitrusResearchMarketingOrder.pdf](http://www.cdfa.ca.gov/mkt/mkt/pdf/Laws/CitrusResearchMarketingOrder.pdf) Questions may be directed to CDFA Marketing Branch Associate Agricultural Economist Kacie Fritz at (916) 900-5095 or CRB President Marcy L. Martin at (559) 738-0246.

Tamara Tollison is the communications specialist at the Citrus Research Board and also serves as the editorial assistant on Citrograph. For additional information, contact tamara@citrusresearch.org.
PUMP MONITORING & CONTROL

- Control two separate pumps, two pressure sensors/transducers, and a downstream flow meter with one device.
- Manually start and stop at the pump or remotely through your smartphone or computer.
- Monitor filter health with pre- and post-pressure transducers mounted to the irrigation line to determine proper flushing.
- Schedule irrigation cycles to T.O.U. off-peak hours for utility cost reductions.
- Have peace of mind with verification on your smartphone or computer that all is well without having to worry at night or drive out to pump sites to confirm pump operation.

FROST PROTECTION

- Connect each wind machine to our cloud through your cellular-powered Altrac device.
- Works with any make or model of wind machine: Diesel, Propane, or Electric.
- View air temp and remotely control equipment using your smartphone or computer.
- Know immediately when a wind machine fails to start. Get alerts through text, phone, or e-mail.
- Expand your Wind Machine Station to include other sensors such as fuel level, soil moisture or leaf wetness.


- Eliminate labor costs while increasing control over your production.
- Check in on team performance with daily run reports.
- Price includes 5 years of cellular data costs.
MEET THE NEW VARIETIES RESEARCH COMMITTEE

CRB BOARD MEMBERS ARE LISTED IN BOLD TYPE.

- Mark McBroom, Chair
- Scott Carlisle, Vice Chair
- Craig Armstrong
- Zac Green
- Henk Griffin
- Ram Uckoo, Ph.D.
- Roger Smith
- Kris Tomlinson
- Georgios Vidalakis, Ph.D.
Committee Introduction

The core responsibility of the New Varieties Research Committee is to provide the California citrus industry with new varieties that meet market demands. Toward that goal, our committee sponsors and oversees research that will discover, review and further develop new citrus scions and rootstocks. We are here to help our state’s growers and producers maintain and surpass the level of expectations that only come from California citrus. The committee wants to push the envelope on new and exciting possibilities in this research area – exploring techniques for speeding up the breeding process and introducing key traits into citrus – that will reflect the work of the New Varieties Research Committee and the Citrus Research Board (CRB) in helping to maintain a viable and profitable industry for California citrus producers.

The committee oversees the Integrated Citrus Breeding and Evaluation Core Program, which is managed by Mikeal Roose, Ph.D., and Tracy Kahn, Ph.D., of the University of California, Riverside. The mandate of this program is to develop and evaluate new scion and rootstock cultivars. They often report on the progress and results of their research in the pages of Citrograph, including in this issue with results from a recently concluded lemon trial (see Lemon Returns on page 36).

Key Recent Accomplishments

We have been moving forward with evaluations of new varieties and improving some of the industry standards. The core program continues to release key varieties for the industry. Following the 2018 Regulatory Summit held in Denver, Colorado, the pathway to introduce citrus varieties into the state has been streamlined. Varieties from Florida with potential HLB tolerance are starting to be released (see Interstate Movement of New and Licensed Citrus Varieties on page 58), and the committee is working on finalizing an evaluation program to rapidly and efficiently screen these and other varieties. Our committee welcomes new challenges to help California remain the premier citrus-producing region.

Current Projects

This year, we are funding seven projects with a total budget of $1,625,228. While more than half of the funding is going to the core breeding program, other continuing research projects managed by this committee are examining greenhouse method standardization for scion and rootstock variety testing, conducting field evaluations of rootstocks for huanglongbing (HLB) mitigation and introducing new non-commercial citrus varieties to CCPP in an effort to stem the introduction of new diseases brought into California by citrus hobbyists via the illegal budwood trade.

Research that got underway in FY20-21 will be continuing conventional breeding efforts to develop and evaluate HLB-tolerant and -resistant citrus hybrids and to create both a range of new, early-flowering citrus varieties and HLB-tolerant and -resistant citrus plants via genetic engineering. (For more information about the work of the New Varieties Research Committee, see “CRB-funded Research Projects for FY20-21 on page 32.)

Goals Moving Forward

While the path to new variety development may be long, we continue to identify, support and encourage research that can both speed the path of development and introduce traits and varieties of value for California growers across the state. Our dedicated group of committee members will never be content with the status quo. It is paramount for us to address the ever-changing interests, preferences and tastes of our consumers.

Mark McBroom is the chair of the New Varieties Research Committee of the Citrus Research Board, where he also serves as secretary-treasurer. Additionally, as vice chair of the Citrus Pest & Disease Prevention Committee and outreach subcommittee chair, he provides quarterly articles of interest to Citrograph’s readers on the committee’s activities. For more information, contact desertcitrus@aol.com

Mark McBroom is the chair of the New Varieties Research Committee of the Citrus Research Board, where he also serves as secretary-treasurer. Additionally, as vice chair of the Citrus Pest & Disease Prevention Committee and outreach subcommittee chair, he provides quarterly articles of interest to Citrograph’s readers on the committee’s activities. For more information, contact desertcitrus@aol.com

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On September 22, the Citrus Research Board (CRB) approved the funding of all new and continuing research projects for the fiscal year (FY) 2020-21 at their annual meeting. This year, 12 new projects, 19 continuing projects, three core programs and one United States Department of Agriculture (USDA) sub-award were approved for funding by the Board for a total of $5,859,529 (Tables 1 and 2).

Huanglongbing (HLB)-related projects remain a focus of the CRB research portfolio, as we continue to work toward creating and evaluating improved citrus varieties and developing potential tools to further those efforts. Work on exploring and refining potential tools for effective bacterial and vector management of the HLB-associated bacterium ‘Candidatus Liberibacter asiaticus’ (CLas) and the Asian citrus psyllid (ACP) - the vectoring pest – continue, as well. Non-HLB related research projects approved this year also cover a range of important research areas for California industry members, including current production issues, potential regulatory requirements and export market needs.

CRB-funded research projects are overseen by one of four CRB Research Committees – New Varieties, Vectored Diseases, Production and Post-harvest Technology, and Pest Management. The Citrus Clonal Protection Program (CCPP), a core program, is overseen by its own CRB committee.

New Varieties Research (5200) Committee

Research projects within this committee address rate-limiting steps in the development and evaluation process necessary for new varieties, while also continuing work to provide the California citrus industry with new varieties that meet market demands. Four continuing and three new research
projects, including the core Integrated Citrus Breeding and Evaluation Program, received support this year through a budget of $1,625,228 (Tables 1 and 2). Continuing projects include standardizing greenhouse methods for scion and rootstock variety testing, field evaluations of rootstocks for HLB mitigation and introducing new, non-commercial citrus varieties in an effort to thwart the introduction of new diseases by citrus hobbyists through illicit budwood trade.

The new projects introduced this year will continue conventional breeding efforts to develop and evaluate HLB tolerant and resistant citrus hybrids and create new citrus varieties via genetic engineering for early flowering and HLB resistance/tolerance. This research committee also oversees the Integrated Citrus Breeding and Evaluation core program, led by Mikeal Roose, Ph.D., and Tracy Kahn, Ph.D., at the University of California, Riverside (UCR) which develops and evaluates new scion and rootstock cultivars for the California citrus industry. (For more information on this committee, see Meet the New Varieties Committee on page 28.)

**Vectored Diseases Research (5300) Committee**

Research projects within this committee focus on detection, eradication, control and management strategies and tools for insect-vectored diseases to minimize crop damage and economic losses. Seven continuing and four new projects are receiving $1,336,427 in support this year (Tables 1 and 2). Continuing projects are looking at improving HLB diagnostics through the development of a low-cost diagnostic tissue processor to increase sample processing, investigating the use of a non-pathogenic bacterium as a biocontrol agent of CLas, identifying the most reliable plant tissues to sample throughout the season for enhanced detection of CLas, evaluating a newly discovered plant-associated RNA as a vector for ACP/HLB control and seeking regulatory approval and implementation of a high-throughput sequencing protocol for citrus pathogen detection.

All new projects are focused on various aspects of HLB. One project focuses on evaluating enzymes derived from prophages (bacterial viruses) present in California strains of CLas which may disrupt biofilm formation. Another project will survey nine citrus growing counties in California for information on the incidence and distribution of citrus tristeza virus (CTV) strains in the state – this information will inform potential efforts to use genetically engineered CTV vectors for HLB mitigation, should that tool become available. Also initiated this year is a project exploring best practices for implementing ACP detector canines in commercial orchards.

With the support of the Citrus Pest and Disease Prevention Program (CPDPP), the CRB continues to support a risk-based model used to identify likely areas of HLB infection across the state. Related CPDPP-supported projects will study the economic benefits of area-wide treatments and create predictive models for ACP and HLB movement between
Table 2: Listing of all CRB-funded research projects for the 2020-21 fiscal year.

