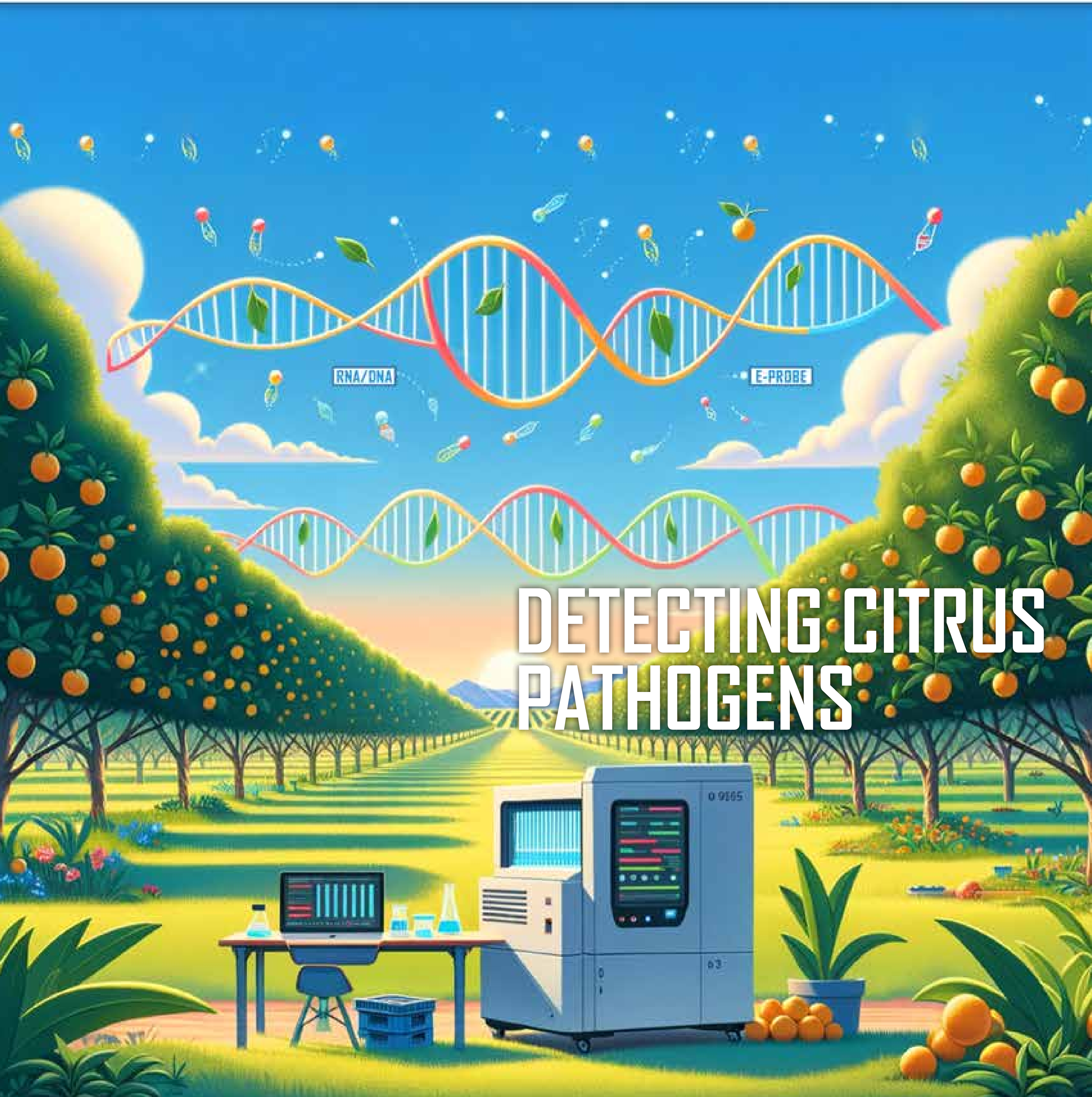
CITRUS RESEARCH BOARD

Citrograph

MAGAZINE

SPRING 2024



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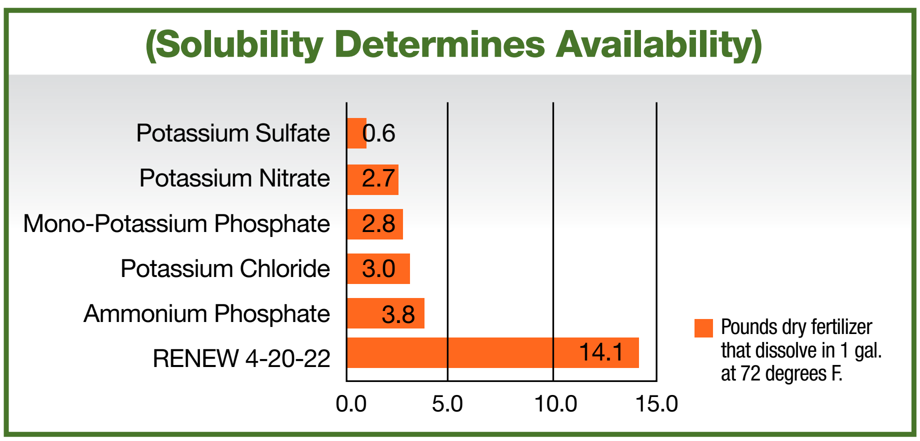

For more information including research results and scientific publications, contact;
Mark Brady, Western Marketing Manager, Plant Food Systems, Inc. (559) 731-1267

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***On the Cover:** The spring 2024 issue of* Citrograph *focuses on vectored citrus disease research and takes a look at some of the latest Citrus Research Board-funded projects in this area. The cover is a conceptual illustration of how advanced molecular biology and computer science are coming together to detect citrus pathogens. To learn more about these exciting new diagnostic tools and potential applications for the California citrus industry, please see "E-Probes Targeting Citrus Pathogens as a New Diagnostic Standard" by Sohrab Bodaghi, Ph.D., et al. on page 44.* (Visual: OpenAI, 2023)

In This Issue

Spring 2024 | Volume 15 • Number 2 The Official Publication of The Citrus Research Board

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

EDITOR'S NOTE: The photos that ran in "California-adapted *Tamarixia Radiata* for ACP Biological Control" in the winter 2024 issue of *Citrograph* on pages 48, 51 and 52 were courtesy of Mike Lewis of the University of California.

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.

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For more information, visit *www.irchlb.com*

MAY 7
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For more information, contact the CRB at (559) 738-0246 or visit *www.citrusresearch.org*

MAY 8
CITRUS PEST AND DISEASE PREVENTION COMMITTEE (CPDPC) MEETING
For more information, visit *www.cdfa.ca.gov/citruscommittee*

JUNE 4
2024 CITRUS RESEARCH BOARD GROWERS EDUCATIONAL WEBINAR SERIES
For more information and additional Webinar Series dates, see page 16.

AUGUST 6
CITRUS RESEARCH BOARD (CRB) MEETING
For more information, contact the CRB at (559) 738-0246 or visit *www.citrusresearch.org*

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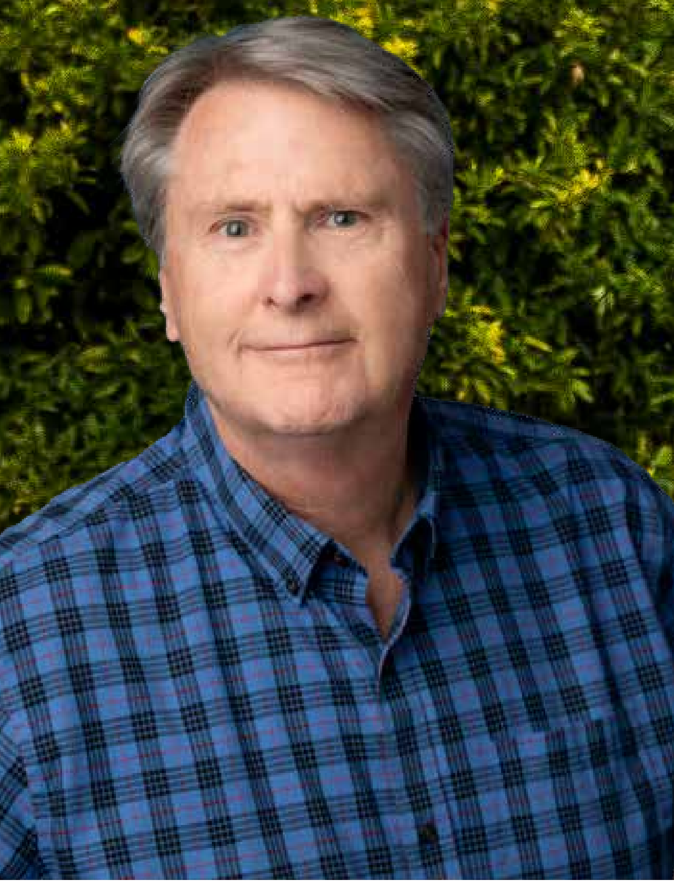
CHAIRMAN'S VIEW

Mark McBroom



First of all, I am incredibly honored and privileged to have the opportunity to chair and serve the Citrus Research Board (CRB) for the 2023-24 fiscal year. It seems we have gone from the frying pan into the fire, as new pests, expanded quarantine zones and new areas are impacted at every turn. We are seeing an increasing number of infected huanglongbing (HLB) trees in the southern regions; but as of this writing, we are still free of commercial groves in that count. How much longer that lasts depends on each of us and how diligent we stay at suppressing Asian citrus psyllid (ACP) and limiting the spread of the HLB bacterium, because the chance to move from infected trees to non-infected trees is limited. I am amazed that we have been as successful as we have been, due mainly to the complete and overwhelming unity within the industry to combat this killer disease. I thank all of you who are part of this effort. In 2008, as ACP finds began, it appeared HLB was where our focus should be. We now know that while HLB is a genuine and constant threat, it is not our only one. We are encountering a plethora of exotic fruit flies and outbreaks that are increasingly overwhelming growers throughout the state's southern region. Not only are pests our main challenge, but we also have the challenge of remaining productive and profitable to maintain California citrus's exceptionalism in the marketplace – not only here at home, but worldwide.

Mark McBroom

Under the leadership of Marcy L. Martin, Melinda Klein and all the staff, the CRB is in excellent hands. The dedication and commitment from our Research, Data, Communication, Accounting and Contracts departments continue to keep pace as we engage in many new concepts and work to support growers, shippers and marketers. We recently had an opportunity to develop a relationship between the U.S. Department of Agriculture (USDA) national breeding program and the USDA Agricultural Research Center in Parlier to expand the federal citrus breeding program into California. This project complements another citrus breeding program with the University of California (UC) system and its dedicated researchers throughout California. Our partners are just as dedicated to the California citrus growers as the CRB. Together, we will support our industry's growers' ability to thrive and continue farming for future generations. While we extend our partnerships with the UC and USDA systems to develop better rootstocks, scions and varieties, we are excited to see what successes remain for growers.

The CRB has support from private enterprises, helping us overcome many of the challenges we face, whether with new chemistry for pests, viruses and diseases that impact our groves or with production-related challenges. The CRB works closely with others to help with labor challenges in the field and the packinghouse. We have several projects exploring the early stages of picking or harvesting devices that we could only dream of years ago. In the shed, researchers are exploring more efficient ways for higher and better throughput into the carton and, ultimately, into the stores for the consumers. The CRB continues seeking novel concepts to enhance our farming practices, aiming to increase efficiency as resources become more difficult to count on. The CRB Board, along with our staff and partners, will continue our diligence in supporting the producers who trust us with their investments to find solutions to the industry's challenges.

We are grateful to have the relationships that continue to work with us, such as Secretary Karen Ross and the California Department of Food and Agriculture, especially Victoria Hornbaker and the staff at the Citrus Pest and Disease Prevention Division (CPDPD), who continue to be reliable in addressing the ongoing challenges that we as California citrus producers face. We are also fortunate to count on Casey Creamer and the California Citrus Mutual (CCM) team to confront legislative challenges, and we rely on their talents to ensure the industry remains viable. The continued partnership with Jim Cranney at the California Citrus Quality Council (CCQC) is essential to augment the export obstacles so that the CRB and the citrus industry can continue to be successful in international markets. There are countless other individuals I have not mentioned to whom we are grateful in this endeavor to conquer the giants we encounter.

It takes all of us as an industry to continue our unified work as the premier citrus producers the world has been accustomed to and relies upon. I will try my best to live up to the expectations and examples that previous chairmen, especially the most recent Justin Brown, have set to maintain and sustain your trust in the CRB.

Finally, I would like to thank you for this privilege, and with the grace of God and His guidance, keep your confidence in the CRB.

Mark McBroom serves as the chairman of the Citrus Research Board. For more information, contact **markm@bloomtobox.com**

VECTORED DISEASES

Caitlin Stanton

The spring issue of *Citrograph* explores the ongoing research in vectored diseases and work being done to combat the spread of huanglongbing (HLB) in California. For this edition of "Industry Views," we spoke with two industry veterans who have been at the forefront of the HLB fight to hear their perspective on the ongoing management of this industry threat.

JUSTIN BROWN
D BAR J ORCHARDS
Orange Cove, California

Fifteen years after HLB was first detected in California, have we made advancements managing an insect-vectored disease?

Yes, we've made advancements. There has been research done, including field trials that are ongoing with Dr. Ivan Milosavljević through the CA-CRaFT Project. We hope to learn a lot from that. From the beginning of HLB, there was the fear of a new thing, and we weren't sure what we were supposed to do. We then moved toward thinking about how [the disease] moves around and figuring out how it may affect California. The area-wide management coordination has been fairly successful in addressing any sort of flare-ups. The proof can be found in the regular reports released by the Citrus Pest Detection and Prevention Program (CPDPP) which detail finds in the Central Valley. In District 1, the number of insects found is very low, so we have done a good job of control measures. Weather also may be a contributing factor, but the vast majority of growers are taking it very seriously. They are treating when they need to, but overall doing a better job farming citrus and fighting other insects, such as California red scale, thrips and more. If there are psyllids present, we are wiping them out with our normal practices to create a beautiful piece of fruit, which the market demands. In total, the citrus industry has upped their game in the last 15 years in producing high-quality citrus; and as a by-product, we have done a good job maintaining our low psyllid product in the Central Valley.

Is the management of quarantines economically affecting citrus growers? If yes, how so?

In the beginning, trucking costs per bin increased a noticeable amount due to quarantine restrictions and the requirement to tarp loads of fruit crossing quarantine boundaries. Also, the spray and move program added an additional per acre cost to treat the grove being harvested and delivered outside of quarantine boundaries. Any grower utilizing the option to field clean and then tarp faced similar increases in costs. Costs from the trucking company, such as increased insurance to tarp trucks, and the cost of the tarps themselves, are passed along to the grower and result in an overall rate increase per bin to ship across quarantine boundaries. Locally, the across-the-board requirement for tarping every load of fruit increased per bin trucking costs; however, it was one of the best things that has happened. Juice trucks previously were exempt from tarping and were moving through heavy citrus growing areas with intact leaves, which I believe resulted in many ACP finds. Since the state-wide tarping mandate has been in effect, District 1 ACP finds fell tremendously because there were no longer psyllids moving freely through major corridors.

Another increased cost is complying with export protocols to satisfy exports to Korea. This is unrelated to psyllids, but I believe an ancillary benefit is that used materials were active on psyllids. Other costs can be seen at the packinghouse level, including better sanitation of equipment and picking bins, county insect monitoring compliance, spray and move coordination with growers and truckers, and new training for workers. The cost of picking a bin of fruit has gone up, mainly because of minimum wage, but there are those additional things that we are required to do to address issues such as food safety, governmental regulation, water issues and also psyllids. The economic impact of psyllid control is a component, but there are other factors that have a bigger impact on growing citrus. Each grower needs to make an individual decision on the procedures that make the most sense economically for their operation. Overall, we should not cut back on psyllid treatments. There may be more we could do, and current procedures for ACP control should continue to be the minimum. Other issues such as water, regulations and labor are what are truly squeezing farm-level returns in California. Ultimately, to grow citrus in California, one must grapple with critical issues on all fronts.

ETIENNE RABE
WONDERFUL CITRUS
Delano, California

Fifteen years after HLB was first detected in California, have we made advancements managing an insect-vectored disease?

This question can be answered in different ways depending on the point of departure one takes. I have the privilege to work across several citrus production regions in north America, including California, Texas and Mexico. I believe the issues and decline that the Florida industry is facing (and to an increasing extent in Texas) have brought the seriousness of the disease closer to home and ensured it is front of mind for many growers, specifically those of us serving the industry on various boards and committees.

From the first ACP finds in southern California (SoCal) and subsequent first finds of HLB in residential areas, we have learned a lot and adapted a lot. I am not sure that some of our adopted regulations have been instrumental in safeguarding our commercial citrus to the extent that we still do not have an HLB find to date. It is my opinion that it is somewhere in a commercial grove, and we just have not detected it yet. The tree removal program in SoCal residential areas, especially in some counties (Orange, Los Angeles), likely is not achieving much to reduce the reservoir of HLB-infected trees. It does not really make sense to spend millions of dollars to scout, diagnose and cull HLB-positive trees without any control of the vector ACP, something which is impossible in the SoCal residential region. It is estimated that approximately six million citrus trees, equivalent to 20,000 acres, are in the backyards of SoCal. The 6,000+ HLB trees removed so far pale in comparison. Has this lowered the HLB reservoir in SoCal? The jury on this is still out. The Science Advisory Panel, convened by the California Department of Food and Agriculture (CDFA) and CPDPP committee, has rightly advised that more effort should be spent on the commercial/residential interface in the risk-based survey, something that now has been implemented.

Can the current absence of HLB in commercial citrus be attributed to the tremendous efforts in time and millions of dollars contributed by the industry (via the CPDPP and CDFA)? Some of it likely is, specifically as it relates to ACP monitoring and quick action to eradicate/manage populations. We, however, also are blessed, specifically in the Central Valley, with a climate not very friendly to ACP and HLB with high summer heat and cold winters. The high heat with accompanying low relative humidity, adversely affects the life cycle of ACP and also may have a detrimental effect on the HLB organism. Additionally, we have less abandoned orchards in our industry, which can serve as reservoirs of infection. Our defined flushes and lack of summer rainfall assist in targeted sprays allowing for effective ACP management. Even if HLB starts showing up in commercial orchards (and we should not be surprised when it does), managing the vector, which we are capable of doing (assuming our chemical arsenal is not depleted soon), will certainly allow us to manage the disease so that it does not automatically sound the death knell to the California industry, as is the case in Florida.

Have we made advancements? YES!

Do we need to stay diligent? YES!

Do we need to start becoming more directed and scalpel-like in how we approach the future and spend our money? Definitely YES!

Is the management of quarantines economically affecting citrus growers? If yes, how so?

The short answer is yes. Various new rules and regulations have been put in place over the last number of years based on the knowledge gained from other regions. I list two of these.

1. **Spray and move program:** this is not just onerous on the growers, but also is sometimes done in the absence of ACP. It really is a safety-first approach. Unfortunately, especially in the case where multiple harvests need to be done on orchards (e.g., lemons and size-picking of mandarins), the 14-day rule between spray and end of "effectiveness" of the application cause our arsenal of chemicals to be put to the test to the extent that we might start seeing resistance. We need to learn from the experience of Brazil where they sprayed every 15 days, in anticipation of a solution which is not there yet, and now is on the precipice of HLB really crippling the industry due to resistance to the chemicals. I advocate against indiscriminate spray and move sprays going forward.

2. **Tarping of loads:** this practice to move fruit from orchard to packinghouse has been put in place due to information related to ACP finds along major transport corridors. Again, the jury likely is still out on this practice. Sceptics might argue that the tarping now keeps the ACP inside the load to be released at the packinghouse!

***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**

CITRUS RESEARCH BOARD

WEBINAR *Series* Returns this Summer

Caitlin Stanton

The Citrus Research Board (CRB) once again will host the Citrus Growers Educational Webinar Series in 2024. This informative series has been held annually since 2020 and provides an opportunity for growers around the state to hear the latest citrus research updates where it is most convenient for them—whether that be at home, in the office or from the groves. Previous webinars have featured an impressive line-up of extension and industry professionals, as well as many researchers funded by the CRB. Past topics included an update on pesticide laws and regulations, a report on California's water availability and specific research within the CRB's Core Research Programs of integrated pest management, variety evaluation, post-harvest disease management and new varieties. Following the live sessions, each webinar is made available on *www.citrusresearch.org* for growers to watch at any time.

Tune in to the four one-hour webinars, scheduled for June 4, 11, 18 and 25. Each session will highlight valuable research and practical discussions for growers. Continuing education units (CEUs) will be available through either the California Department of Pesticide Regulation or Certified Crop Advisers, pending approval. Please check *www.citrusresearch.org* for updates on topics and speakers.

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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CONTINUAL IMPROVEMENT IS CPDPC'S OPERATIONS SUBCOMMITTEE'S GOAL

John C. Gless

The movement of bulk citrus without proper tarping techniques and an ACP compliance agreement is prohibited.

