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Citrograph

MAGAZINE

SUMMER 2026

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VARIETIES**

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SUMMER 2026

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On the Cover: This issue of *Citrograph* focuses on the development and evaluation of new citrus varieties that are helping drive the future of the California citrus industry. Supported by funding from the Citrus Research Board, featured research articles explore advances that enhance resistance and tolerance to huanglongbing (HLB), along with exciting innovations adapted to California's unique citrus growing conditions. These efforts and investments reflect the industry's ongoing commitment to sustainability, resilience, and long-term success. We hope you enjoy this issue. Cover photo credit: Paula Soto, Jeff Orton and Ram Uckoo.



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THE MISSION OF THE CITRUS RESEARCH BOARD

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

CITRUS RESEARCH BOARD MEMBER LIST

By District 2025-2026 (Terms Expire September 30)

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Henk Griffin	2026
Jose Lima	2026
Megan Morreale	2026
Nick Theis	2026
Ram Uckoo	2026
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Greg Galloway	2027
Justin Huffmon	2027
David Mabs	2027
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Justin Golding	2028
Zac Green	2028
Aaron Henderson	2028
Jason Reynolds	2028

DISTRICT 2 – SOUTHERN CALIFORNIA – COASTAL

MEMBER	EXPIRES
John C. Gless III, Secretary/Treasurer	2026
Kevin Ball	2026
Tony Atchley	2027

DISTRICT 3 – CALIFORNIA DESERT

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Craig Armstrong	2026

PUBLIC MEMBER

MEMBER	EXPIRES
Melissa Cregan	2027

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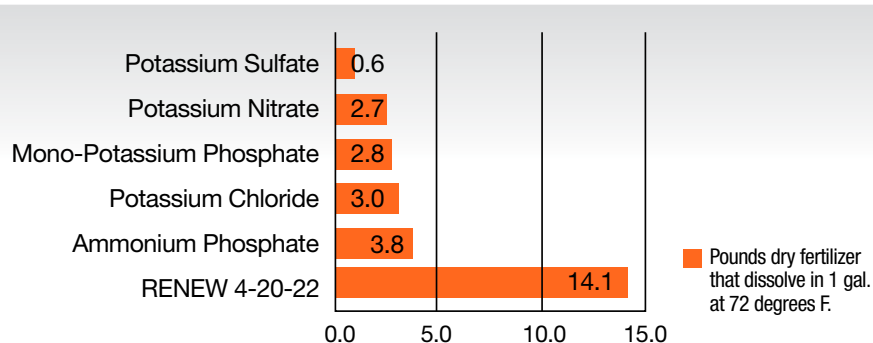
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For more information including research results and scientific publications, contact;
Mark Brady, Western Marketing Manager, Plant Food Systems, Inc. (559) 731-1267





2026

UPCOMING EVENTS

AUGUST 4 **CITRUS RESEARCH BOARD (CRB)** **BOARD MEETING**

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

SEPTEMBER 16 **CITRUS PEST AND DISEASE PREVENTION** **COMMITTEE (CPDPC) MEETING**

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

SEPTEMBER 22 **CITRUS RESEARCH BOARD (CRB)** **ANNUAL BOARD MEETING**

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

OCTOBER 21 **CALIFORNIA CITRUS CONFERENCE**

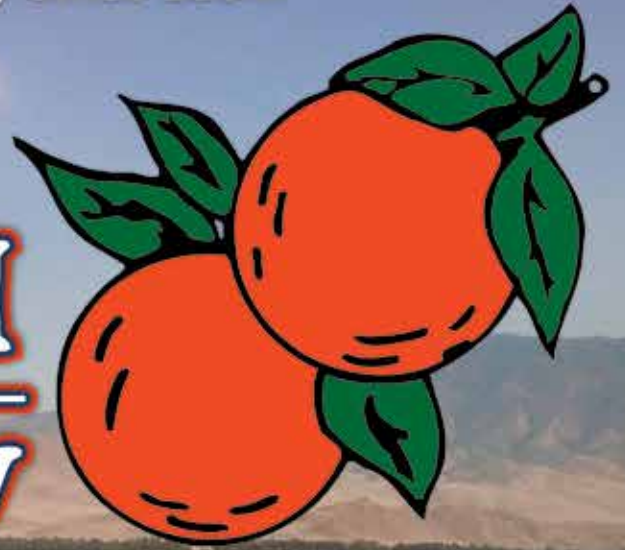
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DECEMBER 9 **CITRUS PEST AND DISEASE PREVENTION** **COMMITTEE (CPDPC) MEETING**

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

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From the PRESIDENT'S DESK

Marcy L. Martin

Welcome to the summer 2026 issue of *Citrograph*, highlighting the ongoing work underway to develop new varieties for our industry. Each of the research projects featured in this issue is designed to expand the options available to California growers by advancing varieties tailored to our state's unique growing conditions and evolving market needs.

As an industry that prides itself on innovation, we work closely with our research partners to develop varieties that will allow California citrus to thrive for generations to come. For many years, the Citrus Research Board (CRB) has partnered with researchers at the University of California, Riverside (UCR) through the Core Breeding Program to advance this work. This program has successfully developed several varieties that are now widely used throughout the California citrus industry.

More recently, CRB has partnered with the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) National Program to expand national citrus breeding efforts into an integrated program with a new location in Parlier, California. While this program has historically focused on varieties for Florida's citrus industry, the California location will allow researchers to develop and evaluate varieties specifically adapted to our environmental conditions. Infrastructure development is currently underway, with trees anticipated to be planted in the coming year.

Throughout the variety development process, researchers account for shifting market preferences, changing growing conditions, and ongoing disease and insect pressures. CRB's New Varieties Research Committee meets multiple times each year to review progress, provide feedback on desired variety traits, and, more recently, to collaborate with the Core Breeding Program to design new grower-site field trials. These trials, conducted in commercial orchards, provide an important opportunity to further evaluate promising selections under real-world production practices. Field trial development is ongoing, with initial grower-site trials expected to begin during the spring of 2027. To learn more about this effort, please read the *Industry Views* article later in this issue on page 24.

Our annual financial report is also included in this issue and serves as one of the primary tools we use to communicate how CRB resources are managed in support of our mission. The report covers the 2024-25 fiscal year and outlines the organization's assets, liabilities, and overall research investment. While informative, this summary does not capture the full scope of all CRB research activities.

Equally important, but not easily reflected on a balance sheet, are the collaborations and partnerships that amplify the impact of our research portfolio. Support from federal and state agencies, universities, and growers extends the value of every research dollar. Likewise, the dedication of staff and researchers is critical to

achieving meaningful outcomes for the industry. We remain committed to improving clarity and transparency in how we communicate our financial position while continuing to tell the broader story behind the numbers.

The strength and long-term prosperity of the California citrus industry remain our top priority. By emphasizing grower involvement and feedback throughout our research efforts, we can ensure that today's investments deliver lasting value. We hope you enjoy the research featured in this issue of *Citrograph*, and we look forward to sharing future updates. 🌍

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



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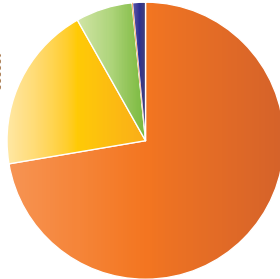
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BY THE NUMBERS: FINANCIAL REPORT

2022/23
ASSETS \$10,818,290
LIABILITIES \$2,658,305
NET ASSETS \$8,219,985

ASSESSMENT RATE: \$0.032



2023 INCOME
\$8,993,563

- 69% ASSESSMENT INCOME:** \$6,248,950
- 17% CPDPP GRANT INCOME:** \$1,498,096
- 12% FEDERAL GRANT INCOME:** \$1,121,355
- 1% INVESTMENT INCOME:** \$65,146
- 1% OTHER INCOME:** \$60,036

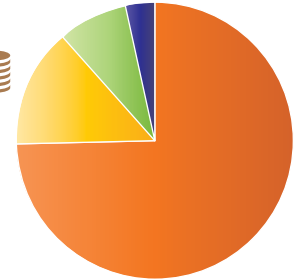
2023 EXPENSES
\$9,242,534

- 77% RESEARCH EXPENSES:** \$7,111,598
- 9% OPERATIONS EXPENSES:** \$846,910
- 2% COMMUNICATIONS EXPENSES:** \$168,812
- 12% ADMINISTRATIVE EXPENSES:** \$1,115,213

2023 INCREASE (DECREASE) IN NET ASSETS
\$248,970 ↓

2023/24
ASSETS \$10,749,134
LIABILITIES \$3,850,703
NET ASSETS \$6,898,431

ASSESSMENT RATE: \$0.032



2024 INCOME
\$8,425,612

- 76% ASSESSMENT INCOME:** \$6,438,050
- 12% CPDPP GRANT INCOME:** \$1,011,038
- 7% FEDERAL GRANT INCOME:** \$571,843
- 1% INVESTMENT INCOME:** \$94,118
- 4% OTHER INCOME:** \$310,563

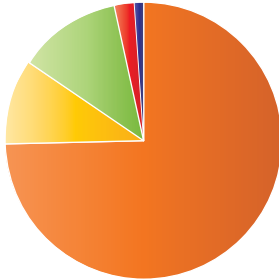
2024 EXPENSES
\$9,747,165

- 75% RESEARCH EXPENSES:** \$7,291,908
- 8% OPERATIONS EXPENSES:** \$800,665
- 4% COMMUNICATIONS EXPENSES:** \$378,517
- 13% ADMINISTRATIVE EXPENSES:** \$1,276,075

2024 INCREASE (DECREASE) IN NET ASSETS
\$1,321,553 ↓

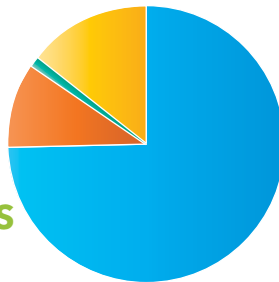
2024/25
ASSETS \$8,855,629
LIABILITIES \$1,953,676
NET ASSETS \$6,901,953

ASSESSMENT RATE: \$0.032



2025 INCOME
\$8,901,512

- 76% ASSESSMENT INCOME: \$6,791,341**
- 9% CPDPP GRANT INCOME: \$846,789**
- 11% FEDERAL GRANT INCOME: \$962,913**
- 3% INVESTMENT INCOME: \$241,238**
- 1% OTHER INCOME: \$59,231**



2025 EXPENSES
\$8,897,990

- 75% RESEARCH EXPENSES: \$6,645,067**
- 10% OPERATIONS EXPENSES: \$868,951**
- 1% COMMUNICATIONS EXPENSES: \$126,045**
- 14% ADMINISTRATIVE EXPENSES: \$1,257,928**

2025 INCREASE (DECREASE) IN NET ASSETS
\$3,522 ↑

These data represent the financial statement of the Citrus Research Board for the years ended September 30, 2023, 2024 and 2025. 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

FY 2024/25
RESEARCH INVESTMENT
\$6.645
MILLION



CITRUS RESEARCH BOARD NOMINATION MEETINGS

Caitlin Stanton

PLEASE NOTE: Information about how the 2026 CRB Board nominations and elections will be conducted will be sent to citrus producers via U.S. mail. Growers may also check the CRB website at citrusresearch.org for updates. We appreciate your participation.

California citrus producers in District 1 (Northern California), District 2 (Southern California - Coastal), and District 3 (California Desert), should make plans to attend the appropriate Citrus Research Board (CRB) nomination meetings. Five positions in District 1, two positions in District 2, and one position in District 3 expire on September 30, 2026. The public nomination meetings will be conducted by officials of the California Department of Food and Agriculture (CDFA) and the CRB.

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 estimating \$10 million. Members are involved in:

- » Developing research priorities and requests for proposals;
- » Prioritizing responses and awarding funds;
- » Devising successful implementation strategies;
- » Assessing progress and
- » Providing critiques of project results.

The 21-member CRB is served by 13 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 four board meetings in various geographic locations, quarterly 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 (except limes) 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: www.cdfa.ca.gov/mkt/mkt/pdf/Laws/CitrusResearchMarketingOrder.pdf. Questions may be directed to CDFA marketing branch economist, Steven Donaldson, at (916) 900-5018 or CRB president, Marcy L. Martin, at (559) 738-0246.

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

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Moving Prevention Efforts Forward in California Citrus

Dahmoon Maesomy

Dahmoon Maesomy pictured with CPDPC's public member, Christopher Greer.

California's citrus industry continues to operate under the persistent pressure of the Asian citrus psyllid (ACP) and huanglongbing (HLB). To meet evolving challenges, the Citrus Pest and Disease Prevention Committee (CPDPC) remains committed to strategies centered on early detection, risk-based response, clear communication, and strong grower involvement. Recent program developments highlight how the state is adapting to new needs while keeping grower support at the forefront.

Strengthening Local Communication Through Grower Liaisons

Following the CPDPC's recommendation at the September 17, 2025, meeting, the CPDPC approved a grower liaison (GL) position for Southern California. The dedicated GL will

serve as a key connector among growers, staff, and local operations, helping ensure rapid communication, timely updates, and coordinated responses during ACP or HLB-related events. This role is designed to directly support growers and navigate regulatory requirements.

Crisis Communication Activation in Imperial County

Imperial County recently experienced its first ACP sample testing positive for *Candidatus Liberibacter asiaticus* (CLas). The insect was collected from a rural residential property outside the city of Imperial; no other citrus or host plants were present on the property. Following protocol, the California Department of Food and Agriculture (CDFA), in coordination with the Imperial County Agricultural Commissioner's office, collected plant samples and the

Imperial County Citrus Pest Control District treated the host tree and coordinated its voluntary removal. No quarantine was established because an HLB quarantine is only triggered by a CLas-positive host plant.

While HLB has not been detected in any plant material in Imperial County, growers and residents are urged to remain vigilant, monitor for pests and disease symptoms, adhere to movement regulations, and report suspicious findings to CDFA's pest hotline at 1-800-491-1899. Both CitrusInsider.org and CaliforniaCitrusThreat.org remain key sources for timely updates.

Committee Membership and Leadership Updates

Tulare County assistant agricultural commissioner, Christopher Greer, has been selected to replace outgoing member Franco Bernardi to serve as the CPDPC's public member.

Additionally, the following new CPDPC officers have been appointed:

- » John C. Gless will serve as chairman.
- » Roger Smith will serve as vice chairman.
- » Kurt Metheny will serve as treasurer/secretary.

The new member and leadership transitions strengthen the committee's governance and support balanced decision-making on issues affecting the citrus industry.

Biocontrol Program Transition

Biological control remains central to long-term ACP management. This year also marks a significant transition with the retirement of Dr. David Morgan, CDFA's primary scientist for biological control. Dr. Morgan began his CDFA career in the mid-1990s as a postdoctoral researcher at University of California, Riverside, having just completed his studies at Imperial College (Master of Science and Ph.D.) in Great Britain. With deep roots in agriculture and decades of experience monitoring invasive pests and developing largescale rearing systems, he played a pivotal role in expanding the use of beneficial insects in California. Under his leadership, more than 35 million beneficial insects were reared, released, and monitored statewide—substantially advancing integrated pest management efforts. His work leaves a lasting legacy that will continue to shape the state's biocontrol strategy.



Secretary Karen Ross and Claudia Vazquez, director of CPDPD

New Director of the CPDPD

Claudia Vazquez was appointed director of the CPDPD at the CDFA by Secretary Karen Ross in September 2025. Over the years, she has served in leadership roles across CDFA's pest detection/emergency projects branch, pest exclusion branch, and citrus pest and disease prevention division. In her previous role, Claudia managed the Comprehensive Pest Prevention Program Analysis (C3PA), a key component of the state's sustainable pest management roadmap. Funded by the California Agricultural Commissioners and Sealers Association (CACASA), the analysis evaluated statewide pest prevention effectiveness and fostered collaboration among academia, industry, and county agricultural commissioners. Claudia holds degrees from University of California, Davis and California State University, Chico. Her agricultural roots run deep. She grew up on her family's cherry farm in Gustine, California, where her family has farmed for more than four decades.

Advancing National Nursery Standards

Statewide regulatory manager, Keith Okasaki, is collaborating with the United States Department of Agriculture (USDA) and the National Plant Board to update the federal citrus nursery stock protocol. The effort aims to modernize the framework with a more risk-based approach to managing nursery detections or structural breaches. This shift seeks to support business continuity while maintaining integrity and confidence in the safeguarding system.