<table>
<thead>
<tr>
<th>Project Number</th>
<th>Project Title</th>
<th>Principal Investigator</th>
<th>Affiliation</th>
<th>Approved Budget</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>5200 - New Varieties</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5200-168</td>
<td>Refinement and application of greenhouse methods to evaluate scion and rootstock tolerance to Clas</td>
<td>Kim Bowman</td>
<td>USDA-ARS</td>
<td>$216,547</td>
</tr>
<tr>
<td>5200-169</td>
<td>Field testing to identify elite rootstocks that can mitigate or prevent HLB in scions commercially</td>
<td>Jude Grosser</td>
<td>Univ. of Florida</td>
<td>$28,000</td>
</tr>
<tr>
<td>5200-171</td>
<td>Reducing disease risk by discovery, introduction and commercialization of new citrus varieties</td>
<td>Dan Willey</td>
<td>Fruitmentor</td>
<td>$67,000</td>
</tr>
<tr>
<td>5200-201</td>
<td>CORE: Integrated citrus breeding and evaluation for California</td>
<td>Mikeal Roose &amp; Tracy Kahn</td>
<td>UC Riverside</td>
<td>$875,170</td>
</tr>
<tr>
<td><strong>5300 - Vectored Diseases</strong></td>
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</tr>
<tr>
<td>5300-173</td>
<td>Breeding for generating HLB resistant citrus, and field evaluation of selected HLB tolerant hybrids.</td>
<td>Chandrika Ramadugu</td>
<td>UC Riverside</td>
<td>$140,000</td>
</tr>
<tr>
<td>5300-174</td>
<td>Inducible flowering technology for citrus speed breeding</td>
<td>Sean Cutler</td>
<td>UC Riverside</td>
<td>$137,277</td>
</tr>
<tr>
<td>5300-175</td>
<td>Engineering citrus for HLB resistance using gene-editing technologies</td>
<td>Vivian Irish</td>
<td>Yale University</td>
<td>$161,234</td>
</tr>
<tr>
<td>5300-182a</td>
<td>DATOC: Data Analysis and Tactical Operations Center (CPDPP)</td>
<td>Neil McRoberts</td>
<td>UC Davis</td>
<td>$175,153</td>
</tr>
<tr>
<td>5300-199a</td>
<td>Risk-based survey for decision making in the management of huanglongbing: Phase II (CPDPP)</td>
<td>Roger Magarey</td>
<td>North Carolina State University</td>
<td>$242,283</td>
</tr>
<tr>
<td>5300-201</td>
<td>Improving HLB diagnosis through instrument engineering</td>
<td>Douglas Hill</td>
<td>Technology Evolving Solutions Inc.</td>
<td>$181,000</td>
</tr>
<tr>
<td>5300-203</td>
<td>Biological control of huanglongbing by the bacterium Paraburkholderia phytofirmans PsJN</td>
<td>Johan Leveau</td>
<td>UC Davis</td>
<td>$2,660</td>
</tr>
<tr>
<td>5300-204</td>
<td>Evaluation of reliable sampling tissue and seasonality for consistent detection of CLas by qPCR</td>
<td>Subhas Hajeri</td>
<td>CCTEAa</td>
<td>$43,000</td>
</tr>
<tr>
<td>5300-205</td>
<td>Phase 2 of high-throughput sequencing as a CCPP routine diagnostic tool for variety introduction</td>
<td>Georgios Vidalakis</td>
<td>UC Riverside</td>
<td>$62,680</td>
</tr>
<tr>
<td>5300-207</td>
<td>Independent-mobile RNA (RNA) expression vector against HLB-Initiate operation &quot;Lab 2 Farm&quot;</td>
<td>Georgios Vidalakis</td>
<td>UC Riverside</td>
<td>$189,360</td>
</tr>
<tr>
<td><strong>New Projects</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>5300-210</td>
<td>California citrus tristeza virus survey</td>
<td>Raymond Yokomi</td>
<td>USDA-ARS</td>
<td>$104,887</td>
</tr>
<tr>
<td>5300-211</td>
<td>Phage hydrolases for Liberibacter biofilm control</td>
<td>Jennie Fagen</td>
<td>North Carolina State University</td>
<td>$35,976</td>
</tr>
<tr>
<td>5300-212a</td>
<td>Predicting the likelihood of ACP/HLB dispersal into California commercial citrus groves via scenario (CPDPP)</td>
<td>Roger Magarey</td>
<td>North Carolina State University</td>
<td>$100,000</td>
</tr>
<tr>
<td>5300-213</td>
<td>Development of best practices for implementation of ACP detector canine technology</td>
<td>Lisa Finke</td>
<td>Canine Detection Services</td>
<td>$199,428</td>
</tr>
</tbody>
</table>

*aFunding provided by the CDFA California Pest & Disease Protection Program (CPDPP) 
bFunding provided by the USDA Technical Assistance for Specialty Crops (TASC)
Table 2: Listing of all CRB-funded research projects for the 2020-21 fiscal year.

<table>
<thead>
<tr>
<th>Project Number</th>
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<th>Affiliation</th>
<th>Approved Budget</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>5400 - Production &amp; Post-Harvest Technology</strong></td>
<td></td>
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<tr>
<td><strong>Continuing Projects</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>5100-154</td>
<td>Citrus dwarfing of commercial varieties using TsnRNAs</td>
<td>Georgios Vidalakis</td>
<td>UC Riverside</td>
<td>$69,381</td>
</tr>
<tr>
<td>5400-155</td>
<td>Epidemiology and management of phytophthora diseases of citrus in California</td>
<td>James Adaskaveg</td>
<td>UC Riverside</td>
<td>$104,000</td>
</tr>
<tr>
<td>5400-156</td>
<td>Forecasting and management of Septoria spot of citrus</td>
<td>James Adaskaveg</td>
<td>UC Riverside</td>
<td>$50,000</td>
</tr>
<tr>
<td>5400-158</td>
<td>Impact of SGMA on competitiveness of California’s citrus industry</td>
<td>Bruce Babcock</td>
<td>UC Riverside</td>
<td>$76,141</td>
</tr>
<tr>
<td><strong>New Projects</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>5400-161</td>
<td>Spray drift study in citrus to support orchard and vineyard airblast drift modeling effort</td>
<td>Peter Larbi</td>
<td>UC ANR</td>
<td>$88,850</td>
</tr>
<tr>
<td>5400-162</td>
<td>Post-harvest disease management</td>
<td>James Adaskaveg</td>
<td>UC Riverside</td>
<td>$61,062</td>
</tr>
<tr>
<td>5400-163</td>
<td>Epidemiology and management of anthracnose dieback and Alternaria Rot in California citrus</td>
<td>Themis Michailides</td>
<td>UC Davis</td>
<td>$67,840</td>
</tr>
<tr>
<td>5400-164</td>
<td>Validating the control of cross-contamination in high-pressure washers and fungicide flooders</td>
<td>Steven Pao</td>
<td>CSU Fresno</td>
<td>$79,632</td>
</tr>
<tr>
<td><strong>5500 - Pest Management</strong></td>
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<tr>
<td><strong>Continuing Projects</strong></td>
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</tr>
<tr>
<td>5500-215ª</td>
<td>Rearing ACP for supply to research projects and assuring genetic quality of <em>Tamarixia radiata</em> (CPDPP)</td>
<td>Richard Stouthamer</td>
<td>UC Riverside</td>
<td>$191,147</td>
</tr>
<tr>
<td>5500-217</td>
<td>Transgenesis and paratransgenesis tools for the control of the Asian citrus psyllid</td>
<td>Omar Akbari</td>
<td>UC San Diego</td>
<td>$100,000</td>
</tr>
<tr>
<td>5500-220</td>
<td>Characterizing earwig damage to citrus fruits, and damage prevention using trunk barrier treatments</td>
<td>Jay Rosenheim</td>
<td>UC Davis</td>
<td>$62,293</td>
</tr>
<tr>
<td>5500-221</td>
<td>Particle films for organic or conventional prevention of Asian citrus psyllid</td>
<td>Christopher Vincent</td>
<td>Univ. of Florida</td>
<td>$72,603</td>
</tr>
<tr>
<td>5500-222</td>
<td>ACP under California conditions: wind, atmospheric pressure, temperature and humidity</td>
<td>Monique Rivera</td>
<td>UC Riverside</td>
<td>$114,594</td>
</tr>
<tr>
<td><strong>New Projects</strong></td>
<td></td>
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</tr>
<tr>
<td>5500-223</td>
<td>Genetically improving the natural enemy of California red scale</td>
<td>Raman Bansal</td>
<td>USDA-ARS</td>
<td>$75,980</td>
</tr>
<tr>
<td>5500-501</td>
<td>CORE: Citrus IPM Program</td>
<td>Monique Rivera</td>
<td>UC Riverside</td>
<td>$511,246</td>
</tr>
<tr>
<td><strong>USDA Sub-awards</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>5050-010b</td>
<td>Breaking critical pest-related trade barriers for California citrus exports (TASC)</td>
<td>Spencer Walse</td>
<td>USDA-ARS</td>
<td>$489,478</td>
</tr>
<tr>
<td><strong>Other Programs</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>6100</td>
<td>Citrus Clonal Protection Program (CCPP)</td>
<td>Georgios Vidalakis</td>
<td>UC Riverside</td>
<td>$683,627</td>
</tr>
<tr>
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<tr>
<td><strong>Total:</strong></td>
<td></td>
<td></td>
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<td>$5,859,529</td>
</tr>
</tbody>
</table>

ªFunding provided by the CDFA California Pest & Disease Protection Program (CPDPP)
bFunding provided by the USDA Technical Assistance for Specialty Crops (TASC)
residential and commercial areas. In coordination with the CPDPP, the Data Analysis and Tactical Operations Center (DATOC) project continues to address operations-based questions for the industry in a data-driven manner.

Production and Post-harvest Technology Research (5400) Committee

Research projects in this committee focus on horticultural factors and production methods (both in the grove and in the packinghouse) that impact fruit quality for the California citrus industry. Maximizing food safety and minimizing trade barriers to maintain foreign and domestic market accessibility remain committee priorities, as well.

Four new and four continuing projects are supported in the amount of $596,906 (Tables 1 and 2). Continuing projects center on evaluating the effects of citrus dwarfing viroid in common California citrus varieties to see how dwarfed varieties might meet the increasing challenges of citrus production (e.g., labor costs, water shortages, land reduction), evaluating new treatments to reduce Phytophthora diseases, forecasting and managing Septoria spot of citrus and estimating the impacts of the Sustainable Groundwater Management Act on citrus acreage and production throughout the state. New projects approved through this committee look at generating citrus-specific spray drift data in California to validate pesticide risk assessment models, assessing and mitigating the impact of anthracnose and Alternaria Rot on production, developing new sanitation treatments for post-harvest decays and validating existing packinghouse sanitation processes to meet audit and/or regulatory requirements.