As pests and diseases continue to threaten commercial citrus production and backyard citrus trees throughout California, it is more important than ever that we continue safeguarding our operations from the deadly impacts that the Asian citrus psyllid (ACP) and huanglongbing (HLB) can pose. This effort has been the sole focus of the Citrus Pest and Disease Prevention Committee (CPDPC) for years, and the operations subcommittee plays a crucial role in identifying ways that we – as an industry – can ensure that Citrus Pest and Disease Prevention Division (CPDPD) staff can continue their work efficiently, all while continuing to embracing scientifically proven methods to ensure that our citrus groves can continue to thrive for years to come. In 2022, the CPDPC commissioned a Science Advisory Panel to help explore innovations and efficiencies of our statewide program and it kick-started a series of improvements in the evolution of our program to ensure it meets our current needs.

Streamlining Mitigations While Minimizing Risk of ACP Movement

Since the beginning of our statewide program, the CPDPC and California Department of Food and Agriculture (CDFA) staff have worked to develop regulations that provide the highest level of safety, while balancing the operational burden on industry members. The operations subcommittee continually collaborates with CPDPD staff and other citrus industry partners to look at how ACP – and ultimately HLB – are moving throughout the state, so we can make better-informed decisions on what mitigation standards

should be in place to best protect citrus operations both inside of the HLB quarantine zone and outside of it. The resulting regulations – which allow for commercial citrus operations to continue operating safely, while avoiding the spread of the pest or disease – are typically developed via recommendations from the operations subcommittee and the CPDPC, which are then approved by the CDFA and enforced by the state or county authorities to curb the spread of the ACP and HLB.

As part of this ongoing evaluation of our regulations and operating procedures, in late 2023, the operations subcommittee and CPDPD staff worked to identify ways that growers can limit extra mitigation steps to move their fruit to packinghouses, while ensuring the risk of ACP or HLB spread is still low. After careful consideration, several updates were made to the CPDPD's official requirements for streamline mitigation steps to move bulk citrus fruit within and from an HLB quarantine area to a packer/processor by providing additional options for mitigation based on the best science available. The updated mitigation requirements may be found on the CDFA's website.

In addition, part of our efforts for continual improvement involves ensuring all industry members are aware of the role they play in safeguarding against the spread of ACP and HLB – not just the grower or packer. There are various compliance agreements set in place to ensure this effort; however, in the spring of 2023, the operations subcommittee supported the CPDPD's recommendation to add a compliance agreement for all harvesters/farm labor contractors (FLCs) in order to harvest citrus groves in California. Since field crews are at the forefront of preventing the spread of ACP between groves, we knew it was important to support any opportunity for additional industry members to stay abreast of ACP and HLB prevention best practices.

As a member of the citrus industry myself, I understand the pressing need to make processes more efficient without compromising on the procedures set in place that ultimately protect our citrus groves. By exploring and implementing easier pathways for bulk citrus movement such as the updated mitigation methods mentioned previously, our goal as an operations subcommittee was to identify ways we could alleviate some of the pressure that growers face daily, while concurrently slowing the spread of ACP across the state.

Communication is Key

We all must work together to save California citrus from ACP and HLB, which continue to be found in residential citrus trees throughout southern California at an increasing rate. Through enhanced collaboration, we aim to streamline processes, share critical information and collectively devise effective mitigation strategies to protect our citrus in an effective and efficient manner. As a committee, we want to see growers throughout California thriving – no matter the circumstance – and we will work to make decisions to make it happen. I look forward to continuing to serve as the operations subcommittee chair as we navigate the challenges of ACP and HLB management as an industry, all while preserving our vibrant citrus landscape for years to come. Many of these improvements begin with suggestions and ideas taken directly from conversations with citrus industry members like you. I encourage you to reach out to your designated committee member who represents your region and share your thoughts about how we – as a committee – can continuously work to improve. Visit *CitrusInsider.org* for a full list of committee members.

***John C. Gless is the operations subcommittee chair for the Citrus Pest and Disease Prevention Committee and also serves as vice chairman of the Citrus Research Board. For more information, contact* Jgless@bagdasarianfarms.com**

Farm labor contractors participate in training to learn how to stop the spread of ACP and teach the rest of the crews how to do the same.

Public service announcement in Spanish spreading awareness about the Asian citrus psyllid and huanglongbing.

Reaching Multicultural Audiences to COMBAT ACP AND HLB

Kevin Ball

California is the most populous state in the U.S. with more than 39 million people, which means reaching all those who play a role in the fight against the Asian citrus psyllid (ACP) and huanglongbing (HLB) is no small feat. For the citrus industry in California, ACP and HLB are top of mind, but the cooperation of California residents is critical in reducing the spread of ACP and HLB; and these diverse audiences are a key element of success in residential areas bordering the commercial citrus industry. This focus on residential tree growers is increasingly important as the intersection between residential communities and agricultural operations inches closer together in key citrus-growing regions. The Citrus Pest and Disease Prevention Program

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以免在無意間
傳播蟲害和疾病

Be careful not to spread insects and disease when gifting citrus this Lunar New Year

3 政府公共資訊 / **Public Information** January 25 - 31, 2023 風報 Wind Newspaper

< Agriculture officials track the presence of pests like the Asian citrus psyllid by placing yellow sticky traps in citrus trees throughout the state. When you allow these traps on your property, you help protect California citrus.
< 加州農業官員透過在全州的柑橘樹上放置黃色黏貼板，來追蹤亞洲柑橘木蝨等害蟲的存在。當您將黃色黏貼板放在您的柑橘樹上時，您就有助於保護加州柑橘。

請在今年農曆春節饋贈親友柑橘產品時特別留意
以免在無意間傳播蟲害和疾病

加州首府沙加緬度 – 2023年1月25 日 – 兔年已經到來！為確保您在今年春節饋贈親友的柑橘水果充分代表所象徵的財富、好運、和吉祥如意，柑橘害蟲及疾病預防計劃（Citrus Pest & Disease Prevention Program，簡稱CPDPP）建議您採取以下做法來保護加州備受關愛的柑橘的美好未來。

名爲亞洲柑橘木虱（Asian citrus psyllid，簡稱ACP)的昆蟲已在加州出現，而且已在全州傳播，威脅到居民自家栽種的果樹和全州的柑橘產業。這種害蟲能夠傳染黃龍病(Huanglongbing，簡稱HLB)，這是一種足以殺死柑橘樹的植物疾病。雖然這種病對人類或動物無害，但可使柑橘樹凋亡而且無藥可醫。

您可以從信譽良好的超市及商店買到健康的橘子、柚子、金橘和其他沒有害蟲和疾病的柑橘類水果 – 這是確保您在佳節期間送給親友的柑橘產品既乾净又優質的最佳方式。

自家樹木生產的柑橘當然也可以送禮，但是卻有傳播害蟲和疾病的較高風險。爲了小心起見，請先清除枝幹和樹葉，並且徹底清洗水果後，再將自家種植的柑橘送人。

遏阻黃龍病繼續蔓延的最佳方式，就是防範亞洲柑橘木虱的進一步傳播。目前有關單位已證實在横跨洛杉磯、橙縣、聖伯納蒂諾、聖地牙哥、和河濱縣超過4000株居民住家的柑橘樹上發現黃龍病的蹤跡。

加州全州自有住宅中，約60%栽種柑橘樹。屋主因此在保護加州柑橘上扮演重要角色，所以請您檢查一下後院的柑橘樹，查看是否有害蟲和疾病的徵兆。

CPDPP另外提供以下要訣，有助於預防亞洲柑橘木虱和黃龍病的傳播：

● 定期檢查樹木。按月檢查亞洲柑橘木虱（經常在新生嫩葉上出現）和黃龍病的徵兆，或是在每次澆水、噴水、和修剪樹木時進行檢查。請登入以下鏈接瞭解應特別注意的徵兆：https://californiacitrusthreat.org/pest-disease/

● 與農業部檢查人員合作。請與可能在您所在地區進行檢查工作的加州農業部官員合作。如果您看到農業部官員，這可能意味著亞洲柑橘木虱或黃龍病已經在您住家附近出現。

● 不要移動柑橘。不要將柑橘植物、莖葉、枝幹等帶進或帶出檢疫地區，甚至進出州界或是國界。請將其留在原地。

● 嫁接時請留意。嫁接柑橘樹時請務必使用已登記並具備來源證明文件的芽條。

● 不要讓螞蟻爬上您的柑橘樹。螞蟻會保護例如亞洲柑橘木虱等害蟲。請在柑橘樹附近放置捕捉螞蟻的誘餌，並遵照產品説明書使用。

黃龍病會影響所有柑橘類植物和部分柑橘類植物的親屬，例如月橘（Orange Jasmine）和咖喱葉。如果您發現亞洲柑橘木虱或黃龍病的蹤跡，請打全州熱綫電話：800-491-1899 通報。

有關如何預防亞洲柑橘木虱和黃龍病的進一步詳情，請登入 CaliforniaCitrusThreat.org，查閱柑橘害蟲及疾病預防計劃説明

* 柑橘害蟲及疾病預防計劃是由加州柑橘果農出資成立的計劃，由加州食品和農業部負責管理，目的在對抗威脅加州柑橘樹的嚴重蟲害與疾病。

自家樹木生產的柑橘當然也可以送禮，但是

Lunar New Year advertorial placements in English and Chinese bilingual *Wind Newspaper* urging residents to be cautious while celebrating with citrus.

(CPDPP) outreach team understands the diverse makeup of California residents and the importance of reaching residents throughout the state in an authentic way to spread awareness about ACP and HLB.

The CPDPP outreach team aligns its multicultural outreach strategies with culturally relevant themes, traditions, experiences and channels of communication in a variety of languages including English, Spanish, Vietnamese, Chinese and more, as these are among the top languages spoken in California. By making information regarding ACP and HLB readily available, relevant and easy to consume, the CPDPP is able to ensure residents are well equipped to protect California's citrus.

Celebrating Cultural Milestones to Connect with California's Asian Communities

The Lunar New Year and Mid-Autumn Festival are widely celebrated throughout the state by Asian-American residents; and for many Asian cultures, citrus fruits such as oranges, tangerines and pomelos are traditional gifts and decorations used to celebrate these holidays as they signify wealth, luck and prosperity. Seeing these as timely, culturally-relevant opportunities to reach these audiences and remind them of the dangers of ACP and HLB, the CPDPP outreach team developed proactive campaigns around each celebration to ensure residents celebrate safely with citrus as they gift or display the symbolic fruit.

The team developed articles that spread awareness about ACP and HLB and the dangers that come along with the pest and disease if homegrown citrus fruit is not carefully handled while celebrating. Best practices were shared on how to prevent the spread of ACP and HLB, and the articles encouraged residents to consider buying citrus fruit gifts from reputable grocers as another way to ensure they don't spread the pest and disease.

The articles were translated into Chinese and Vietnamese and complemented with symbolic imagery in alignment with each respective celebration. The advertorials were placed in notable Chinese and Vietnamese print and digital outlets throughout the state, including *World Journal, Nguoi Viet, Viet Bao, Wind Newspaper, Chinese L.A. Daily News, Nhật báo Cali Today and Sing Tao Daily*, all of which boast loyal readership.

B8 VIỆT BÁO THỨ SÁU 22-9-2023

HÃY CẨN THẬN KHÔNG GÂY LÂY LAN SÂU BỆNH
KHI TẶNG CAM QUÝT VÀO DỊP TRUNG THU NĂM NAY

Tết Trung Thu đã gần kề! Trong khi bánh trung thu là quà tặng phổ biến nhất trong mùa lễ này, thì các loại trái cây họ cam quýt như bưởi cũng rất phổ biến vì hình dạng của nó tượng trưng cho mặt trăng và tên của nó trong tiếng Trung Hoa có âm tương tự như "phúc lành". Chúng cũng rất được mùa vào thời điểm này trong năm.

Để đảm bảo trái cây giống cam quýt quý vị cắt hái đem đi tặng người thân thể hiện phước lành sung túc như những lời chúc lành, hãy bảo đảm rằng quý vị làm theo đúng phương pháp khi thu hoạch chúng để bảo vệ giống cây cam cây quýt yêu quý của California trong nhiều năm tới.

Một loại côn trùng có tên là rầy chổng cánh châu Á (ACP) đã được phát hiện ở California trên khắp tiểu bang, đe dọa giống cây có múi trong nước và ngành công nghiệp cam quýt của tiểu bang. Loài côn trùng gây hại này có thể lây lan một loại bệnh thực vật nguy hiểm có tên là Huanglongbing (HLB), làm chết cây có múi. Mặc dù không gây hại cho con người và động vật nhưng căn bệnh này gây tử vong cho cây có múi và hiện không có thuốc chữa.

Quý vị có thể mua quýt, bưởi, quất và các loại trái cây có múi khỏe mạnh khác không có côn trùng và bệnh tật từ các cửa hàng tạp hóa có uy tín - đây là cách tốt nhất để đảm bảo rằng quý vị đang tặng những quả cam quýt sạch, chất lượng cao cho bạn bè hoặc người thân trong gia đình của mình trong dịp mùa lễ này. Cây có múi trồng tại nhà cũng có thể được tặng nhưng có nguy cơ truyền sâu bệnh cao hơn. Để thận trọng, hãy loại bỏ lá và thân của những quả có múi trồng tại nhà và rửa thật sạch trước khi đem đi tặng.

Cách tốt nhất để ngăn chặn sự lây lan của căn bệnh HLB là ngăn chặn sự lây lan của giống sâu bệnh ACP. HLB đã được xác nhận ở hơn 6,000 cây có múi ở các quận Los Angeles, Orange, Riverside, San Bernardino và San Diego.

Chủ nhà ở California – 60% trong số họ sở hữu cây có múi – đóng vai trò quan trọng trong việc bảo vệ cây có múi ở tiểu bang của chúng ta và họ được yêu cầu phải bảo vệ cây ở sân sau của họ bằng cách tìm kiếm các dấu hiệu của sâu bệnh.

Chương trình Phòng Chống Dịch Bệnh và Sâu Bệnh Trên Giống Cây Có Múi cung cấp cho quý vị các hướng dẫn sau đây để giúp ngăn chặn sự lây lan của ACP và HLB:

- **Kiểm tra cây thường xuyên.** Kiểm tra các triệu chứng ACP (thường thấy khi lá mới mọc) và các triệu chứng HLB hàng tháng hoặc bất cứ khi nào bạn tưới nước, phun thuốc hoặc tỉa cây. Tìm hiểu thông tin cần thiết ở đây: https://californiacitrustthreat.org/pest-disease/
- **Hợp tác với các quan chức nông nghiệp.** Hợp tác với các quan chức nông nghiệp của tiểu bang và quận, những người có thể đang kiểm tra cây cối trong khu vực của quý vị. Nếu quý vị thấy một quan chức nông nghiệp, điều đó có thể có nghĩa là loài sâu bệnh ACP hoặc căn bệnh HLB đã được tìm thấy gần nhà quý vị.
- **Đừng di chuyển cam quýt.** Không di chuyển cây, lá hoặc tán lá của giống cây có múi vào hoặc ra khỏi khu vực cách ly hoặc xuyên biên giới tiểu bang hoặc quốc tế. Giữ cây trong phạm vi địa phương.
- **Ghép thận trọng.** Chỉ sử dụng những chồi đã ghi danh có tài liệu nguồn gốc rõ ràng khi cấy ghép cây có múi.
- **Đuổi kiến khỏi cây cam quýt.** Kiến bảo vệ các giống sâu bệnh gây hại như ACP. Đặt mồi kiến xung quanh cây có múi và làm theo hướng dẫn trên nhãn sản phẩm.

HLB gây ảnh hưởng đến tất cả các loại cây cam quýt có múi và một số họ hàng của cây có múi, bao gồm cả cây hoa nhài màu cam. Nếu quý vị nghĩ rằng mình đã tìm thấy loại sâu bệnh ACP hoặc bệnh HLB, hãy gọi đến đường dây nóng toàn tiểu bang theo số 800-491-1899.

Để biết thêm thông tin về cách phòng ngừa ACP và HLB, hãy truy cập https://californiacitrusthreat.org/vietnamese/

Mid-Autumn Festival advertorial placement in Vietnamese-language daily newspaper *Viet Bao* encouraging residents to celebrate with citrus safely.

To further amplify these messages, the outreach team coordinated the development of targeted social media ads further encouraging residents to celebrate safely with citrus during culturally significant celebrations.

Recognizing Citrus Significance During Hispanic Heritage Month

In honor of Hispanic Heritage Month, the CPDPP outreach team developed a radio campaign to secure placements in Spanish-language stations throughout the state with a key focus on the significance of citrus in the Hispanic culture and the level of importance residents should place on being vigilant in looking for ACP and HLB to keep citrus thriving for years to come.

A radio ad was developed from the point of view of an abuelita (grandma), written with the audience's humor in mind while highlighting our key messages of encouraging residents to protect their trees against ACP and HLB. The radio ad ran on Spanish radio stations throughout the state, focusing on markets that broadcast to major citrus-growing regions, and regions with prominent ag-urban interfaces including Bakersfield, Ventura, Santa Barbara, Riverside and San Bernardino.