Conclusion

California citrus growers rely on prevention efforts that are responsive, transparent, and grounded in practical solutions. This year's updates—from expanded field communication and rapid response work in Imperial County to leadership transitions and national coordination—strengthen the state's ability to protect citrus health. By maintaining readiness and leveraging collaborative expertise, the program supports the long-term viability of California's citrus industry. 🍊

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



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CALIFORNIA CITRUS CONFERENCE RETURNS TO VISALIA IN OCTOBER



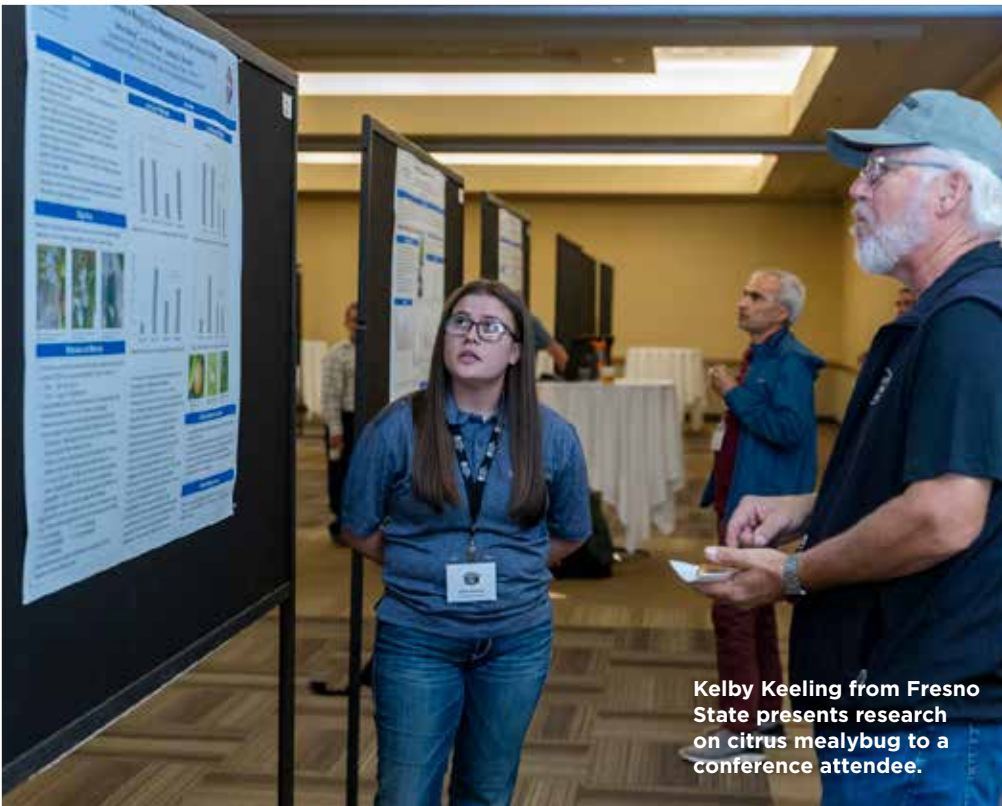
Caitlin Stanton

Dr. James (Jim) Adaskaveg presents post-harvest citrus disease research results at the 2024 California Citrus Conference

The Citrus Research Board (CRB) is excited to announce that the California Citrus Conference will be held October 21, 2026, in Visalia, California, at the Visalia Convention Center. The citrus industry is invited to participate in this in-person only conference which will showcase the best of the best in citrus research. Highlighted topics will include updates on the fight against the Asian citrus psyllid (ACP) and huanglongbing (HLB), integrated pest management (IPM) strategies, production technology, post-harvest food safety, and more. The last California Citrus Conference was held in 2024 and welcomed nearly 350 attendees for a full day of research talks and scientific poster presentations.



The California Citrus Conference brings together growers, packers, and researchers to share the latest citrus industry information and research findings.



Kelby Keeling from Fresno State presents research on citrus mealybug to a conference attendee.

These scientific research posters will be displayed for attendees to look over, and researchers will be available to answer questions. Continuing education units will also be available to conference attendees, pending approval by the California Department of Pesticide Regulation (CDPR) and Certified Crop Advisors (CCA).



The California Citrus Conference provides networking opportunities with fellow industry members as Terry Peltzer (left) and Franco Bernardi (right) engage in conversation.

Registration for the conference will open later this summer. To learn more about the conference, please visit www.citrusresearch.org. Please contact the CRB communications department at events@citrusresearch.org with any questions about the California Citrus Conference.

Caitlin Stanton is the director of communications with the Citrus Research Board and also serves as the editorial assistant on Citrograph. For more information, please contact events@citrusresearch.org

We are thrilled to showcase the latest citrus research and look forward to seeing you all in October. 🍊

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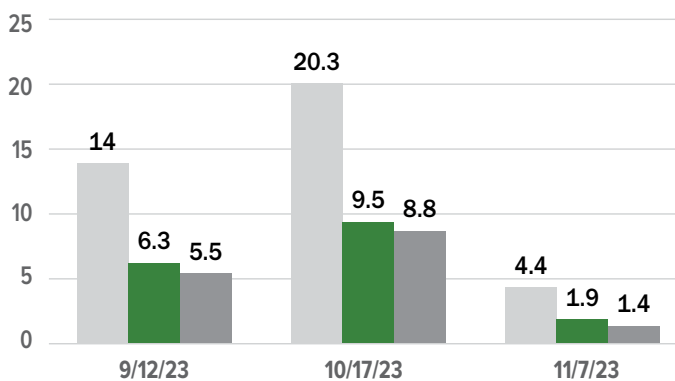


GRAPES

CITRUS – SUNBURN AND YIELD

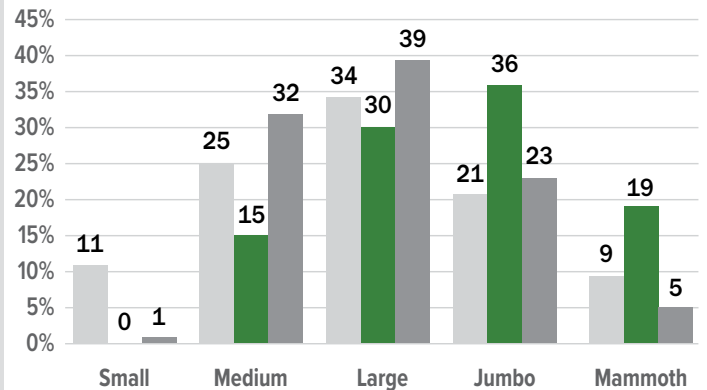
Var. Miho Wase – Sawtooth Ag Research, Farmersville, CA 2023

Count of Sunburn-Damaged Fruit at 3 Evaluation Dates



Untreated Check
 Eckosil Shield
 16 fl oz/A | ABCD
 Surround
 50 lb/A | AC

Yield – % Grade Size



Application Dates

A: 6/14/23 **B:** 6/29/23 **C:** 7/15/23 **D:** 7/28/23



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INDUSTRY VIEWS

Caitlin Stanton

The summer issue of Citrograph is focused on new varieties. As part of our Core Breeding Program with researchers at the University of California, Riverside (UCR), the Citrus Research Board's (CRB) New Varieties Research Committee has initiated enhanced grower field trials. These trials will begin in spring 2027, and several of the committee members have shared their thoughts on its benefit for the industry.

In addition to views on the grower field trials, we have several vectored disease-related opinions to share as well. In early 2026, the growers below shared their feedback on disease challenges in conventional and organic fields.



JOSE LIMA
Citrolima US Citrus Nursery

What is your perspective on the benefits of the grower field trials, including the evaluation of materials CRB has been investing in and our partnership with UC Riverside for these trials?

The development of a new variety from inception to market is a long process, requiring many “boxes” to be checked—maturity window, color, brix, acidity, shape, flavor,

production, and growth habit, among others. Each of these characteristics carries its own level of importance and must meet defined standards. While a new variety may exceed expectations in several key areas, it ultimately must satisfy the needs of the California citrus grower, who is responsible for producing the supply needed to meet market demand.

This is where grower field trials play a critical role. The candidate varieties entering these trials have already been rigorously screened over multiple years by the UC Riverside team and have demonstrated strong potential. By evaluating them under standard commercial practices with California growers, we can validate that potential and better understand the key factors that drive performance.

This process ensures that when a variety is advanced for official release, we have both the data and the confidence to support its likelihood of success in real-world conditions.



JUSTIN BROWN
D Bar J Orchards

What is your perspective on the benefits of the grower field trials, including the evaluation of materials CRB has been investing in and our partnership with UC Riverside for these trials?

CRB and UCR are taking a big step forward in the industry/ researcher relationship with the soon-implemented grower trials of UCR varietal selections that show promise for public release. Why is this important? First off, implementing grower trials furthers the research effort to develop new, improved, different, and/or novel citrus varieties for the benefit of the industry. We now have the opportunity to dial in the always nagging questions growers face: “If I plant this, will it work?”, “Are there any pitfalls to consider with this variety?” and “What can I expect under commercial growing conditions, including its long-term performance?” These and other questions are top of mind when evaluating the next best variety to plant.

The second important aspect of grower trials is engagement of industry with the research and the plant breeding process. Industry members will have better opportunities to provide real-time feedback to the UCR breeding program of what we are observing in the field and what we want to see developed in the future. Researchers are best at informing growers what is possible, while growers are best at informing researchers what is practical. I see a bright future and a strong relationship between the two moving forward.



RAM UCKOO
Wonderful Citrus

What is your perspective on the benefits of the grower field trials, including the evaluation of materials CRB has been investing in and our partnership with UC Riverside for these trials?

Conducting commercial field evaluations of new citrus cultivars developed by the UCR in collaboration with CRB is essential to sustaining the competitiveness and long-term viability of California's citrus industry. Innovation remains

a cornerstone of California industry success, particularly in the face of evolving production challenges, market pressures, and threats such as disease and climate variability. At the same time, shifting consumer preferences and price sensitivity demand cultivars that consistently deliver superior eating quality, visual appeal, and value.

Modern cultivar development is therefore not only focused on yield and productivity, but also on key quality attributes such as flavor, seedlessness, peelability, and shelf life. However, the true performance of these selections can only be validated under real-world commercial conditions. Field trials enable growers to assess adaptability, management requirements, and economic returns, ensuring that promising varieties meet both production and market expectations.

Over the long term, these evaluations serve as a critical feedback loop, helping refine breeding priorities and accelerate innovation. Importantly, they also foster strong collaboration between citrus breeders and industry stakeholders, creating a unified effort to develop and adopt cultivars that will drive the future success of California citrus.

Industry Views – Vectored Diseases



JUSTIN HUFFMON
Bee Sweet Citrus

What were your main disease challenges in 2025, and what do you expect this year? How do you think the industry is handling disease issues that occur during production?

One of our main disease challenges in 2025 was brown rot, which increased due to early rainfall and frequent fog. We also observed clear rot, primarily associated with mechanical damage, harvest wounds, and snail activity. The use of longer-lasting fungicides in the field, along with immediate post-harvest applications of fungicides or sanitizers, has helped reduce overall disease pressure.

With costs rising, it is becoming harder to justify managing pests that do not directly affect production. How can the industry address these needs to minimize the spread of vectored diseases?

Our most significant threat continues to be huanglongbing (HLB), which is spread by the Asian citrus psyllid (ACP) and remains devastating to the citrus industry. Strict adherence to harvest protocols, along with strong industry-wide

cooperation, is critical to slowing its spread. As production costs continue to rise, it is increasingly important that treatments targeting other pests also suppress ACP when possible. Maintaining ACP populations at or near zero is essential to protecting citrus. Encouragingly, ongoing research into ethyl formate for post-harvest disinfection, ant control strategies and self-releasing Tamarixia cages shows promise in sustaining beneficial insect populations.

How should IPM strategies for insects that vector diseases improve to support the industry? What advancements or tools should be prioritized?

Integrated pest management is essential, as no single tool can prevent insect-vector diseases. Until a cure or resistant citrus variety is available, we must use every available strategy to slow the spread of HLB. Improved early detection of ACP and HLB, combined with strong predictive modeling, is critical to targeting resources where they will have the greatest impact.



CHARLOTTE DAVIDSON
Organic Grower

What were your main disease challenges in 2025, and what do you expect this year? What differences have you seen between organic and conventional groves?

Our main challenge is ACP, especially since we are now in a quarantine area. We are fortunate to have a Tamarixia research project going on here on the property.

How do you think the industry is handling disease issues that occur during production?

I think that the industry does the best it can with the resources available. That said, communication with neighboring growers is important, and any time that the industry can facilitate getting growers together is value added.

With costs rising, it is becoming harder to justify managing pests that do not directly affect production. How can the industry address these needs to minimize the spread of vectored diseases?

For pest management, and there seem to be more and more new pests, I have put in swaths of native plants. It is true that our property is small, and we live here, so the native landscaping serves a variety of functions, but I do think that less monoculture and more native plantings might help some. I also release beneficials on a regular basis.

How should IPM strategies for insects that vector diseases improve to support the industry? What advancements or tools should be prioritized?

As an organic grower, I would say that IPM strategies need to include thoughtful and realistic alternatives to spraying, including native plants and the release of beneficial insects. More education is always good.

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

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CRB-FUNDED RESEARCH PROJECTS FOR FISCAL YEAR 2025-26

Meagan Iott and Melinda Klein

On September 23, 2025, the Citrus Research Board (CRB) approved the funding of research projects for the 2025-26 fiscal year at its annual meeting. This year, sixteen projects and four core programs received board support in the amount of \$4,913,396 (**Table 1, 2**). Projects approved address ongoing and emerging needs for the California citrus industry including new variety development, post-harvest diseases, and pest management. Huanglongbing (HLB)-related projects remain a key component of the CRB research portfolio, with research underway to create and evaluate potentially tolerant and resistant citrus varieties and to improve HLB-vector control efforts.

CRB-funded research projects are overseen by CRB Research Committees. These committees include New Varieties, Vected 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.

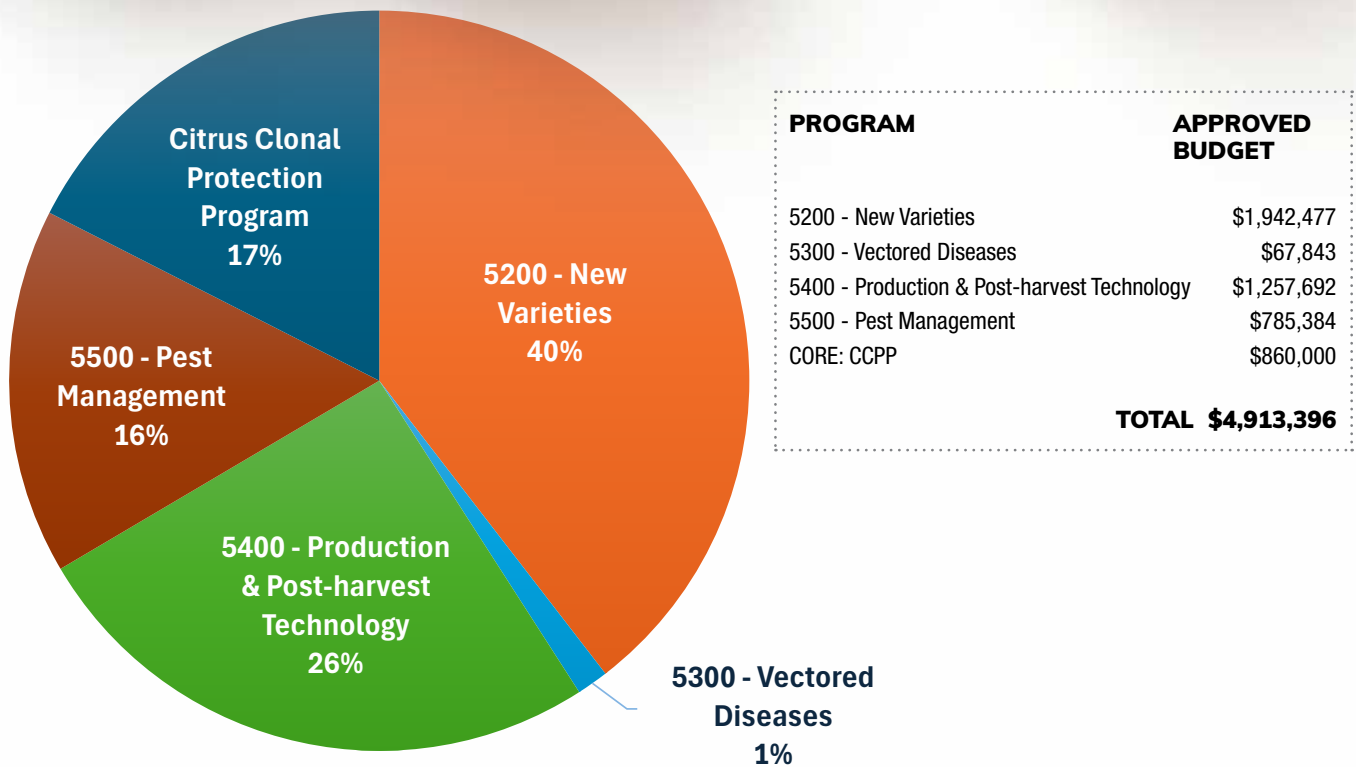
New Varieties Research (5200) Committee

Research projects overseen by this committee focus on providing the California citrus industry with novel, commercially viable varieties that meet market demands. Four research projects and the Integrated Citrus Breeding and Evaluation core program are receiving support this year in the amount of \$1,942,477 (**Table 1, 2**).

Research efforts in this area include work to develop a graft transmissible gene editing system for citrus, with a second project developing HLB resistant or tolerant citrus varieties for the California fresh citrus industry using traditional breeding methods. Citrus variety evaluation efforts continue at the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) San Joaquin Valley Agricultural Center, with the initiation of in field scion and rootstock evaluation trials for USDA-ARS material developed in Florida.



Table 1. Listing of Citrus Research Board-funded research projects for the 2025-26 fiscal year by category.



A research project initiated this year will work to target and modify regions within a citrus susceptibility gene (SWEET 15) to reduce HLB-associated bacterial growth while minimizing negative impacts to the host plant.

The Integrated Citrus Breeding and Evaluation core program is led by Danelle Seymour, Ph.D., at the University of California, Riverside (UCR) along with Tracy Kahn, Ph.D., and Mikeal Roose, Ph.D., at UCR and Glenn Wright, Ph.D., at the University of Arizona. The core breeding program will continue to develop and evaluate scion and rootstock cultivars with HLB tolerance/resistance while incorporating other commercially important traits to improve production efficiency and enhance flavor. The New Varieties Research Committee is working with core program researchers to initiate grower field trials to evaluate experimental varieties at locations using standard commercial production practices. More information on this program can be found in this issue on page 46.

Vectored Diseases Research (5300) Committee

Research projects overseen by this committee focus on detection, eradication, control and management strategies and tools for insect-vectored diseases to minimize crop damage and economic losses. The machine learning model, a two-year project designed to efficiently identify regions in state with the highest probability of HLB, wrapped up validation activities at the end of 2025 using unspent funds from the previous year. Work is currently underway to coordinate use of this tool with the California Department of Food and Agriculture Citrus Program activities to optimize HLB-survey site selection. A second project is supporting statewide HLB operations using data engineering and statistical best practices. These projects bring a combined budget of \$67,843 (Table 1, 2).

Table 2. Listing of all Citrus Research Board-funded research projects for the 2025-26 fiscal year.