Pest Management Research (5500) Committee

Pest Management research projects concentrate on eradication, control or management strategies and tools against insect pests to minimize crop damage and to maintain foreign and domestic market accessibility. Five continuing projects, one new project and the recently renewed Integrated Pest Management core program were funded for the FY20-21 at $1,127,863 (Tables 1 and 2). ACP projects include infrastructure support for ACP colonies at UCR for research projects and biocontrol programs (with CPDPP assistance), developing methods for genetic engineering of ACP, field evaluation of particle films to

<table>
<thead>
<tr>
<th>Project Number</th>
<th>Project Title</th>
<th>Principal Investigator</th>
<th>Affiliation</th>
</tr>
</thead>
<tbody>
<tr>
<td>5100-154</td>
<td>Citrus dwarfing of commercial varieties using TsnRNAs</td>
<td>Georgios Vidalakis</td>
<td>UC Riverside</td>
</tr>
<tr>
<td>5400-103</td>
<td>Evaluation of new post-harvest treatments to reduce post-harvest decays in packinghouse operations</td>
<td>James Adaskaveg</td>
<td>UC Riverside</td>
</tr>
<tr>
<td>5400-155</td>
<td>Epidemiology and management of phytophthora diseases of citrus in California</td>
<td>James Adaskaveg</td>
<td>UC Riverside</td>
</tr>
</tbody>
</table>

The following projects were missing from our Summer 2020 table:
reduce ACP levels in commercial orange and lemon groves and determining how the California climate influences ACP dispersal and survival.

Non-ACP projects include a continuing project to characterize earwig damage and host preference, as well as develop management practices for earwigs, and a new project looking to enhance biological control of California red scale through the development of a non-genetically modified insecticide resistant red scale predator - *Aphytis melinus*. The core Citrus IPM Program led by Monique Rivera, Ph.D., at UCR conducts long-term research applying IPM strategies to manage major citrus pests in California, including ACP. The CRB continues to oversee a cost-sharing grant with the USDA Technical Assistance for Specialty Crops (TASC) Program providing additional research funds to researchers to develop systems-based control measures for pests of export concern in Korea, China, Australia and New Zealand.

### Citrus Clonal Protection Program (CCPP)

The CCPP, led by Georgios Vidalakis, Ph.D., at UCR continues their work providing a safe mechanism for the introduction and distribution of clean citrus varieties to the California citrus industry and residents. The 2020-21 budget disbursement to CCPP is $683,627 (Tables 1 and 2).

### Summary

The CRB remains committed to prioritizing, investing and promoting research that improves the sustainability and profitability of the California citrus industry. At this time, HLB is a significant area of focus with 23 of these new and continuing CRB research projects actively engaged in HLB and ACP or related research projects. Other CRB-funded projects support the ongoing core programs and address current export and production needs of the California industry. The CRB also continues to identify, develop and join collaborations with other funding agencies to maximize California citrus grower investments in research. The projects underway support a sustainable citrus industry in our state by taking a proactive stance on identifying and implementing short-, medium- and long-term solutions to the threats and concerns of the California citrus environments and markets.

*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. Melinda Klein, Ph.D., is the chief research scientist at the Citrus Research Board in Visalia, California, where she also serves as scientific editor of Citrograph. For additional information, contact melinda@citrusresearch.org*
Project Summary

Deciding which lemon selection to grow is an important factor in maximizing returns. This lemon trial for the California Desert, conducted from 2005-06 through 2018-19, was the first trial that collected fruit quality characteristics, yield, pack-out data and estimated cumulative returns per acre to compare the commercial potential of ten lemon selections against two California commercial standards.

Leading the nation in acreage and production, California lemons are grown predominantly in the coastal and desert regions of the state with smaller numbers of acres in the San Joaquin Valley. Although the bearing acreage of navel oranges was approximately five times that of lemon acreage in 2019, the value of lemon production in packinghouse-door equivalents was about 70 percent of that for navel oranges in 2018-19 in California (USDA NASS 2019).

Desert lemons occupy an important early-season market niche with high returns relative to other growing regions in California. In 2018-19, the highest freight on board (FOB) returns for fresh-packed lemons occurred during August and September (USDA NASS 2019). Lemon trees grown in the California desert have a shorter bearing season with a higher percentage of the crop produced in late summer and fall and serve as an important supplemental source of fruit.
for packinghouses located in Ventura, California. Choosing lemon selections that yield well, occupy important market niches and meet changing market needs are crucial in addressing global competition and maximize returns.

This project was the first lemon trial designed to collect yield and pack-out data to evaluate the commercial potential of these selections in the California desert and suggest which selections might be suitable for future multi-location replicated trials in other parts of the state.

Trial History

Eleven tree selections were planted in May 2006, and the twelfth, Limonero Fino Largo (95), was planted in April 2007 on a 3.2-acre site at the Coachella Valley Agricultural Research Station (CVARS), near Thermal, California (Table 1). Overall, the trial had 20 trees of each selection budded to Citrus macrophylla rootstock in five groupings of four trees per selection with 23 feet by 18 feet spacing in a randomized complete block experimental design.

The first of two prior progress reports published in Citrograph compared the 12 lemon selections (Table 1) for freeze tolerance, tree growth based on canopy volume, fruit yield and earliness (Wright et al. 2014). The second reported results of experimental and commercial fruit pack-out, exterior and interior fruit quality, nutrient issues and fruit storage life (Kahn et al. 2015). This final report provides a summary comparing these 12 selections over the entire trial for yield, packout, commercial returns and key points from previous trials into summary descriptions of each selection.

Table 1: Description of 12 cultivars in trial planted in Thermal, California.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>CCPP Variety Introduction (VI) Number</th>
<th>Background</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allen Eureka</td>
<td>227</td>
<td>One of two standards in this trial and the most commonly planted nucellar Eureka selection in California and Arizona, especially in coastal California due to its propensity to set fruit over a wide range of the season.</td>
</tr>
<tr>
<td>Corona Foothills¹</td>
<td>871</td>
<td>A limb sport selection of the Villafranca cultivar, which is neither a Lisbon or Eureka lemon, but has characteristics intermediate between the two. This selection is the most commonly planted lemon in Arizona, but not currently common in California.</td>
</tr>
<tr>
<td>Femminello Santa Teresa</td>
<td>929</td>
<td>A selection of Femminello Comune originating from Italy with a purportedly greater percentage of its fruit maturing in late summer compared to other Femminello selections. VI 929 is available from the CCPP as part of their early release budwood distribution.</td>
</tr>
<tr>
<td>Interdonate</td>
<td>667</td>
<td>Originated in Italy, and considered to be a lemon x citron hybrid. This variety was not previously tested in California or Arizona.</td>
</tr>
<tr>
<td>Limoneira 8A</td>
<td>380</td>
<td>Other commercial standard for this trial and the most commonly planted Lisbon selection in California and Arizona. Limoneira 8A Lisbon trees are considered to be vigorous and produce early season fruit.</td>
</tr>
<tr>
<td>Limonero Fino 49</td>
<td>480</td>
<td>The earliest yielding cultivar and chief winter lemon of Spain. First introduced into California in 1987, this variety has not previously been evaluated in California, but performed well in Arizona trials beginning in 1995.</td>
</tr>
<tr>
<td>Limonero Fino 95</td>
<td>674</td>
<td>In Spain, this cultivar performs similar to Limonero Fino 49, but harvest is about two weeks earlier and productivity lower. Introduced in 2003 but not previously tested in Arizona or California.</td>
</tr>
<tr>
<td>Messina</td>
<td>661</td>
<td>Introduced from Spain to California in 2002 and likely to be very similar to the Italian selection Femminello Messina.</td>
</tr>
<tr>
<td>Seedless Lemon</td>
<td>492</td>
<td>Introduced to California from Lassocks Nursery, South Australia, in 1939, but did not become a registered variety until 1985. Formerly known as Seedless Lisbon, but now listed as Seedless Lemon since it is not thought to be a Lisbon selection.</td>
</tr>
<tr>
<td>Variegated Pink Eureka</td>
<td>486</td>
<td>A limb sport of a Eureka lemon introduced in 1931, this selection has less vigorous growth, unique fruit and foliage with green and white splotches on leaves and fruit that is striped green and cream, turning to yellow with pink oil glands and a pink blush at maturity.</td>
</tr>
<tr>
<td>Walker Lisbon</td>
<td>415</td>
<td>A Lisbon selection, Walker, an older cultivar reported to be of lesser importance in the Citrus Industry Volume I (Hodgson 1967), but has performed well in Arizona trials.</td>
</tr>
<tr>
<td>Yen Ben</td>
<td>586</td>
<td>A Lisbon lemon sport selection that originated in Queensland, Australia, in the 1930s.</td>
</tr>
</tbody>
</table>

All cultivars listed here, either introduced or developed through the breeding program go through a rigorous “Variety Introduction-VI” disease testing and therapy program under quarantine at the Citrus Clonal Protection Program (CCPP). Varieties that successfully complete the VI process receive a unique VI identification number that permanently accompanies the budwood that is made available as Protected Foundation Block or Early Release budwood from the CCPP.

¹The Corona Foothills budwood source (VI 871) was selected from the best performing Corona Foothills tree in this Desert Lemon trial based on yield and health by a group of growers and T. Kahn in the summer of 2014. The original registered source of budwood for Corona Foothills for the Desert Lemon trial was from Young’s Nursery in Thermal, California, because there was no CCPP Variety Introduction source of this cultivar available when the trial was initiated. Young’s Nursery obtained their original budwood source from Willits and Newcomb Nursery (now Lyn Citrus Seed, Arvin, California) many years ago. Two years ago, Lyn Citrus Seed sent budwood of the original “Foothill Lisbon” lemon to the CCPP, which became VI 868. Even though VI 868 is a Villafranca selection and not a true Lisbon lemon, the W&B records refer to it as Foothill Lisbon for historical reasons, and the CCPP has kept this name (Hodgson 1967).
Yield Data

From 2008-09 through 2018–19, harvest dates were based on market conditions and determined by officials at Richard Bagdasarian, Inc. (RBI). Due to large fruit loads beginning in the 2010-11 season, we shifted from one to two harvests per season at the recommendation of the packinghouse. However, the 2015-16 and 2018-19 seasons were exceptions because there was not enough fruit to justify a second harvest. For the first harvest in a season (the actual date ranged from September 1 through November 15), fruit was size-picked with a #10 ring by pickers from Coachella Valley Citrus (CVC). For the second harvest of the season (from November 16 through January 31), pickers stripped the remaining fruit from the trees. Yield data from each group of four trees were collected by counting the numbers of whole and fractional picking sacks harvested from each group. Yield was determined as pounds per tree for each of the seven harvest seasons. Yields also are presented as bins of fruit per acre.