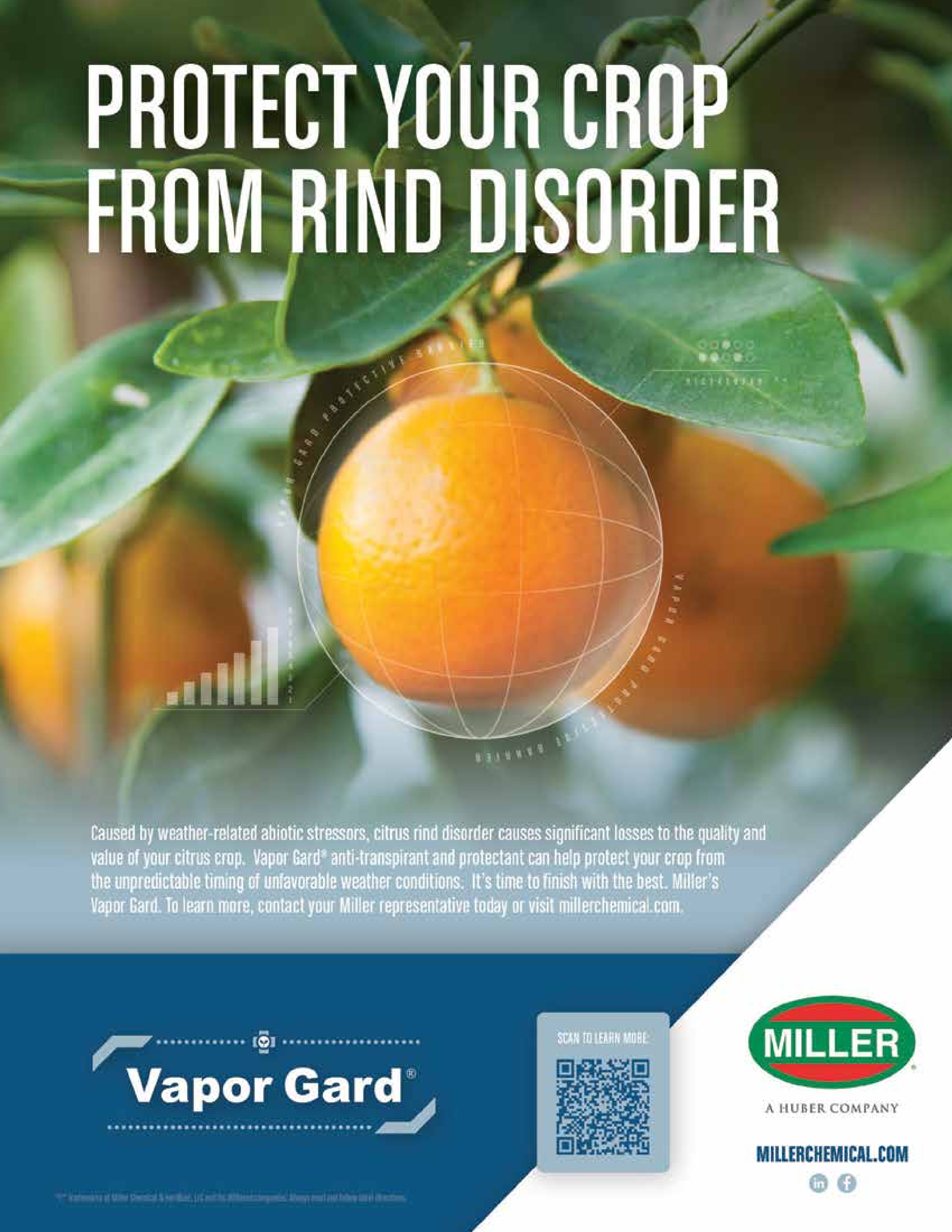
Casting a Wider Net with Broadcast

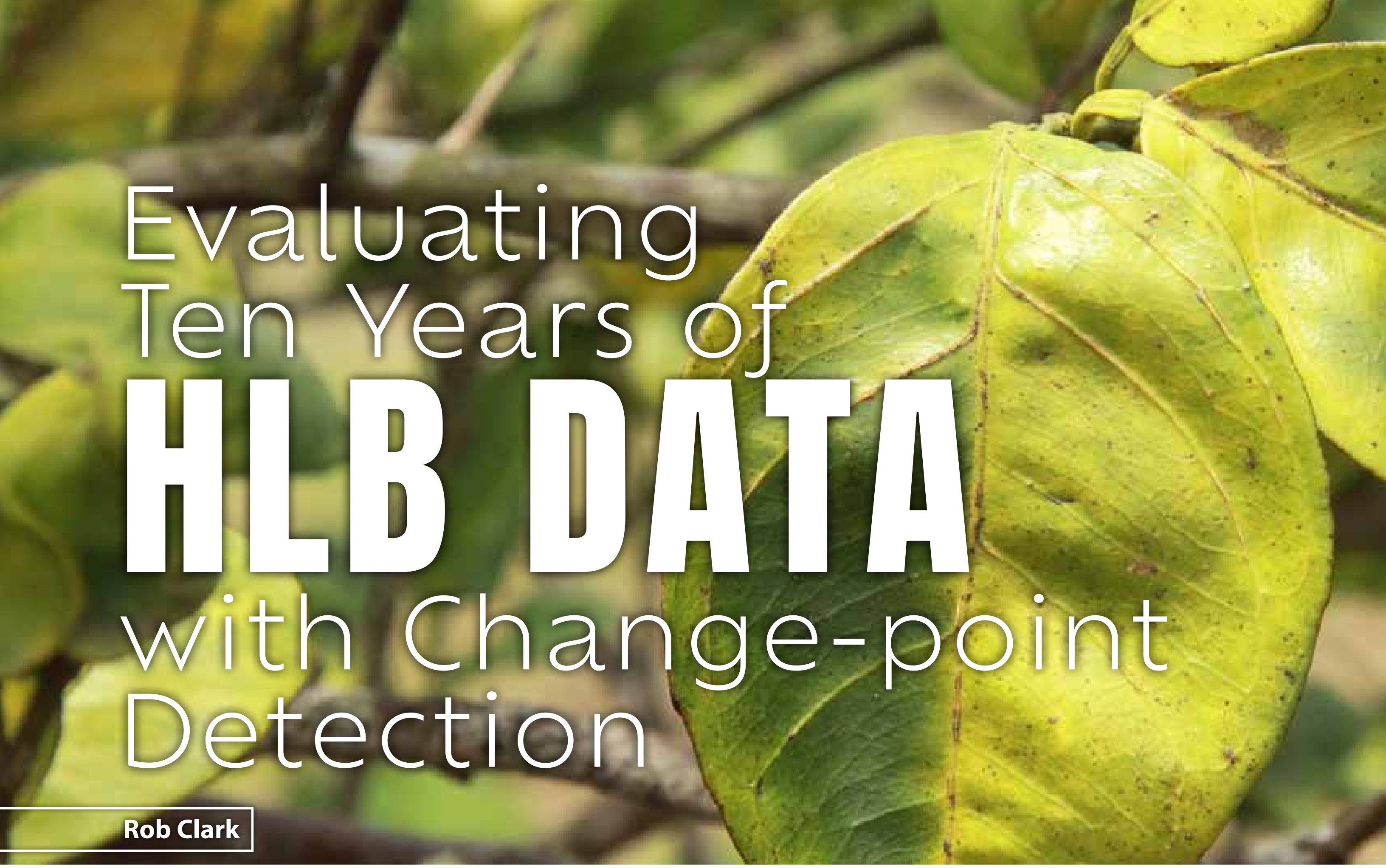
According to Nielsen, Hispanic individuals are avid audio consumers as 94 percent of Hispanic individuals ages 18-49 listen to AM/FM radio each month. Mexican and Spanish Adult Contemporary are the top two formats among Hispanic adults 18 and older. To reach this key audience – representing more than 39 percent of the state's population – the outreach team coordinated the distribution of a statewide in-language audio news release to Spanish broadcast outlets. The release focused on a 2023 U.S. Department of Agriculture report that predicted California's orange production numbers are expected to exceed Florida's this crop year and using this as a warning signal for residents to stay vigilant for ACP and HLB. Additionally, the CPDPP's television public service announcement was distributed to Spanish broadcast outlets throughout the state, spreading general awareness about ACP and HLB.

Combating the spread of ACP and HLB is a collaborative effort that requires buy-in from all stakeholders. Communicating with key audiences in an accessible, digestible way is critical to getting the diverse residential population of California on board to keeping the state's citrus healthy and thriving for years to come. By working together, we can all save California's citrus.

***Kevin Ball is the outreach subcommittee chair for the Citrus Pest and Disease Prevention Committee. For additional information, contact Kevin at* kevin.ball@aglandca.com**

Targeted social media posts on Facebook and Instagram wishing residents a happy Lunar New Year and sharing best practices for how to celebrate safely with citrus.


PROTECT YOUR CROP
FROM RIND DISORDER
Caused by weather-related abiotic stressors, citrus rind disorder causes significant losses to the quality and value of your citrus crop. Vapor Gard® anti-transpirant and protectant can help protect your crop from the unpredictable timing of unfavorable weather conditions. It's time to finish with the best. Miller's Vapor Gard. To learn more, contact your Miller representative today or visit millerchemical.com.
Vapor Gard®
SCAN TO LEARN MORE:
MILLER®
A HUBER COMPANY
MILLERCHEMICAL.COM



Evaluating Ten Years of HLB DATA with Change-point Detection

Rob Clark

Project Summary

*More than ten years of survey data have been collected on huanglongbing (HLB) and the Asian citrus psyllid (ACP), which vectors the presumed causal agent of HLB, '*Candidatus *Liberibacter asiaticus' (CLas), as part of monitoring programs. Since detecting the first CLas-infected citrus tree in California, monitoring programs have yielded a complex dataset that can be used to evaluate long-term trends in HLB and the timing of increased disease incidence. The use of change-point detection as a statistical tool is proposed to evaluate when HLB detection rates change over time and if these increases coincide with any new lab or field methods. While there always are more variables to be examined, it is the author's opinion that current increases in disease incidence are not the byproduct of sampling methods and may represent a recent increase in the prevalence of HLB.*

About the Data

Extensive management efforts were enacted to reduce the incidence of HLB to commercial citrus groves after it was first detected in March 2012 in an urban citrus tree in Los Angeles County. As part of these management efforts, regulatory diagnostic tests were conducted by the state of California. Both commercial and residential citrus trees have been surveyed for CLas, as well as ACP; and since December 2012, CLas-positive diagnostic tests have been reported in more than 800 ACP and 6,000 residential trees as part of a large-scale program. Linking these data to ten years of surveys and lab testing, we can pinpoint the specific dates at which HLB disease incidence has increased. Distinguishing the "signal from the noise" allows us to identify if increases in HLB detections legitimately indicate the outbreak is expanding or are the result of other factors, such as modifications to protocol that have occurred since this survey program began.

Change-point Detection

As a statistical tool, change-point detection informs us when rates of disease incidence change with breakpoints (Kim et al. 2008). Breakpoints are a single time point plus or minus a margin of error. In epidemiology, change-point detection helps determine upward or downward trends in disease incidence through time (e.g., Boudou et al. 2021). The timing of these upward or downward trends can be compared to the known dates of critical events like the discovery of new counties with CLas-positive trees or the start of a new survey protocol, such as resampling of leaf tissue. New county detections, in particular, are important inflation points in change-point detection since they represent an uncovering of CLas-positive trees that contributes to the total number of reported cases occurring as part of testing during delimitation surveys when new quarantine zones are established. These delimitation surveys can contribute a large number of new CLas-positive trees over a short time period when they occur. Change-point detection was used to examine CLas-positive detections in ACP and plant tissue samples at monthly timesteps as part of the modeling process. Change-point detection discovered four significant trend changes in rates of CLas-positive ACP and trees.

Sequence of Events

The first documented detection of ACP in California occurred in 2008 in San Diego County (Byrne et al. 2018). For ACP, the first upward trend in cases started with two CLas-positive ACPs found in Orange County in March 2017 (**Figure 1**). Prior detections of CLas-positive ACP were limited to Los Angeles County. CLas-positive ACP detections increased at this time in early 2017 before leveling off later in the year (**Figure 1**). Change-point detection identified more detections starting in early 2022 (**Figure 1**) before going back to a lower baseline in late 2023 **(Figure 1**).

The first detection of a CLas-positive tree in a residential area did not occur until 2012 in Los Angeles County (CDFA 2012), with all subsequent detections occurring in residential areas, as well. Five years later, in 2017, CLas-positive trees further were identified in Orange and Riverside counties (CDFA 2017a,b). In 2019, a CLas-positive tree was identified in San Bernardino County. Shortly thereafter, the number of CLas-positive trees steadily increased until early 2019 (CDFA 2019), followed by another CLas-positive tree in San Diego County in 2021 (CDFA 2021). It has been conjectured that this increase was the byproduct of increased survey efforts; however, through this time period, surveys and total number of tests actually decreased between 2018 and 2019 (CDFA 2023b). This occurred prior to the impacts of COVID-19 lockdowns (**Figure 1**, **Figure 2**). An increase in detection of CLas-positive trees started in January 2021. Increased subsampling of leaves, roots and peduncle tissue began as part of diagnostic testing by the state of California in December 2020 (**Figure 2**). While this event may explain a flat increase in reported disease incidence, it does not fully explain the subsequent increases in CLas-positive trees starting in December 2022 (**Figure 2**). Most recently, a CLas-positive tree was identified in Ventura County in September 2023 (CDFA 2023a).

Conclusions

Change-point detection has provided an opportunity to evaluate major events in HLB disease incidence in California, particularly in the last few years. The proposition that 2017's higher rates of CLas-positive ACP (**Figure 1**) and CLas-positive tree detections (**Figure 2**) coincide with Orange County is supported. However, lab resampling of plant tissue may explain an increase that started in 2021, but this does not coincide with the current spike in detections that started in December 2022 (**Figure 2**). In fact, 2023 looked to be a record year, contributing 30 percent of all known detections in California with the highest CLas-positive tree monthly total of 307 in June (**Figure 2**), and 76 Clas-positive ACP detected in February (**Figure 1**). All potential factors causing more CLas-positive tree detections have not yet been examined,

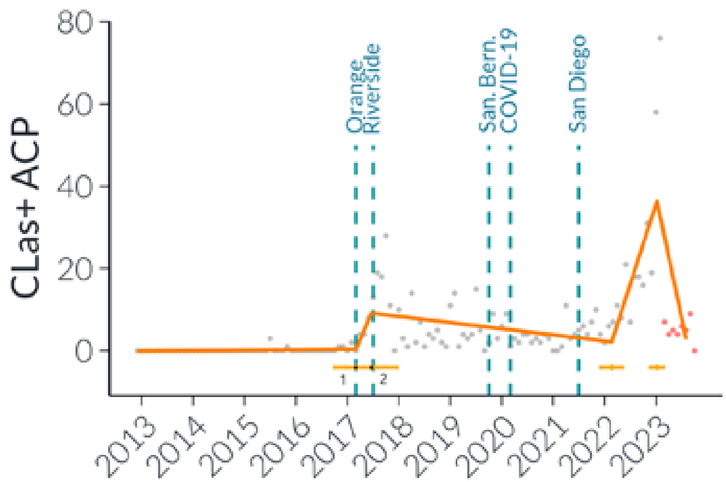


Figure 1. Total monthly counts of *'Candidatus* Liberibacter asiaticus'-positive (CLas+) Asian citrus psyllid (ACP) [Y-axis] from December 2012 - October 2023 [X-axis]. Vertical lines indicate the first CLas-positive tree detection for each county and the first COVID-19 lockdowns in California. Circles are the monthly number of detections, with the orange line indicating the change-point detection model. Change-points are shown with horizontal bars at the bottom of the figure. The first two change-points have overlapping error bars and are labeled "1" and "2". San Bernardino = San. Bern.

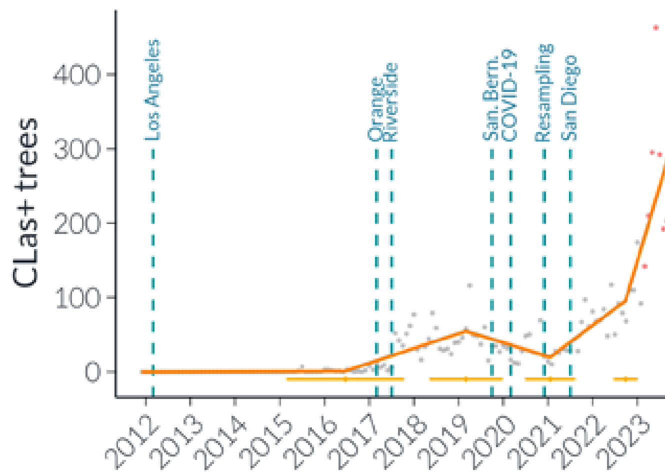


Figure 2. Total monthly counts of *'Candidatus* Liberibacter asiaticus'-positive (CLas+) trees [Y-axis] from December 2011 - October 2023 [X-axis]. Vertical lines indicate CLas-positive detection in trees for each county, the first COVID-19 lockdowns in California, and the adoption of plant tissue resampling alongside peduncle and root tissue sampling ("Resampling"). Circles are the monthly number of detections, with the orange line indicating the change-point detection model. Change-points are shown with horizontal bars at the bottom of the figure., with red points being 2023 data currently under analysis. San Bernardino = San. Bern.

such as the relative contribution of delimitation surveys to total counts following the discovery of new CLas-positive trees. Further data will be collected into 2024, so it remains to be seen if the recent spike in detections will go back down to a baseline like Clas-positive ACP (**Figure 1**) or if the upward trend will continue.

References

Boudou M.; ÓhAiseadha, C.; Garvey, P.; et al. 2021. Breakpoint modelling of temporal associations between non-pharmaceutical interventions and symptomatic COVID-19 incidence in the Republic of Ireland. PLoS ONE 16(7):e0255254. *https://doi.org/10.1371/journal.pone.0255254*

Byrne F.J.; Grafton-Cardwell, E.E.; Morse, J.G.; et al. 2018. Assessing the risk of containerized citrus contributing to Asian citrus psyllid (*Diaphorina citri*) spread in California: Residence times and insecticide residues at retail nursery outlets. *Crop Protection* 109:33–41.

CDFA. (2012) "Citrus disease huanglongbing detected in Hacienda Heights area of Los Angeles County." Available at: *https://www.cdfa.ca.gov/egov/Press_Releases/Press_Release.asp?PRnum=12-012* (Accessed: December 21, 2023).

CDFA. (2017a) "Citrus disease huanglongbing detection and quarantine in Orange County." Available at: *https://www.cdfa.ca.gov/egov/Press_Releases/Press_Release*.asp?PRnum=17-025 (Accessed: December 21, 2023).

CDFA. (2017b) "Quarantine for citrus disease huanglongbing extends to portions of Riverside and San Bernardino counties." Available at: *https://www.cdfa.ca.gov/egov/Press_Releases/Press_Release.asp?PRnum=17-051* (Accessed: December 21, 2023).

CDFA. (2019) "Detection of citrus disease huanglongbing triggers quarantine expansion in portions of San Bernardino and Los Angeles counties." Available at: https:/*/www.cdfa.ca.gov/egov/Press_Releases/Press_Release.asp?PRnum=19-114* (Accessed: December 21, 2023).

CDFA. (2021) "Detection of citrus disease, huanglongbing, in San Diego County establishes new quarantine area." Available at: *https://www.cdfa.ca.gov/egov/Press_Releases/Press_Release.asp?PRnum=21-091* (Accessed: December 21, 2023).

CDFA. (2023a) "Detection of citrus disease in Ventura County establishes new quarantine area." Available at: *https://www.cdfa.ca.gov/egov/press_releases/Press_Release.asp?PRnum=23-173* (Accessed: December 21, 2023).

CDFA. (2023b) "Meeting of the California Department of Food and Agriculture California Citrus Pest and Disease Prevention Committee." Available at: *https://www.cdfa.ca.gov/citrus/docs/committee/2023/20231108_Supporting_Materials-CPDPC.pdf* (Accessed: December 21, 2023).

Kim H.J.; Yu, B.; Feuer, E.J. 2008. Inference in segmented line regression: as simulation study. *Journal of Statistical Computation and Simulation* 78:1087-1103.

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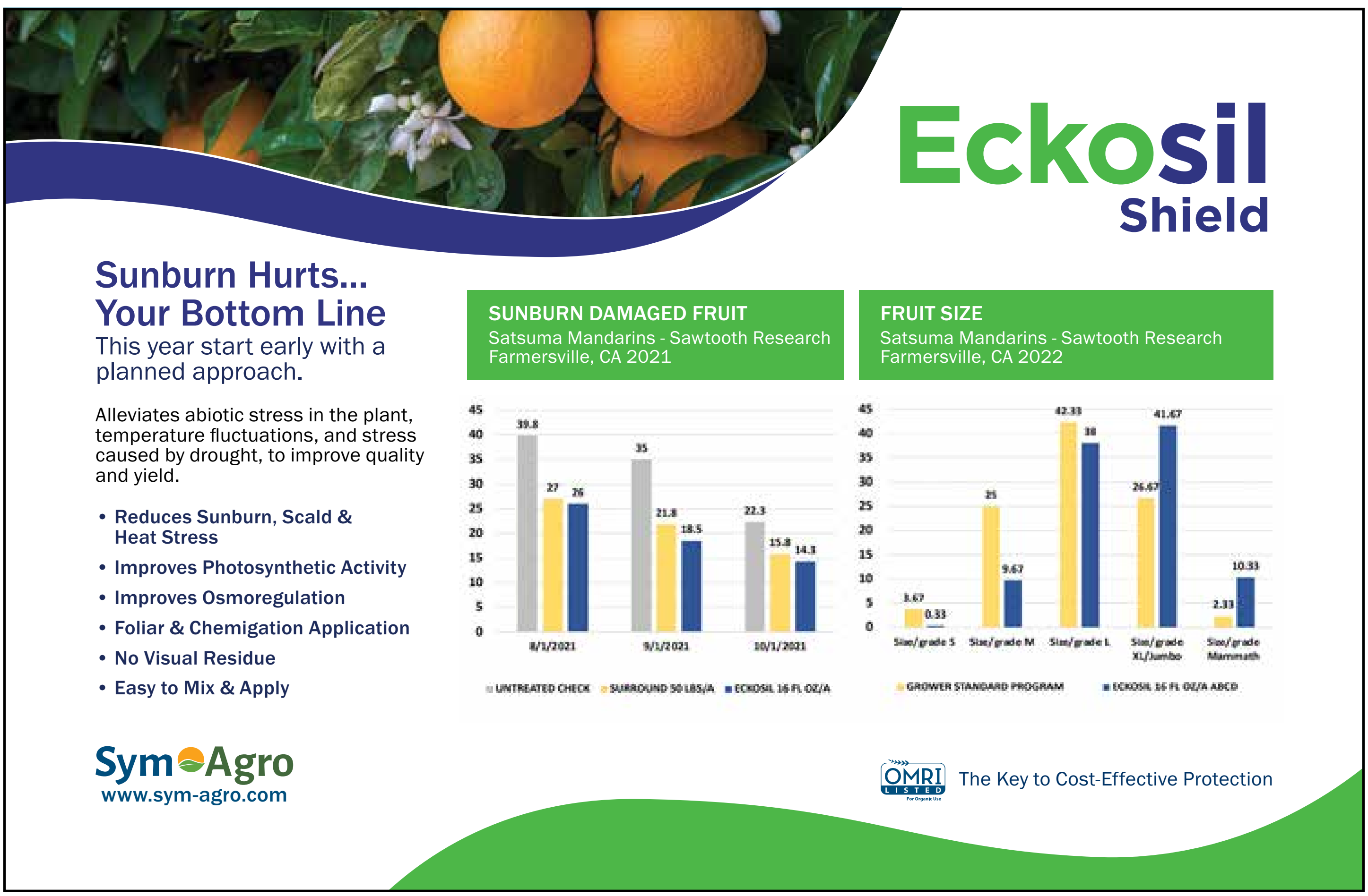

CITRUS YELLOW VEIN CLEARING VIRUS RESEARCH IN CALIFORNIA

UPDATE ON WORK AT THE SAN JOAQUIN VALLEY AGRICULTURAL SCIENCES CENTER

Yongduo Sun, Sydney Helm Rodriguez and Raymond Yokomi

Figure 1. Symptoms of *Citrus yellow vein clearing virus* in a dooryard lemon tree in Tulare, California, clearly showing yellow veins and leaf distortion.