Project Title	Principal Investigator	Affiliation	Approved Budget
5200 - NEW VARIETIES			
Breeding to introgress HLB resistance traits in Citrus	Chandrika Ramadugu	UC Riverside	\$140,000
Developing graft-transmissible gene editing in citrus	Vivian Irish	Yale University	\$176,166
Defining effector response regions in SWEET15 to combat disease and maintain citrus quality	Vivian Irish	Yale University	\$145,715
New California citrus cultivars through integrated breeding and evaluation	Danelle Seymour	UC Riverside	\$980,596
Citrus Breeding Research Project at USDA-ARS San Joaquin Valley Agricultural Sciences Center in Parlier, CA	Summaira Riaz	USDA-ARS	\$500,000
5300 - VECTORED DISEASES			
A machine-learning platform to optimize detection of citrus greening disease	Robert Clark	EcoData Technology LLC	\$30,403
Data engineering and statistical best practices support for statewide HLB operations*	Robert Clark	EcoData Technology LLC	\$37,440
5400 - PRODUCTION & POST-HARVEST TECHNOLOGIES			
Control of Lemon Peel Pitting in the Central Valley	Ashraf El-kereamy	UC Riverside	\$122,735
Field efficacy trials of fungicides that will reduce wood rot diseases in citrus	Glenn Wright	Univ. of Arizona	\$109,275
Robotic Citrus Harvesting Solution	Tal Fogelman	Nanovel LTD	\$199,449
Understanding the Infection of Grapefruit by <i>Neoscytalidium dimidiatum</i> causing Citrus Gummosis and Disease Management *	Themis Michailides	UC ANR	\$59,940

Table 2. Listing of all Citrus Research Board-funded research projects for the 2025-26 fiscal year.

Project Title	Principal Investigator	Affiliation	Approved Budget
5400 - PRODUCTION & POST-HARVEST TECHNOLOGIES (CONTINUED)			
Rapid high throughput diagnostic method for <i>Elsinoë australis</i> .*	Madhurababu Kunta	Texas A&M Kingsville	\$92,752
Identifying a citrus-specific surrogate organism to validate peroxyacetic acid use in washing system	Laura Rolon	Cal Poly SLO	\$86,764
Optimizing postharvest ethylene management strategies to improve degreening and storage practices	Mary Lu Arpaia	UC Riverside	\$87,652
Evaluation of efficacy of sodium methylparaben in controlling major postharvest citrus diseases.	Seiya Saito	USDA-ARS	\$40,630
Data Management Support, project 5400-172	Robert Clark	EcoData Technology LLC	\$26,432
Pre- and Postharvest Citrus Disease Management - Core Project	Jim Adaskaveg	UC Riverside	\$432,063
5500 - PEST MANAGEMENT			
California adapted <i>Tamarixia radiata</i> to support ACP biological control **	Raju Pandey	CRB	\$182,649
Novel Treatments for Quarantine Insect Pests	Spencer Walse	USDA-ARS	\$55,000
Pioneering sustainable ACP control in California's citrus orchards using self-release enemy cages	Ivan Milosavljević	CRB	\$177,649
Evaluation of a new trapping system and attractant for snail management in California citrus orchards	Xavier Martini	Univ. of Florida	\$15,511
Core Citrus Integrated Pest Management	Sandipa Gautam	UC Riverside	\$354,575
CITRUS CLONAL PROTECTION PROGRAM			
Citrus Clonal Protection Program (CCPP)	Georgios Vidalakis	UC Riverside	\$860,000
			Total: \$4,916,396

* Off-cycle project initiated in the 2024-25 fiscal year, continuing into the 2025-26 fiscal year.

**Funding provided by the CDFA Citrus Pest & Disease Prevention Program.

Production and Post-harvest Technology Research (5400) Committee

Research projects overseen by 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 are committee priorities as well. Nine research projects, and the Pre- and Post-harvest Citrus Disease Management core program are receiving support this year in the amount of \$1,257,692 (Table 1, 2).

Research efforts underway continue to investigate the cause of lemon pitting in the Central Valley with additional data analysis support. Fungicide efficacy against wood root diseases is being evaluated in the southern desert region. Research projects active this year are also investigating the infection cycle of *Neoscytalidium dimidiatus*, the source of citrus gummosis in Star Ruby grapefruit. Work is underway to develop high throughput PCR protocols to successfully identify *Elsinoë australis*, the causal agent for sweet orange scab, from field samples. Field evaluation continues on a mechanical citrus harvester. The CRB Research Department is also conducting survey activities in coordination with

UC researchers to understand the extent and causes of the premature fruit drop seen in mandarins at the end of 2025.

Packinghouse-related projects are focused on several key areas: identifying a citrus-specific surrogate to validate antimicrobials in post-harvest washing systems; optimizing ethylene management to improve degreening and storage practices; and evaluating the efficacy of sodium methylparaben as an alternative tool for post-harvest disease control in navel oranges.

This research committee also oversees the Pre- and Post-harvest Citrus Disease Management core program, led by Jim Adaskaveg, Ph.D., at UCR, which focuses on management of pre- and post-harvest diseases of citrus caused by fungi, fungal-like organisms and non-fastidious bacteria. This program continues to work closely with the CRB and California Citrus Quality Council to define the deficiencies in the current regulatory protocols used by federal and state agencies to identify *E. australis* during state survey activities.

Pest Management Research (5500) Committee

Research projects overseen by this committee focus on eradication, control or management strategies and tools



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against pests to minimize crop damage and to maintain foreign and domestic market accessibility. Four research projects and the Integrated Pest Management (IPM) core program are receiving support this year in the amount of \$785,384 (**Table 1, 2**). Two projects involve *Tamarixia radiata*, a parasitoid wasp that attacks Asian citrus psyllid (ACP). The first project collects locally derived *T. radiata* populations for use in the state's ACP biocontrol program, and the second project is developing and evaluating the use of self-release cages for in-grove production of *T. radiata* as a potential option for ACP control. A third project continues to develop phytosanitary tools to control various quarantine insect pests. The fourth project initiated this year will evaluate a snail trapping system developed in Florida under California citrus orchard conditions.

The Core Citrus IPM Program is led by Sandipa Gautam, Ph.D., and is based at the Lindcove Research and Extension Center. This core program conducts research applying IPM strategies to manage major citrus pests in California. Citrus mealybug, thrips and California red scale are key pests of study for the current year.

Citrus Clonal Protection Program (CCPP)

The CCPP, led by Georgios Vidalakis, Ph.D., at UCR continues its work providing a mechanism for the safe introduction and distribution of clean citrus varieties. The 2025-26 budget disbursement to CCPP is \$860,000 (**Table 1, 2**).

Summary

The CRB remains committed to prioritizing, investing and promoting research that improves the sustainability and profitability of the California citrus industry. The projects underway support a sustainable California citrus industry by taking a proactive stance on identifying and implementing short, medium and long-term solutions to the threats and concerns of the California citrus environment and markets. 🌱

Meagan Iott is an associate research scientist at 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, where she also serves as scientific editor of Citrograph. For additional information, contact melinda@citrusresearch.org



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Evaluation of Carrizo and its Gene-edited Lines for Resistance to Huanglongbing

Ronald Tapia, Hamza Ashfaq, Fred Gmitter, and Zhanao Deng

Summary

Rootstocks play a critical role in protecting citrus scion cultivars from devastating damage caused by huanglongbing (HLB). Carrizo is one of the most widely used rootstocks in California. Understanding and improving Carrizo's resistance¹ to HLB is needed to face HLB's imminent threat to the California citrus industry. Previously, we created three gene-edited Carrizo lines (i.e., D7, D10, and D12) with different mutations, or genetic changes, in a single disease susceptibility gene, Downy Mildew Resistant 6 (DMR6). In this project, we tested these mutants along with the original Carrizo rootstock for their ability to handle HLB over 27-29 months in a greenhouse setting. Carrizo itself showed strong natural tolerance² to HLB, with low infection levels and steady plant growth. The D7 line grew well, even when laboratory tests showed higher levels of HLB-causing bacteria, indicating strong tolerance. The D12 line behaved differently depending on the rootstock used. Plants grafted on Kuharske citrange showed relatively higher infection, while plants grafted on sour orange showed no detectable infection for the entire study, suggesting the D12 line could work as a dwarfing rootstock or interstock³ and might block bacterial movement. Compared to our other work showing more consistent improved HLB tolerance in DMR6-edited 'Duncan' grapefruit, Carrizo lines showed more variable outcomes from DMR6 editing, suggesting strong genetic background effects on the impact of gene editing in citrus. Further trials are needed to validate the performance of these gene-edited lines under natural endemic HLB pressure.

Combating HLB with Rootstock Resistance

Huanglongbing (HLB) is posing an imminent threat to the California citrus industry. The HLB-associated bacterium, *Candidatus Liberibacter asiaticus* (CLAs), infects and destroys both the scion and rootstock parts of citrus trees. Soon after Asian citrus psyllid (ACP) transmits CLAs into citrus trees, the bacteria move to the roots and multiplies within root tissues. CLAs can cause severe root damage even before it disrupts the scion phloem and incites disease symptoms on citrus leaves and stems. Commercial citrus trees also need HLB resistance or tolerance in their rootstock to survive HLB and stay productive.

The *DMR6* disease susceptibility gene plays a critical role in citrus susceptibility to HLB. This gene encodes an enzyme that converts the plant defense hormone, salicylic acid (SA), into a chemical that cannot activate plant defenses. Editing *DMR6* and disrupting its function has resulted in disease resistance in tomato (Thomzella et al. 2021) and several other horticultural crops. Carrizo citrange is one of the most widely planted and economically important citrus rootstocks in California. Understanding how Carrizo responds to HLB and improving its resistance to HLB are essential for continued use of Carrizo and for developing new generations of rootstocks to mitigate HLB's damage.

Our lab was the first group to successfully edit the *DMR6* gene in citrus, and we produced three gene-edited Carrizo lines (D7, D10, and D12). These mutant lines showed strong resistance to another bacterial disease, citrus canker (Parajuli et al. 2022). The objectives of this project were to determine the resistance of these lines to HLB after two inoculation methods, bud-grafting and ACP feeding, which mimic nursery and field transmission of HLB bacteria, respectively.

Procedures

Gene-edited lines and Carrizo were propagated by grafting buds on HLB-tolerant Kuharske citrange and HLB-susceptible sour orange rootstock. This grafting step, rather than using rooted cuttings, was included to avoid potential influence of these lines' differences in rooting capability on plant growth and/or response to CLAs inoculation. When propagated plants reached a sufficient stem size, the rootstocks were inoculated with CLAs by grafting two infected buds from 'LB8-9' Sugar Belle® (hereafter SB) or rough lemon onto each plant. This was done to allow for a relatively uniform level of CLAs bacteria to infect Carrizo and the mutants to be evaluated. Prior to the inoculation, the SB and rough lemon plants were tested to confirm they carried high levels of CLAs. Throughout this study, the inoculum source plants maintained a Ct value⁴ between 21 and 30, indicating continuous presence of high levels of CLAs in their leaf tissues. For each gene-edited line, eight to 12 plants were

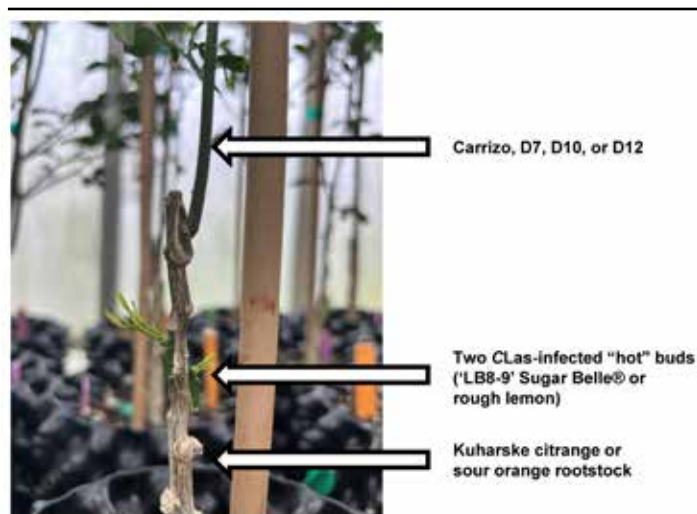


Figure 1. Inoculation of Carrizo and gene-edited lines by grafting two CLAs-infected buds onto each clonally propagated plant (Photo credit: R. Tapia).



Figure 2. Re-inoculation of Carrizo and gene-edited lines by exposing tender shoots to CLAs-infected Asian citrus psyllids in bags (Photo credit: R. Tapia).

first inoculated by bud grafting. Due to insufficient number of buds available from the source plants, bud grafting on gene-edited lines with Kuharske was completed in April 2023, while bud grafting on gene-edited lines with sour orange was completed in June 2023 (**Figure 1**). They were then re-inoculated by exposing the tender shoots of each plant to 20 infected ACPs (10 male and 10 female) in December 2024 (**Figure 2**). Additionally, at the same timepoints, two plants per line were grafted with two "clean" non-infected buds and exposed to "clean" non-infected ACPs as controls. All plants were grown in a temperature-controlled greenhouse with double doors at the University of Florida (UF)/Institute of Food and Agricultural Sciences' (IFAS) Citrus Research and Education Center in Lake Alfred, Florida.

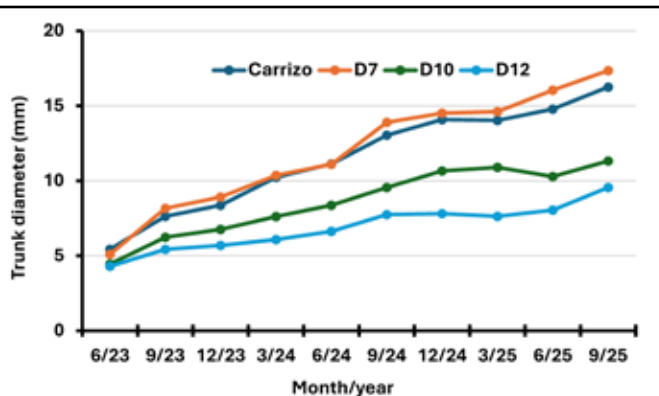


Figure 3. Average trunk diameter (mm) of Carrizo and gene-edited lines grafted on Kuharske after CLAs inoculation and re-inoculation.

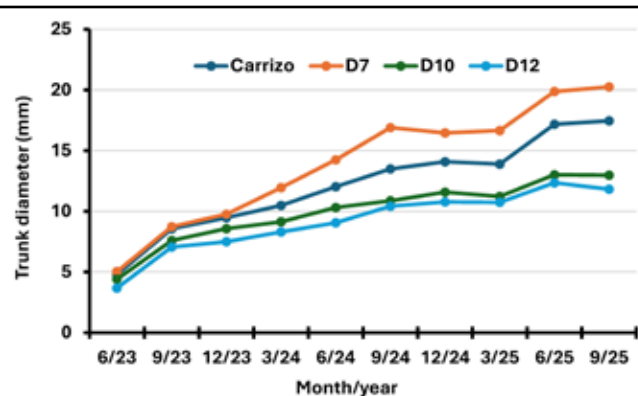


Figure 4. Average trunk diameter (mm) of Carrizo and gene-edited lines grafted on sour orange after CLAs inoculation and re-inoculation.

Four parameters, including trunk diameter, plant height, foliar symptom, and CLAs titer⁵, were used to assess HLB resistance. CLAs titers were determined using quantitative real-time polymerase chain reaction (qRT-PCR)⁶. Plant evaluations and lab tests were started in April 2023 and repeated every three months over 29 months for those plants on Kuharske and 27 months for those plants on sour orange. This was done for a total of six times after bud graft inoculation (late June 2023 through early October 2024) and four times after ACP re-inoculation (late December 2024 through September 2025). When trees reached the top of the greenhouse, they were cut back twice during the evaluation. This was unplanned and likely confounded height comparisons, reducing the plant height differences among mutants. Thus, plant height data were excluded in this report.

At the last evaluation (September 2025), leaves were sampled from each mutant and from Carrizo to confirm the type and frequency of DNA sequence changes at the targeted site in the *DMR6* gene and to determine the content of plant defense hormone SA after the *DMR6* gene editing.

Carrizo Plant Growth and HLB Progression

Carrizo is widely used in California’s citrus groves as well as in research laboratories for testing gene editing strategies for HLB resistance. We closely monitored its responses to inoculation with CLAs. After CLAs inoculation and re-inoculation, wild-type Carrizo plants grew well in the greenhouse. Their trunk size increased by approximately 3 to 3.6 times between June 2023 and September 2025 (Figure 3 and 4). Typical HLB symptoms (blotchy mottled leaves) did not appear on these Carrizo plants. However, 20 to 40 percent of the inoculated Carrizo plants showed chlorotic and/or small leaves beginning in March 2024. In subsequent evaluations, 40 to 100 percent of plants exhibited these types of leaves. Nonetheless, most plants with such leaves remained negative for CLAs.

None of the Carrizo plants tested positive until 12-14 months after the initial inoculation. Over the 27- to 29-month evaluation, 40 to 60 percent of Carrizo plants tested positive once (with Ct values equal to or below 34), but none were positive more than once. In the last evaluation, 20% of Carrizo plants on Kuharske and sour orange rootstock tested positive.

Plant Growth of Gene-edited Lines and their Responses to CLAs Inoculation

Trunk diameter: The D7 line plants were similar in size with wild type Carrizo, while the D10 and D12 line had smaller trunk diameters (Figure 3 and Figure 4).

Leaf symptoms: Blotchy mottled leaves were not observed on any gene-edited plants nor on wild-type Carrizo. Similar to Carrizo, the D7, D10, and D12 plants on Kuharske began to show chlorotic and/or small leaves in March 2024. In subsequent evaluations, the percentage of plants with these symptoms ranged from 25 to 100. The appearance of small leaves on D7 grafted on sour orange was delayed by about nine months compared to wild-type Carrizo on sour orange.

CLAs titer: Compared to Carrizo, a higher percentage of D7 samples (18.4% on Kuharske and 16.7% on sour orange) tested positive for CLAs. D7 positive leaf samples had lower Ct values (21.78 on Kuharske and 21.72 on sour orange), indicating higher CLAs titers in D7 plants. In the last evaluation, 50% of D7 plants on Kuharske and 40% of plants on sour orange tested positive for CLAs. One D7 plant had very low Ct values (15.21 – 16.40) over nine months and then became negative and grew well. Another D7 plant also grew well, even though it showed low Ct values (18.70 – 18.75).