Despite yearly variation, for the past 11 seasons, Corona Foothills, Walker Lisbon, Limonero Fino 49, Femminello Santa Teresa and the two commercial standards, Allen Eureka and Limoneira 8A Lisbon, had the greater yields; while Yen Ben, Interdonato, Seedless Lemon, Limonero Fino 95, Messina and Variegated Pink Eureka had the lesser yields (Figure 1). During the past seven seasons, Corona Foothills generally had the greatest yield. Variegated Pink Eureka had the lowest yield for all seasons except one (2014-15), which was not surprising since the variegated leaves for this selection contains less chlorophyll and have lower photosynthetic capacity. The greatest yields for most of the selections during the 11 seasons occurred in 2012-13 for all selections, except
for Variegated Pink Eureka and Yen Ben. Although there was a slight recovery in 2017-18, the overall reduction in yield from 2012-13 may be due to several possible causes. Initially, yield reduction after 2012-13 might be due to lack of carbohydrates needed to sustain a second large crop in 2013-14. However, a yield rebound would have been expected in 2014-15, but it was not seen. The warm fall in 2014 led to greater than normal fruit drop, another possible cause. But the likely main cause of reduced yield was shading and reduced flower production due to overgrowth, accentuated by the close tree spacing. In spring 2015, we conducted substantial pruning between and inside trees, which reduced 2015-16 yield, but allowed additional light into the interior and led to recovery. This is thought to explain the upswing in yield in 2017-18.

A yield summary of the 2008-09 to 2018-19 seasons demonstrated that Corona Foothills had the greatest cumulative yield followed by Walker Lisbon, Limonero Fino 49 and Femminello Santa Teresa (Figure 2). The two standards for the trial, Limoneira 8A Lisbon and Allen Eureka, as well as Yen Ben, had lesser cumulative yields, but these were not significantly different from those of Walker Lisbon, Limonero Fino 49 and Femminello Santa Teresa. Limonero Fino 95, Seedless Lemon and Messina had even lesser cumulative yields, and Variegated Pink Eureka had the least.

**Pack-out Data**

For each season from 2008-09 through 2017-18, 30-35 lb. sub-samples of fruit of each selection were collected and passed through a portable single-line fruit sizer to collect...
pack-out data. From the early harvests, Messina had the largest sized fruit, peaking on sizes 75 and 95, while Corona Foothills, Interdonato, Limonero Fino 95, Walker Lisbon, Allen Eureka, Seedless Lemon and Femminello Santa Teresa fruit size peaked on 95 and 115 (Figure 3). Variegated Pink Eureka and Yen Ben had the smallest sized fruit, peaking on sizes 140 and 165 (Figure 3).

From the later harvests, Messina again had the largest size fruit, peaking at size 75 (Figure 4). Allen Eureka, Corona
Values within the bar segments are estimated annual returns per variety x 1000. Values at the bar are cumulative returns for the seven year period. Annual returns for segments that are too narrow for values are less than $1,500 per acre.

2017-18 data not available for 'Limonero Fino 95'.

2016-17 and 2017-18 data not available for 'Variegated Pink Eureka'.

Foothills, Femminello Santa Teresa, Interdonato, Limoneira 8A, Limonero Fino 49 and Limonero Fino 95, Seedless Lemon and Walker Lisbon peaked on size 95 (Figure 4). Variegated Pink Eureka and Yen Ben again had the smallest fruit, peaking on sizes 140 and 165 (Figure 4).

Commercial Lemon Returns

RBI provided commercial returns data for the 2011-12 through 2017-18 harvest seasons. Commercial returns data included the number of standard cartons in the three fresh fruit quality grades – Sunkist (#1), Choice (#2) and Standard (#3) – as well as the equivalent cartons sent for processing. Within each quality grade, the returns provide specifics about the numbers of cartons in each fruit size category (75, 95, 115, 140, 165, 200 and 235) and the price for which each carton was sold based on quality and size. Marketing, hauling, packing and other charges are deducted from the total price, and a net return is calculated. Numbers of field bins, standard cartons, field boxes (60 lb.) and a net return per field box also were provided. Based on these data and the number of trees per acre of each selection, we estimated commercial return ($USD) per acre.

In this trial, Corona Foothills had the greatest over-all cumulative returns per acre during the seven seasons, but not the greatest return per acre for all seasons (Figure 5). Limonero Fino 49 had the greatest returns per acre in 2011-12 and 2016-17, and Walker Lisbon had greater or identical returns per acre in 2015-16 and 2017-18. The selection with the lowest total cumulative returns per acre was Variegated Pink Eureka; however, returns per acre were unavailable for two seasons. In those seasons when returns per acre were available for all selections, Seedless Lemon, Messina and Interdonato also had lesser returns per acre. Given that Variegated Pink Eureka had the greatest return
per field box for three years (2011-2014) of almost $40 per field box in 2013-2014, but only 16 boxes total, while Corona Foothills and Limonero Fino 49 had returns of more than $20 per field box with more than 140 field boxes (Kahn et al. 2015), the consistently low yields of Variegated Pink Eureka had a greater effect on the cumulative returns per acre than did the returns per field box.

The ability of RBI management to provide the packinghouse return data – despite the difficulty keeping the fruit of each of the 12 lemon selections separate during the picking, hauling and packing process – makes the results of this study particularly valuable and relevant. It enabled us to estimate cumulative returns per acre for each selection, for each season and for the seven seasons together.

Summary of Each Selection’s Attributes

The selections are organized below based on whether they were top yielding selections or lower yielding ones with other valuable attributes. Results on fruit storage life, interior fruit quality characteristics including seed number per fruit listed below were based on data from 2009-10 through 2014-15 which reflect a general trend for the entire trial (Kahn et al. 2015).
Top Yielding Selections

Corona Foothills had the best first harvest yield and the greatest cumulative yield and cumulative returns per acre of all selections. Corona Foothills matured relatively early, had good fruit size and excellent exterior fruit quality. The fruit were oblong with average juice content, average of 14.2 seeds per fruit, high peel thickness and average peel smoothness with a little less juice than the others.

Walker Lisbon had a good first harvest yield and the second most cumulative yield and total returns per acre in this trial. The exterior fruit quality of oblong-shaped fruit was good, and the fruit had average juice content, the greatest seed count per fruit of all selections with 18.2 seeds per fruit and average peel thickness and peel smoothness.

Limonero Fino 49 had excellent first harvest yield, good second harvest yield, third most cumulative yield and total returns per acre, which were very close to Walker Lisbon. Along with Corona Foothills, this was the earliest-maturing selection in this trial. Limonero Fino 49 had excellent first and second harvest fruit size and good exterior quality. Fruit is oblong with average juice content, 12.8 seeds per fruit, average peel thickness and not a particularly smooth peel. Trees were thorny.

Limoneira 8A Lisbon was one of the commercial standards in this trial and is the most planted lemon selection in California. Relative to the other selections in this trial, Limoneira 8A yield for the first harvest and second harvest periods was in the mid-range and had the fifth most cumulative yield over the trial. Based on cumulative returns per acre estimates, this standard ranked fourth behind Corona Foothills, Walker Lisbon and Limonero Fino 49. Limoneira 8A was not particularly early in terms of the size distribution of fruit for the early harvest period. Fruit had good fruit size and exterior quality with rounder fruit, average juice content, 12.4 seeds per fruit, average peel thickness and a not particularly smooth peel.

Allen Eureka was the other commercial standard in the trial and had the sixth most cumulative yield and cumulative returns per acre. Oblong Allen Eureka fruit had good size with average exterior quality that had average juice content, 10.8 seeds per fruit, and average peel thickness smoothness.

Femminello Santa Teresa had good first harvest and second harvest yields, the fourth most cumulative yield and fifth most cumulative returns per acre just behind Limoneira 8A. Fruit maturity was not quite as early as that of some of the other selections and had a smaller first harvest fruit size and average exterior quality. Fruit is round in shape with average juice content, 15 seeds per fruit, average peel thickness and not particularly smooth rind.
Lower Yielding Selections with Other Valuable Attributes

Yen Ben had poor first harvest and below average second harvest yields. The total yields per season were forth to sixth least per season with the sixth most cumulative yield of the 12 selections. Yen Ben was not an early maturing selection. Fruit were small but had good exterior quality. Relatively round, the fruit had low juice content and 2.5 seeds per fruit. The peel was relatively thin, but quite smooth, which probably accounts for results that demonstrated that Yen Ben was highly susceptible to post-harvest diseases.

Interdonato yield varied from lowest to sixth least depending upon the season with the fifth least cumulative yield. Fruit size was usually good, with excellent exterior quality, round fruit with average juice content and 11.4 seeds per fruit. Peel is extremely thin and attractively smooth, which is one of the fruit’s strengths, but fruit have a pointed mammilla, which can be easily damaged during picking and packing which may account for Interdonato fruit being very susceptible to post-harvest diseases.

Limonero Fino 95 trees were a year younger than other selections since they were planted a year later. Total yield varied per season but ranked from the least to the sixth least yielding selection with the fourth least cumulative yield. The fruit were early maturing and had excellent fruit size and exterior fruit quality. The fruit were oblong with average juice content, 5.5 seeds per fruit and a relatively thick peel that was not particularly smooth.

Seedless Lemon had an average first harvest yield, but poor second harvest, with total yields that were third or fourth least each season and third least cumulative yield of 12 selections. Seedless Lemon is not an early selection. The fruit were small and oblong in this trial but had good exterior quality with high juice content and were the least seedy with just one seed per fruit. The peel was relatively thick and had average smoothness.

Messina had below average first and second harvest yields. Total yields were second or third least each season and second least cumulative yields. This selection was earliest maturing of all selections, which was reflected in large fruit size and packout. Fruit had excellent exterior quality, but some individual fruit were almost the size of small melons; too large for the fresh fruit market. The fruit were round with average juice content, 2.5 seeds per fruit and had a thick, smooth peel.
Variegated Pink Eureka had poor first and second harvest yields, the least or second least total yield per season and the very least cumulative yield. Fruit were not early maturing and were the smallest of all selections with average exterior fruit quality. This selection had exceptional return per field box, but the least estimated return per acre. The fruit were round with good juice content and 5.5 seeds per fruit with a thin but not smooth peel.