Project Summary

Citrus yellow vein clearing virus *(CYVCV) is the causal agent of an exotic citrus disease found in 2022 in citrus trees on residential properties in Tulare, California. CYVCV has been reported to induce 20 to 80 percent yield loss in lemon production in China (Zhang et al. 2019). Although the impact of CYVCV in California is unknown, the spread of CYVCV may pose a threat to the California citrus industry. The objective of this project is to characterize the CYVCV California strain and to determine its origin and mode of transmission. The CYVCV strain collected in California was propagated successfully in six different citrus cultivars in the greenhouse. Based on real-time Reverse Transcription quantitative Polymerase Chain Reaction[1] (RT-qPCR), all inoculated cultivars supported CYVCV replication. Eureka lemon and sour orange showed typical symptoms of yellow vein clearing, whereas sweet orange, grapefruit, mandarin, Alemow (*Citrus macrophylla*) and citron were asymptomatic. We collected six isolates of CYVCV from different properties and maintained them in the greenhouse. Whole virus genomes and coat protein sequences of three isolates were determined and compared with the global public database of CYVCV sequences. The California CYVCV isolates[2] sequenced formed a distinct group that shares the most recent common ancestry with Indian CYVCV isolates. Spirea and cotton aphids transmit the California CYVCV strains, while studies into citrus whitefly transmission are currently in progress. An infectious cDNA clone[3] named 'CYVCV-CA1' has been developed that will accelerate our research on the etiology and host range of CYVCV.*

CYVCV Disease Status

The first occurrence of citrus yellow vein clearing disease worldwide was reported in lemons and sour oranges from Pakistan in 1988. Since then, it has been detected in Turkey, India, Iran, China and South Korea (Liu et al. 2020). CYVCV was detected in Tulare (**Figure 1**) by the California Department of Food and Agriculture (CDFA) while conducting the Multi-Pest Survey, a part of the U.S. Department of Agriculture (USDA) Citrus Health Response Program (CHRP); and the virus was confirmed by the USDA on April 1, 2022. This discovery is the first detection of CYVCV in the Western Hemisphere. Currently, the CDFA is following CHRP protocols by conducting delimiting surveys to determine the extent of the CYVCV infestation. So far, CYVCV has not been detected in commercial citrus orchards. According to a CDFA report presented at a Citrus Pest and Disease Prevention Committee meeting held on November 8, 2023, a CYVCV Regulatory Response and State Interior Quarantine will be proposed through the regular rulemaking process.

CYVCV Research Activities Underway

Research on the California CYVCV isolates was initiated in October 2022 to examine vector transmission and spread of CYVCV and to assess the disease potential of CYVCV. Six CYVCV isolates from different citrus cultivars and Tulare properties identified by the CDFA were established and maintained in the containment greenhouse at the USDA-Agricultural Research Service - San Joaquin Valley Agricultural Sciences Center in Parlier, California. The virus was propagated in six different citrus cultivars, and all inoculated plants supported CYVCV replication based on RT-qPCR. Eureka lemon and sour orange showed typical strong symptoms of CYVCV (**Figure 2**); whereas sweet orange, grapefruit, Alemow and citron were asymptomatic. In the greenhouse, RT-qPCR testing of symptomatic hosts like Eureka lemon and sour orange produced the highest (20,000 and 17,000 copies, respectively) levels of target viral genomic RNA upon quantitative testing. Asymptomatic

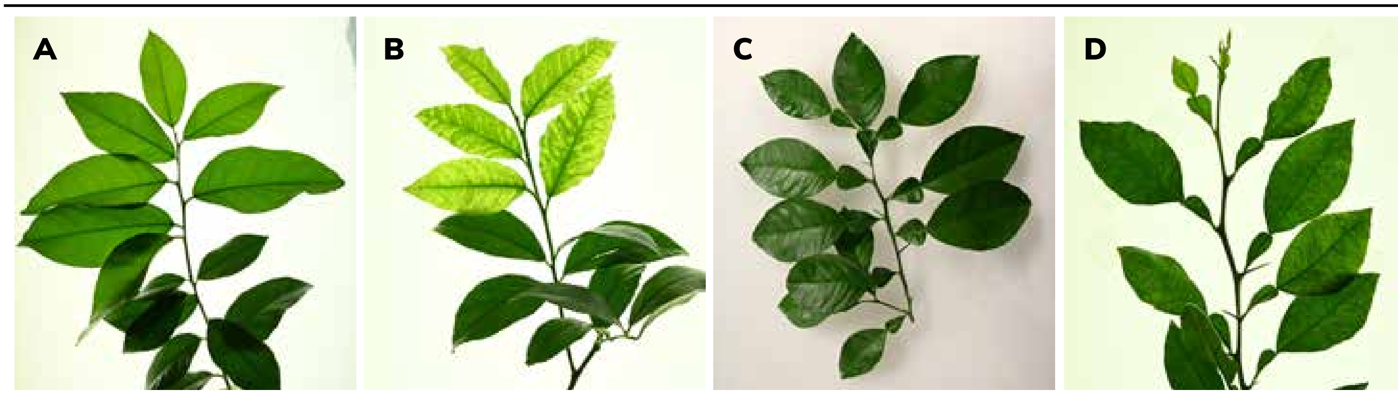

Figure 2. Greenhouse symptoms of *Citrus yellow vein clearing virus* in Eureka lemon (A. healthy; B. infected); and Sour orange (C. healthy; D. infected).

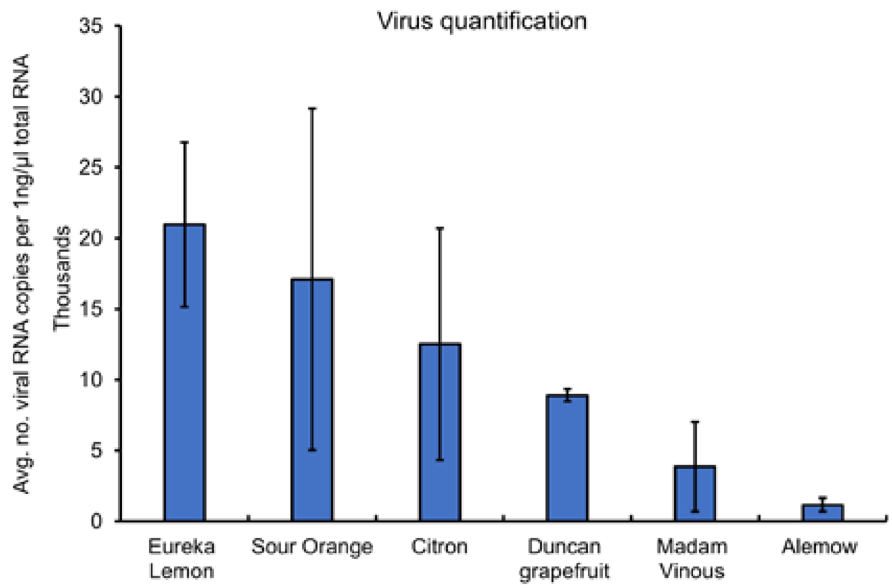

Figure 3. Quantitation in copies of target *Citrus yellow vein clearing virus* RNA from 150 milligrams of fresh tissue from different citrus hosts tested one year after graft inoculation in the greenhouse. Error bars = Standard deviation.

hosts like citron and Duncan grapefruit produced lower (10,000 to 15,000 copies) levels of CYVCV, whereas Madam Vinous sweet orange and Alemow produced the least (5,000 copies) amount of CYVCV (**Figure 3**). We currently are expanding this research to include mandarin and many more commercial cultivars. However, our data so far shows that many citrus cultivars can support CYVCV replication, regardless of the presence or absence of symptoms. This is significant because these hosts can serve as a virus reservoir for CYVCV acquisition and spread by insect vectors.

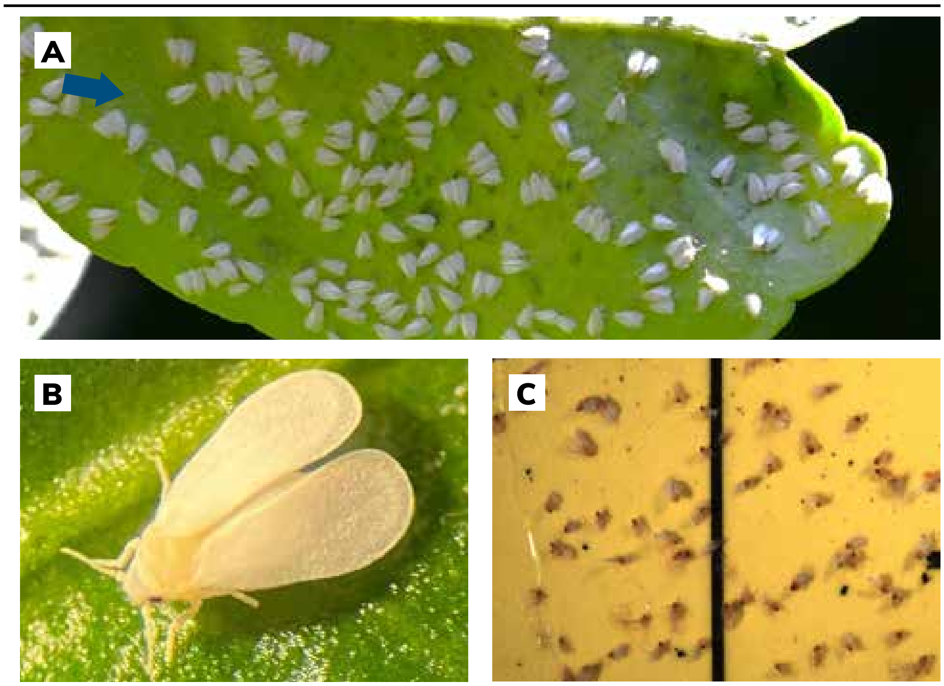

Figure 4. Citrus whitefly (*Dialeurodes citri*). A. Adults and small whitish eggs (arrow) of the citrus whitefly. B. Citrus whitefly adult. C. Citrus whiteflies trapped on yellow sticky trap.

Since the CYVCV is exotic and known to occur in east and south Asia and Middle East regions, it is important to ascertain the origin of the virus found in Tulare. Therefore, we determined the complete genome sequence of three CYVCV isolates collected from different hosts and properties in Tulare (Sun and Yokomi 2023). The sequences of our isolates ranged from 95.4 percent to 97.4 percent identity to the 54 complete CYVCV genome sequences listed in GenBank. This indicated a relatively high genetic similarity. However, the CYVCV isolates we sequenced formed a distinct group when aligned against other complete CYVCV genomes and CYVCV coat protein gene sequences. Upon further in-depth analysis, our CYVCV isolates shared a common recent ancestry with isolates from India, which suggests they may have originated and diverged from CYVCV Indian isolates.

CYVCV can be transmitted through grafting, by mechanical means, and by aphids and whiteflies. In California citrus orchards, young colonies of spirea aphid (*Aphis spiraecola*) and cotton aphid (*A. gossypii*) occur seasonally on young flush and disappear when flush matures unless other new citrus flush is available. In growth chamber transmission experiments using Eureka lemon and Sour orange for CYVCV acquisition and Alemow and Eureka lemon as inoculation hosts, the spirea aphid transmitted CYVCV at a rate of 39.3 percent compared to that of the cotton aphid at 14.3 percent. Although citrus whitefly (*Dialeurodes citri*) in the San Joaquin Valley were difficult to find, a localized population was found in an organic mandarin orchard in central California (**Figure 4**). A laboratory colony of the whitefly is now being established, and CYVCV vector tests with the whitefly are underway. Citrus whiteflies are readily attracted to yellow, so we are now using yellow sticky traps to monitor seasonal population levels in the orchard (**Figure 4C**).

In addition to infecting most citrus species, cultivars and hybrids, CYVCV has been reported to infect a wide range of non-citrus plants including grapevines, common bean, cowpea and an herbaceous laboratory plant *Nicotiana benthamiana* (Afloukou and Önelge 2020; Liu et al. 2020). Additionally, CYVCV has been detected in weed species such as *Malva sylvestris*, *Solanum nigrum*, *Sinapis arvensis* and *Ranunculus arvensis* (Önelge et al. 2016). This non-citrus host range needs to be confirmed. The non-citrus host range for the CYVCV found in Tulare is unknown and is under investigation by our lab. If the non-citrus host range of our CYVCV isolates is as wide as has been reported in the literature, managing field spread of CYVCV may be challenging due to the presence of vectors having many hosts. Currently, an infectious clone named CYVCV CA1 has been developed that produces infectious virus particles in Eureka lemon and *N. benthamiana* plants. This infectious clone will be an important tool to accelerate our research critical to verify the non-citrus host range of CYVCV.

Looking Forward

Understanding the CYVCV genomic organization, pathogenicity and epidemiology is the foundation for developing suitable measures to control and/or manage this disease. Therefore, we are continuing our studies on the host range of CYVCV, vector transmission and dispersal. Our current experiments on sequencing and using an infectious cDNA clone of CYVCV to examine virus movement and replication within cells and organelles of the various hosts of the virus will expand our knowledge of this emerging virus and help determine the potential impact of CYVCV to our citrus industry.

References

Afloukou, F.M.; Önelge, N. 2020. First report of natural infection of grapevine (*Vitis vinifera*) by *Citrus yellow vein clearing virus*. *New Disease Reports* 42(1):5-5.

Liu, C.; Liu, H.; Hurst, J.; et al. 2020. Recent Advances on *Citrus yellow vein clearing virus* in Citrus. *Horticultural Plant Journal* 6(4):216-222.

Sun, Y.-D.; Yokomi, R. 2023. Whole genome sequence of *Citrus yellow vein clearing virus* CA1 isolate. *BMC Research Notes*. 16: 166. *https://doi.org/10.1186/s13104-023-06443-7*

Önelge, N.; Bozan, O.; Gök-Güler, P. 2016. First report of *Citrus yellow vein clearing virus* infecting new natural host plants in Turkey. *Journal of Plant Pathology* 98(2):373.

Zhang, Y.H.; Liu, C.H.; Wang, Q.; et al. 2019. Identification of *Dialeurodes citri* as a vector of *Citrus yellow vein clearing virus* in China. *Plant Disease* 103(1):65-68.

Glossary

[1]**Reverse Transcription quantitative Polymerase Chain Reaction:** A variant of PCR that is used to quantify amounts of a target RNA transcript in a sample.

[2]**Isolates:** A representative collection of a virus separated from its parent source by graft transmission to a host organism in the greenhouse or an isolation of the genomic material from the field source for the purpose of biological and molecular characterization.

[3]**Infectious cDNA clone:** A double-stranded DNA copy of an RNA virus genome that can be used to artificially infect plants.

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NEW MICROBIAL TOOLS to Combat HLB-associated Citrus Pathogens

Flavia Campos Vieira, Christopher Drozd and M. Caroline Roper

Bacteria, isolated from field-grown citrus trees, growing in a petri dish. What metabolic secrets do they hold?

Project Summary

*Our research focuses on characterizing citrus-associated microorganisms to find new antimicrobial compounds with the potential to suppress the growth of '*Candidatus *Liberibacter asiaticus' (CLas), the huanglongbing (HLB)-associated pathogen, and secondary citrus root pathogens that may aggravate root decline in HLB-affected trees. We have identified several antimicrobial compounds produced by fungi and bacteria isolated from citrus that have anti-Liberibacter activity. From these, we have a lead compound that shows inhibitory activity to CLas. We also have identified several citrus microbiome-derived bacteria that inhibit both Liberibacter and citrus root pathogens. Our continued research goal is to develop antimicrobial natural products and the microbes that produce them to manage HLB and the root decline associated with it.*

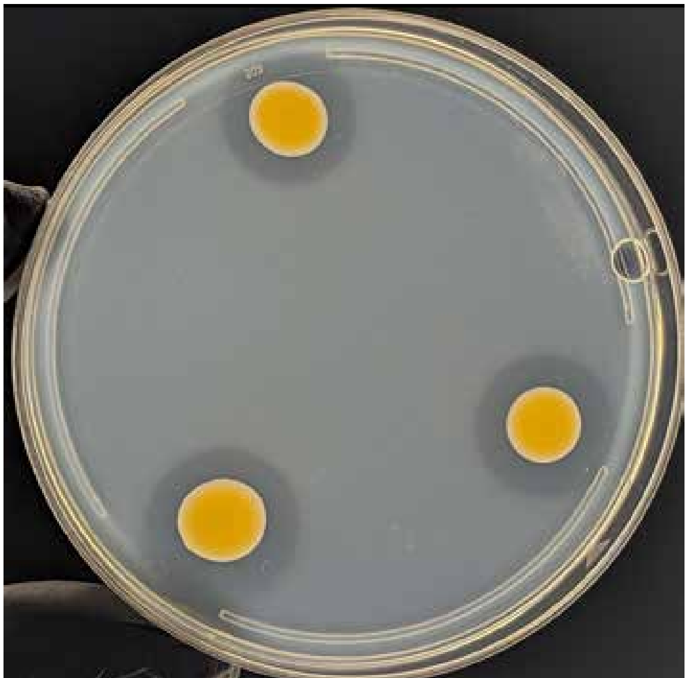

Figure 1. A bacterium native to the citrus microbiome (yellow colonies) secreting a substance that is able to stop the growth of *Liberibacter crescens* (white haze) in bacterial growth media.

Introduction

Plants live in close association with microorganisms (bacteria, fungi, protists, nematodes and viruses), with what is called the plant microbiome. Plant–microbiome interactions can contribute to disease suppression by stimulating the plant immune system, producing antibiotic compounds and competing for resources with pathogens (Zhang et al. 2021).

To better understand how the citrus microbiome influences tree health and disease suppression in the context of HLB, a collaborative effort was made to characterize the citrus-associated microbiome of HLB-affected trees in Florida. We described the distribution and diversity of bacteria and fungi associated with citrus trees in the different plant compartments (leaf, stem and roots) using culture-independent methods (high throughput sequencing)[1] and traditional microbial culturing[2], from which we established a citrus-associated microbial culture collection (Blacutt et al. 2020).

Complementary to this effort, we also have characterized the microbiomes of non-HLB-impacted citrus trees in California and have used novel culturing tools to capture bacteria from the environment in more efficient ways. Between these two microbial culture collections isolated from both low (CA) and high (FL) HLB disease pressure, we have more than 3,000 microbial isolates, giving us a very powerful tool to study the citrus microbiome and its ability to suppress disease.

Screening Citrus Microbes to Fight HLB

One of the challenges of studying HLB is the fact that CLas cannot be cultured in artificial growth media, which prevents direct study of the bacteria's biology. Thus, *Liberibacter crescens*, a closely related but culturable bacteria, is used as a surrogate for laboratory studies. To discover beneficial properties for plant protection, specifically the potential to suppress CLas, we used a laboratory inhibition bioassay to screen recovered microorganisms for their ability to inhibit the growth of *L. crescens* in a petri dish (**Figure 1**). We have identified several bacteria and fungi that produce compounds that serve as promising leads for new antimicrobials to mitigate HLB.

Testing Potential Microbes and Antimicrobial Compounds to Fight HLB

A subset of the bacteria that have shown antimicrobial activity to *Liberibacter crescens* have been selected to be tested in plant bioassays in the California Citrus Research Foundation Biosafety Level 3 Plant facility, a quarantine facility where we can work with CLas-infected plants and psyllids, located in Riverside, California. We have tested different bacterial species in singlets and in consortia for preventive treatment (before the disease is established), following the workflow representation in **Figure 2**. These studies are ongoing.

In pursuit of a more efficient screening assay with a higher throughput and mindful of the difficulties associated with evaluating CLas titer in entire plants relying on visual symptoms, we have transitioned our *in planta* screening methodology to adopt the detached leaf assay developed by the Heck lab (Igwe et al. 2022; Higgins et al. 2023). In this setup, detached leaves are immersed in control or treatment solutions overnight then sampled after seven days to test for changes in bacterial presence/growth (**Figure 3**). CLas copy number (CN) was measured by the ratio of rRNA to rDNA (CLas rRNA: CLas rDNA) of the CLas 16S gene, where the lower value for the ratio indicates higher antibacterial activity (Igwe et al. 2022; Higgins et al. 2023).