D10 plants tended to exhibit higher percentages of positive leaf samples (21.9% on Kuharske and 25.7% on sour orange)

when compared to wild-type Carrizo (8.6% on Kuharske and 5.7% on sour orange). Positive D10 samples showed an average Ct value of 33.10 (on Kuharske) or 23.85 (on sour orange). In the last evaluation, 40% of D10 plants on Kuharske and 20% of plants on sour orange tested positive for CLas.

D12 plants on Kuharske and sour orange behaved completely differently. D12 plants on Kuharske showed a higher percentage of positive leaf samples (26.1%) and a low Ct value (21.60), indicating higher CLas titers in D12 plants on Kuharske. Surprisingly, in contrast, none of the D12 plants on sour orange had any positive leaf samples over the whole 27-month evaluation. In the last evaluation, 33.3% of D12 plants on Kuharske and zero percent of plants on sour orange tested positive for CLas.

Impact of Gene Editing on Plant Defense Hormone Content

The D7, D10, and D12 lines are chimeras⁷ and carry different percentages of both the mutated *DMR6* gene and the wild type *DMR6* gene. Due to the unstable nature of chimeras, the portion of mutated *DMR6* gene within a plant could change over time. To identify how much was present at the end of the experimental trial, four plants per line were sampled at the end of the study in September 2025 to confirm the changes gene editing introduced to the *DMR6*. DNA sequencing confirmed that the D7 plants sampled had the lowest frequency of edited sequences (approximately 10 percent), while the D12 plants sampled had the highest frequency of edited sequences (close to 75 percent) (Table 1). The frequency of edited sequences in the D10 plants sampled was close to 34 percent (Table 1).

Wild-type Carrizo and D7 plants shared similar SA contents, while D10 and D12 mutants contained several times more SA. After CLas inoculation, wild-type Carrizo and D7 plants exhibited a reduction in SA content. In contrast, both D10 and D12 displayed increases in SA content.

Conclusions and Discussion

In this study, inoculated wild-type Carrizo plants grew well in containers in the greenhouse, although 40 to 80 percent of them had chlorotic and/or small leaves beginning in March 2024. These plants exhibited relatively high Ct values (27.76 to 32.87) and contained only low to medium CLas titers. Overall, wild-type Carrizo showed strong tolerance or partial resistance to CLas.

D7 plants grew as well as wild-type Carrizo for plant growth and trunk diameter. Positive D7 plants remained vigorous, resembling HLB-tolerant cultivars such as 'LB8-9' Sugar Belle®. Some of the positive D7 plants turned negative. These data suggest that D7 possesses strong tolerance to CLas.

D12 on sour orange remained negative for CLas over the full 27-29-month period. D12 also carried the highest frequency of genetic changes, dominated by high impact deletions and accumulated high amounts of SA. These results suggest the possibility that high-edited D12 could function as an effective dwarfing rootstock or interstock to suppress or limit systemic transmission of CLas bacteria.

These findings can benefit the California citrus industry in a number of ways. These data provide the first biological resistance/tolerance dataset for wild-type Carrizo and *DMR6*-edited lines inoculated through graft and ACP inoculations, offering an improved benchmark for HLB resistance evaluation. In responding to CLas inoculation, D7 retained its vigor, grew well, and tolerated relatively high CLas titers, while D12 strongly suppressed CLas bacteria on HLB-susceptible sour orange, offering valuable materials for further testing in the field.

Overall, data collected from wild-type Carrizo and gene-edited lines showed variable response to CLas inoculation. The inconsistency of disease progression in these plants remains to be understood and will need further investigation. The natural tolerance of Carrizo might have impacted the results of the current study. In other experiments involving 'Duncan' grapefruit, *DMR6*-edited lines showed significant resistance/

Table 1. Types and frequencies of genetic changes for each test mutant introduced by gene editing in the *DMR6* gene.

GENETIC CHANGES AND FREQUENCIES (AVERAGED OVER FOUR CLONAL PLANTS)		
	At the first site targeted for gene editing	At the second site targeted for gene editing
D7	Deletion of one base (10.40%), Deletion of 39 bases (2.50%)	Insertion of 3 bases (10.10%)
D10	Deletion of 69 bases (33.85%), Deletion of 3 bases or insertion of two bases (5.73%)	N/A
D12	Deletion of four bases (64.47%), insertion of one base (7.87%)	Deletion of 7 bases (64.70%)

tolerance to CLas. We suspect that genetic background has affected the outcome of *DMR6* editing. It appears that this gene editing strategy may work more effectively in HLB-susceptible types of citrus such as grapefruit. To address these questions, field evaluations of these edited lines and additional new lines with different genetic backgrounds (e.g. sweet orange) are warranted. Such evaluations are especially critical considering the long production life of citrus rootstock and scion cultivars. 🍊

CRB Research Project #5200-177

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Glossary

¹Resistance: Trees can prevent or limit CLas infection and damage.

²Tolerance: Trees stay healthy and productive even if the CLas is present.

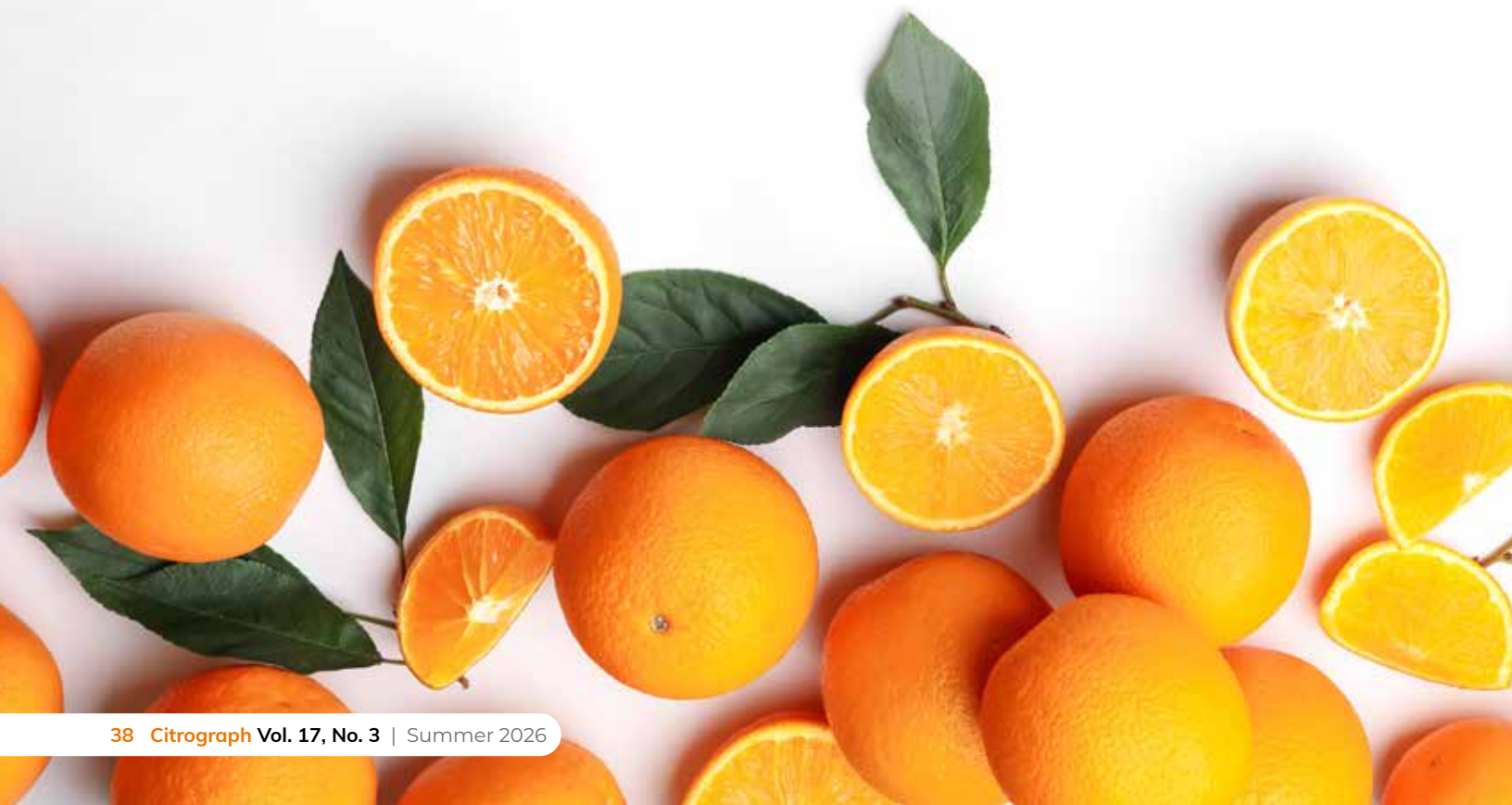
³Interstock: A short piece of stem wood placed between rootstock and scion to add benefits.

⁴Ct value: Abbreviation for cycle threshold in qRT-PCR. Lower numbers mean more bacteria detected; higher numbers mean less bacteria detected. Generally, when a citrus leaf sample has a Ct value equal to or smaller than 34, the sample is considered positive for CLas bacteria.

⁵CLas titer: A relative measure of the amount of *Candidatus Liberibacter asiaticus* bacteria in a sample.

⁶qRT-PCR: A highly specific laboratory test used to determine the relative abundance of CLas bacterial cells in citrus leaf samples.

⁷Chimera: A plant that is a mixture of two or more genetically different types of cells.





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Use of Genomic Tools in Breeding Citrus Varieties with Huanglongbing Resistance

Chandrika Ramadugu and Mikeal Roose

Project Summary

*Citrus breeding is a time-consuming process due to juvenility and long generation times. In our project, we are breeding citrus to develop resistance to huanglongbing (HLB) by crossing with *Microcitrus* species. The goal is to select hybrids from the breeding program that may be useful as novel varieties with HLB resistance traits and edible fruit quality. We use genomic tools developed by our team and other researchers to screen the breeding population to verify parentage, identify and discard nucellar seedlings, and pre-select hybrids with putative resistance. Genotyping is used as a basic tool for screening hybrids generated in the breeding program. We source information from the Affymetrix citrus chip, the citrus genome database, genomic sequences of resistant and susceptible varieties used as parents in breeding, and the presence of genomic fragments known to have resistance-associated genes.*

Introduction

Citrus is cultivated in tropical and subtropical climates (40°N to 40°S), where environmental conditions favor disease development. Cultivation of most crops typically involves a few varieties selected for their superior horticultural qualities that consumers prefer. While this is convenient for agricultural management, the uniformity of the cultivated variety makes it vulnerable to new diseases. Huanglongbing (HLB) has caused substantial financial damage to citrus industries in many regions, including Florida and Brazil. In Southern California, the California Department of Food and Agriculture (CDFA) has reported removing 10,550 HLB-positive residential trees (CDFA 2026). However, the pathogen may be present in adjacent trees that appear non-symptomatic. In our view, the development of genetic resistance is vital to disease management once the prolific insect vector that spreads HLB has been established, and the disease is present in the area. Wild crop relatives and landraces¹ are often useful for introducing desirable traits, such as disease resistance, into cultivated crops. In this project, we have utilized *Microcitrus* and *Eremocitrus* species to incorporate HLB resistance traits into cultivated citrus. In the first generation (F1), many novel hybrids are disease-resistant, proving that we can develop HLB-resistant citrus. Using *Microcitrus* and *Eremocitrus* as breeding parents increases the genetic diversity of the citrus types resulting from our breeding and may be helpful when new plant diseases emerge. Many genes determine the HLB resistance trait and are therefore complicated to study, quantify, and incorporate into citrus through biotechnological methods.

Citrus breeding in general is slow due to juvenility, with certain *Eremocitrus* and *Microcitrus* hybrids showing an extended juvenility period. Confirming HLB resistance in new

hybrids is difficult, but identifying hybrids with field resistance is essential, given the decades-long production window expected for citrus groves. To assist in breeding activities, several molecular tools can be used to analyze breeding populations to identify hybrids, discard nucellar seedlings that are clones of the female parent, correlate resistance traits with specific genomic fragments, and preselect putatively valuable hybrid progeny. We routinely use various genomic tools to identify seemingly useful hybrids. Brief descriptions of some of the tools used in our project and how they assist in our breeding efforts are included here.

Genotyping of Hybrid Populations

All citrus and related genera (including *Eremocitrus* and *Microcitrus*) have nine pairs of chromosomes. The length of the individual chromosomes varies from about 21 million to 59 million bases. Most of the DNA is identical across diverse citrus species. However, some changes in the DNA sequence may cause the observed differences in horticultural traits. These include sequence changes where just one 'letter' or nucleotide in the citrus DNA code is changed, referred to as single-nucleotide polymorphisms² (SNPs). Loss of a few nucleotides in a sequence or loss of larger regions of the chromosome constitutes a 'deletion.' Inverted stretches of sequences are 'inversions,' and the presence of a stretch of nucleotides in a different region of the genome (compared to the normal location) is referred to as a 'translocation.' These sequence alterations can explain many of the physical or functional differences observed among various citrus types. Sometimes, obvious physical differences are not apparent, but by analyzing sequence differences, breeders can better understand the underlying genetic variation and select hybrids with genetic traits of potential value.

Hybrid no.	Seedling no.	Seed Prnt	Pollen Prnt	Generation	Sequence
15001	1	ML_6592_06	AD2	AX_160441010	AATTTAAACCCCGGAAGGTTGGTTCGGGAACCAAACTTACTTTTAAAAGGGGACGGCCGGAAACCCCGGAACCCCTAATTT
15002	2	ML_6592_06	AD2	RC102_KASP_LGC	CAATCAACCCCTGGAAGGTTGGTTCGGGACAGAACTAATTTGAAAAGGGGACGGCCGGAAACCCCGGAACCCCTAATTT
15003	3	ML_6592_06	AD2	RC104_KASP_LGC	CAATTAACCCCGGAAGGTTGGTTCGGGAACCAAACTTACTTTTAAAAGGGGACGGCCGGAAACCCCGGAACCCCTAATTT
15009	4	ML_6592_06	AD2	RC106_KASP_LGC	CAATCAAGCCCGGAAGGTTGGTTCGGGACCAAACTTACTTTTAAAAGGGGACGGCTGGAACCCCGGAACCCCTGACTAATTT
15103	4	ML_6592_06	AD2	RC108_KASP_LGC	AATTTAAACCCCGGAAGGTTGGTTCGGGAACCAAACTTACTTTTAAAAGGGGACGGCCGGAAACCCCGGAACCCCTAATTT
15118	7	ML_6592_06	AD2	RC110_KASP_LGC	AATTTAGCCCTGGAAGGTTGGTTCGGGAACCAAACTTACTTTTAAAAGGGGACGACTGGAACCCCGGAACCCCTAATTT
				RC111_KASP_LGC	
				RC112_KASP_LGC	
				AX_160100969	
				RC_126_KASP_LGC	
				RC_127_KASP_LGC	
				RC_128_KASP_LGC	
				RC_129_KASP_LGC	
				RC_130_KASP_LGC	
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				RC_155_KASP_LGC	
				RC_156_KASP_LGC	
				RC_158_KASP_LGC	
				RC_159_KASP_LGC	
				RC_160_KASP_LGC	
				RC_152_KASP_LGC	

Figure 2. Identification of nucellar seedlings in the population. Of the six hybrids shown here, two were nucellar and identical to the maternal parent. The hybrids confirmed through genotyping are highlighted in green.

advanced hybrids (second generation and beyond), molecular markers are required to confirm parentage. Some citrus varieties have nucellar embryony³ and produce progeny identical to the seed parent. We identify nucellar seedlings through genotyping and discard them. We utilize KASP genotyping in both cases, to confirm pollen parents or to identify and discard nucellar progeny and focus our evaluation efforts on novel hybrids of interest.

Another role of molecular tools is to select hybrids with traits of interest. Since there are no defined markers associated with HLB resistance, preselecting the breeding population for further evaluation is difficult. With genomics tools, it is possible to use molecular data to pre-select putatively resistant types. In a study conducted by our team to identify resistance-associated (R) genes in the genomes of the Australian lime parents used in our project, we identified over 600 R genes.

Figure 3 shows the locations of some R genes in the genome of *Microcitrus australasica* (Finger lime). In the nine chromosomes of the *Microcitrus* parent, we have identified eight clusters of resistance-associated R-genes in chromosome one. **Figure 3** shows seven, 18, seven, six, zero, 11, 13, and four sets of R-genes in chromosomes 2 to 9, respectively (Liu et al. 2025). It is not clear which of these R genes confers resistance. By designing genotyping markers at strategic genomic locations, we can identify large chromosome fragments of the parental genomes that may be associated with disease resistance. Confirmation of our data will be possible once many hybrids with HLB disease phenotyping are available and a positive correlation between resistance and the presence of specific R genes is documented.