Catalyst for Future Trials Throughout State

Based on initial results of this trial, a set of second-generation multi-location lemon trials were planted in spring 2015 at four sites: CVARS, Santa Paula, Lindcove Research and Extension Center (LREC) and University of California, Riverside (UCR). These trials include Corona Foothills, Walker Lisbon, Limonero Fino 49, Yen Ben, Interdonato, Limoneira 8A Lisbon (as a commercial standard) and a selection designated as Limoneira 8A IR1 a temporary name for a low seeded, irradiated selection of Limoneira 8A by the UCR Breeding Program. The rootstocks vary in these trials based on location with Carrizo citrange, C35 citrange and Rich 16-6 trifoliate at LREC and UCR; Carrizo, Citrus macrophylla and Swingle at Santa Paula; and Carrizo, C. macrophylla and C. volkameriana (Volk) at CVARS. In addition, there is a demonstration block adjacent to the trial at LREC with two trees of each scion rootstock combination next to each other. We expect these new trials will provide valuable information for growers across the state interested in commercial lemon production and look forward to highlighting results in future Citrograph issues.

CRB Research Project #5200-201

References


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MEASURING SCION AND ROOTSTOCK TOLERANCE TO HLB IN THE GREENHOUSE

Project Summary

The presumptive pathogen ‘Candidatus Liberibacter asiaticus’ (CLas) can infect all citrus species and relatives, but expression of the disease huanglongbing (HLB) caused by this infection differs greatly between different types of citrus. Good tolerance¹ to HLB has been identified in some citrus species and citrus relatives, including clones that are rootstocks. We are inoculating an assortment of citrus cultivars and relatives, both grafted and as seedlings, with CLas under controlled greenhouse conditions to evaluate relative sensitivity² or tolerance of different scion and rootstock cultivars, as well as potential parents to use in breeding. These studies will identify the best rootstocks available now to allow Washington Navel, Valencia and Tango trees to tolerate HLB if they become infected, and they also will improve knowledge of HLB tolerance and resistance³ to enable development of cultivars with enhanced resistance in the future. The information obtained will immediately help growers make informed decisions to prepare for the HLB disease threat, improve the prospects for continued profitability following tree infection and advance our ability to develop fully HLB-resistant or tolerant scions and rootstocks for the future success of the California industry.
The success of commercial citrus production depends on the combination of superior scions with fruit quality traits in demand by the markets and rootstocks with good adaption to the environmental conditions, along with resistance or tolerance to disease and pest threats. HLB, which is associated with the bacterium CLas and spread by the Asian citrus psyllid, is severely affecting the production of citrus worldwide, and has been detected in some residential areas of California. Infected trees of the common sweet orange, grapefruit and mandarin crops exhibit reduced vigor, thinner canopies, lower fruit production and poorer fruit quality. In regions with widespread HLB, like Florida, several strategies are being used in citrus groves to reduce the disease’s impact on profitability; but the most economical strategy, and the one most effective to implement at tree planting, is the use of HLB-tolerant scions and rootstocks.

Evaluation of cultivar tolerance to CLas infection is complicated due to the erratic spread of CLas under natural conditions, erratic distribution of CLas and disease within infected trees, complex genetic scion/rootstock systems and a variable timeline for tree decline due to only partially understood interactions of environment and management practices. Our studies are being conducted in controlled greenhouse conditions with controlled graft-inoculation of CLas for a more precise and accurate assessment of the effect of HLB on each cultivar and scion/rootstock combination.

Our project involves testing an assortment of different scion and rootstock cultivars in greenhouse experiments in Florida and the Biosafety Level-3 Lab (BSL-3P) containment facility in Riverside California, to assess the sensitivity, tolerance or resistance of those cultivars to CLas infection. After plants are grown to a suitable size, they are graft inoculated with CLas and then evaluated in the greenhouse for 18-21 months to assess the effects of infection on plant growth and health. Previous studies have shown good correlation between this type of greenhouse evaluation and field studies in an HLB-endemic environment (Bowman et al. 2016; Bowman and Albrecht 2020). In the Florida studies, each experiment involves the inoculation and evaluation of about 400 potted one- to two-year-old citrus seedlings or grafted trees (Figure 1). Similar experiments in the Riverside BSL-3P recently were started, so this report focuses on the results from the Florida studies.

Our greenhouse studies are divided into two types of experiments with controlled infection of trees by CLas: to evaluate scion cultivars and breeding germplasm as seedlings and to evaluate rootstock effects on grafted trees with Washington Navel, Valencia and Tango scions.

This report describes more detail on the individual experiments and provides some preliminary results from the experiments in progress.

To evaluate scion cultivars and germplasm with CLas strains from both California and Florida, we are using seedlings from selections that can be uniformly propagated by seed. The cultivars and species represent a range of both HLB-sensitive and more tolerant or resistant selections from several different types of citrus germplasm, including Duncan grapefruit, Olima Valencia, Cleopatra, Kinnow, Sunburst, W. Murcott, Assad Citron, Poncirus trifoliata, US-897 and Microcitrus inodora. Large differences are being observed between the disease effects on the different cultivars. At 12 months after inoculation in the seedling study, the growth of Valencias was reduced by infection with CLas to about 30 percent of the growth of non-infected Valencias, while other cultivars and species were much less affected by CLas infection (Figures 2 and 3). Seedlings of Assad Citron and US-897 showed no growth reduction resulting from infection with CLas (infection was verified by qPCR). While the cultivars with highest tolerance, like citron and US-897, do not have fruit that are suitable for use as scion cultivars, they demonstrate that similar high levels of HLB-tolerance can be developed through breeding of future cultivars that combine tolerance with good fruit quality.

Of more immediate benefit to growers, results from the seedling study with scion cultivars can help identify meaningful differences in resistance or tolerance that can affect success of commercial plantings in areas affected by HLB. For example, common sweet orange and grapefruit cultivars generally show severe symptoms

![Figure 1](image1)  
![Figure 2](image2)
from HLB, while some of the mandarin scions appear to be only moderately damaged. It appears that plantings of CLas-tolerant mandarins are more likely to remain commercially successful after infection with CLas than are plantings of sweet orange or grapefruit, which would agree with some previous observations (McCollum and Baldwin 2016).

In addition to choice of scion, growers also can improve tree tolerance to HLB by using a rootstock that is more resistant or tolerant than other common rootstocks. A previous study demonstrated that sweet orange trees on Swingle citrumelo that became infected with CLas lost nearly 60-70 percent more of their leaf area than trees on the new rootstocks US-942 and US-812 that became infected (Bowman and Albrecht 2020). In some of the greenhouse experiments, we are comparing different rootstocks with Washington Navel and Tang orange scions to determine relative HLB tolerance of different scion and rootstock combinations. There is clear evidence that some rootstocks have a significantly better tolerance to CLas infection than other rootstocks, and when used with a sweet orange scion, will result in a healthier tree canopy and better cropping (Albrecht and Bowman 2012; Bowman et al. 2016; Bowman and Albrecht 2020). In this project, we are using 12 rootstocks, including those of commercial interest in California like Carrizo, C22, C57, C146 and trifoliate orange, and others that have demonstrated better tolerance to HLB in Florida (like US-942 and US-812).

Once complete, the results from these studies will help California growers anticipate the potential disease responses of different scion/rootstock combinations that may develop if their existing field trees become infected with CLas. The results also can be used in decisions about which of the rootstock options are best to plan for use in future tree planting.

Scion and rootstock cultivars separately and in combination may have large effects on tree resistance or tolerance to CLas infection and the potential for continuing profitability if HLB becomes widespread in California. This project will help provide information that enables growers to make good decisions about current plantings and planned future plantings, as well as essential knowledge to direct the breeding of future scion and rootstock cultivars with resistance and tolerance to HLB.
THERE’S NO ROOM FOR MITES OR ACP.

NOW! 3-DAY PHI
Development of engineered citrus varieties that flower after chemical treatments

Sean Cutler, Mikeal Roose and Sang-Youl Park

Project Summary

Citrus breeders have brought many exciting new varieties to market over the years, but the biology of citrus limits how quickly geneticists can respond to market needs. Citrus plants grown from seed possess a juvenile period of five or more years before they reach sexual maturity, adding many years to each breeding cycle compared to annual crops. In this project, we have used state-of-the-art protein engineering methods and concepts from the growing field of synthetic biology¹ to build a chemically activated genetic circuit that allows citrus to flower early. After introducing this circuit into citrus by Agrobacterium-mediated transformation, we have shown that flowering can be induced in juvenile citrus (Carrizo citrange) plants (less than one year old) and produce fruit and seeds. Our newly developed genetic “switch” is controlled by an inexpensive agrochemical called Revus®. The immediate benefit of this project is faster generation times for citrus breeders. We also envision our new genetic switch may be used to design cultivars with traits that growers can activate as needed, as a long-term benefit.
Citrus breeding programs face lengthy timelines for new variety development due to the extended juvenility of citrus, a biological feature shared by most long-lived perennial tree crops. As a result, citrus breeders develop new varieties more slowly than breeders who work with annual crops. Each cycle of crossing and selecting new and exciting traits, the actual breeding work, takes many years for citrus species. It would be beneficial to accelerate citrus breeding timelines—to develop methods for fast-tracking citrus generation times so that breeders can respond to emergent threats such as huanglongbing (HLB) and other diseases, as well as other horticultural traits, as quickly as possible. In this project, we engineered new transgenic varieties that can be induced to flower as juveniles using a low-cost agrochemical. This means that citrus breeders now can use our engineered “inducible flowering time” (iFT) strains to speed up breeding efforts. The iFT technology also will allow breeders to acquire fruit quality data in juvenile transgenic plants, which will hasten selection timelines.