We also have performed chemical analysis to identify the compounds responsible for the antimicrobial activity we observed in the petri dish. Our lead compound is a known antimicrobial compound with antibiotic activity against a myriad of pathogens and already has a background of research in the pharmaceutical field that we can leverage for

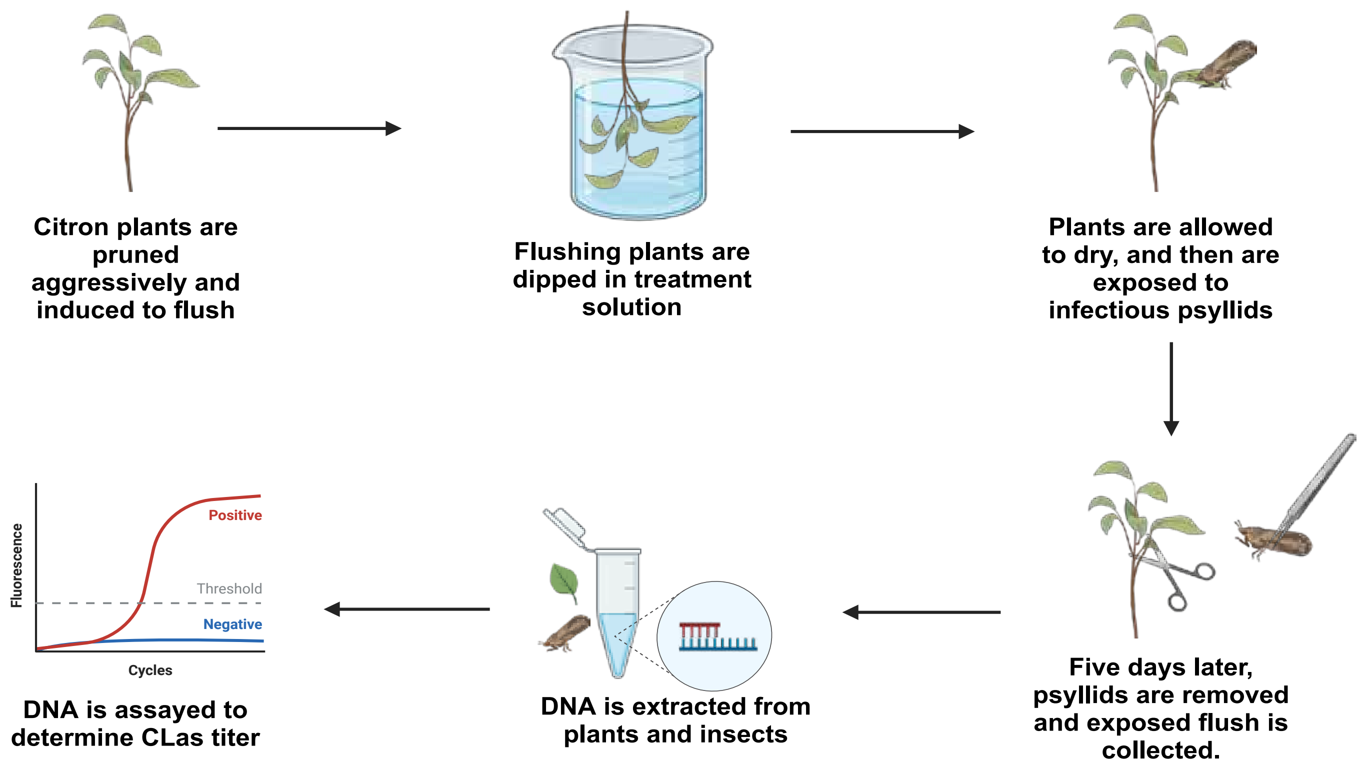

Figure 2 - Preventive treatment of huanglongbing using beneficial microbes. Citron plants were pruned to induce synchronized flushing and establish homogeneous plant height (around 15 cm). Plants with flushes of around one millimeter in diameter were dip inoculated in a 1X phosphate buffered saline solution or in a bacterial solution. Plants were exposed to '*Candidatus* Liberibacter asiaticus' (CLas)-infected Asian citrus psyllid (ACP) and after feeding period, ACPs were removed and plants were treated with insecticide. The ACP-free plants were evaluated and sampled by quantitative polymerase chain reaction (CLas titer quantification) over time.

development. This compound was identified in our screening pipeline as having activity against *L. crescens*. Further testing revealed that microbial extracts enriched in this compound also were effective in reducing CLas titer within infected citrus tissue. Our lab is working toward scaling up production and further purification of this compound to better define the dose necessary for CLas suppression, which would later contribute to application rates needed for CLas suppression in trees.

Conclusion

With this strategy, we aim to create microbiome-informed disease management tools that are able not only to suppress the growth of the pathogen associated with HLB, but also to suppress other pathogens that further exacerbate the disease. These tools would be in the form of purified natural products, or as living microbes, that could be applied to orchards to provide holistic disease control that works with the microbiome rather than against it.

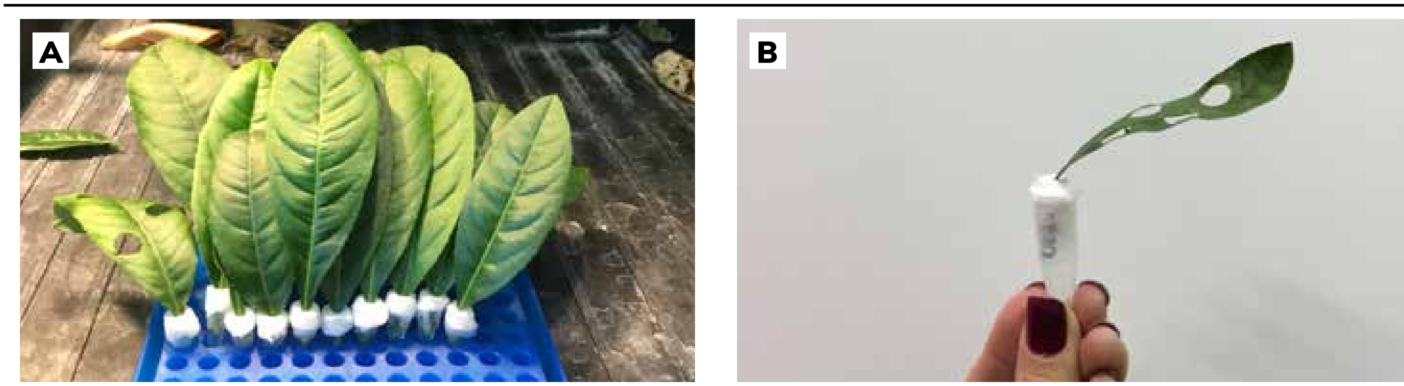

Figure 3 - Detached leaf assay setup. (A) Detached leaves immersed in control or treatment solution at the beginning of the incubation period. (B) Leaf punched and transferred to a tube with buffer solution until the end of the incubation period.

Acknowledgements

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Glossary

[1]**Culture-independent methods (high throughput sequencing):** Directly sequencing DNA from environmental samples, instead of initially growing the microorganisms on a petri dish.

[2]**Traditional microbial culturing:** Spreading environmental samples onto bacterial growth media and isolating the microorganisms that grow.

References

Blacutt, A.; Ginnan, N.; Dang, T.; et al. 2020. An *in vitro* pipeline for screening and selection of citrus-associated microbiota with potential anti-"*Candidatus* Liberibacter asiaticus" properties. *Applied and Environmental Microbiology* 86(8):e02883-19. *https://doi.org/10.1128/AEM.02883-19*

Ginnan, N.A.; Dang, T.; Bodaghi, S.; et al. 2020. Disease-induced microbial shifts in citrus indicate microbiome-derived responses to huanglongbing across the disease severity spectrum. *Phytobiomes Journal* 4(4):375–387.

Higgins, S.A.; Igwe, D.O.; Ramsey, J.S.; et al. 2023. Plant-derived, nodule-specific cysteine rich peptides inhibit growth and psyllid acquisition of '*Candidatus* Liberibacter asiaticus', the citrus Huanglongbing bacterium. *BioRxiv. https://doi.org/10.1101/2023.06.18.545457*

Igwe, D.O.; Higgins, S.A.; Heck, M. 2022. An excised leaf assay to measure acquisition of '*Candidatus* Liberibacter asiaticus' by psyllids associated with citrus huanglongbing disease. *Phytopathology* 112(1):69-75.

Zhang, J.; Cook, J.; Nearing, J.T.; et al. 2021. Harnessing the plant microbiome to promote the growth of agricultural crops. *Microbiological Research* 245:126690. *https://doi.org/10.1016/j.micres.2020.126690*

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THE CITRUS CLONAL PROTECTION PROGRAM

Ten Stages of Budwood Production and Distribution and Performance Metrics

Georgios Vidalakis

Figure 1. Citrus Clonal Protection Program (CCPP) staff performing a step in the therapy process on a high-risk citrus introduction from an area infested with citrus canker and huanglongbing, in isolation from other citrus materials at the Citrus Therapy, Diagnostic and Research Laboratory at the University of California, Riverside campus.

Project Summary

The availability of pathogen-tested citrus propagative materials is fundamental to the establishment and maintenance of a sustainable and competitive citrus industry. For this reason, the Citrus Research Board (CRB) has continually supported the Citrus Clonal Protection Program (CCPP) since the inception of the CRB marketing order in 1968. Established in 1957, the CCPP today is a cooperative program with the University of California, Riverside (UCR), UC Agriculture and Natural Resources (UC ANR), the California Department of Food and Agriculture (CDFA), the U.S. Department of Agriculture-Animal and Plant Health Inspection Service (USDA-APHIS) and the California citrus industry represented by the California Citrus Nursery Board (CCNB) and the CRB. The CCPP provides a safe mechanism for the introduction of citrus germplasm into California and distributes pathogen-tested and true-to-type citrus propagative material to the California citrus industry and, via the National Clean Plant Network (NCPN), to the entire U.S. Reports on the CCPP's activities from October 2017 through September 2021 were given previously (Vidalakis 2022).

The CCPP introduces citrus varieties into California using proper federal[A] and state[B] permits following a comprehensive process that allows for introduction under quarantine, therapy, pathogen testing and quarantine release for distribution of pathogen-tested budwood to the industry, scientists and the public (Vidalakis 2022).

The CCPP citrus variety introduction process includes ten major stages:

1. receiving budwood and recording (i.e., introductory plant propagation number [IPPN]);
2. preliminary pathogen testing (i.e., pre-index);
3. *in vitro* tissue culture therapy for the production of therapied plantlets (i.e., shoot-tip grafting [STG]);
4. *in planta* growth of STGed plantlets (i.e., propagation of STGed plantlets on a citrus rootstock);
5. therapy success test (i.e., test the STG/rootstock tree for pathogens identified in pre-index);
6. variety index (VI)[1] (i.e., VI that includes testing for known graft-transmissible pathogens of citrus);
7. request to regulatory agencies for variety release from quarantine (i.e., CDFA and USDA-APHIS, depending on origin of the variety, domestic or international);
8. establishment of budwood sources;
9. testing of budwood sources for endemic graft-transmissible pathogens of citrus in California; and
10. distribution of pathogen-tested budwood from established budwood sources to the industry, scientists or the public (Vidalakis 2022).

From October 2021 through September 2023, the CCPP had a total of 355 individual accessions at different stages of the introductory process (**Tables 1** and **2**, real time updates on accessions under quarantine at *https://ccppdms.ucr.edu/ccppdms/upcoming_varieties*). It is important to note that as an accession is moving through the introductory process, its presence is recorded and counted in all the different stages for the reporting period. For example, in the two-years reported in this article and given that the introductory process lasts approximately 2-2.5 years, it is quite possible for one accession to be recorded up to four times – i.e., once as received [Stages 1-2]; once as therapied [Stages 3-5]; and once at the variety index [Stage 6] or even as a quarantine release [Stage 7-8]. Therefore, even though there were 355 individual accessions in the introductory process, the CCPP executed 524 processes towards safe introduction into California (**Tables 1** and **2**).

In this reporting period, the CCPP received 200 budwood shipments and performed 4,403 pre-index tests for 169 citrus accessions (**Table 1** and **Figure 2**). These tests identified 100 accessions infected with viruses such as citrus tristeza, citrus tatter leaf, citrus leaf blotch, citrus vein enation, citrus variegation, citrus psorosis and citrus virus A; different citrus viroid species (exocortis and cachexia); and the bacteria *Xanthomonas citri* subsp. *citri* and *'Candidatus* Liberibacter asiaticus' [Stages 1-2].

The therapy procedure used at the CCPP for pathogen elimination from citrus budwood (**Figure 1**) is STG and is performed at a microscopic scale using a few plant cells from the apical meristems of feather flushes produced by the introduced budwood in culture. Between October 2021 and September 2023, the CCPP performed 1,949 therapy procedures for 144 citrus accessions (**Table 1** and **Figure 3**) [Stage 3].

A few weeks past STG, the plantlets growing *in vitro* are removed from the growth medium. After removal of their roots, they are grafted onto a mature and fast-growing

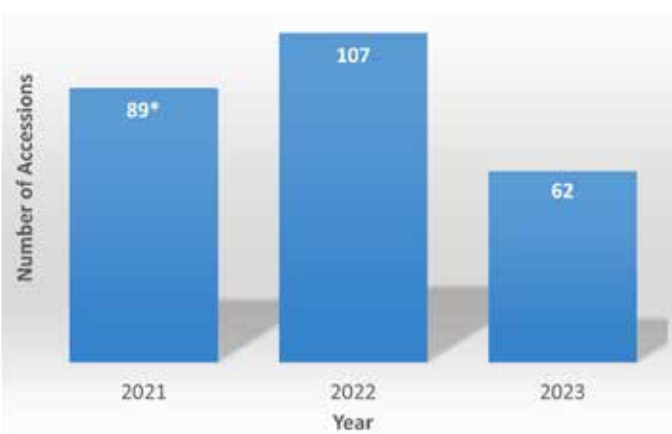
Table 1. Number of citrus accessions at different stages of the Citrus Clonal Protection Program introductory process from October 2021 through September 2023. A total of 355 individual accessions entered 524 processes at the eight variety introduction stages. For accession type descriptions, see Table 2.

ACCESSION TYPE	NUMBER OF ACCESSIONS			
	RECEIVED [STAGES 1-2]	THERAPIED [STAGES 3-5]	VARIETY INDEX [STAGE 6]	QUARANTINE RELEASE [STAGES 7-8]
Proprietary	20	21	8	16
Public Domain	25	22	17	15
Breeding Programs	45	26	10	12
Re-Index	41	50	23	12
Research Material	35	21	7	2
Specific Agreement	3	4	53	36
Total: 524	**169**	**144**	**118**	**93**

[A]*CFR Title 7, § 319.19 Notice of quarantine-Citrus Canker and Other Citrus Diseases.*
[B]*Cal. Admin. Code tit. 3, § 3250 Citrus pests exterior quarantine*

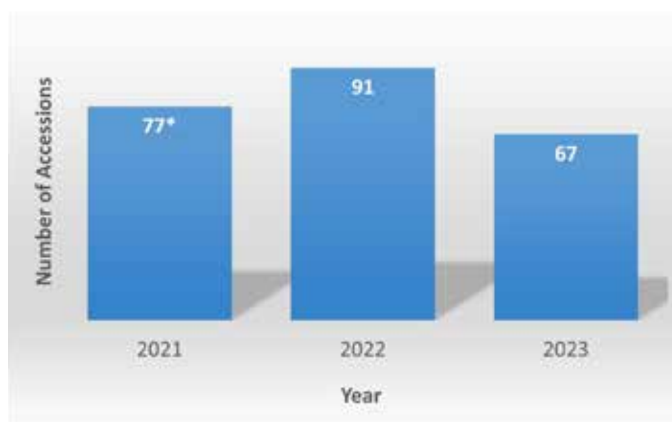
Table 2. Description of different accession types handled within the Citrus Clonal Protection Program (CCPP) introductory process from October 2021 through September 2023.

ACCESSION TYPE	DESCRIPTION
Proprietary	Proprietary introductions where the CCPP service user pays for the cost of therapy and testing and receives the produced budwood source exclusively.
Public Domain	Includes public domain accessions submitted to the CCPP after a request from an individual for accessions in the National Clean Plant Network, U.S. Department of Agriculture-Agricultural Research Service (USDA-ARS); USDA-ARS-National Clonal Germplasm Repository for Citrus and Dates, Givaudan Citrus Variety Collection at UCR or other public domain sources. There is no cost to the requester and the varieties become available to any interested party only for the cost of budwood, without any license or other fees applied by the requester.
Breeding Programs	Includes accessions from the UCR Core Breeding Program, as well as other breeding programs such as University of Florida or USDA-ARS. There is no cost to the breeding programs if the materials are part of a specific research project, in which case there are budgeted funds that can be used to recover costs of the CCPP activities. If a variety becomes proprietary, or becomes available to the public, but a license fee is applied, the CCPP engages in discussions for the implementation of a cost reimbursement plan.
Re-index	Includes accessions being therapied and tested for pathogens due to loss of original CCPP budwood source (e.g., death or old accession with outdated testing). Cost is part of the CCPP operational budget to produce and maintain therapied and pathogen-tested budwood sources.
Research Material	Includes USDA-ARS and other citrus germplasm, research accessions. Such accessions are typically produced by genetic engineering for further downstream use by scientists. Other cases include accessions sent to CCPP by visiting scholars who wish to be trained on citrus therapeutic and diagnostic protocols; and in the process, the accession can be cleaned up for use in California and the area of origin. There is no cost to the research programs unless the materials are part of a specific research project or a scholarship program for the visiting scholar, in which case there is a budget or bench fees that can be used to recover costs of the CCPP activities. If a variety becomes proprietary, or a variety becomes available to the public but a license fee is applied, the CCPP engages in discussions for the implementation of a cost recovery plan.
Specific Agreement	Includes accessions under specific agreements such as material transfer agreements (MTA) (e.g., USDA-ARS and University of Florida); CRB-funded projects (e.g., Fruitmentor™); CCPP variety index service agreement (e.g., the user pays for half of the cost of therapy and testing and receives the produced budwood source exclusively for seven years, before the accession enters the public domain). If a variety under MTA becomes proprietary, or a variety becomes available to the public but a license fee is applied, the CCPP engages in discussions for the implementation of a cost recovery plan.



* 28 accessions received after October 2021.

Figure 2. Number of citrus accessions received under quarantine from October 2021 through September 2023.

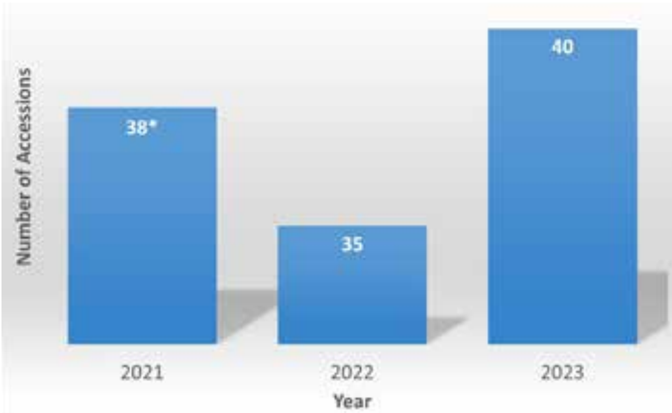


* 22 accessions were therapied after October 2021.

Figure 3. Number of citrus accessions therapied from October 2021 through September 2023.

citrus rootstock, such as rough lemon, to produce the first generation of source trees of the accession. Between October 2021 and September 2023, the CCPP produced 254 first generation sources trees for 92 accessions and performed 5,547 therapy success tests to verify that any pathogens detected during pre-index in stage 2 are now removed via the STG from the introduced varieties [Stages 4-5].

After negative therapy success tests, the STGed source plants must test negative for the known graft-transmissible pathogens of citrus at the Variety Index (VI) protocol to become eligible for release from quarantine. Upon successful completion of the VI indexing, each variety is assigned a unique VI number that always accompanies and identifies each variety that tested negative for known citrus pathogens



* 18 accessions were released from quarantine after October 2021.

Figure 4. Number of citrus accessions released from state and federal quarantine from October 2021 through September 2023.

by the CCPP. In this reporting period, the CCPP executed 8,108 VI tests for 118 citrus accessions (**Table 1**) [Stage 6]. After the VI is completed with negative results, the director of the CCPP writes a letter to state and federal regulatory agencies requesting release of the tested varieties from quarantine. Between October 2021 and September 2023, the CCPP performed 1,166 STGs and successfully completed 7,013 VI tests that resulted in the release of 93 citrus accessions from quarantine for use by the citrus industry and researchers (**Table 1** and **Figure 4**) [Stage 7].

Budwood sources of the quarantine-released varieties are established so that the various stakeholders (i.e., industry, scientists, public) can access the germplasm. The public domain citrus varieties released from quarantine (**Table 3**) are deposited in the CCPP Lindcove Foundation Facility (LFF) at the UC-ANR Lindcove Research and Extension Center (LREC) for distribution (protected foundation block) and trueness-to-type evaluations (evaluation foundation block). In this reporting period, the CCPP established 84 budwood sources for 74 accessions and supplied 4,212 buds for propagation of budwood sources [Stage 8]. All CCPP trees at the CCPP-LFF (protected and evaluation blocks) are tested annually for several viral and bacterial pathogens endemic to California as required by CDFA regulations[C]. In this reporting period, the CCPP executed 42,531 pathogen tests for 2,146 budwood tree sources [Stage 9].

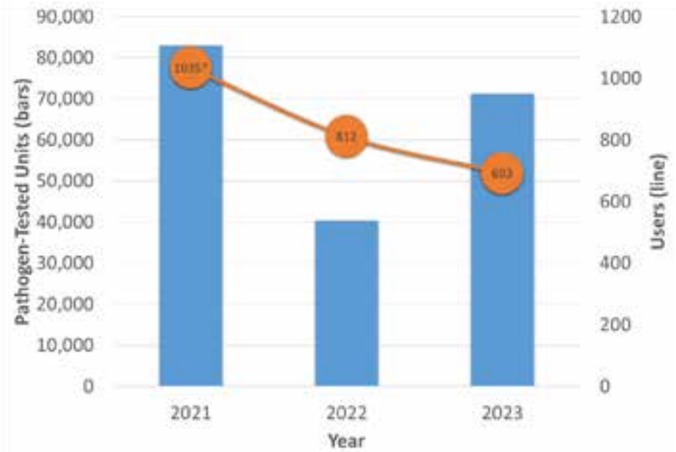
Between October 2021 and September 2023, the CCPP executed 2,266 budwood orders and distributed 121,939 buds of 374 different citrus VIs to 1,468 users from its LREC CCPP-LFF protected foundation block (**Figure 5**) [Stage 10]. Mandarins, navel oranges and specialty varieties represented 30, 15 and 10 percent of the budwood requests, respectively. Limes, lemons, blood oranges, Valencia oranges and pummelos ranged between nine and five percent of the budwood distributed, while grapefruits, sour oranges, sweet oranges and rootstocks ranged between one and three percent. For

Table 3. Public domain citrus varieties which completed variety indexing (VI) between October 2021 and September 2023.