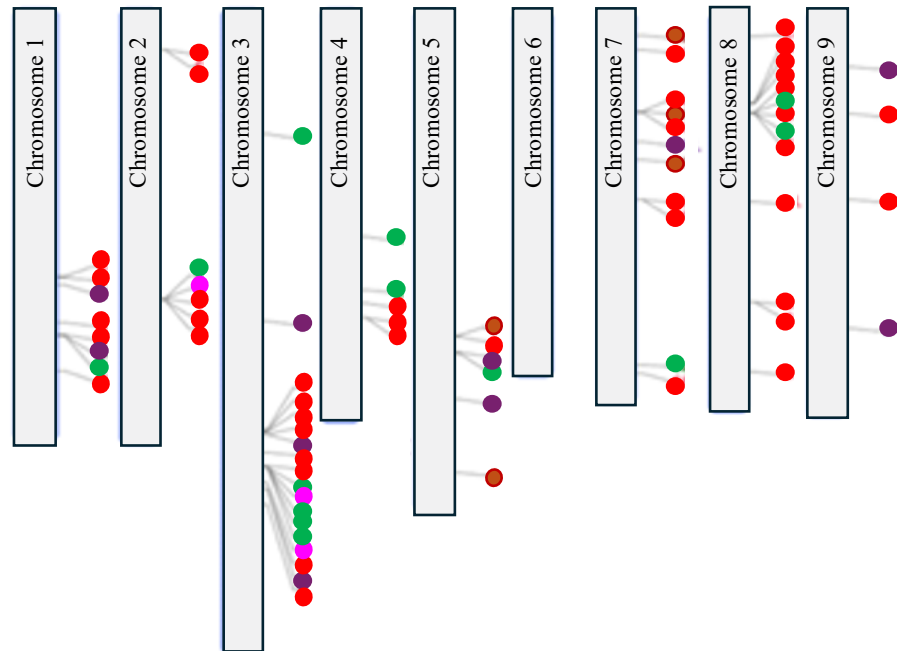


Figure 3. The *Microcitrus* genome sequence was analyzed using bioinformatic programs to identify resistance-associated R genes on each chromosome. The dots represent locations on the chromosomes where R genes were identified. The colors indicate distinct types of R genes. Information from Liu et al. 2025.

Conclusion

The development of molecular markers to accelerate hybrid breeding and selection is essential for improving tree crops such as citrus. It enables us to analyze and screen a larger population of hybrids for desired traits, which drastically reduces the breeding timeline. It also enables a more targeted approach in identifying genes that could be associated with HLB tolerance or resistance. In our projects, we have determined the complete genomic sequences of the breeding parents and certain selected hybrids. Identification of candidate resistance-associated genes will help elucidate the mechanisms of resistance in the various *Microcitrus* parents used in the study and may lead to the development of technologies that will hasten the process. 🌱

Funding Acknowledgment

Sequencing of breeding parents, identification of resistance-associated genes (through bioinformatic analysis), pattern of introgression⁴, and other molecular analysis through short-read sequencing were made possible through funding from USDA NIFA projects #2019-70016-29068; 2020-70029-33201; and 2023-70029-41315.

Glossary:

¹ **Landrace:** A local plant variety that is adapted to a region and typically has greater genetic diversity compared to a cultivar.

² **Single-nucleotide polymorphisms:** Genetic variation at a single nucleotide in the DNA of an organism.

³ **Nucellar embryony:** A form of asexual seed reproduction where embryos develop only from maternal seed tissue rather than through fertilization.

⁴ **Introgression:** The process of introducing genetic material from a wild species into a cultivated plant through a series of back crosses.

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Evaluating the Potential of New Huanglongbing-tolerant ROOTSTOCKS FOR USE IN CALIFORNIA

Danelle K. Seymour, Philippe Rolshausen, M. Imran Hamid,
Paulina Quijia-Lamina, German Villalba-Salazar,
Emmanuel Avila de Dios, Christopher Wallis,
Georgios Vidalakis, and Kim D. Bowman

Photo by Philippe Rolshausen.

Project Summary

Our team is evaluating new huanglongbing (HLB)-tolerant rootstocks developed by the United States Department of Agriculture (USDA) Citrus Rootstock Breeding Program in Florida for their potential use in California. Newly released HLB-tolerant rootstocks, and the most promising of those not yet released, have been submitted to the Citrus Clonal Protection Program and are being propagated for research purposes. Evaluation of HLB-tolerant rootstocks for their response to stresses and pathogens relevant to California is ongoing. Our goal is to provide California growers with access to new HLB-tolerant rootstocks along with evidence-based guidance for their use in California.

Research Overview

Huanglongbing (HLB) continues to spread in Southern California, predominantly in dooryard citrus trees. Through diligent surveillance of the pathogen and its insect vector, major citrus production regions in California have been protected from this disease for more than a decade. Despite this success, commercial citrus production regions are still at risk. Our team, including scientists based in California, Texas, and Florida, is working to develop new rootstock cultivars with tolerance to HLB and evaluate their commercial potential in

regions affected by or at risk of HLB disease. This collaborative effort is supported by federal funds from the Emergency Citrus Disease Research and Extension program. This article will discuss three aspects of our research that are relevant to California citrus growers: the development of new HLB-tolerant cultivars in Florida where HLB disease pressure is high, a new regulatory pathway to introduce germplasm into California for research purposes, and focused evaluation of new rootstocks for their response to soilborne pathogens and other stresses.



Figure 1. Twelve-year-old trees of Valencia sweet orange on Swingle rootstock (left), and US SuperSour 5 rootstock (right) in a Florida field trial before harvest in March 2026.

Developing New HLB-tolerant Rootstocks

The HLB disease epidemic that began in Florida in 2005 sparked extensive efforts to develop new citrus rootstock cultivars that were more tolerant of HLB and other diseases, while supporting a tree that produced large quantities of high-quality fruit. The USDA Citrus Rootstock Breeding Program in Florida, led by Dr. Kim Bowman, had previously begun an effort to develop a superior new rootstock with the good attributes of sour orange rootstock, but without the Achilles heel of citrus tristeza virus (CTV) sensitivity. This effort, termed the 'SuperSour Project,' was re-tooled to include increased tolerance or resistance to HLB, along with other critical citrus rootstock traits (Bowman et al. 2021). At present, about 350 SuperSour hybrid rootstocks are included in 20 replicated field trials in Florida with sweet orange scion, and they are being evaluated seasonally for tree health, tree size, fruit productivity, and fruit quality. So far, five rootstocks have been released from the effort (US SuperSour 1, 2, 3, 4, and 5) based on superior yield and other field performance metrics, compared to Swingle and sour orange rootstocks, in multiyear Florida trials severely affected by HLB (Bowman 2023) (**Figure 1**). Some of the new USDA rootstocks also appeared to possess other desirable attributes, such as good tolerance or resistance to CTV, phytophthora disease, citrus nematodes, high pH soil, and salinity, so it is anticipated that they may also prove to be superior rootstocks in citrus production regions that are not affected by HLB.

A New Regulatory Pathway for the Introduction of HLB-tolerant Rootstocks

To address the urgent need for solutions to HLB disease, the Citrus Clonal Protection Program (CCPP) has implemented a newly amended regulatory protocol designed to drastically shorten the timeline for introducing citrus germplasm into California for research purposes. Historically, introducing varieties from outside California required a lengthy process of therapy and testing. Under the new "Research-Only-Permit" track, the CCPP can now accept germplasm that has already undergone therapy at the National Clean Plant Network (NCPN) Center in Florida. Upon arrival in Riverside, these materials undergo rigorous laboratory testing for all known graft-transmissible pathogens and are propagated *in vitro* for a mandatory observation period, but they do not need to repeat the time-consuming therapy step. This streamlined process reduces the introduction timeline to approximately 12 months, facilitating faster establishment of greenhouse and field trials while maintaining strict phytosanitary security. Materials introduced via this pathway are restricted to research use until they undergo the full Variety Index (VI) required for commercial release.

Table 1. Citrus Clonal Protection Program status of budwood sources with variety index (VI) not restricted to research use.

IPPN	NAME OF VARIETY	STATUS	VI
1708	US SuperSour 1	Variety Index	1708
1709	US SuperSour 2	Variety Index	1709
1710	US SuperSour 3	Therapy	N/A
1705	US SuperSour 4	Variety Index	1705
1694	US SuperSour 5	Variety Index	1694
674	US-802	Released From Quarantine	927
767	US-812	Released From Quarantine	965
673	US-897	Released From Quarantine	985
1554	US-942 (VI928-ReIndex)	Released From Quarantine	1554
604	US-1283	Released From Quarantine	916

Leveraging this expedited pathway, the CCPP has successfully introduced and is actively propagating a wide range of US SuperSour rootstock hybrids. As of late 2025, a micropropagation protocol to produce tissue culture cuttings for their acclimatization and rooting in the greenhouse was successfully established. Multiple key accessions, including the US SuperSour series (1, 3, 4, and 5), have been propagated using this method. Significant progress has also been made with other promising unreleased hybrids. These varieties are now moving into the greenhouse phase to generate plants for both greenhouse testing and field trials planned for California. In addition to the “Research-Only-Permit” track, all US SuperSour rootstocks currently available for commercial use in Florida were submitted for full VI in 2023 and 2024, and clean plant material will be available for commercial use in California when VI is completed (Table 1).

Evaluation of New HLB-tolerant Rootstocks in Response to Soilborne Pathogens and Other Stresses

Selecting rootstocks with tolerance or resistance to multiple pathogens and stresses is instrumental to long-term industry success. One important aspect of our project is to evaluate rootstocks released by the USDA Citrus Rootstock Breeding Program, including the US SuperSour series as well as earlier releases, like the popular HLB-tolerant US-942 rootstock, for their response to soilborne diseases. Those include citrus slow decline caused by the nematode *Tylenchulus semipenetrans*, dry root rot caused by *Fusarium solani*, and Phytophthora root rot caused by *Phytophthora citrophthora* and *P. nicotianae*. Results showed that three rootstocks, including two of the most popular Florida rootstocks (US-897 and US-942) are resistant to the nematode *T. semipenetrans* (Hamid et al. 2025). Data from rootstock evaluation against *F. solani* will be published later this year, and the experiments on Phytophthora root rot are ongoing. Evaluations of ungrafted rootstocks are conducted in a growth chamber, but ultimately these HLB-tolerant rootstocks will be grafted with Washington navel orange or Tango mandarin, and their horticultural performance

will be evaluated in the field at the Lindcove Research and Extension Center starting in 2027. Rootstocks will also be evaluated in the greenhouse for their ability to tolerate water deficit and salinity.

Conclusion

Our team is evaluating the best of the new HLB-tolerant rootstocks from the USDA in Florida with scions important in California and under California conditions. Based on our research, growers will be able to make informed decisions about rootstock use in production regions at risk of HLB disease. 🌱

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CITRUS RELATIVES AS NAVEL ROOTSTOCKS: EFFECTS ON FRUIT QUALITY AND GROWTH

Zachary Thomas and Mikeal Roose

Project Summary

The use of HLB-tolerant or resistant citrus relatives in the University of California, Riverside (UCR) rootstock breeding program will affect the scions grafted onto the new rootstocks. This trial is intended to discover what effects certain relatives will have on Washington Navel scions. It is important to understand how different citrus relatives impact tree health, productivity, and disease responses so that the correct crosses can be made to offset negative effects and lead to a rootstock that will grow well, produce high yields, and be HLB-tolerant or resistant.

Introduction

The University of California, Riverside (UCR) rootstock breeding program has been improving rootstocks through conventional breeding with releases like C35, Bitters, Carpenter, and Furr. Citrus greening, or huanglongbing (HLB), is a major problem, causing millions in losses annually worldwide. In Ramadugu et al., citrus species of Australian origin that have a natural tolerance/resistance to HLB were identified (2016). Those species include *Microcitrus australis*, *M. australasica*, *M. inodora*, and *Eremocitrus glauca*. Rootstocks have long been known to

affect scion fruit quality and tree growth traits (Roose 2014). Our program has started breeding with Australian species to integrate tolerance/resistance into advanced rootstock germplasm. A trial was started in 2019 to help understand what effects these relatives would have on the scion when used as rootstocks. This data could then be used to choose the best parents to create elite rootstock hybrids using the HLB-resistant/tolerant relatives.

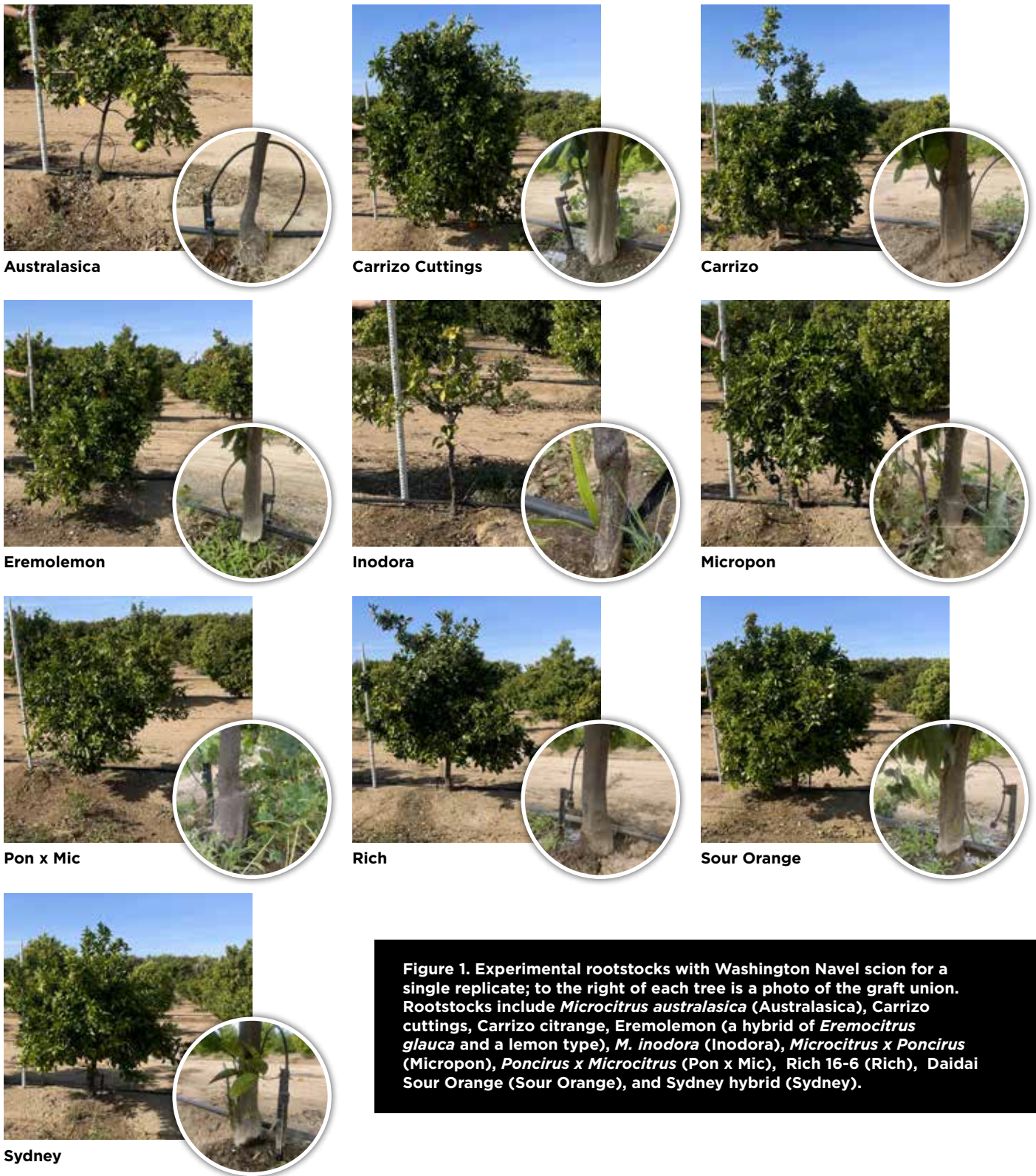


Figure 1. Experimental rootstocks with Washington Navel scion for a single replicate; to the right of each tree is a photo of the graft union. Rootstocks include *Microcitrus australasica* (Australasica), Carrizo cuttings, Carrizo citrange, Eremolemon (a hybrid of *Eremocitrus glauca* and a lemon type), *M. inodora* (Inodora), *Microcitrus x Poncirus* (Micropon), *Poncirus x Microcitrus* (Pon x Mic), Rich 16-6 (Rich), Daidai Sour Orange (Sour Orange), and Sydney hybrid (Sydney).

Rootstock Selection and Propagation

Seven citrus relatives were initially chosen for this trial: *E. glauca*, *M. australasica* (Aust), *M. inodora* (Ino), Eremolemon (Elem, a hybrid of *E. glauca* and a lemon type), Sydney hybrid (Syd, *M. australis* × *M. australasica*), *Microcitrus x Poncirus* (Micropon-C), and *Poncirus x Microcitrus* (Pon x Mic). Carrizo citrange, Daidai Sour Orange (Sour), and Rich 16-6 were also included as controls. Seed was collected and planted in spring 2019 for all except the Micropon-C, which produces no seed and was grown from cuttings started in the same month as the seed was planted. We propagated Carrizo using cuttings as a control for the cutting-derived relative hybrid and to determine if Carrizo itself would perform differently if derived from cuttings. It should be noted that Aust,

Ino, and Syd do not produce nucellar seedlings, and individuals selected for this trial are the offspring that most resemble the initial parent, based on leaf shape, leaf color, bark color, growth habits, and vigor. All other cultivars were visually rogued using the same criteria, and apparent nucellar seedlings were used for the rootstocks. *M. australis* did not set any seed that year, and the cuttings we attempted failed to root.

The rootstocks were grafted with Parent Washington Navel (budwood received from the Citrus Clonal Protection Program) in spring 2020. The trees were kept in the greenhouse for a year to attain sufficient size for field planting. During this time, most of the trees on *E. glauca* rootstocks died. The two survivors died within months of being field-planted. Six replicates of each remaining rootstock were planted in Riverside in June 2021. The scion of one Ino died one year after planting. Tree height, two tree widths (taken 90° apart), health, union rating, and sucker counts were measured annually from 2021-2025. Canopy volume was calculated as ((height x width1 x width2)/4). Yield, Brix, titratable acid, fruit neck, fruit diameter, weight of a fruit, rind color, internal color, core type (i.e. solid, semi-solid, hollow), core width, segment membrane thickness, seed number, peelability, segment separation, peel thickness, internal texture, juice percentage, rind texture, puffing, and granulation were assessed annually starting in 2023 based on a 10-fruit sample from each tree (when available). Ino and Aust had fewer than 10 fruit per tree and were removed from statistical analysis for fruit traits. The fruit samples were collected during the first two weeks in January.

Results

The relatives had several significant differences from the standard (control) rootstocks. Carrizo plants grown from cuttings did not statistically differ from seed-derived Carrizo plants in any metric measured.