The genetic control of flowering is well-understood at the molecular level. When the right environmental conditions exist, which vary from species to species, plants activate a gene called Flowering locus T (FT)2. Scientists have known about FT’s role as a regulator of flowering for more than a decade (Turck et al. 2008). FT encodes a protein that moves long distances within a plant to signal flowering. It is synthesized in leaves at high levels immediately before flowering and then moves throughout the plant body in the phloem, the specialized plant tissue that transports sugars from leaves to apical meristems3 and fruit. FT protein is transported in the phloem to cells in the shoot apical meristem, instructing the meristem to make flowers. FT protein is unique because it acts far away from its site of synthesis. In this way, FT is like a hormonal signal, a property that makes FT very powerful for manipulating flowering.

Others have shown that transgenic citrus plants engineered to produce high FT gene and protein levels flower as juvenile plants (Endo et al. 2005). Even though the strategy has been previously tried by others, there is one big problem; the engineered plants also have several abnormal developmental traits, including reduced growth because they express FT at all growth stages. Ideally, one would like to produce FT and initiate flowering when flowering is desired, but keep it off the rest of the time, mimicking how plants in nature usually control the FT gene. Unfortunately, the tools needed to do this in plants under real-world field conditions were not well developed when we started our project. Therefore, we set out to build a system for inducible control of gene expression that performs well in citrus. Inducible gene expression4 systems are used extensively in microbial biotechnology. For example, the insulin used by people with diabetes is mass-produced in bacterial strains that synthesize insulin by inducible expression. Such systems are critical for insulin production. If the engineered bacteria produced insulin all of the time, they would grow too slowly since producing insulin consumes many cellular resources (Farmer and Liao, 2000). Inducible systems get around this dilemma by allowing organisms to live two lives—one as healthy and fast-growing hosts that can be grown to high densities and a second life as production factories.

Although microbial engineers have used inducible systems for many years, there are few options for regulating gene expression in plants and none with the right combination of properties that are practical for field application. The current gene induction systems that exist are tailored for laboratory research and use toxic and expensive inducers, which prohibits their use in agriculture. We have developed a new gene induction system that addresses these issues. Our system is regulated by mandipropamid, an inexpensive agrochemical sold commercially as Revus that is used for controlling oomycete pathogens, fungus-like microorganisms, such as Phytophthora species. Our new system could theoretically be used in real-world production environments to regulate gene expression.

To engineer iFT citrus plants, we built a simple transformation vector that contains all components for Revus-induced gene

![Figure 1. (A) Inducible flowering in juvenile citrus. Roughly eight-month old iFT Carrizo flowering shoot, about two weeks post-inducer treatment. (B) Fruit formation in an iFT strain. An approximately 12-month old juvenile iFT transgenic with fruit. (C) Successful use of iFT juvenile pollen in crosses. Pollen collected from iFT Carrizo was used to pollinate a low acid pummelo; shown is one of five fruit obtained, about nine months after pollination. (D) High-level FT gene expression can be observed 24 hours after treatment with the inducer (mandipropamid; shown at right “+mandi”) in comparison to uninduced control plants (“mock”).]
expression. Genes of interest can be easily inserted into this construct and then introduced into plants by Agrobacterium-mediated transformation. Since FT protein moves into apical meristems via the phloem, we investigated a phloem companion cell-specific promoter for controlling gene induction using a viral gene expression controlling element derived from the Rice Tungro Bacilliform Virus (RTBV). In our experiments, the RTBV-controlled system performs well.

To date, we have created multiple juvenile Carrizo citrange iFT transgenic plants by transforming seedling tissue and selected two transgenic lines with robust growth and Revus-inducible flowering (Figure 1A). These lines have produced multiple fruit after Revus-treatment (Figure 1B). In total, we have collected about 100 seeds from 18 Carrizo fruit and validated that the seedlings harbor the transgene by genotyping⁵. It is essential that our iFT lines produce viable pollen if they are to be useful for breeding efforts. To test this, we collected pollen from approximately ten-month old iFT plants induced to flower by Revus treatments and then used this pollen to cross onto a pummelo female. Fruit have been obtained, one of which is shown (Figure 1C); the first two fruit yielded roughly 140 seeds. Since pummelos are self-incompatible, it is highly likely the seeds produced resulted from successful crosses, and that the iFT-pollen is functional, but this requires further validation.

We investigated levels of FT gene in young iFT transgenic Carrizo leaves both pre- and post-inducer treatment. There was more than a 20-fold increase in gene induction after the Revus treatments (Figure 1D). Thus, our new system allows for rapid FT induction in citrus.

Collectively, our data demonstrate that our iFT system works and is poised to accelerate breeding programs. To empower these efforts, we currently are introducing our iFT construct into several important cultivars, including HLB-resistant citrus relatives and mandarin cultivars closely related to sweet oranges. More generally, this work delvers a new and powerful tool for inducible gene expression to the citrus biotechnology community. Synthetic biology is rapidly changing how biological engineers approach many problems. To be useful to the citrus industry, a whole new suite of tools to control gene expression is needed. Over the long term, we are optimistic that these tools will allow biotechnologists to develop new inducible horticultural traits. 🌸

CRB Research Project #5200-156

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**Glossary**

1 *Synthetic Biology:* A field of study that merges principles from biology and engineering to design organisms with specific traits.

2 *FT:* *Flowering Locus T* is a gene encoding a small protein that moves throughout the plant to induce flowering.

3 *Apical meristem:* Small clusters of cells at the growing tips of organs, including shoots and roots, that produce structures such as leaves and/or flowers.

4 *Inducible gene expression:* Rapid and specific expression of genes in response to an external stimulus.

5 *Genotyping:* Determining the genetic makeup of an organism.

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**References**


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Citrus Orchard Security

The citrus industry has experienced rapid growth over the last several years and we want to ensure longevity of the valley’s commodity by providing protection against ag theft. Thieves have access to harvested products, equipment, machinery, and vehicles. There are materials like palladium and platinum that make catalytic converters located in vehicles and citrus wind machines more desirable. The Pipkin Detective Agency has extensive experience in agriculture security. Our team leverages the power of technology to provide modern security strategies capable of monitoring and protecting citrus orchards 24 hours by 7 days a week, and 365 days per year. We encourage you to contact us today for a no obligation security consultation.

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**Mature Citrus Budwood Transformation Technology**

**Project Summary**

When resistance genes to huanglongbing (HLB) are found, how do we get them into existing cultivars without losing the original quality traits (e.g., flavor, easy peel, etc.)? Conventional breeding to incorporate desired traits of resistance without losing valuable traits in commercial varieties could take decades. Transformation – the genetic modification of citrus in a lab – is an option. However, successful transformation has been limited to a few cultivars and often results in juvenile trees requiring years of development before cropping.

The technique we examined used mature budwood tissue that was trimmed down to a small number of cells capable of regeneration. A similar technique is routinely used on numerous citrus cultivars to produce pathogen-free plantlets and regenerate trees that remain capable of flowering and fruiting. Specifically, our goal was to adapt this technique for citrus transformation purposes.

A number of modifications to the existing technique can be used to improve mature citrus tissue transformation. These include the method of wounding the tissue to start the process, use of surfactants (soaps), modifying the types and concentrations of sugars and salts in the growth medium for the plant tissue and the type and timing of hormones applied. These changes to the mature citrus transformation protocol currently are being combined and applied toward the modification of Washington Navel, Mexican lime, Lisbon lemon and Cocktail grapefruit, allowing us to (hopefully) add any genes of interest for any trait without changing the cultivars’ attributes.
HLB is a bacterial plant disease, associated with ‘Candidatus Liberibacter asiaticus’ (CLas), that greatly compromises citrus trees and currently has no cure. HLB-resistant trees are essential for citrus industry survival. Once identified, resistance genes will need to be introduced into a range of commercial citrus cultivars. Current transformation technology is not amenable to most citrus cultivars important in California. Shoot tip grafting (STG) technology, developed to produce pathogen-free citrus, is a technique we are trying to implement for citrus transformation. The technique uses the tip tissue from axillary shoot buds (found at the junction of the leaf and stem) of mature trees and is routinely used for successful clean-up of a wide range of cultivars (Volk et al. 2012). We studied the modification of this technique for citrus transformation and examined other methods for improving the entire process. Our goals were to:

1. optimize the budwood transformation regeneration protocol in citrus tissue;
2. develop enhanced transformation methods with the ability to rapidly identify transformed tissue; and
3. introduce potential HLB resistance genes for later cultivar evaluation.

A number of steps were needed to repurpose the STG protocol for transformation of mature citrus tissue. The first question was whether the tip tissue (a cube 2-3 mm wide) from axillary shoot buds could be transformed to contain the desired genes. When the tip tissue was directly transformed, followed immediately by tip grafting, most tips died. Even when they did survive, the resulting plant was a mosaic – of mixed genetic background – and useless for plant production. Alternatively, axillary shoot buds that were induced to grow in vitro before use provided a more robust tissue that could survive the transformation process and yield fully transgenic plants. Twenty-eight scion and four rootstock cultivars were evaluated for axillary shoot bud induction.

Four different hormone pre-treatments for stimulating bud growth were tested. Of the 32 cultivars examined, we were able to induce bud growth in 27 of the lines.

With this source of healthy material, our next objective was to determine if the tissue could be used to produce transgenic citrus. To do this, we used a technique called Agrobacterium-mediated transformation. This technique was discovered in the 1980s when scientists found that a native soil microbe (Agrobacterium) inserted its DNA into plant cell nuclei to produce galls and that this process could be manipulated. These scientists determined how the system worked and removed the gall-forming genes. They further learned how to add DNA of interest in its place, such as disease resistance genes. Using Agrobacterium-mediated transformation, we examined all 27 cultivars in which axillary bud growth was induced. Genetic transformation was observed in eight cultivars (Carrizo, Troyer, C-22, Moro Blood Orange, Sour Lemon, Lisbon lemon 8A, Clemenules Clementine and Persian lime). We were able to visualize the added DNA due to the presence of the DSRed gene. This gene produces a non-toxic compound that fluoresces in the modified cell providing a way to quickly identify modified tissue (Figure 1).