VI	VARIETY	CITRUS TYPE	ORIGIN
1000	Bidwell's Bar Mother Orange (CRC 1512)	Sweet Oranges	UC Riverside Citrus Variety Collection
1013	RICO 5-50	Sweet Oranges	Puerto Rico
1016	UENO IVIA-406	Mandarins	Spain
1028	Large	Specialty Fruit	China
1315	Pumpkin	Specialty Fruit	China
1356	Salustiana	Sweet Oranges	Puerto Rico
1358	FRONTON	Sweet Oranges	Puerto Rico
1359	RICO 7-56	Sweet Oranges	Puerto Rico
1378	Matsuyama Pummelo	Pummelos	Hawaii, United States
1394	Nine Pound Lemon DPI-201-21	Other Lemons	USDA-ARS-NCGRCD
1468	Citrus yuko (CRC 3146)	Specialty Fruit	UC Riverside Citrus Variety Collection
1469	Orbon Lemon hybrid (CRC 4140)	Other Lemons	UC Riverside Citrus Variety Collection
1470	Femminello lemon (CRC 3500)	Eureka Lemons	UC Riverside Citrus Variety Collection
1504	Variegated Valencia	Valencia Oranges	Georgia, United States
1552	US-942 (USDA-ARS-NCGRCD Source)	Rootstocks	USDA-ARS-NCGRCD

USDA-ARS-NCGRCD: United States Department of Agriculture-Agricultural Research Service-National Clonal Germplasm Repository for Citrus and Dates; UC: University of California

[C] *Section 3701, Citrus Nursery Stock Pest Cleanliness Program*



* 10,371 buds were distributed to 202 users after October 2021.

Figure 5. Pathogen-tested citrus propagative units (i.e., buds) distributed and number of users receiving buds from October 2021 through September 2023.

budwood availability, licensed varieties and orders, please visit *http://ccpp.ucr.edu/budwood/budwood.php*.

The CCPP is dedicated to helping maintain California at the forefront of high-quality citrus nursery and fruit production. The progress reported here in the ten stages of production and distribution of pathogen-tested citrus propagative materials from the CCPP is essential for the continued protection and viability of California's citrus industry.

CRB Research Project #6100

Glossary

[1]**Variety index (VI):** A comprehensive set of laboratory and biological tests targeting all known graft-transmissible pathogens of citrus. When all tests are negative for a citrus variety, it receives its unique VI number that accompanies the variety for life and indicates that is has been produced or tested by the CCPP and is eligible for commercial or other use in California.

References

Vidalakis, G. 2022. Citrus Clonal Protection Program. *Citrograph* 13(4):36-40.

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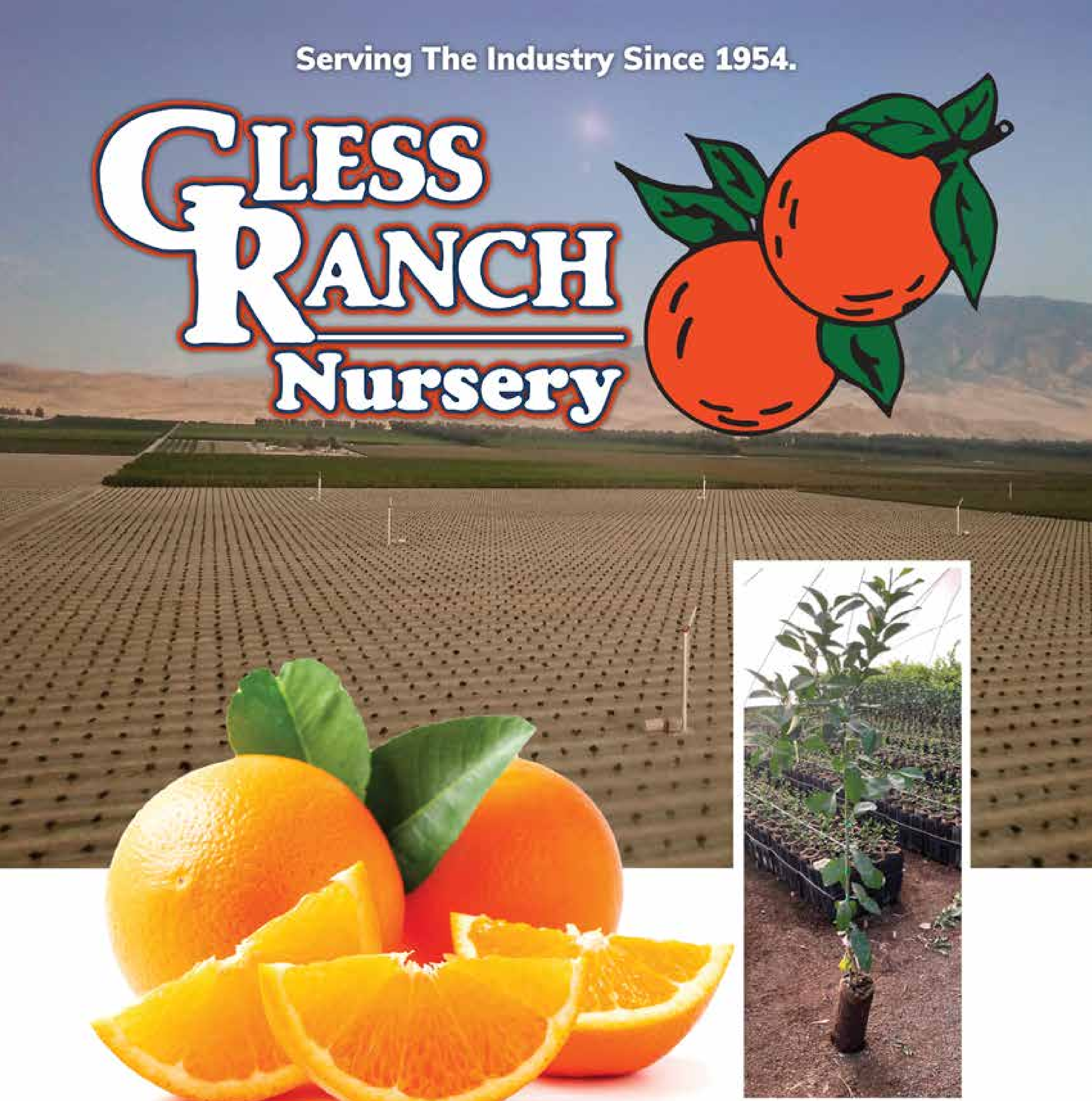
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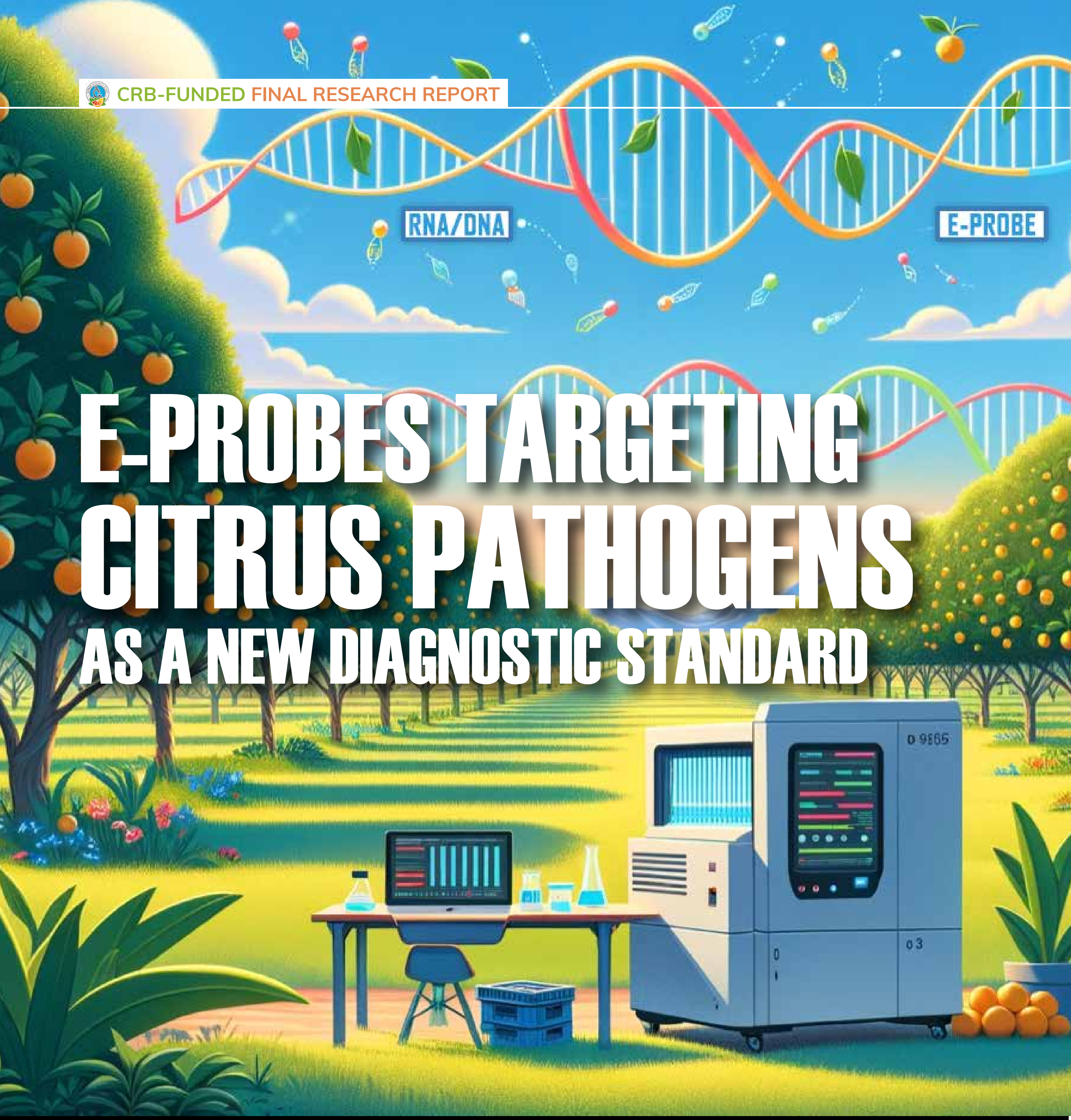
E-PROBES TARGETING CITRUS PATHOGENS AS A NEW DIAGNOSTIC STANDARD



Conceptual illustration showcasing the application of advanced molecular biology and computer programming to detect citrus pathogens.

Sohrab Bodaghi, Tyler Dang, Huizi Wang, Andres Espindola, Irene Lavagi-Craddock, Fatima Osman, Marcos Ribeiro, Danielle Do Nascimento, Arunabha Mitra, Josh Habiger, Kitty Cardwell and Georgios Vidalakis

Project Summary

At the conclusion of the project, we demonstrated the use of high-throughput sequencing (HTS), a groundbreaking technology that merges molecular biology and computer science for citrus pathogen detection and identification. Our primary objective was to simplify HTS data analysis, making it accessible to individuals without a bioinformatics background. The Electronic (E)-probe Diagnostic Nucleic Acid Analysis (EDNA) technology, developed by the Oklahoma State University (OSU) Institute of Biosecurity and Microbial Forensics, proves to be a promising tool for achieving this objective. In the project's final stages, we conducted a comprehensive assessment of EDNA analysis alongside existing diagnostics within the Citrus Clonal Protection Program (CCPP) variety index (VI) process.

Our achievements include the successful calculation of the statistical limits of detection for E-probes targeting citrus pathogens, the design of E-probes for internal controls and the organization of workshops tailored for regulators and future EDNA users. Additionally, we published EDNA protocols and validation data, expected to serve as a foundational basis for regulatory acceptance of the technology. EDNA analysis has the potential to simplify the diagnostic tests required for the release of citrus varieties from quarantine. Furthermore, the user-friendly HTS/EDNA technology online platform holds considerable potential. This project marks a significant step toward the widespread adoption of EDNA technology in citrus pathogen detection.

Background

The California citrus industry, valued at approximately $3.6 billion with an economic impact of $7.6 billion in the state (Babcock 2022), faces threats from various pathogens spread through tree grafting with infected materials. In its ongoing efforts to protect California's citrus, the CCPP-associated scientists are working toward incorporating advanced pathogen detection methods like HTS/EDNA in the citrus variety introduction process. HTS, integrating molecular biology and computer science, allows simultaneous detection of multiple pathogens in plant samples. Due to significant reductions in HTS costs and the increased affordability and well-documented nature of EDNA (Dang et al. 2022a and b), these technologies are expected to become valuable for citrus germplasm trade partners worldwide, bolstering biosecurity measures for the citrus industry.

In the case of the CCPP, the variety introduction process includes diagnostics based on biological and laboratory assays complimenting each other for the detection of various pathogens and diseases. Biological indexing[1] is based on seven species of citrus indicators while the laboratory assays are based primarily on 30 individual polymerase chain reaction (PCR) tests. Combined, these diagnostic approaches comprise CCPP's variety index (VI), which requires approximately eight to ten months to be completed. New technologies such as HTS/EDNA provide an opportunity to consolidate the molecular tests of the VI process by consolidating the 30 PCR tests into a single multiplex assay, thus generating savings in effort, cost and time.

Table 1. High-throughput-Electronic (E)-probe Diagnostic Nucleic Acid Analysis list of targeted citrus pathogens.

A. TARGET PATHOGENS FOR WHICH VALIDATION IS COMPLETE	
1	Citrus tristeza virus
2	*'Candidatus* Liberibacter asiaticus'
3	Citrus exocortis viroid
4	*Spiroplasma citri*
5	Citrus vein enation virus
6	Citrus psorosis virus
7	Citrus leaf blotch virus
8	Citrus tatter leaf virus
9	Citrus variegation virus
10	Citrus concave gum associated virus and Citrus virus A
11	Hop stunt viroid (citrus isolates)
B. TARGET PATHOGENS FOR WHICH *IN PLANTA* VALIDATION IS IN PROGRESS	
12	*Xanthomonas citri* subsp. *citri*
13	*Xylella fastidiosa* subsp. *pauca*
14	*'Candidatus* Phytoplasma aurantifolia'

Ongoing testing of HTS/EDNA compared with currently established standards will continue for several years to ensure the sustained reliability of E-probes over time. Moreover, the cost-effectiveness for detecting multiple citrus pathogens in a single sample is likely to drive regulatory acceptance and laboratory adoption in the near future. In our previous studies, we have demonstrated that HTS/EDNA was sensitive and specific, eliminating the need for personnel with a bioinformatics background or high-cost servers conducting extended analyses to acquire diagnostic results (Dang et al., 2019, 2021, 2022 a, b, 2023). The publications resulting from our studies serve as crucial documentation for the ongoing citrus HTS/EDNA regulatory acceptance proceedings.

Summary of Results

We successfully designed, curated and statistically validated E-probes targeting the citrus pathogens outlined in **Table 1**. We have addressed the challenge of calculating the limit of detection (LOD) for E-probes targeting citrus pathogens by incorporating housekeeping genes from non-citrus woody hosts. This ensures unique reads for statistical analyses and helps prevent false positives (Dang et al. 2023). Our recent E-probe validation has emphasized specificity, computerized analytical sensitivity and diagnostic sensitivity, entailing testing against both confirmed positive and healthy specimens. These E-probes are now available for implementation in the CCPP's VI process (**Table 1, A**).

Since June 2021, the HTS/EDNA protocol has been incorporated successfully into the CCPP VI testing pipeline. HTS/EDNA results were reported to regulatory agencies in parallel with bioindexing and laboratory tests, for seven VIs and 67 citrus varieties released from state and federal quarantine. This reporting process is integral to facilitating the regulatory acceptance of the HTS/EDNA protocol for the introduction and quarantine release of citrus varieties into California by the CCPP. Moreover, an *in silico* validation study was initiated between OSU and CCPP, covering metrics such as robustness, reliability and transferability, with comparisons made across various operators to report to the regulatory agencies in the future. In the past four years, we also conducted five workshops and training sessions for regulators and future EDNA users. These events were essential for evaluating the user-friendliness of the HTS-EDNA online platform and fostering interest in its adoption for citrus diagnostics. These activities, alongside our existing publications, showcase the alignment of HTS/EDNA results with the CCPP's established and regulatorily accepted bioindexing and laboratory protocols and laid the foundation towards regulatory acceptance of the new diagnostic technology.

Final Steps

For the remaining three target pathogens, all E-probe design and validation steps have been completed, except for the *in planta*[2] validation tests (**Table 1, B**). Due to the exotic nature of these pathogens to California, *in planta* validation was delayed by the unavailability of infected samples. To overcome this challenge, we acquired the necessary federal and state permits and initiated collaboration with both domestic and international scientists to obtain infected plant tissue samples. The final *in planta* validation steps for these bacterial pathogens are anticipated to conclude in the coming months. Based on recent data accumulated from this project, along with upcoming publications, we further anticipate adoption of the HTS/EDNA assays for all 14 targeted citrus pathogens of this project.

Conclusion

As we conclude our data collection and present our findings to regulatory agencies, it is evident that HTS and EDNA are potent diagnostic tools, that can streamline the CCPP's citrus variety introduction and quarantine release processes. The widespread acceptance of HTS within scientific communities, alongside EDNA, leads us to confidently anticipate that this technology will become a mainstream tool for citrus pathogen detection. Moreover, the transferability of HTS/EDNA technology to various diagnostic laboratories, facilitated by the MicrobeFinder (MiFi®) online platform, and ease of use, holds significant promise.

This technology establishes a structured pipeline for the inclusion of potential emerging citrus pathogens into the CCPP's existing diagnostic pathogen detection program. Moreover, this will help reduce the overall duration of the variety indexing pipeline for the CCPP. The CCPP and other citrus germplasm programs can leverage this technology to benefit the citrus industry by providing access to pathogen-tested, certified budwood for citrus grove establishment. These advancements collectively enable a more efficient pathway for the introduction and distribution of new citrus varieties in California.

CRB Research Project #5300-205

Glossary

[1]**Biological indexing (or Bioindexing):** Plant-based diagnostic technique employed to identify specific plant pathogens causing disease under controlled environmental conditions. In citrus, this involves grafting a blind bud/bark piece from a suspected diseased plant onto a young, greenhouse-grown indicator plant, followed by monitoring for the development of characteristic symptoms. Indicator plants chosen for bioindexing usually are susceptible to a particular pathogen, exhibiting noticeable disease symptoms shortly after being graft-inoculated with the infected plant tissue.

[2]***In planta* (Latin):** Processes, experiments or analyses conducted within a living plant or using a sample from a living plant. In the context of this article, the term refers to analyses of high-throughput data generated from citrus samples infected with citrus pathogens.

Reference

Dang, T.; Bodaghi, S.; Lavagi, I.; et al., 2019. Next generation sequencing as a routine diagnostic tool. *Citrograph* 10(4): 58-61.

Dang, T.; Espindola, A.; Bodaghi, S.; et al., 2021. High-throughput sequencing: a potential diagnostic tool. *Citrograph* 12(2):58-62.

Babcock, B.A. 2022. Economic impact of California's citrus industry in 2020. *Journal of Citrus Pathology* 9(1). *https://doi.org/10.5070/C49156433*

Dang T.; Wang H.; Espindola A.; et al., 2022a. Development and statistical validation of E-probe Diagnostic Nucleic Acid Analysis (EDNA) detection assays for the detection of citrus pathogens from raw high-throughput sequencing data. *PhytoFrontiers* 3:113-123. *https://doi.org/10.1094/PHYTOFR-05-22-0047-FI*

Dang, T.; Espindola, A.; Vidalakis, G.; et al., 2022b. An *in silico* detection of a citrus viroid from raw high-throughput sequencing data. pg. 275-286 *in Viroids: Methods and Protocols, Methods in Molecular Biology*. Editors: Rao, A.; Lavagi-Craddock, I.; Vidalakis, G. (eds.), Springer, Vol. 2316. *https://doi.org/10.1007/978-1-0716-1464-8_23*

Dang, T.; Espindola, A.; Bodaghi, S.; et al., 2023. High-throughput sequencing as a routine diagnostic tool: the final steps. *Citrograph* 14(2):40-43.

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A SMARTER WAY TO SURVEY FOR HLB

EVALUATING A RISK-BASED MODEL IN SOUTHERN CALIFORNIA

Weiqi Luo, Drew Posny and Neil McRoberts

Project Summary

*To manage the spread of huanglongbing (HLB) in California, stakeholders and decision-makers need to make informed decisions based on reliable evidence and develop risk-based survey (RBS) models to support surveillance efforts. As of October 2023, more than 7,000 '*Candidatus *Liberibacter asiaticus' (CLas)-positive trees and Asian citrus psyllids (ACP) have been detected across southern California. In particular, HLB has been detected frequently in some urban neighborhoods of Los Angeles and Orange counties, and it is gradually appearing in Riverside, San Bernardino and San Diego counties. As the epidemic evolves, various factors in the RBS design and development are likely to change, including the risk of introduction from international travel, the distribution of ACP, progression of detected CLas-positive hosts and ACP, citrus transportation corridors and other potential human-mediated factors contributing to HLB epidemic development, as well as available resources for flexible and efficient survey program implementation. Continued evaluation of the effectiveness of the RBS models allows us to refine the early detection and predictive ability for HLB in diverse residential landscapes and monitor any potential threat to nearby commercial citrus. Additionally, we have conducted comprehensive spatio-temporal analyses to gain a deeper understanding of HLB outbreaks and improve early detection efforts. This includes refining the residential citrus host map, estimating HLB prevalence and positivity rate, as well as evaluating the reliability of RBS relative to other approaches.*

Introduction

As the dynamics of ACP/HLB change, the scope of the survey program in California needs to adapt to meet any new demands and effectively support HLB detection efforts. Risk factors contributing to the current and expected dynamics of ACP/HLB across California are evaluated and assessed regularly to optimize the RBS models including updated risk maps and survey deployment strategies. These surveys are designed based on known and biologically based risk factors for early detection and HLB mitigation. The risk maps identify areas with a high risk of pathogen introduction or spread intended to help prioritize surveillance, monitoring and control strategies. Key features of the model design, including the improved refinement of the residential citrus map and appropriate balance of risk factors, are continuously analyzed to enhance program capabilities in conducting effective surveys. Moreover, the proximity of elevated risk to commercial citrus groves is also a cause for concern and serves as an early warning indicator for growers. To ensure that the risk-based surveys remain effective over time, regular maintenance and updating of the risk factors and model components are required.