Height and Volume: The height and overall size of the trees on many of the

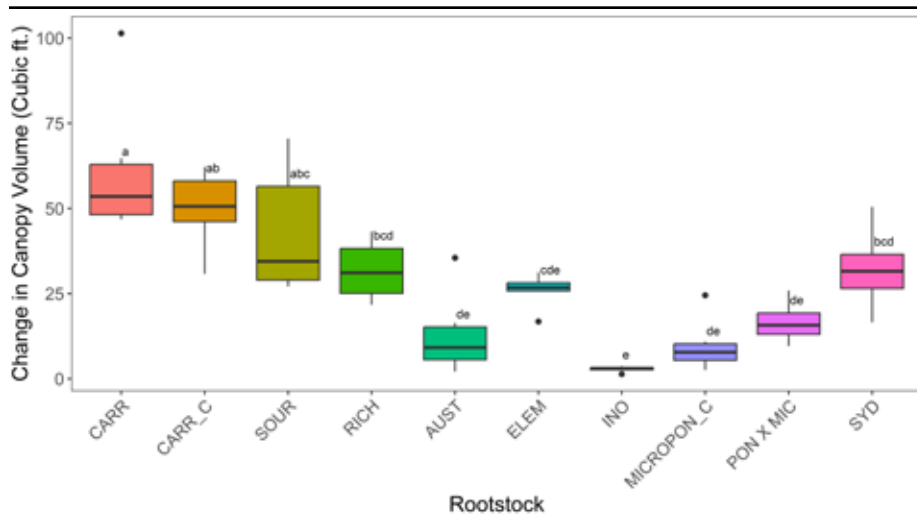


Figure 2. Change in canopy volume in cubic feet from 2022 to 2025 for navels on 10 rootstocks. No shared letters indicate significance for that rootstock pair using Tukey's test at $p < 0.05$. Rootstocks include Carrizo citrange (CARR), Carrizo cuttings (CARR-C), Daidai Sour Orange (SOUR), Rich 16-6 (RICH), *Microcitrus australasica* (AUST), Eremolemon (ELEM), *M. inodora* (INO), *Microcitrus x Poncirus* (MICROPON_C), *Poncirus x Microcitrus* (PON x MIC), and Sydney hybrid (SYD).

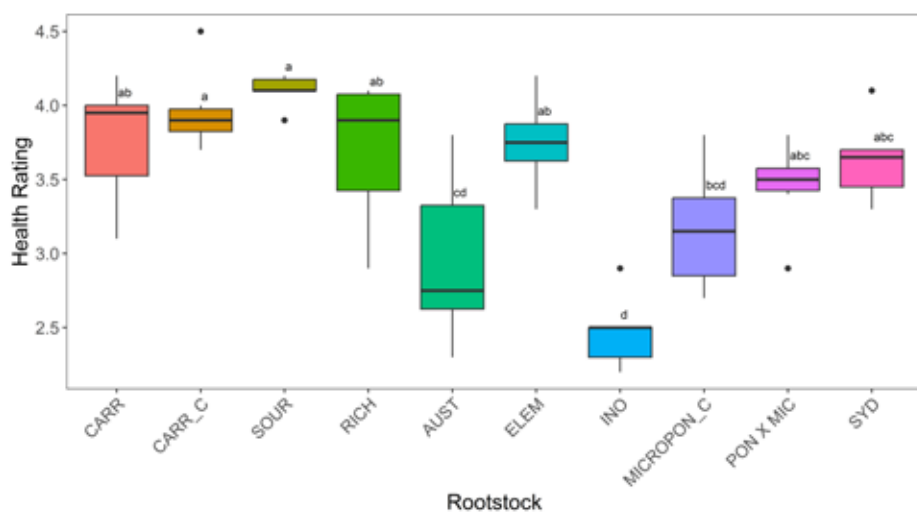


Figure 3. Effects of rootstocks on average health ratings in 2025. No shared letters indicate significance for that rootstock pair using Tukey's test at $p < 0.05$. Rootstocks include Carrizo citrange (CARR), Carrizo cuttings (CARR-C), Daidai Sour Orange (SOUR), Rich 16-6 (RICH), *Microcitrus australasica* (AUST), Eremolemon (ELEM), *M. inodora* (INO), *Microcitrus x Poncirus* (MICROPON_C), *Poncirus x Microcitrus* (PON x MIC), Sydney hybrid (SYD).

relatives were smaller than the standards, averaging 20-50 percent the size of Carrizo (Figures 1 and 2).

Health: Health was measured on a one through five scale, with five being the best and one being nearly dead. Tree health ratings were based on canopy density, leaf color, and amount of branch dieback. Ino, Aust, Micropon-C, and Pon x Mic had low health during the trial (Figure 3). Ino and Aust were significantly lower than standards.

Bud Union: Bud union is measured as a ratio of the scion to the rootstock and is estimated visually. Ino showed a ratio of 1.25:1 scion to rootstock diameter, and Aust showed a ratio as low as 1:2 scion to rootstock diameter. The other rootstocks showed some rootstock overgrowth with a ratio between 2:3 and 3:4 scion to rootstock

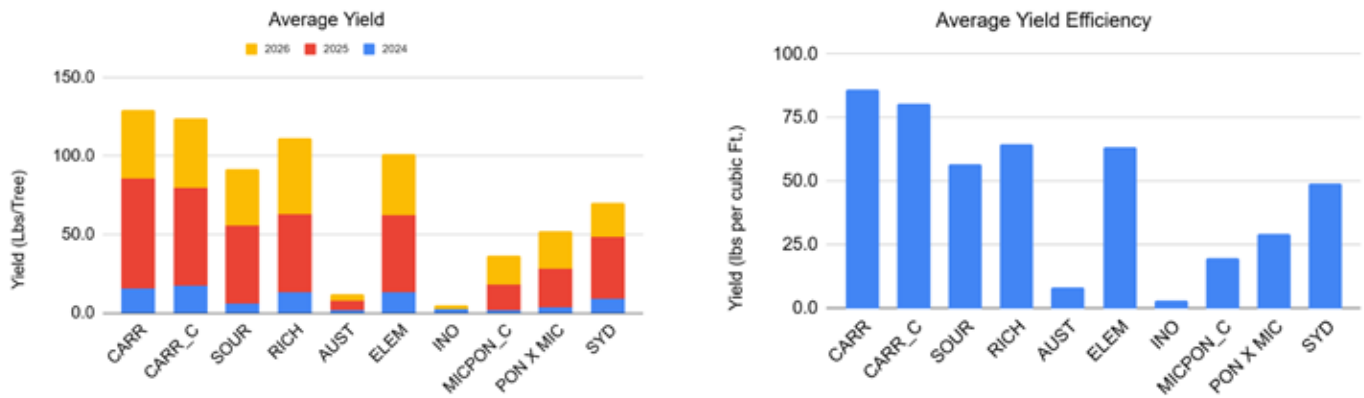


Figure 4. The graph on the left shows the average cumulative yield for 2023-2025. The graph on the right shows average yield efficiency as 2023-2025 yield/2025 canopy volume in pounds of fruit per cubic foot of canopy. No shared letters indicate significance for that rootstock pair using Tukey’s test at $p < 0.05$. Rootstocks include Carrizo citrange (CARR), Carrizo cuttings (CARR-C), Daidai Sour Orange (SOUR), Rich 16-6 (RICH), *Microcitrus australasica* (AUST), Eremolemon (ELEM), *M. inodora* (INO), *Microcitrus x Poncirus* (MICPON_C), *Poncirus x Microcitrus* (PON x MIC) and, Sydney hybrid (SYD).

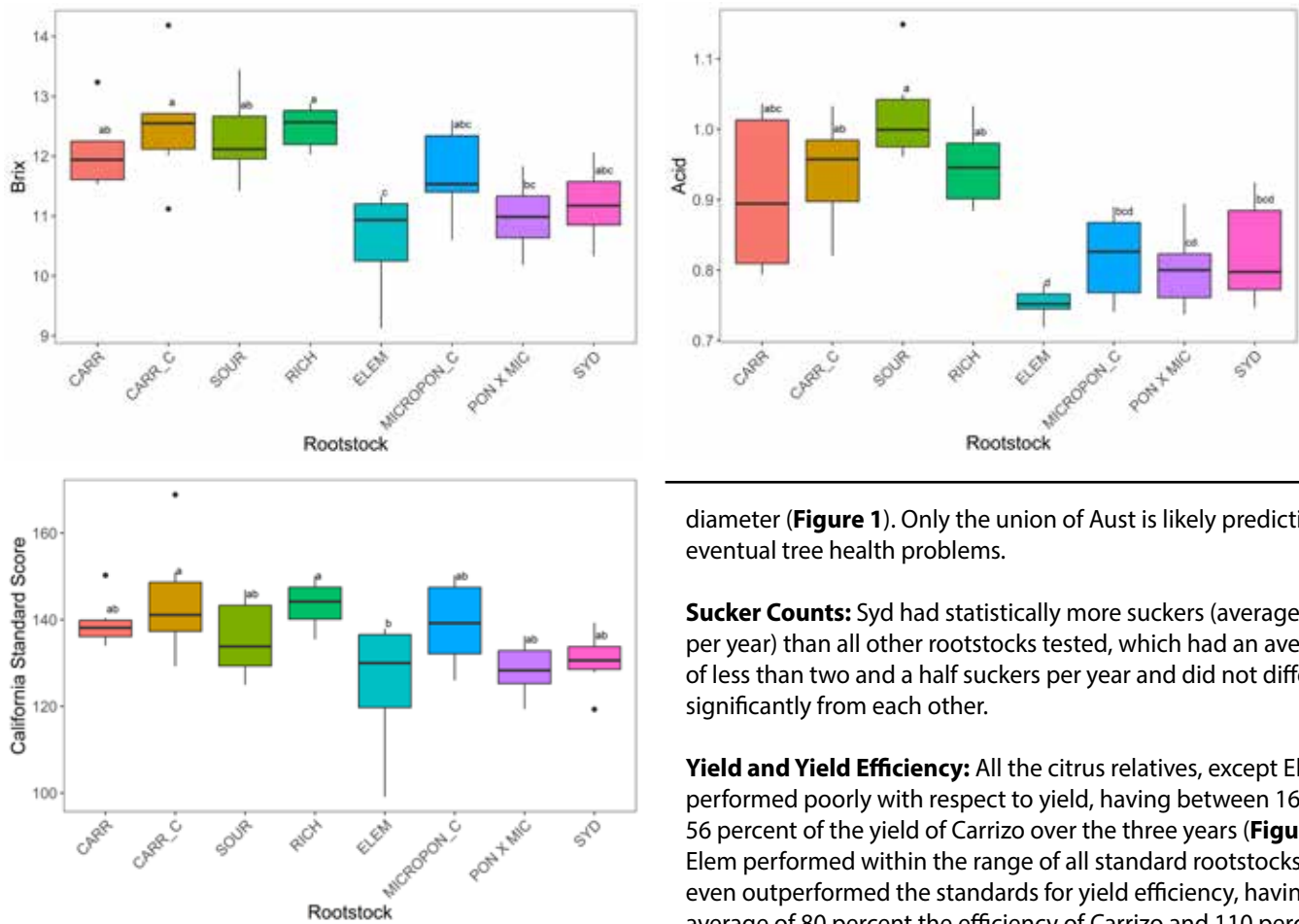


Figure 5. The graph on the top left shows average Brix over three years. The graph on the top right shows average acid percentages. The bottom graph shows the California Standard Index Score. No shared letters indicate significance for that rootstock pair using Tukey’s test at $p < 0.05$. Rootstocks include Carrizo citrange (CARR), Carrizo cuttings (CARR-C), Daidai Sour Orange (SOUR), Rich 16-6 (RICH), Eremolemon (ELEM), *Microcitrus x Poncirus* (MICROPON_C), *Poncirus x Microcitrus* (PON x MIC), and Sydney hybrid (SYD).

diameter (**Figure 1**). Only the union of Aust is likely predictive of eventual tree health problems.

Sucker Counts: Syd had statistically more suckers (average five per year) than all other rootstocks tested, which had an average of less than two and a half suckers per year and did not differ significantly from each other.

Yield and Yield Efficiency: All the citrus relatives, except Elem, performed poorly with respect to yield, having between 16 and 56 percent of the yield of Carrizo over the three years (**Figure 4**). Elem performed within the range of all standard rootstocks, and even outperformed the standards for yield efficiency, having an average of 80 percent the efficiency of Carrizo and 110 percent the efficiency of Sour over the three years (**Figure 4**). Yield efficiency was calculated as the cumulative yield from 2023 through 2025 divided by the 2025 canopy volume.

Brix and Acid: In comparison to standard rootstocks, juice of most relatives showed a slight (at least 0.5 units) decrease in the Brix (**Figure 5**) and a larger decrease in the acid content of 0.1 to 0.2 units (**Figure 5**). Differences in Brix and acid were statistically significant only for Aust and Elem.

Table 1. Average scores for four rootstocks from the taste survey at UC Riverside Citrus Day 2026. Sweetness, richness and texture scores are based on a 1-10 scale; Tastes like navel from the Citrus Variety Collection (CVC) were scored with an agree (1) to disagree (-1) scale. Micropon-C - *Microcitrus x Poncirus*.

Traits \ Rootstocks	Carrizo	Sydney	Eremolemon	Micropon-C
Sweetness Score	7.00	6.76	5.76	6.22
Richness Score	6.38	5.29	5.16	5.96
Texture Score	5.46	5.31	5.84	5.65
Tastes like navel from CVC	0.33	-0.29	-0.35	0.26

Solids-to-Acid Ratio and California Standard: The relatives showed a higher solids-to-acid ratio than Sour, which is likely due to lower acid levels. The California Standard index score (derived from BrimA and calculated using the formula $[Brix - (titratable\ acid * 4)] * 16.5$) varied, but all fruits scored within the proper maturity index (greater than 90), and the variation was slight (**Figure 5**).

Taste Survey: A blind tasting of navels with 50 participants was performed on UCR Citrus Day in 2026. Four of our rootstocks were included: Carrizo, Syd, Elem, and Micropon-C. Our survey indicated that, on average, people thought fruit from trees on relatives were less sweet and less rich in flavor when compared to those on Carrizo. Scores were determined on a 1-10 scale for sweetness, richness, and texture. How the taste of fruit from trial trees on different rootstocks compared to Navel on Carrizo from the Citrus Variety Collection was measured using an agree or disagree response, which was converted to a numeric score; 1 for agree and -1 for disagree.

Other Fruit Traits: All other measured traits (fruit neck, fruit diameter, fruit weight, rind color, internal color, core type, core width, segment membrane, seed number, peelability, segment separation, peel thickness, internal texture, juice percentage, rind texture, puffing, and granulation) showed no statistically significant differences among rootstocks.

Discussion

Australian species as rootstocks appear to negatively impact yield, internal fruit quality, and tree growth of navels. Elem (a first-generation hybrid of Eremocitrus and a lemon) is an exception in that navels on this rootstock have greater yield efficiency, which may be useful in high-density plantings, although Brix and acids are negatively affected. Elem's performance indicates that even one-two generations of hybridization with Eremocitrus and a lemon can result in some hybrids that mitigate some negative effects, whereas the fruit quality issue will require breeding with parents that confer exceptional fruit quality. We have not yet tested many of these cultivars for their performance under common diseases such as *Phytophthora* and CTV. A CTV test on the field trees showed that all were negative as of March 2026. We plan to collect data over the next couple of years to determine if the quality of fruit

and yield remain consistent. We also intend to collect fruit at three different time points to determine if the relatives have any effect on the maturation of the fruit. The negative impacts of using these relatives as rootstocks on fruit quality are fairly small, while effects on tree growth and yield are relatively large, suggesting future crossing should focus on improving yield, tree growth, and fruit quality, while maintaining HLB-resistance. 🌱

CRB Project #: 5200-201

Acknowledgements

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Recently Released Cultivars Enhances California Citrus Diversity

Toni Siebert Wooldridge, Karene Trunnelle, Robert Krueger, Brittany Moreland, Paulina Quijia-Lamina, German Villaiba-Salazar, Georgios Vidalakis, and Tracy Kahn

New citrus scion and rootstock cultivars that improve disease resistance and/or tolerance, meet evolving market demands, enhance flavor, and increase production efficiency are essential for the long-term sustainability of the California citrus industry. Potential new cultivars arise through three primary pathways: (1) introductions of commercial cultivars from outside California, (2) discovery of novel types within the state by growers or researchers, and (3) development of new cultivars through the University of California, Riverside (UCR) Citrus Breeding Program.

This article compiles background information on scion varieties recently released from quarantine by the Citrus Clonal Protection Program (CCPP) (Vidalakis 2026). The new introductions described here may be evaluated for fruit quality and trueness-to-type as part of the Citrus Research

Board (CRB)- funded project, *New California citrus cultivars through integrated breeding and evaluation*, led by Danelle Seymour, Ph.D., Tracy Kahn, Ph.D., Glenn Wright, Ph.D., and Mikeal Roose, Ph.D. Additional information on these varieties is available through the UCR Givaudan Citrus Variety Collection (GCVC; citrusvariety.ucr.edu).

How Does the GCVC Utilize Newly Introduced Citrus Varieties from CCPP?

Once a citrus variety is released from quarantine, it may be established in the GCVC as a living reference accession. GCVC staff propagates and plants the variety in field or

in protected greenhouse blocks to ensure long-term preservation and availability for research and evaluation. Tree growth, phenology, productivity, and overall horticultural performance are monitored under California conditions.

For commercially promising new introductions, structured, multi-season evaluations of fruit quality are undertaken. These assessments determine maturity timing, internal and external quality, and overall commercial potential compared to existing industry standards. Results are then disseminated to growers, nurseries, and researchers through presentations and publications to inform decisions regarding the variety's suitability for California citrus production. Though not all introduced varieties are suitable for fruit quality evaluation, retaining these accessions can still have significant scientific and strategic value for genetic diversity.

Here, we summarize background information on recent introductions, including the rationale for their importation and key horticultural traits observed under California conditions.