We were able to observe transformation of mature tissue from induced axillary bud tissue. However, using current transformation technology, the number of cultivars successfully modified was low (eight out of 27), and regeneration of a plant from this material was not possible. In other words, we could get the new DNA into the tissue but were unable to produce a plant from this tissue. However, some rootstocks like Carrizo, C-22, Troyer and, surprisingly, the scion Blood Orange (Moro) (Figure 2) were capable of regeneration shoots, although the efficiencies were low and not viable for commercial use.

Figure 1. The DSRed gene was added by Agrobacterium-mediated transformation to Lisbon lemon 8A. Panel A shows the tissue under white light. Panel B shows the tissue under green light that causes DSRed to fluoresce allowing the modified cells to be visualized.
In addition to studying repurposing of STG, a number of modifications to conventional transformation were examined in an attempt to increase the efficiency. These included different media and hormones for plant growth, ways to wound tissue, types of surfactants and use of genes that enhance plant survival. The media modifications involved changing the ratio and types of sugars and salts given to the plant as food for growth in vitro. After many trials, it was determined the plant medium defined as Driver and Kuniyuki Walnut (DKW) was superior to anything previously used for regenerating mature citrus tissue. When DKW medium was supplemented with the hormones 6-benzylaminopurine (BA), naphthalene acetic acid (NAA) and 6-furfurylaminopurine (Kinetin), healthy shoot production resulted from mature tissue of Washington Navel (De Oliveira et al., 2016). As such, this has become the most commonly used medium in our lab.

When plant tissue is wounded, it releases chemical compounds that stimulate Agrobacterium-mediated DNA transfer. By maximizing the plant’s wound responses, we increase the effectiveness of the Agrobacterium-mediated gene transfer, which in turn increases the rate of transformation. We examined wounding by exposing tissue to the following stresses: physical (e.g., mechanical and sonication) and chemical (e.g., enzymatic and dehydration). Although all methods worked to some degree, the enzymatic method provided the most robust, reproducible response. The enzymes used are known for their ability to break down plant tissue, and essentially strip off the outer layers of cells from the plant tissue. By modifying the enzyme amounts and exposure time, we were able to demonstrate a 135 percent increase in transformation efficiency over the current method.

Types of surfactants used in transformation also were examined. The basis for this is that the surfactant allows the Agrobacterium to more easily penetrate the waxy outer layer of the plant surface, providing better binding to the plant tissue, thus allowing and enhancing transfer of DNA to the citrus cell. Seven novel surfactants and a control were tested for increased transformation using the model plant Arabidopsis to limit the number of surfactant trials with citrus. All of the surfactants tested increased transformation during the process. Trials were moved to citrus with the commonly used Agrobacterium strain EHA105 and a novel strain 1416. In these tests, strain EHA105 was unaffected by the presence or absence of the surfactant, while strain 1416 resulted in an 18 percent increase in effective DNA transfer to citrus tissue. Taken together, the results appear to be Agrobacterium strain-specific and provide a positive boost in DNA transformation efficiency.

Another aspect being examined to increase citrus transformation was the use of genes to increase plant survival during the transformation process. Plants will fight off infections (such as bacterial colonization) by immediately killing off the infected cells to prevent spread of the bacteria. Therefore, to increase the ability of transforming citrus cells, we attempt to convince the cells not to die when colonized by Agrobacterium. We did this by adding anti-cell death genes to the DNA injected into the plant cell. A total of four anti-death genes (BAG4, BI-1, DAD1 – derived from Arabidopsis; P35 - derived from a virus) were examined for increasing cell survival. Our studies determined that two of the genes (BAG4, P35) regularly increased the survival of the plant tissue, which we observed as an increase in transformation by up to ten percent. One of the genes (DAD1) did not affect transformation and the final gene (BI-1) actually lowered the rates of survival. From the two genes that increased cell survival, we chose to use the gene BAG4 derived from Arabidopsis.

With these results, we were able to construct an Agrobacterium transformation vector with enhanced transformation capacity. This construct included selection (antibiotic) genes optimized for citrus. The early detection gene, DSRed, was included to visually distinguish between

**Figure 2.** The DSRed gene was added by Agrobacterium-mediated transformation and shoot regeneration to the rootstock Troyer. Panel A shows the tissue under white light. Panel B shows the tissue under green light that causes DSRed to fluoresce enabling the modified plantlet to be visualized.
transformed tissue and untransformed “escape” plants. In addition, the anti-death gene **BAG4** was added to reduce the rate of cell death during transformation, thereby increasing the number of cells surviving the *Agrobacterium* infection/transformation process. Once completed, this construct was used to introduce potential HLB disease resistance genes and defensin peptides (defense related antimicrobial peptides) into citrus. The 15 disease resistance genes (**PME1, PDR1.2, NPR1, mThionin, harpin, SCAMPPS333, CSM-1, SoiSN1, SoiSN2, CapA1, CapA2, CapG1, CitGrp1, CitGrp2, BP178**) have been constructed into single and multiple combinations. The rootstock Carrizo was used to produce more than 300 transgenic plantlets from all constructs mentioned. Transformation frequencies ranged from 4-50 percent among different constructs and conditions examined. More than 200 plantlets were prepared for CLas challenge. Unfortunately, a pest infestation destroyed most material before it could be tested, and backup material was not available. However, a few plants containing the **CSM-1** and **harpin** genes survived. The **CSM-1** gene showed an increase in hypersensitivity (localized death when infected by CLas), while the **harpin** displayed an increase tolerance to CLas infection as determined by surviving longer in the greenhouse than the control trees and by the exceptionally high titer recorded before plant death.

In conclusion, citrus transformation offers a unique method of modifying a citrus tree whereby genes of interest (in this case, for disease resistance) can be added to its genome. The process is faster than breeding and doesn’t change the existing desirable characteristics of each cultivar. However, the technology requires adapting techniques to many commercial cultivars. A number of aspects of the transformation and tissue regeneration process were examined such as media, hormones, wounding, addition of genes to help the plant survive and the strain of *Agrobacterium* used. These modifications all have shown increases in the ability to transform mature citrus tissue. Currently, efforts now are underway to combine all these aspects into a single protocol for use on mature citrus tissue. When research is completed, it will be made publicly available to benefit the entire industry.

**References**


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INTERSTATE MOVEMENT OF NEW AND LICENSED CITRUS VARIETIES
A CASE STUDY FROM FLORIDA

Irene Lavagi-Craddock, Greg Greer, Jude Grosser, Fred Gmitter Jr., Kim Bowman, Ed Stover, Greg McCollum, Ben Rosson, MaryLou Polek, Robert Krueger and Georgios Vidalakis
The sustainability, competitiveness and economic success of the California citrus industry depend on the availability to the citrus growers of newly developed citrus varieties with important horticultural characteristics including, but not limited to, resistance or tolerance to pests. However, new varieties are often protected, patented or licensed, and movement of citrus propagative material among states remains a complex regulatory issue. In 2015, California citrus growers visited citrus breeders and scientists from the University of Florida (UF) and U.S. Department of Agriculture-Agricultural Research Service (USDA-ARS) in Florida to observe different citrus varieties and hybrids growing in a huanglongbing (HLB) endemic environment and assess their potential value for the California citrus industry.

The lack of a well-defined protocol (e.g. material transfer agreements, variety introduction cost payments, terms for maintenance and distribution of propagative materials) for the introduction and commercialization of protected/licensed citrus varieties – coupled with the spread of ‘Candidatus Liberibacter asiaticus,’ the HLB-associated bacterium and its vector, the Asian citrus psyllid (ACP) – to almost all citrus-producing areas in the world provided an opportunity to address the regulatory complexity of the interstate movement of citrus scions and rootstocks.

Since 2015, the collaborative efforts of university, government and industry organizations supported by one Citrus Research Board (CRB) and two HLB Multi-Agency Coordination (HLB-MAC) Group grants, overcame the regulatory and material transfer gridlock that hindered the flow of citrus varieties from Florida citrus breeding and evaluation programs into California (Lavagi et al. 2017).

Currently, the Citrus Clonal Protection Program (CCPP) has 68 Florida-developed rootstock and scion varieties in its citrus variety introduction pipeline. Thirteen of these have been released from quarantine (Table 1), seven varieties are currently in the last variety index (VI) step, and one variety has entered an evaluation agreement with a private California entity. The remaining 48 varieties are in various stages of therapy and disease indexing1.

The introduced varieties include 58 scion and 10 rootstock accessions, with 38 varieties developed by UF and 30 by the USDA-ARS. The imported rootstocks are of particular relevance in the fight against HLB, as initial observations in Florida indicated some level of HLB tolerance. In addition, some scion varieties also appear to have some level of HLB tolerance (e.g., US 6-49-96 Orange Hybrid-SunDragon, and LB 8-9 Sugar Belle®).

The introductions released from quarantine since the last Citrograph update (Lavagi-Craddock et al. 2020) include four mandarin varieties of particular commercial interest in California (i.e., Seedless Snack UF N40W-6-3, UF 411, UF-950, US 6-15-89/US Ortanique LS), one sweet orange hybrid variety reported to have very high HLB tolerance in Florida (US 6-49-96 SunDragon) and one rootstock (US-812).

As material introduced from outside of California completes CCPP therapy and indexing and is released from quarantine, budwood sources will be propagated at the CCPP Lindcove

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Table 1: 13 Florida-developed citrus accessions released from Federal and State quarantine; Therapy and VI (Variety Index) completed at the Citrus Clonal Protection Program.

<table>
<thead>
<tr>
<th>Variety Name</th>
<th>Type</th>
<th>Project*</th>
<th>VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>SL UF C4-15-21 (19)</td>
<td>Mandarin</td>
<td>CRB</td>
<td>943</td>
</tr>
<tr>
<td>LB 8-9 Sugar Belle®</td>
<td>Mandarin</td>
<td>CRB</td>
<td>952</td>
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<tr>
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<td>Mandarin</td>
<td>CRB</td>
<td>961</td>
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<tr>
<td>UF 950</td>
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<td>CRB</td>
<td>962</td>
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<tr>
<td>Seedless Snack UF N40W-6-3</td>
<td>Mandarins</td>
<td>CRB</td>
<td>953</td>
</tr>
<tr>
<td>US 6-49-96 (US SunDragon)</td>
<td>Sweet Orange-like</td>
<td>MAC-I</td>
<td>964</td>
</tr>
<tr>
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*Funding agency providing support for material.