Residential Citrus Host Population

Accurate estimation of residential citrus host locations is crucial for understanding HLB epidemiology and implementing appropriate management measures such as surveys, control and delimitation. Leveraging land parcel data for all California counties from the California Department of Food and Agriculture (CDFA) has significantly improved our ability to estimate the residential citrus host population. To estimate potential residential citrus host locations, we specifically classified residential parcels with sufficient areas for dooryard citrus, encompassing single-family residences, townhomes, duplexes and mobile homes. The final residential citrus host distribution was estimated at one-square mile grids (STR – Section Township and Range) based on the identified residential parcels and the delimitation survey-derived estimates of both the percentage of properties with dooryard citrus and the average number of citrus trees planted per property within each county. In southern California, we estimate residential citrus accounts for nearly half of the total number of citrus trees (**Table 1**). This underscores the significance of residential HLB surveys for effective HLB management.

HLB Prevalence and Positivity Rate

With finite resources, optimizing efficiency in surveying citrus locations is important. Therefore, we employed statistical modeling methods to estimate the overall HLB prevalence (percent areas infected) and HLB-positive rate (percent residential citrus tree population infected) for each county. Together, these metrics can assist in identifying those areas that are at greater risk. This will greatly aid in determining what effective mitigation method is needed at different scales and what specific areas require the most urgent attention.

Our analysis revealed an exponential increase in HLB prevalence across southern California from 2015 to 2022. Interestingly, Los Angeles and Orange counties experienced a reduction in HLB prevalence in 2020 and 2021 while Riverside, San Bernardino and San Diego observed small increases. In 2022, there was an increase in HLB prevalence in Orange County exceeding 25 percent with 7.5 percent of the residential citrus population infected, suggesting a need to prioritize additional mitigation methods to deter further spread outside this region. In contrast, Riverside, San Diego and San Bernardino showed increases in HLB prevalence, but the HLB-positive rates remained below five percent within these counties.

Risk Model Evaluation

We conducted an extensive retrospective analysis to evaluate the effectiveness of each risk factor in our model to enhance its predictive ability. The outcomes of this analysis formed the basis for determining the appropriate weight assigned to each risk factor in the comprehensive model construction to improve its accuracy in light of the evolving ACP/HLB epidemic in California. **Figure 1** demonstrates the substantial predictive power of the introduction risk, derived from the census travel model (Gottwald et al. 2019), in identifying initial instances of new HLB detections in southern California counties. We also observed that areas with the highest risk values for previous ACP density closely correspond to established psyllid populations, encompassing the majority of confirmed HLB-positive detections between 2016 and 2022.

Additionally, we acknowledge the significance of previous HLB locations, which tend to cluster in the late epidemic phase. These locations are crucial risk indicators for conducting follow-up delimitation surveys across all

Table 1. Comparison of estimated residential and commercial citrus tree populations by county in southern California.

COUNTY	TOTAL # RESIDENTIAL PROPERTIES (LAND PARCEL DATA FROM CDFA)	RESIDENTIAL PROPERTIES WITH CITRUS (DELIMITATION SURVEY-DERIVED)	TOTAL # RESIDENTIAL CITRUS PROPERTIES (ESTIMATED)	AVERAGE CITRUS TREE PLANTED PER PROPERTY (DELIMITATION SURVEY-DERIVED)	RESIDENTIAL CITRUS TREE POPULATION (ESTIMATED)	COMMERCIAL CITRUS POPULATION (ESTIMATED, ASSUMING 100 TREES/ACRE; CITRUS LAYER FROM CDFA)
LA	1,589,829	63.25%	1,005,544	1.90	1,910,534	0
Orange	563,373	41.61%	263,914	2.17	572,693	56,700
Riverside	611,347	55.97%	342,167	3.04	1,040,188	1,647,300
San Diego	590,277	77.91%	459,897	3.06	1,407,285	1,007,800
Imperial	36,706	62.71%	23,017	2.39	55,010	722,400
San Bernardino	485,689	68.19%	331,169	2.42	801,429	271,000
Ventura	174,682	62.71%	109,539	2.40	262,893	2,604,900
Total					**6,050,032**	**6,310,100**

Table 2: The estimated huanglongbing (HLB) prevalence and positivity rate for five southern California counties from 2015 to 2022. Note, there are null values (--) due to no new HLB tree detections for that county/year.

County	2015	2016	2017	2018	2019	2020	2021	2022
HLB PREVALENCE (% AREAS INFECTED)								
Los Angeles	0.4%	0.5%	2.4%	3.6%	4.0%	2.6%	2.9%	**13.5%**
Orange	--	--	16.2%	13.7%	20.5%	14.9%	17.9%	**26.5%**
Riverside	--	--	0.9%	--	0.8%	2.0%	2.0%	**5.8%**
San Bernardino	--	--	--	--	3.0%	3.4%	5.6%	**4.9%**
San Diego	--	--	--	--	--	0.2%	0.2%	**0.6%**
HLB POSITIVITY RATE (% RESIDENTIAL CITRUS POPULATION INFECTED)								
Los Angeles	0.6%	0.3%	0.7%	0.5%	0.6%	0.7%	1.4%	**2.6%**
Orange	--	--	1.3%	0.7%	1.8%	1.1%	3.9%	**7.5%**
Riverside	--	--	0.2%	--	0.6%	0.4%	0.7%	**1.8%**
San Bernardino	--	--	--	--	0.6%	0.5%	1.1%	**2.7%**
San Diego	--	--	--	--	--	1.3%	3.3%	**0.8%**

counties. In the model, we also consider the impact of various other risk factors such as plant nurseries, big box stores, citrus transport corridors, packinghouses, farmers' markets (i.e., swap meets and flea markets) and proximity to military installations and Native American lands. However, we have found that the impact of data availability on the model is not as robust or consistent compared to the three most significant risk factors previously identified. The data for these factors are considerably variable across different counties, likely due to inherent uncertainty and collinearity (a strong correlation between variables), as well as landscape diversity. Next, we can conduct more comprehensive analyses and assessments to determine their significance in predicting the spread and impact of the ACP/HLB epidemic.

Risk Factor Weighting Optimization

There is no doubt that these risk factors are interconnected, necessitating creation of a weighted composite risk model to predict HLB infection accurately. This model began with the optimization of the three most critical risk factors: census travel, ACP density and previous HLB locations. These factors initially were optimized exhaustively through simulations to determine the most optimal weight combination with the highest predictive power. After establishing the optimal weighting range for the initial three risk factors, additional critical factors were systematically incorporated based on importance to improve the predictive efficacy of the model. We standardized the model to avoid overfitting and to ensure resilience from noise. **Figure 2** highlights the most significant contributions to the model. Initially, ACP predominantly contributed to the model, which reflects the initiation of HLB infection through ACP transmission. After 2017, the contributions shifted to previous HLB hotspots, as these were pivotal in the spread of psyllids to nearby susceptible trees. Interestingly, farmer's markets, citrus nurseries and big box stores exhibited higher weights in the early years, possibly due to human-mediated dispersal and disposal practices in the absence of robust disease management protocols in these settings. Census travel (i.e., introduction risk from international travel) was considered one of the most important factors in the early epidemic for each county, but its contribution gradually reduced after 2017. Citrus transport corridor starts to show an increased impact on HLB spread in the last two years, highlighting the need for monitoring human-mediated pathways for spread. Notably, our analysis revealed higher HLB detections near packinghouses in 2017. This highlights the urgent need to increase sampling around these facilities to better understand and mitigate potential risks. Throughout the epidemic survey, there was no apparent connection between military installations and Native American lands contributing to the increased HLB prevalence in the landscape.

After this comprehensive analysis, we conducted a full model predictive performance evaluation for all new HLB finds 2015-22 (**Figure 3**). The predictive accuracy of the model was more than 95 percent for 2015 and 2016 and the overall predictive accuracy has consistently been higher than 85 percent since 2017. Any fluctuations observed may be attributed to underlying factors for which our model has not yet accounted. As we accumulate more data, we will continue to refine the accuracy of the HLB epidemic predictions.

Conclusions and Next Steps

The development of risk-based survey design and protocols is essential for effective HLB management in California. These provide evidence-based decision-making tools, survey efficacy evaluations and deployment strategies to

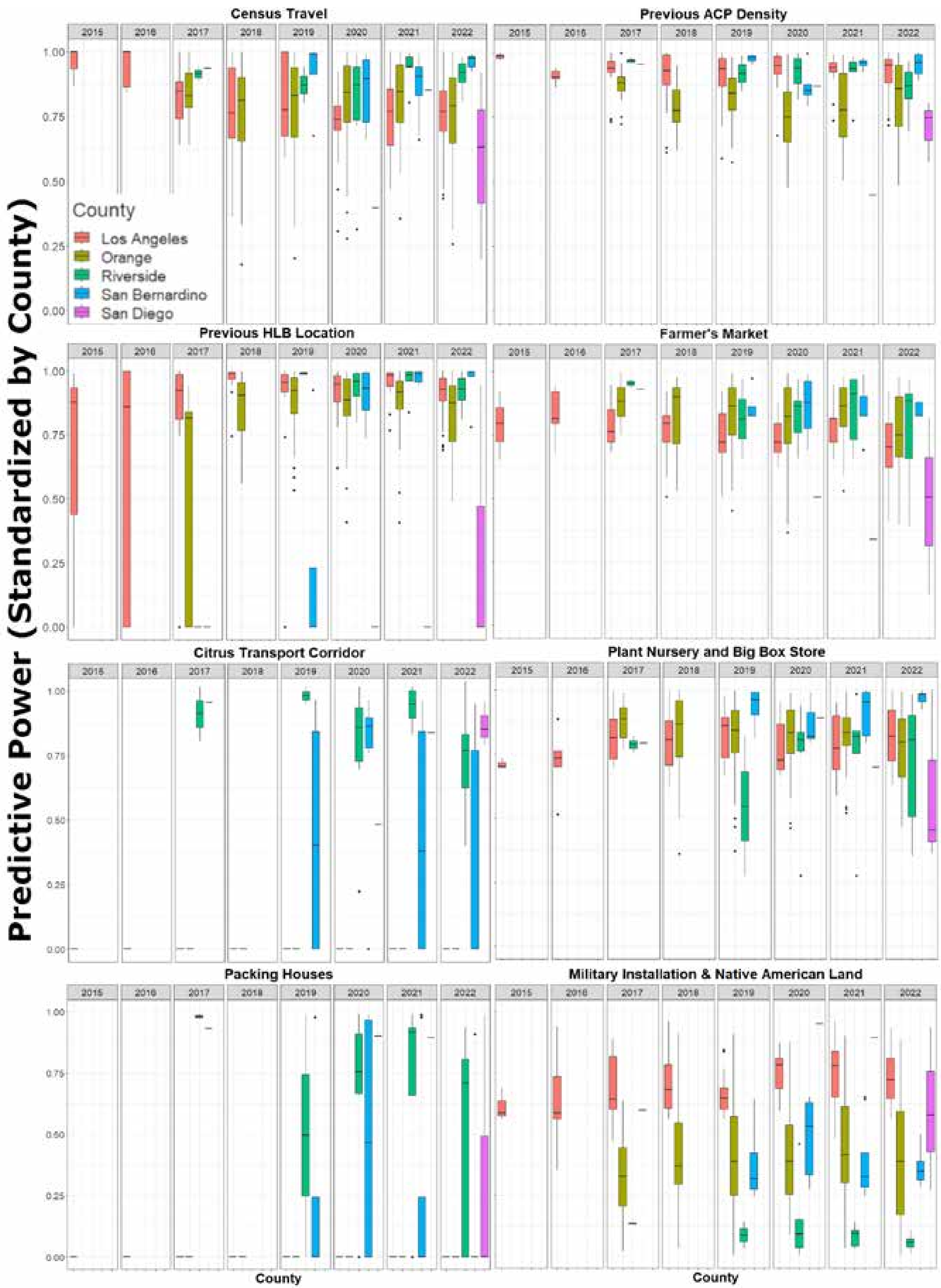

Figure 1. Evaluation of eight different risk factors on new huanglongbing detections using boxplots between 2015 and 2022, standardized by each county. The median and middle 50 percent of the predictive power for each factor are highlighted in the box. The whiskers show the high/low spreads from the middle part of the box, and the dots represent potential outliers for overall performance.

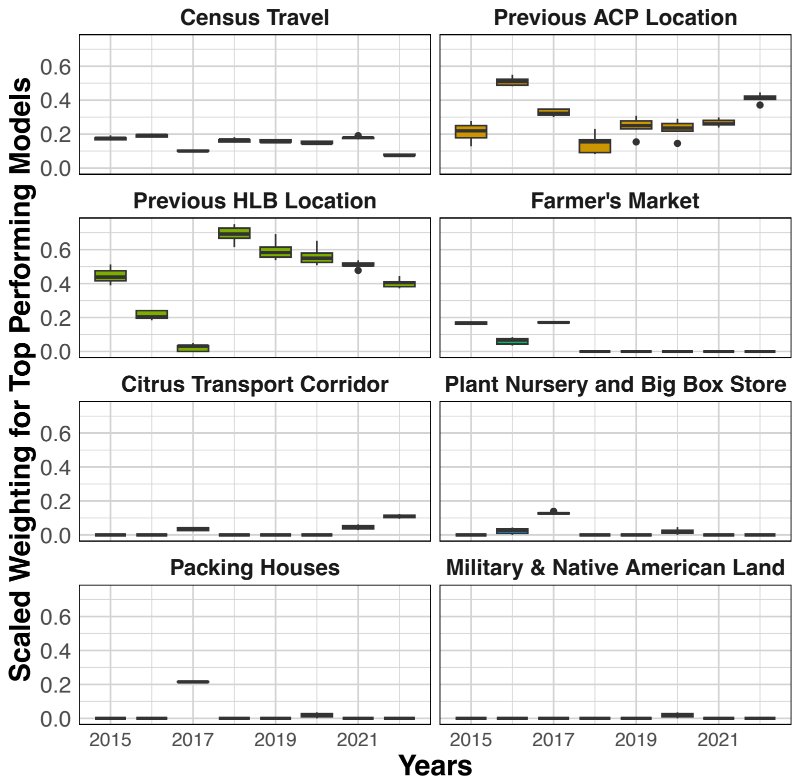

Figure 2. The optimized weighting distribution of selected risk factors for the top five percent performing risk models.

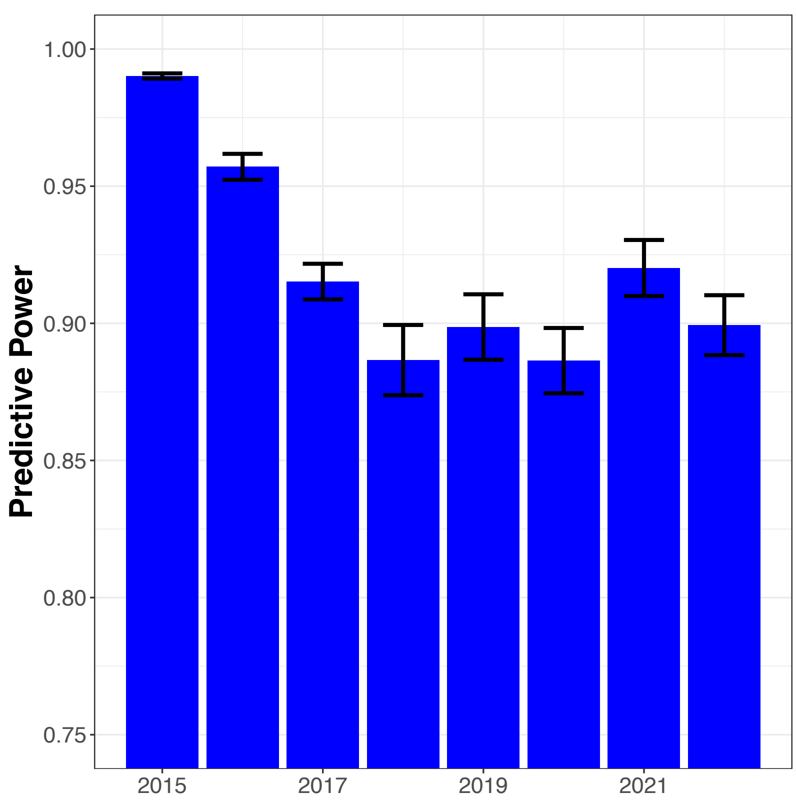

Figure 3. The mean performance (with standard error) of the full weighted model using the optimized weight distribution (i.e., from Figure 2) in predicting new huanglongbing locations, 2015-22.

optimize HLB control efforts. In collaboration with surveillance decision-makers, we regularly assess the HLB situation in California using up-to-date risk maps, prioritizing sampling efforts in HLB hotspots during subsequent survey cycles. These residential HLB risk maps can serve as regional early warning systems for growers and regulatory agencies, enhancing survey resource allocation to swiftly mitigate disease pressure beyond epidemic zones.

Furthermore, our research has explored cost-effective approaches to optimize risk factor weighting to improve early detection through numerous model evaluations. Assigning the surveyors to the right location can free up valuable manpower and resources for other program needs. Integrating the spatiotemporal characteristics of HLB dynamics using survey data also can help prioritize survey deployment and sampling resources for different delimitation survey protocols. Our research has estimated HLB prevalence and positivity rates in each county by combining risk maps with actual sampling density and detection techniques, facilitating further investigations into sampling effectiveness and allocation based on various outbreak risks and detection probabilities.

CRB Research Project #5300-199

References

Gottwald, T.; Luo, W.; Posny, D.; et al. 2019. A probabilistic census-travel model to predict introduction sites of exotic plant, animal and human pathogens. *Philosophical Transactions of the Royal Society* B. 374: 20180260. *https://doi.org/10.1098/rstb.2018.0260*

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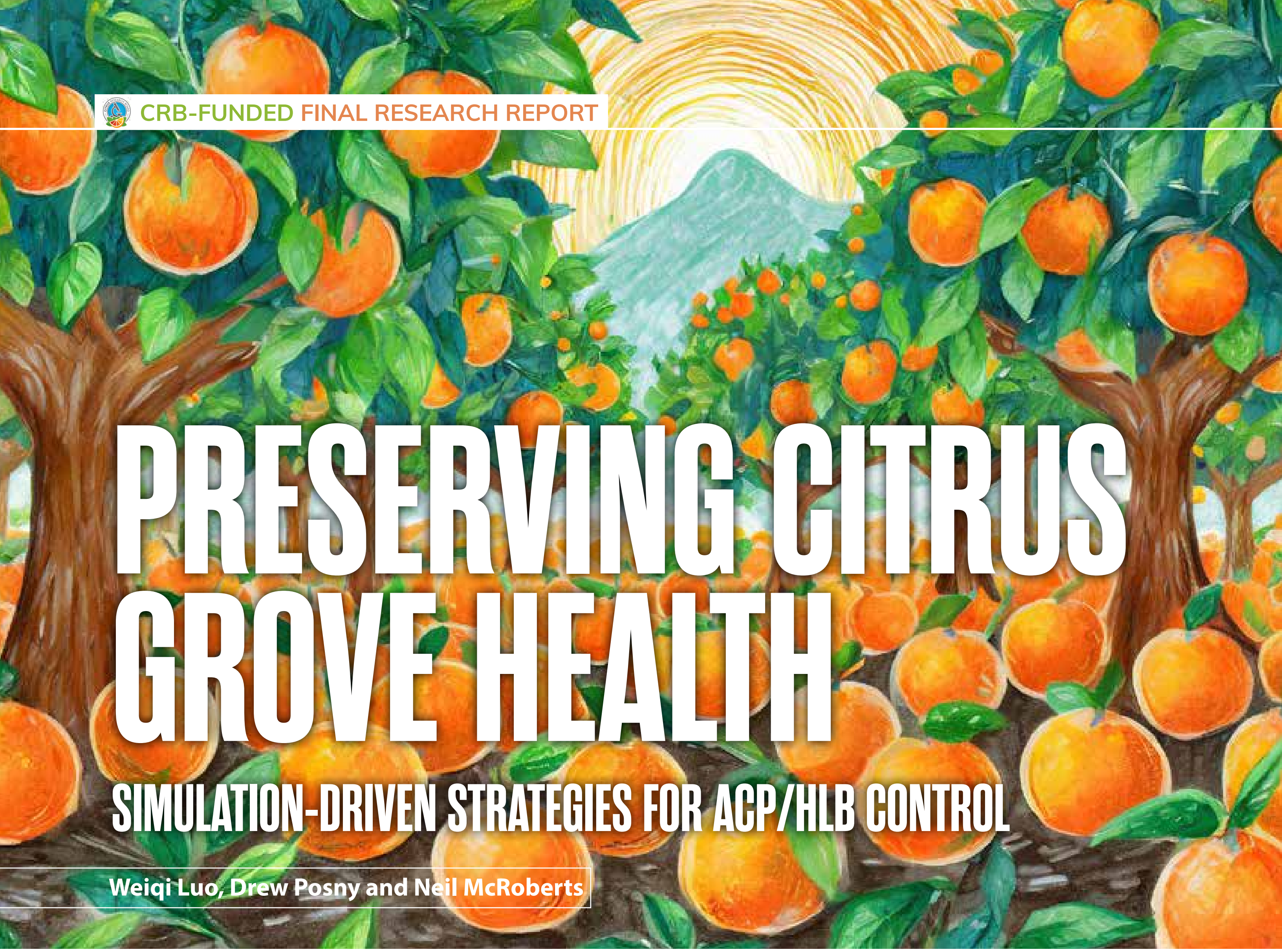

CRB-FUNDED FINAL RESEARCH REPORT

PRESERVING CITRUS GROVE HEALTH

SIMULATION-DRIVEN STRATEGIES FOR ACP/HLB CONTROL

Weiqi Luo, Drew Posny and Neil McRoberts

Project Summary

*As the Asian citrus psyllid (ACP) and huanglongbing (HLB) distributions continue to advance, it is crucial to consistently reevaluate our management approaches to ensure their effectiveness and efficient use of resources. Through the past few years, there has been a significant increase in residential HLB cases in southern California. This is an alarming progression of the incidence of HLB, posing serious threats to the citrus industry's sustainability in California. It is imperative that we deepen our understanding of the factors influencing ACP population dynamics and HLB development, including pathways and landscape drivers. To address these critical needs, we continued to improve our developed interactive online ACP/HLB simulation system (*https://epi-models.shinyapps.io/AgentBasedModel/*).*

California's citrus landscape is characterized by a diverse range of land uses, including purely residential areas, mixed residential and commercial citrus, and predominantly commercial citrus regions. Each of these land use types exhibits a distinct distribution of host plants, leading to variations in the factors influencing the development and spread of ACP and HLB. During the past decade, we have witnessed the emergence and progression of ACP and HLB in southern California. This has been coupled with sporadic detections and local eradication efforts of ACP in central and northern California. These developments have necessitated a significant expansion of ACP and HLB response areas. Given the differences in geography, climate, disease pressure and grower compliance levels across these regions, adopting a one-size-fits-all control strategy may prove ineffective in managing the disease and its vector within both residential and commercial landscapes.