Figure 1

VI¹ 1562 *Citrus leiocarpa* (Koji orange)

This new introduction (**Figure 1**) originated from a public request through the CCPP Variety Introduction System (ccpp.ucr.edu/cvis.html) and was sourced from the GCVC. The accession was received as seed in 1956 from H. Yoshimura, University of Osaka, Japan. Field notes from Dr. Willard Bitters, GCVC Curator (1946–1982), following a 1963 trip to Japan, described the fruit as very small, oblate, and bright orange, with a flattened stem end and 15–20 seeds. In 1922, Tyōzaburō Tanaka described the Koji orange in *Citrus Fruits of Japan* as “very early maturing small fruits of extremely smooth surface.”



Figure 2

VI 941 Soheila citron hybrid

Soheila (**Figure 2**) was introduced from Paradise Nursery, Chatsworth, California, in October 2020. Limited initial information was available about the pedigree of this variety, however, based on recent in-field observations

of the accession under orchard conditions at the Lindcove Research and Extension Center, it is presumed to be a citron hybrid. The fruit is notably large, yellow, and with a highly textured, pebbly rind. The aromatics of the external rind are consistent with those of citron. The leaves are marginally smaller than typical pummelo varieties, yet less characteristic of true citron. Young flushes display a purple-red coloration. The albedo² is slightly spongy in texture, with vesicles³ less coarse than those observed in true citrons. The flavor profile is sour with a slight bitterness.



Figure 3

VI 1035 Bower mandarin

Bower mandarin (**Figure 3**), developed by United States Department of Agriculture (USDA) researchers in Orlando, Florida, is the result of a cross between the Clementine mandarin and the

Orlando tangelo. Although this cultivar offers a good flavor profile and matures relatively early (typically in early December), it faces significant horticultural challenges such as strong alternate bearing⁴, resulting in inconsistent annual crop yields and poor peeling quality. Recent research indicates that Bower is tolerant to huanglongbing, but has been described as having high sourness and a slight metallic off-flavor (Jeffries 2024).



Figure 4

VI 1469 Orbon lemon hybrid

Orbon (**Figure 4**) originated from a hybridization experiment of “the Lisbon lemon by a variety of blood orange” conducted by Howard B. Frost at the UCR California Citrus Experiment Station during the early

decades of the 20th century (Frost, 1948). This hybrid is best described as a horticultural curiosity. Trees exhibit relatively low vigor and modest yield. The fruit is orange-shaped with a smooth, light yellow rind and produces few seeds based on field observations. Flesh color ranges from light to medium yellow, with no anthocyanin⁵ pigmentation observed internally; however, anthocyanin coloration may develop on the rind if fruit are left on the tree. The flavor is pleasantly sub-acid with subtle orange-like notes.



Figure 5

VI 1468 *Citrus yuko*

Received as seed from H. Yoshimura, University of Osaka, Japan, in 1956, this accession (**Figure 5**) is believed to be a yuzu-related hybrid involving *Citrus ichangensis* and mandarin, with yuzu confirmed as the seed

parent (Yamamoto and Kobayashi, 1996). Tyōzaburō Tanaka described it in *Citrologia* (1961) as a yuzu-type hybrid with fruit size comparable to Satsuma with a somewhat rough rind. The fruits are round to slightly flattened, yellow to orange at maturity, aromatic, and moderately acidic but milder than yuzu or sudachi, and reportedly hang well on the tree for harvest from early fall through winter.



Figure 6

VI 1000 Bidwell's Bar sweet orange

Historically, the Bidwell's Bar variety (**Figure 6**) is representative of the seedy, mid-season sweet oranges prevalent in California prior to the widespread cultivation of the Washington navel orange. This variety

constitutes a vital genetic resource and serves as a tangible link to the renowned mother orange tree situated near the Bidwell's Bar bridge in Oroville, California, planted in 1856. Its successful growth demonstrated that citrus cultivation was viable in Northern California, thereby initiating the local industry. It is widely considered to be the oldest living citrus tree in the state.



Figure 7

The tree is of good vigor and size, though slow-growing and somewhat irregular, with distinctive light-green foliage. It tends to produce out-of-season bloom and fruit and is sensitive to cold, heat, and desert winds, but is productive in favorable environments.

VI 1581 Ovale Calabrese sweet orange

The Ovale Calabrese sweet orange (Figure 7) is a prominent, well-regarded Italian variety, once considered the top common sweet orange in Italy. Its fruit is medium-large, oval, and mostly seedless, with a medium-thick, tight rind and juicy, well-flavored flesh. It is late-maturing (similar to Valencia), colors well when ripe, and holds well on the tree, while also shipping and storing effectively.



Figure 8

Visitors can see it on a boat ride or the "Behind the Seeds" tour. From Florida, it is potentially a lemon-citron hybrid and reported to have been originally collected from the Snively Collection in 1977 for the Florida Citrus Arboretum. Public interest, boosted by its Epcot display, has increased its visibility. Epcot employees maintain the Nine-pound variety, and it is distinct from Ponderosa lemon.

VI 1394 Nine Pound lemon

Nine-Pound lemon (Figure 8) is a massive ornamental citrus fruit, reportedly weighing up to 15 pounds, and is currently featured at Epcot's Living with the Land exhibit within the Walt Disney World Resort complex.



Figure 9A



Figure 9B

VI 1449 UCR 1958 Batchelor Memorial tree navel orange

The Batchelor memorial tree, formally dedicated in 1958, is a culturally and historically significant specimen to UC Riverside. The tree serves as a living tribute, specifically honoring the invaluable contributions of Dr. Leon D. Batchelor, a towering figure in citrus research. The health of the Batchelor Memorial tree had been in noticeable decline since 2016, despite comprehensive and persistent efforts to maintain its vitality. Even

with continuous oversight treatment of inarching by certified arborists, the tree's overall condition continued on a downward trajectory. As a proactive measure for preservation, budwood⁶ was collected and submitted to the CCPP in 2020 to ensure the genetic material of this important tree is safeguarded for the future. In May 2022 (Figure 9A), and again in 2023 and 2025, the CCPP performed inarchings using various rootstock types, and the tree now appears to be responding to the treatment (Figure 9B), producing new flushes and slowly restoring its canopy to health.

Enhanced Finger Lime Germplasm Enter the CCPP Pipeline

In addition to introductions initiated through the CCPP Variety Introduction process, recent submissions from the USDA-ARS National Clonal Germplasm Repository for Citrus & Dates represent an important expansion of available germplasm⁷. With CRB funding supporting structured fruit quality evaluations, the GCVC characterized selections that exhibited improved pigmentation and larger fruit size relative to previously available material, and eight new finger lime varieties are now listed on both the Protected Foundation Block and Early Release budwood lists (Figure 10).

Citrus Research Board Project 5200-201

Glossary

¹**VI:** Refers to variety index number, a unique identifier assigned by the CCPP to citrus varieties that have successfully completed quarantine processing.

²**Albedo:** The white inner layer of the citrus peel located between the outer rind (flavedo) and the fruit pulp.

³**Vesicles:** Juice-filled sacs within citrus pulp that contain the edible portion of the fruit.

⁴**Alternate Bearing:** A production pattern in which a citrus tree yields a heavy crop one year followed by a lighter crop the next.

⁵**Anthocyanin:** A plant pigment responsible for red or purple coloration in plant tissues, including the rind or flesh of some citrus fruits.

⁶**Budwood:** Young citrus shoots containing buds used for vegetative propagation to produce genetically identical trees.

⁷**Germplasm:** Genetic plant material preserved for breeding, conservation, and scientific research.

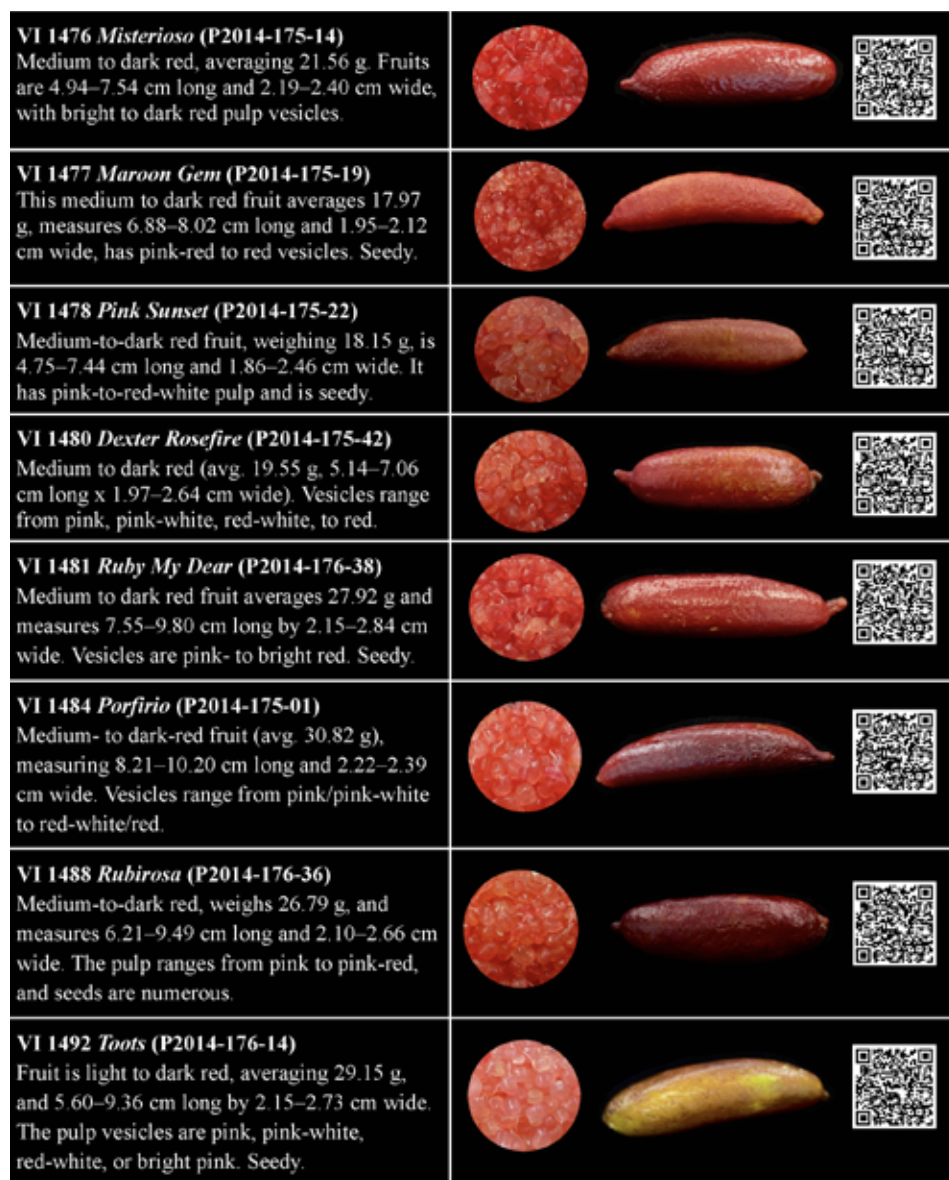


Figure 10

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Toni Siebert-Wooldridge is a senior museum scientist; Karene Trunelle is a museum scientist; and Tracy Kahn, Ph.D., is an endowed chair and curator of the Givaudan Citrus Variety Collection, all in the Department of Botany and Plant Sciences at UCR. Robert Krueger, research leader and Brittany Moreland, biological science technician, are at the USDA-ARS National Clonal Germplasm Repository for Citrus & Dates. Paulina Quijia-Lamiña is an assistant project scientist at CCPP. German Villalba-Salazar is a junior specialist at CCPP, and Georgios Vidalakis, Ph.D., is a professor and extension specialist in plant pathology and the director of the CCPP at UCR.



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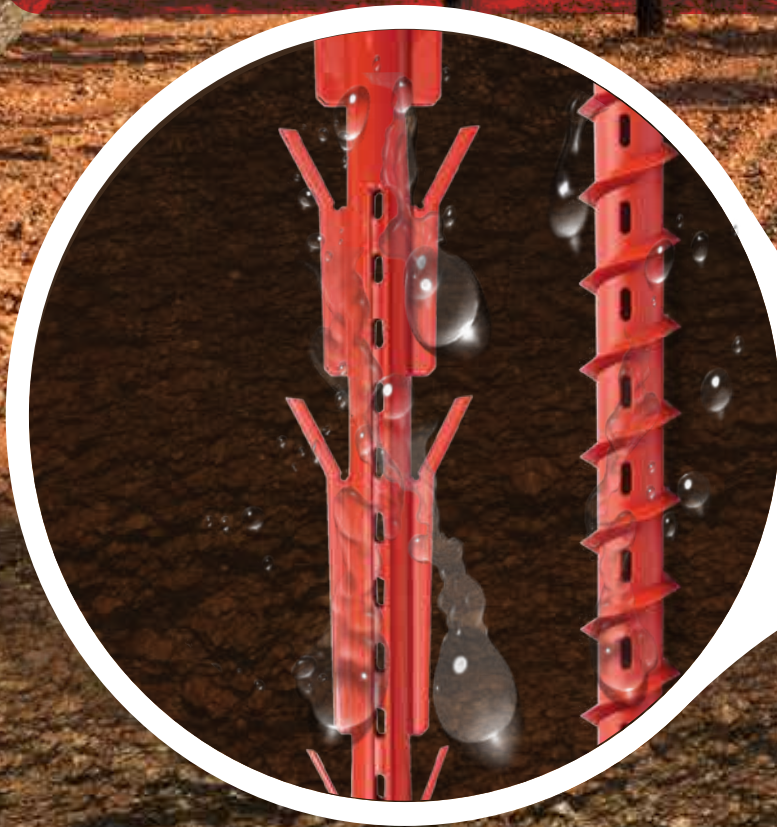
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UCR BREEDING PROGRAM GROWER TRIAL UPDATE

Tracy Kahn, Toni Siebert-Wooldridge, Karene Trunnelle,
Danelle Seymour, Claire Federici, and Mikeal Roose

Attendees at the 2026 UC Riverside Citrus Day (February 19, 2026) sample a diverse range of new introductions and UC Riverside-developed selections while reviewing varietal descriptions.

Project Summary

This article provides an update on the new grower trials developed in collaboration with the Citrus Research Board to evaluate unreleased University of California, Riverside (UCR) Breeding Program advanced selections under commercial conditions. The summer 2025 issue of Citrograph (Siebert-Wooldridge et al. 2025) described the initial set of mandarins, lemons and hybrid selections planned. This article provides additional information, including updated plans regarding selections and controls to be included.

The UCR breeding program, supported by and in collaboration with the Citrus Research Board (CRB), is developing supplementary grower trials to test advanced unreleased selections, previously described in Siebert-Wooldridge et al. (2025), under commercial growing conditions. These selections will be planted in unreplicated

plots at two locations on property acquired by the CRB; one in Strathmore and the other in Maricopa. These selections are also currently in multi-location replicated research field trials at other locations in California. Since these selections have not been released, they are protected by non-propagation and test agreements which prohibit additional propagation,

Table 1. List of advanced selection type and identification code for each of the selections in the grower trials.

ADVANCED SELECTION TYPE	SELECTION IDENTIFICATION CODE
Orange-Grapefruit Hybrid	RS6-15
Grapefruit-Pummelo Hybrid	RS98.10-4
Grapefruit-Pummelo Hybrid	RS95.14-13
Lemon Selection	LTL001
Lemon Selection	ALE001
Lemon Selection	LFN001
Lemon Selection	WKR001
Lemon Selection	WKR003
Lemon Selection	LFN004
Mandarin-Pummelo Hybrid	CPH001
Mandarin Selection	SAC001
Mandarin Selection	PGM001
Valencia Selection	OLV001
Valencia Selection	OLV002

distribution, or sale of trees and fruit, as well as public showcasing of unreleased varieties—all of which remain the property of the Regents of the University of California.

We previously described the selections planned for the grower trials (Siebert-Wooldridge et al. 2025), but based on further evaluation, one lemon (LFN003) and one mandarin (PGM002) selection were removed due to trueness-to-type issues. Two Valencia selections from mutation breeding will now be included in the grower trials. The two Valencia selections are being evaluated in replicated trials planted in 2019 on two rootstocks. Both of the selections have less than 1.0 seeds per fruit, and one selection has less than 0.5 seeds per fruit (50 fruit per selection per year) based on five years of fruit production. This selection also appears to mature earlier in the season. Altogether, there will be 14 selections planted (Table 1) and evaluated at each of the two locations.

To provide a meaningful comparison, standard commercial varieties will also be included in the grower trials including Limoneira 8A and Eureka for the lemon selections, Star Ruby grapefruit for the grapefruit-pummelo and orange-grapefruit hybrids, Page and Clementine Sidi Aissa for the mandarin selections and Olinda for the Valencia selections. Each selection and commercial standard will be represented by 20 trees, with 10 trees on each of the two rootstocks. The rootstocks chosen for the trials are Macrophylla and X639 for the lemons, Carrizo and X639 for the grapefruit-pummelo and orange-pummelo hybrids and the mandarins, and Carrizo and Rubidoux trifoliolate for the Valencia selections.

The current plan is for the trees to be propagated this spring and planted in spring 2027 after the field sites are prepared. Please stay tuned for future updates. 🌱

CRB Research Project #5200-201

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Siebert-Wooldridge, T. et al; 2025. UCR Breeding Program and Growers Partner to Evaluate New Selections. *Citrograph* 16(3): 58-61.

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Re-birth of the USDA-ARS Citrus Evaluation Program in California

Rodrigo Krugner, Christopher Wallis,
and Sumaira Riaz

Citrus growers in California are navigating a convergence of pressures: prolonged drought and salinity challenges, evolving pest and disease threats including the looming risk of huanglongbing (HLB), increasing labor costs, and fast-shifting consumer preferences for seedless fruit, vibrant color, and consistent flavor. To address these challenges, a new partnership linking the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) San Joaquin Valley Agricultural Sciences Center (SJVASC) in Parlier, California with the Citrus Research Board (CRB), is accelerating the evaluation of fresh-market citrus specifically adapted to California's production realities.