1See “Research-HLB Resistant Varieties-CRB and USDA MAC Funded” at https://ccppdms.ucr.edu/ccppdms/upcoming_varieties
Research and Extension Center (LREC) Foundation Facility (LFF), and a small number of field trees will be planted at a CCPP-LREC field block for preliminary horticultural observations.

How these varieties perform in California remains to be determined. There is an impending need to create an evaluation pipeline for these varieties that meets current industry requirements and priorities with a special interest in HLB prevention and management (Lavagi-Craddock et al. 2020).

This very successful effort now has entered a new phase. Various stakeholders are discussing the routine movement of citrus budwood from different citrus programs into Citrus Centers of the National Clean Plant Network. Future protocols also are being discussed for the safe interstate movement of other citrus materials such as pollen and seed, and the formulation of a national program that is customizable for the needs of each citrus-producing state to evaluate and commercialize citrus varieties independent of their point of origin.

This project focusing on the safe interstate movement of citrus propagative material clearly demonstrates the value of the CRB’s leadership in facilitating partnerships among scientists, regulators and the California citrus industry (Klein 2019). As a result of this project, the CCPP now can introduce citrus material from anywhere in the U.S. into California, regardless of the HLB status of that area.

We hope the benefits of the transformation achieved by this project in the interstate and international movement of citrus varieties will be long lasting, and the opportunity to shape citrus variety evaluation platforms with emphasis on current industry needs will be of great benefit to the citrus industry.

CRB Research Project #5200-149
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Project Summary

During the last ten years, much progress has been made in the development of new techniques to modify the genomes of living organisms. In contrast to the process of plant transgenesis¹ that came out in the 1980s, these new gene-editing techniques do not rely on the introduction of large pieces of foreign DNA to a new organism. Instead, gene editing works by creating modifications to genes already present in an organism of interest. CRISPR² is probably the most well-known of these new gene-editing techniques. We and other research groups have demonstrated that CRISPR can be applied successfully to citrus for creating small DNA modifications to specific genes. The goals of this project were to keep improving CRISPR for use in citrus and to demonstrate whether the technique could be taken a step further by not only targeting specific genes for modification, but also by controlling the type of modification made at these genes. Precise engineering of citrus genes is a critical step needed to create new cultivars that can, for example, resist huanglongbing (HLB) and other diseases.
**Targeted Mutagenesis and Gene Targeting**

The gene-editing CRISPR/Cas9 technique relies on a gene-protein complex (CRISPR, Cas9 and a guide RNA [gRNA]) that act together to cut DNA at specific locations in the genome (Jinek et al. 2021). The most common application of CRISPR/Cas9 is targeted mutagenesis, which results from mistakes during the repair process of cut DNA made by CRISPR/Cas9 (Figure 1A). The main DNA repair pathway inside living organisms is error-prone and typically leads to random small insertions or deletions of DNA sequences at the location where the DNA is cut by CRISPR/Cas9. These modifications created by targeted mutagenesis usually will completely inactivate a gene so that the function of the protein encoded by this gene is eliminated.

In addition to targeted mutagenesis, CRISPR/Cas9 also can be used to precisely modify the DNA of specific genes by gene targeting. Like targeted mutagenesis, gene targeting is initiated by a specific DNA cut induced by CRISPR/Cas9. However, gene targeting relies on a different DNA repair pathway inside living organisms that uses a donor DNA template to repair the DNA break (Figure 1A). The DNA sequence of the donor template is transferred during the repair process to the location where the DNA cut was made by CRISPR/Cas9. Therefore, by controlling which template is used to repair a cut, precise changes can be made to a gene that was targeted by CRISPR/Cas9.

The goal of this project was to improve upon CRISPR/Cas9 for use in citrus by establishing for the first time an effective citrus gene targeting system. We also wanted to test optimized expression systems for Cas9 and gRNA that could lead to more efficient CRISPR activity in citrus.

**Assessing Gene Targeting Efficiency in Citrus**

We measured gene targeting using an assay based on protoplast cells collected from citrus leaves (Carrizo citrange). Protoplasts are live cells (extracted from plant tissues) that lack cell walls. These protoplasts are suspended in a liquid...
solution, and foreign DNA can be introduced into these cells under certain conditions. We exposed protoplasts to 1) DNA coding for Cas9 and a gRNA targeting the PDS gene of citrus to demonstrate targeted mutagenesis and 2) a DNA template that could potentially be used to repair the DNA cut at the PDS gene made by CRISPR to demonstrate gene targeting. We included in the DNA template a specific mutation that would be expected to be copied into the PDS gene of citrus if gene targeting were used to repair the DNA cut. This specific mutation would thus serve as a marker to measure the efficiency of gene targeting in the protoplast assays. Results from these experiments showed that we were successful at inducing targeted mutagenesis of the PDS gene in protoplasts due to the cutting activity of Cas9 and the gRNA. However, we did not detect evidence of the specific mutation at the PDS gene, which indicates that gene targeting is not occurring in citrus protoplasts.

Next, we decided to test if gene targeting could work in citrus by applying a different technology based on plant DNA viruses. Modified, non-pathogenic forms of these DNA viruses are able to carry additional DNA in their genome for Cas9, gRNA and a DNA template required for gene targeting (Baltes et al. 2014). In a few select plant systems (e.g., tobacco and tomato), low levels of gene targeting activity can occur when these modified DNA viruses were transformed into plants (Baltes et al. 2014; Čermák et al. 2015). We tried to apply this technology in citrus by first designing plant DNA viruses containing Cas9, a gRNA targeting the PDS gene of citrus and a gene for green fluorescent protein (GFP), and then transforming them into citrus plants (Carrizo citrange). In these experiments, expression of the GFP protein indicates that plants have been transformed successfully. The GFP protein was expressed in regenerated citrus plants, indicating that the modified DNA viruses were present in these plants and producing the proteins required for CRISPR activity. However, our results showed that mutations at the PDS gene were not observed in these experiments.

Enhancing CRISPR Activity in Citrus

Another research direction for our project was to improve CRISPR/Cas9 activity in citrus by testing the efficacy of genetic elements known as promoters. Promoters control the levels of expression of genes in living organisms. Different promoters induce different levels of expression. Depending on the experimental goal, promoter activity can have a large impact on the result of the experiment. In the case of CRISPR/Cas9, both the Cas9 protein and the gRNA need to be expressed at very high levels inside cells to produce the required molecules that will cut DNA. We tested various promoters to drive expression of Cas9 and the gRNA in citrus and measured CRISPR activity in stably transformed citrus plants (Carrizo citrange). For these CRISPR experiments, we targeted the PDS gene of citrus, as pds mutants produce white tissues and thus indicate CRISPR activity in these plants (Zhang et al. 2017).

Results from our experiments have shown that the RPS8 promoter (from the plant RPS8 gene) is extremely efficient for inducing high levels of gRNA appropriate for CRISPR activity in stably transformed citrus plants (Figure 1B). We have confirmed the efficiency of the RPS8 promoter in experiments where four gRNAs are expressed simultaneously in the same transformed plant. In these plants, high levels of CRISPR/Cas9-mediated mutations could be detected at the four genes targeted by the different gRNAs expressed from the same RPS8 promoter.

Future Directions

Gene targeting remains a challenge in citrus, as in most other plant systems. However, new technologies recently have come forward (e.g., Prime editing) that offer new hope for precisely modifying the genome of important crops like citrus (Anzalone et al. 2019). Aside from gene targeting, our work has revealed that CRISPR activity can be further enhanced in citrus by choosing better promoters to drive expression of the gRNA. This result will make it much easier to perform targeted mutagenesis in citrus and will help in creating large collections of citrus genetic variants in the near future that can be used to identify plants with enhanced tolerance or resistance to the HLB pathogen. Developing new citrus varieties via CRISPR could provide a comprehensive solution to the HLB problem for growers.
Glossary

1Transgenesis: Introducing a gene from one organism into the genome of another organism.

2CRISPR: Acronym for Clustered Regularly Interspaced Short Palindromic Repeats, which is a molecular tool used to create a targeted cut in the DNA of an organism.

3Cas9: Enzyme that cuts DNA complementary to a guide RNA sequence.

4Targeted mutagenesis: Process by which a specific gene is modified in a random manner leading to its inactivation.

5Gene targeting: Process by which a specific gene is modified in a precise way resulting in a new function.

References


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In the 1870s, a new variety of orange revolutionized the citrus industry in California. It reached the Golden State when two homesteaders from the eastern United States, Eliza and Luther Tibbets, brought new trees that they received from a friend in Washington, D.C., to the new colony of Riverside. The fruit on these trees, which had a small belly button-like protrusion that contained the embryo of a small second fruit within, was eventually dubbed the Washington navel orange. These trees came into bearing around January, complementing citrus fruit that Californians harvested in other parts of the year. When Eliza Tibbets exhibited her oranges at local fairs, they won top prizes, leading many Californians to ask for cuttings to bud onto their own trees. Within 20 years, the Washington navel orange had become a predominant crop in California.

The success of Washington navels at citrus fairs from southern California to Chicago convinced consumers nationwide that this fruit tasted better than other varieties of oranges. By 1886, San Bernardino County, of which Riverside was then a part, counted 214,000 orange trees. Los Angeles County boasted 729,000 the same year, which increased to 2,500,000 orange trees by 1910. Riverside County, which broke away from San Bernardino County in 1893, had 1,300,000 orange trees in 1910, which bore $1,200,000 of fruit. The Washington navel orange had truly boomed, becoming one of the leading crops within California’s lucrative agricultural sector.

These black and white images from a bulletin on the citrus industry, published in 1929, show the importance of Washington navel oranges. The cross-section of the orange shows the small second fruit growing within the rind. One of the parent Washington navel orange trees planted by Eliza Tibbets appears in another image. Although it was later replanted, this tree is still preserved in the city of Riverside, almost a century and a half after Tibbets initially planted it. The parent tree also appears on this color image of a citrus crate label from Riverside.

For more information, contact bjenkins@laverne.edu

— Courtesy of Benjamin Jenkins, Ph.D.
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Pounds dry fertilizer that dissolve in 1 gal. at 72 degrees F.

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

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

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