The new program blends federal appropriations with sustained support and guidance from the CRB, aligning national research capacity with local needs and delivering next-generation scions and rootstocks for California citrus growers.

This effort builds on a rich legacy. Commercial citrus production in the United States began at about the same time in California and Florida during the late 19th century, with California leading orange production for many years. In the 1940s, USDA scientists played a crucial role in the development of frozen orange juice concentrate, which made citrus production in Florida an economic powerhouse and boosted consumer appreciation and health. Consequently, citrus production increased in Florida whereas in the 1960s, the California fresh orange industry decreased due to urbanization (Cooper et al. 1964). Today, citrus is grown commercially in California, Florida, Texas, and Arizona.

During the 2024-25 season, California accounted for 84% of the total United States citrus production. Florida produced 13%, and Texas and Arizona produced the remaining 3%. Collectively, the industry's annual income in the 2024-25 season surpassed \$2.8 billion, with California's production value estimated at about \$2.5 billion (USDA-NASS 2025). This economic footprint underscores why breeding and evaluation programs tailored to California's conditions are critical for sustaining profitability and competitiveness of the citrus industry in the United States.

Brief History of USDA-ARS Citrus Research

Plant breeding is a multidisciplinary science that includes plant geneticists, horticulturists, physiologists, pathologists, and food quality scientists to meet research goals and enable technology transfer. By the early 1930s, the USDA in California and Florida had several lines of research on citrus bud variation, nutrition, and pathology including foliar, fruit, and trunk diseases. By the 1940s, citrus breeding, nutrition, and pathology research became coordinated as a single USDA project based in Orlando, Florida, that included research in California, Texas, and Arizona. Research on citrus irrigation was conducted at the United States Salinity Laboratory in Riverside, California. In 1946, USDA citrus breeding research in California was transferred from Riverside to the United States Date & Citrus Station in Indio, California (Cooper et al. 1964). However, the USDA-ARS citrus breeding program in California was terminated in 1983, leaving a gap in region-specific varietal development by the USDA.

Today, the USDA-ARS conducts a wide range of research with citrus in 12 locations across the United States: Albany, California; Riverside, California; Parlier, California; Fort Collins, Colorado; Fort Pierce, Florida; Gainesville, Florida; Miami, Florida; Hilo, Hawaii; Beltsville, Maryland; Fredrick, Maryland; Ithaca, New York; and Wyndmoor, Pennsylvania. In Riverside,

research at the National Clonal Germplasm Repository include optimizing cryopreservation of citrus accessions, screening for ploidy and polyembryony, advancing micropropagation protocols, and generating and verifying pathogen-free materials prior to global dissemination. In Parlier, research focuses on 1) the development of applied strategies to combat vector-borne diseases that include HLB, citrus tristeza virus, citrus stubborn, and citrus yellow vein clearing virus, 2) the development of integrated preharvest and postharvest technologies to maintain or improve fresh citrus fruit quality and reduce postharvest decay, and 3) pioneering postharvest interventions such as fumigant alternatives to ensure compliance with phytosanitary standards, reduce pesticide residues, and maintain fresh fruit quality for domestic and export markets. The research portfolio at Fort Pierce is similarly impressive, but particularly, it plays a unique role in breeding the next generation of high-performing citrus cultivars by providing superior citrus trees that support grower profitability. The new citrus evaluation program established in 2023 at the Parlier location has similar expectations, to deliver varieties adapted to California's climate and fresh market needs.

Program Launch

Establishing and accelerating the Parlier program requires a source of evaluation material. To address this requirement, a partnership was formed among the United States Horticultural Research Laboratory in Fort Pierce, the National Germplasm Repository for Citrus and Dates in Riverside, and the SJVASC in Parlier. This collaboration is building and evaluating a collection of promising materials of oranges, tangerines, grapefruit, and lemons in Parlier. Transfer of materials within California and out-of-state required completion of quarantine protocols regulated by the USDA-Animal Plant Health Inspection Service (APHIS) and the California Department of Food and Agriculture (CDFA) and conducted by the Citrus Clonal Protection Program (CCPP) in Riverside. Parlier is evaluating scion and rootstock breeding lines developed within the USDA, while simultaneously developing resources to expand evaluation efforts beyond USDA-developed material. This two-track approach ensures growers benefit from proven genetics being tested in the environment relevant to California commercial citrus production.

Development of new varieties of a perennial fruit crop such as citrus requires a minimum of 10 years from hybridization to release. When the Parlier program was established in 2023, basic infrastructure was non-existent. Since then, laboratory space has been assigned to the new citrus breeding research program. A controlled environment room was built to store plant and fruit samples, and a new 2,000 square foot greenhouse was built to accommodate incoming scion and rootstock materials for propagation. Field evaluation in California is required to fully determine the commercial potential of a new variety for California growers because a hybrid desirable for Florida may not necessarily be valuable



Citrus trees grown in the greenhouse above for field evaluation of scions.

in California. In preparation for field evaluations, grapevines in a five-acre trellised vineyard plot in Parlier were removed. The plot was deep-ripped, fumigated, and a new irrigation system was installed for establishing a scion evaluation trial that includes 10 materials developed in Fort Pierce.

Field testing of new rootstocks may require as much as 20 years to evaluate each planting. An additional 20 acres were secured at the nearby Reedley College in Reedley, California, through a land lease agreement established between the USDA-ARS and Reedley College after extensive environmental assessments and a boundary survey were conducted as part of conformance with the National Environmental Policy Act. The land at the Reedley site is being prepared for a rootstock trial expected to be planted in spring 2027 with about 14 different rootstock varieties. Other advanced scions and rootstocks from the USDA-ARS in Fort Pierce are expected to clear quarantine and be planted in following years. These initial scion and rootstock selections will be evaluated for horticultural traits including vigor, size, scion compatibility, yield, fruit maturity period, and fruit characteristics such as size, peel, flavor, color, and seedlessness. Such information will aid personnel in determining which lines are suitable for culture in California.

Critically, this is not a duplication of Florida’s work—it is an expansion and adaptation. The Florida program has demonstrated the power of genetics combined with rigorous evaluation in delivering varieties with higher yields, improved disease resistance, and better flavor and consistency. Parlier’s mission is to build on that success by tailoring these advances to California’s distinct climates, soils, and market demands. This collaboration is pivotal, as it allows Parlier to access Florida’s extensive selection pipeline and rapidly test “best-of-class” candidates under California conditions, while discoveries originating in California can inform Florida and other regions facing similar challenges.

The result of this partnership is a national evaluation effort coupled with regional precision—leveraging shared expertise to accelerate innovation for growers across the United States citrus industry. This coordinated and targeted approach will speed up breeding effort success and novel cultivar deployment that will ensure a bountiful citrus harvest for many years to come.

Author’s Note

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CRISPR Coming to Fruition for California Citrus Growers

Yianni Lagos and Quinton Allen

Most growers have heard of CRISPR. It seems to have taken on a nebulous form as a panacea for all that plagues agriculture. It can be. The whole story of how CRISPR can benefit citrus growers in California is far more complex with significant development timelines, difficulties with certain varieties, and regulatory hurdles. With the help of funding from the National Science Foundation (NSF) and the United States Department of Agriculture (USDA), Soilcea, a CRISPR Innovator based in Tampa, Florida, has navigated the different obstacles to take the concept of CRISPR from great potential to tangible field trees that can help growers.

CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats, which does little to explain its use as a tool for breeding. Over billions of years, bacteria have developed sophisticated molecular tools—far superior to any man-made inventions—to protect against invading pathogens. Researchers have adapted the CRISPR tool to improve plants. The cutting tool in the CRISPR system, the Cas9 (CRISPR-associated protein 9), does an extremely precise cut that makes CRISPR editing one of the most versatile tools for breeding new resistant citrus cultivars. Rather than introducing foreign traits, CRISPR can restore the plant's existing biological defenses to create healthier citrus.



Figure 1. Example of the vigor displayed by Soilcea’s HLB-resistant CRISPR-edited rootstocks. Two-year-old mandarin hybrid scions grafted onto wild-type (left) and CRISPR-edited (right) Carrizo rootstocks that were exposed to ACP in a hoophouse for eight weeks before planting and grown under normal Florida field conditions.

CRISPR, however, needs the right targets to cut to become actionable. In the sweet orange genome, there are 319 million base pairs and 25,376 protein-coding genes (Wu et al. 2018). Even with all the citrus labs combined, there is not enough capacity to generate and test CRISPR edits across the entire citrus genome. In fact, testing more than a few different genes in a single experiment is difficult due to the necessity of multiple replicates and natural variability in psyllid infection and tree performance. That is why Soilcea looked to the research of Dr. Nian Wang and his team at the University of Florida (UF), who uncovered the underlying mechanisms of huanglongbing (HLB) or citrus greening disease (Wang et al. 2017). Other researchers in California, such as Dr. Wenbo Ma (formerly at University of California, Riverside) and Dr. Gitta Coaker (University of California, Davis), also provided significant contributions to these developments (Clark et al. 2018).

The mechanism of HLB infection involves the HLB-associated bacteria, *Candidatus Liberibacter asiaticus* (CLAs), using effector proteins that disrupt the citrus tree's natural defense pathways to generate the optimal environment for bacterial proliferation. We think of effector proteins as weapons that precisely attack citrus defenses to make a citrus tree the ideal home for CLAs to thrive. HLB is spread by the Asian citrus psyllid (ACP), when it transmits CLAs.

The Wang lab identified potential targets in citrus to disrupt CLAs effector proteins and restore the tree’s natural defenses and optimized CRISPR transformation methods for citrus. Soilcea exclusively licensed those technologies from UF and then went to work. Over the next eight years, Soilcea explored hundreds of CRISPR targets, created thousands of CRISPR-

edited trees, and tested those trees by reverse grafting¹ them on HLB-infected trees. Promising trees were then tested through traditional grafting of HLB-infected budwood onto edited rootstocks, ACP inoculation of trees, and Florida field trials in ACP infested locations, with many potential targets failing. Yet, this narrowed down our research to the best targets, eventually bringing together edits from the three most promising targets to develop a tree ready for growers: a CRISPR-edited Carrizo rootstock—CarriCea T1—that resists CLAs by targeting the effector interacting proteins to restore the natural plant defense of citrus. Soilcea has sent the CarriCea T1 rootstock to the California Citrus Clonal Protection Program in anticipation of research being conducted in California and commercial release.

The CRISPR-edited Carrizo rootstocks have undergone almost three years' worth of Florida-based field trials. The field trial in **Figure 1** shows how promising CRISPR-edited lines outperform the conventional or wild-type controls after almost three years in the grove. Results further show the CRISPR-edited Carrizo lines have significantly reduced bacterial levels compared to control trees (**Figure 2**). We have

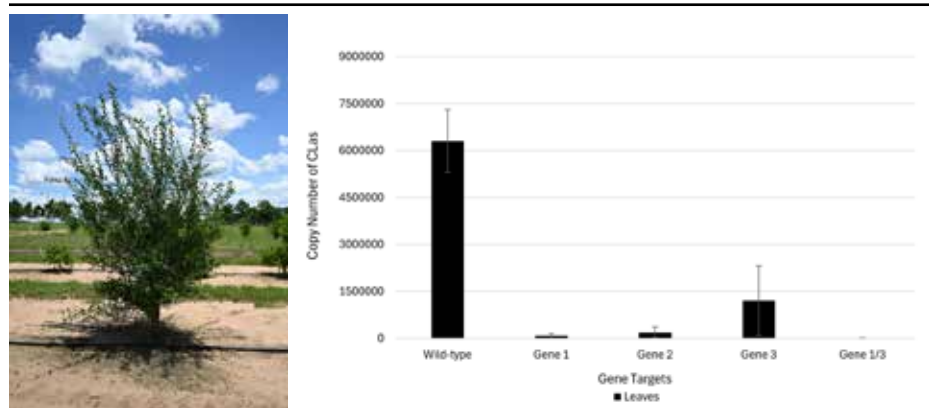


Figure 2. Photo of Carrizo rootstock with CRISPR editing (left). Graph shows the reduced CLAs copy number in several of Soilcea’s lines of HLB-resistant CRISPR-edited rootstocks that were exposed to ACP in a hoophouse for eight weeks before planting and grown under normal Florida field conditions (right).

extended the trial to five other locations in Florida and hope to secure additional funding to commence trials in Texas and California to evaluate the CarriCea T1 rootstock in different soils and different environmental conditions.

Our CRISPR research is ongoing with new rootstock and scion varieties and additional citrus targets. Soilcea is CRISPR-editing grapefruit to create new HLB and canker-resistant varieties for the entire United States citrus industry. Soilcea has successfully edited and regenerated Ray Ruby grapefruit for HLB resistance and is working in conjunction with Dr. Ahmad Omar at UF to establish callus lines and to use CRISPR to develop disease resistance traits in Star Ruby, Rio Red, and Ruby Red (**Figure 3**). Soilcea is also developing new sweet orange, mandarin, and rootstock varieties.

Additionally, Soilcea is the commercialization partner for Dr. Nian Wang’s research focused on CRISPR-editing a class of reactive oxygen-related proteins in citrus to assess their HLB-resistance and a project aimed at accelerating the development and release of HLB-resistant varieties. Soilcea also continues to discover and test new promising CRISPR targets across the CLas effector interactome in collaboration with Dr. Zhiyong Wang at the Carnegie Institution for Science located at Stanford University.

CRISPR has promise. CRISPR has potential. CRISPR has produced. Soilcea’s CRISPR trees are entering grower hands in Florida and will soon be available to growers in California. Soilcea is developing new CRISPR varieties important to the California industry and will continue using this powerful tool to ensure the future of the California citrus industry remains bright.

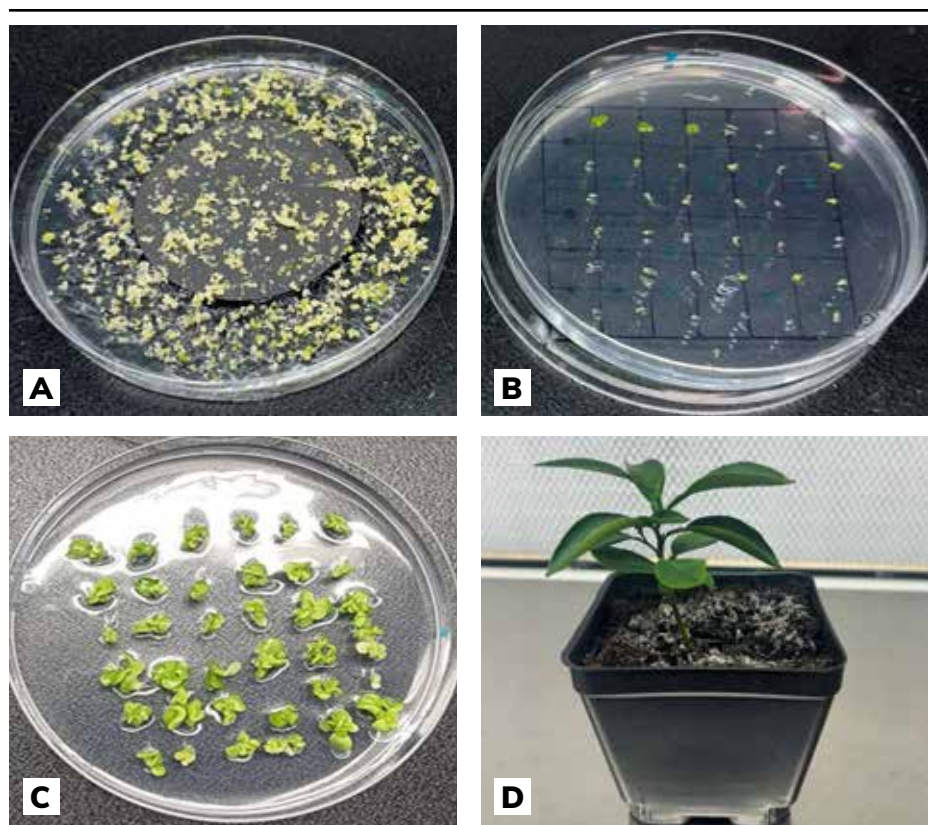


Figure 3. Tissue culture regeneration of CRISPR-edited grapefruit. (A) Callus. (B) Developing embryos. (C) Plantlets. (D) Plants in soil. Figure provided by Dr. Brendan Mormile, senior scientist at Soilcea.

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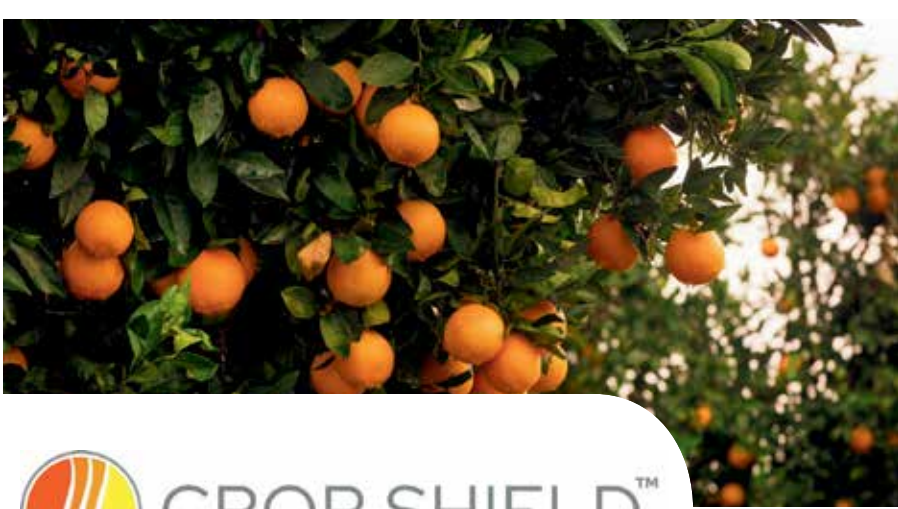
Glossary

Reverse Grafting: Horticulture technique where a rootstock is grafted to a tree that has HLB disease to test disease resistance of CRISPR-edited Carrizo rootstocks.

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