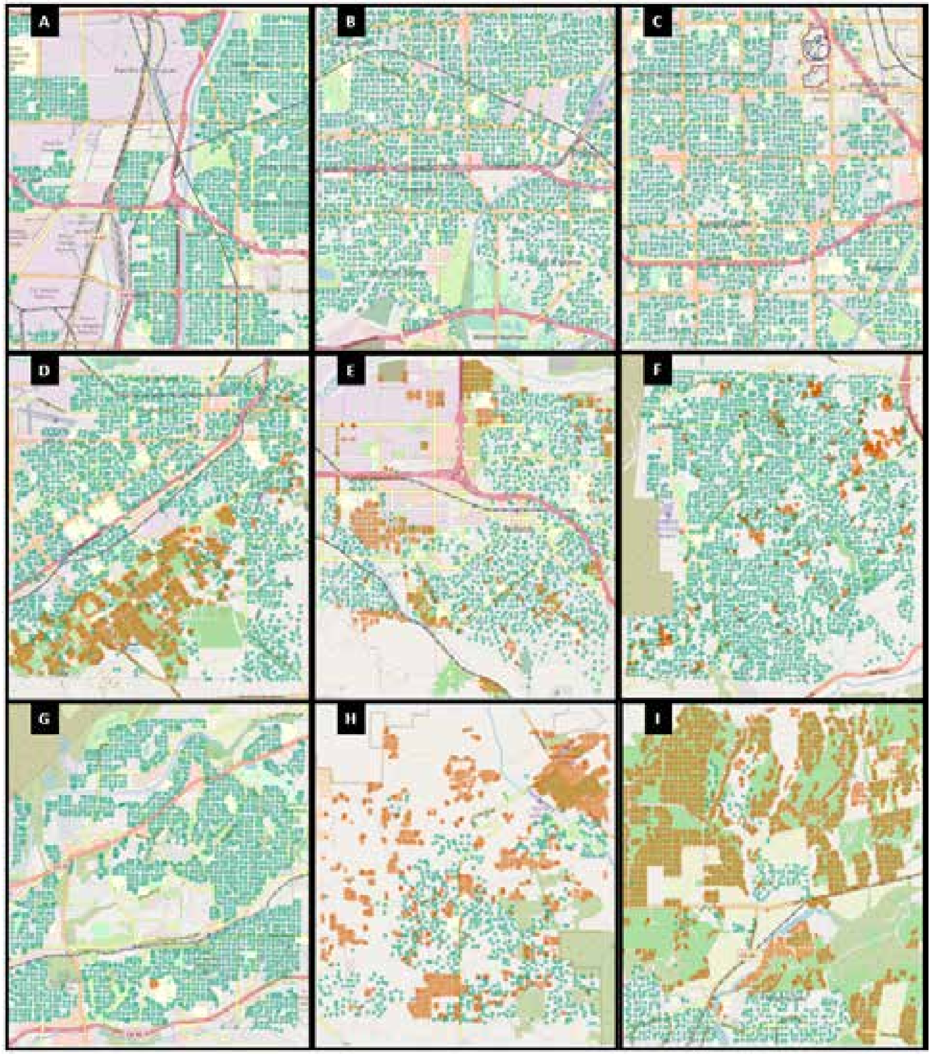

Figure 1. Available 25-square mile southern California citrus landscapes in (A) Long Beach [Los Angeles County], (B) San Gabriel [Los Angeles County], (C) Garden Grove [Orange County], (D) Riverside [Riverside County], (E) Redlands [San Bernardino County], (F) Fallbrook [San Diego County], (G) Oceanside [San Diego County], (H) Valley Center [San Diego County] and (I) Camarillo/Las Posas Valley [Ventura County] for simulation case analyses. Residential citrus properties are indicated in green, while commercial blocks are displayed in orange. These selected regions highlight the complexity of citrus landscapes in southern California.

This research aims to identify management strategies that align most effectively with the specific landscape characteristics and the current phase of the HLB distribution. To address this complex challenge, modeling tools have been employed to provide guidance on optimal control and management strategies through comprehensive statistical analyses and evaluations (internal and historical data validation) within various landscape contexts. To accomplish this, we have extended our previously developed agent-based model, which simulates changes in various locations over time. This model allows us to investigate how ACP and HLB may spread in real-world landscapes. In addition, this allows us to conduct in-depth analyses of epidemiology, risk assessment and the performance of management programs with scenario-based simulations.

Southern California Citrus Landscapes

The configuration of the citrus host landscape plays a pivotal role in our assessment of ACP/HLB dispersal dynamics and the development of location-specific management strategies. We have included additional citrus landscapes with ACP/HLB detections in our data processing and simulation analyses. As illustrated in **Figure 1**, we have selected nine distinct citrus landscapes, each encompassing a 25-square mile area, that are available online for scenario-based simulation studies. These selected areas represent a diverse spectrum of citrus compositions and the interplay between residential and commercial host types.

Climate Data

We have leveraged the National Oceanic and Atmospheric Administration's National Centers for Environmental Information as a valuable resource for the collection of climatological data, enabling us to assess their influence on the ACP/HLB distribution. Within California, a total of 276 weather stations are available, although they do not all operate simultaneously. Typically, there are approximately 160-170 active weather stations in operation each year that provide us with a robust dataset for summarizing weather conditions at the county level. While numerous weather variables are recorded, our study primarily focuses on the number of freezing days (i.e. daily minimum temperature less than 0°C) and total rainfall, as they are critical to the dynamics of ACP/HLB.

Climate Analysis

Timely issuance of alerts regarding latest favorable climate events that could promote ACP development across various regions in California is crucial. This functionality becomes particularly valuable in identifying counties with potential elevated risk of ACP/HLB infestations due to factors such as warm winters and other climatic variables. Accordingly, we are processing and scrutinizing data from most major citrus counties across southern, coastal and central California that, encompass areas with a historical prevalence of high ACP infestations and those with minimal ACP presence.

Noteworthy trends based on climate data from the 2001 season through the 2023 season, are depicted in the boxplots in **Figure 2**. For instance, Fresno, Monterey,

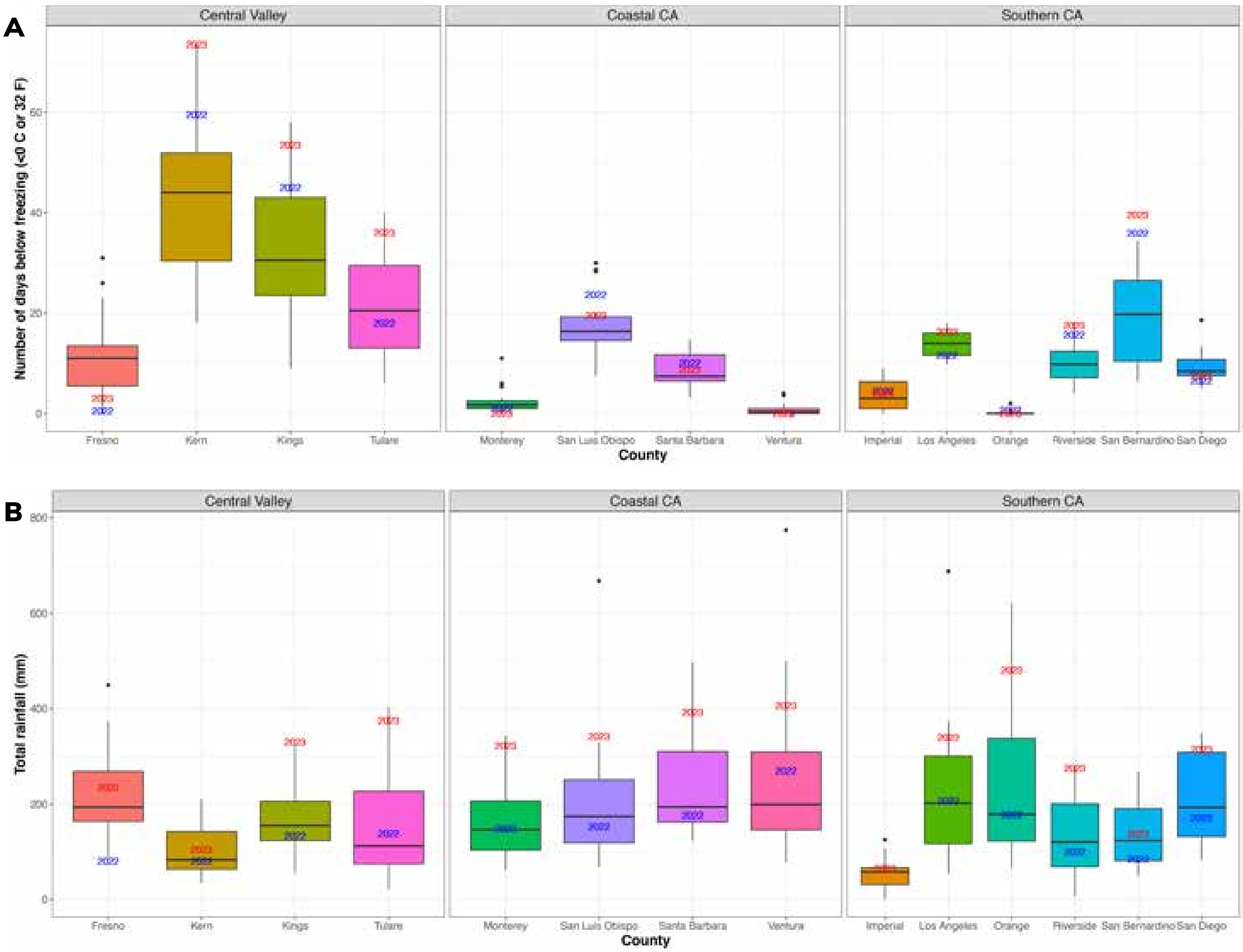

Figure 2. Number of freezing days (A) and total rainfall (B) summarized annually for southern, coastal and central California, in boxplot format seasons 2001-2023. Instead of calendar year, we are using season (2023 season: 07/2022 – 06/2023) to capture over-winter climate events. Box limits indicate the range of the central 50 percent of the data, with a central line marking the median value. Lines extend from each box to capture the range of the remaining data, with dots placed past the line edges to indicate outliers. The position of 2022 and 2023 seasons are marked on the boxplot accordingly.

Ventura and Orange counties experienced a near absence of freezing days in both 2022 and 2023, whereas Kern, Riverside and San Bernardino counties endured more extended periods of freezing temperatures. This suggests that milder winters may elevate the likelihood of ACP surviving through the winter months. Moreover, 2023 was one of the wettest years for most counties in California. Imperial County, owing to its desert climate, continued to receive minimal rainfall. Rainfall patterns can impact citrus flush production significantly, potentially correlating with ACP development, although this relationship occasionally can be obscured by irrigation practices.

Climate Impact Simulation

We now are investigating the influence of climate on ACP populations, with a focus on how temperature shifts might affect their long-term distribution and, consequently, the HLB spread. To gain insights into this intricate relationship, we selected three distinct landscapes (Camarillo, Ventura County; Fallbrook, San Diego County; and Redlands, San Bernardino County) from **Figure 1** for simulation.

In **Figure 3**, we present a snapshot of the general ACP and HLB dynamics within residential and commercial citrus settings for each of these landscapes, with no control measures applied, as deduced from a total of 100 simulations. There was rapid spread of ACP in regions characterized by warm winters, contrasting with a considerably slower rate in areas with colder climates. Notably, it took nearly 15 years for ACP to become established across the entire landscape in Redlands, whereas in Camarillo and Fallbrook, this spread occurred in less than three and seven years, respectively. By the end of the simulation, more than half of the commercial citrus in Camarillo will be infected, whereas only 25 percent of commercial citrus will be infected in Redlands.

It's crucial to align these simulation results regarding climate impact with effective management decisions. Stakeholders

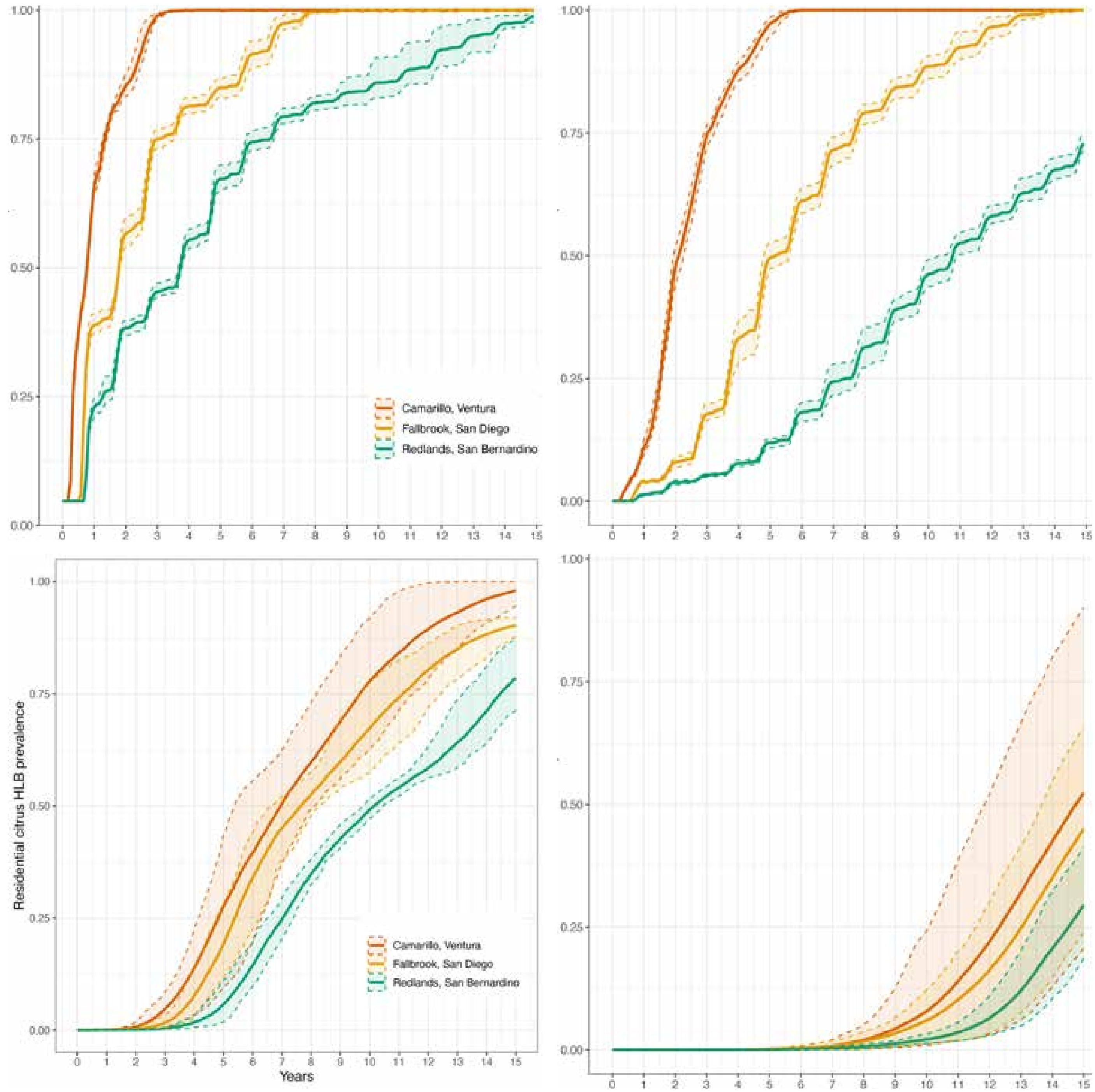

Figure 3. Assuming a five percent random distribution of Asian citrus psyllid (ACP) and initializing one cluster of five huanglongbing (HLB)-positive trees in the residential area to simulate the progression of ACP and HLB under different climate scenarios without implementing any survey or control measures. The solid lines represent the baselines (average behavior of the system) across 100 simulations, and the shaded areas represent the 95 percent confidence interval.

and regulatory agencies can utilize climate data to anticipate periods of heightened ACP activity in warmer winter regions. This enables them to implement targeted monitoring and surveillance during these times for the early detection of ACP infestations. So far, the slower progression of ACP in the Central Valley can be attributed, in part, to the longer freezing period during winter and ongoing eradication efforts. However, there is a need to alert citrus growers and residents in the Central Valley to raise awareness about the increased risk of ACP spread during warmer climate periods.

Detection Accuracy Simulation

Simulation of the spatiotemporal dynamics of HLB in commercial citrus groves offers us a valuable tool for assessing various management strategies related to surveys, pesticide application, biological control, tree removal and replanting. Survey and early detection play pivotal roles in any adaptive disease and vector management program. Therefore, we are evaluating the effectiveness and sustainability of HLB

Table 1. Asian citrus psyllid (ACP) and huanglongbing (HLB) management scenario setting and parameters.

PARAMETER	VALUE
Simulation landscape	Camarillo, Ventura
Simulation Time	15 years
Initial ACP Incidence	5% residential citrus, randomly distributed
Initial HLB Incidence	1 cluster of 5 HLB positive residential trees
Residential Survey Scheme	10% residential citrus/year, randomly selected
Commercial Survey Scheme	10% commercial citrus groves/year, 10% sampling density
Residential citrus delimitation	250 meter radius
Commercial citrus spray scheme	3 times/year (March, April & December)
Spray efficacy	90%
Removal Strategy	Individual
Removal Delay	Within 60 days
HLB detection accuracy	0.5, 0.7, 0.9 for asymptomatic trees

surveillance programs that incorporate early detection methods across a range of detection accuracy levels.

For illustrative purposes, we are examining the impact of three threshold values of HLB detection accuracy (0.5, 0.7 and 0.9 for asymptomatic trees) on HLB dynamics. Detailed settings for detection accuracy scenarios are presented in **Table 1**. In residential citrus landscapes, management protocols involve the deployment of routine surveys where plant and ACP samples are collected and tested for the presence of CLas. Any HLB-positive findings lead to the subsequent removal of infected trees, followed by a 250-meter management intervention delimitation response to contain. In commercial settings, growers routinely apply chemical treatments to mitigate ACP infestations. This simulation allows us to assess the consequences of varying detection accuracy levels on the dynamics of HLB infection.

In our scenario analysis focused on the Ventura landscape (**Figure 4**), the implementation of simulated management strategies has had a profound impact on slowing down the increased incidence of HLB in both residential and commercial areas compared to a scenario with no control measures. Notably, there are significant variations in the HLB epidemic dynamics across three different HLB detection accuracy levels.

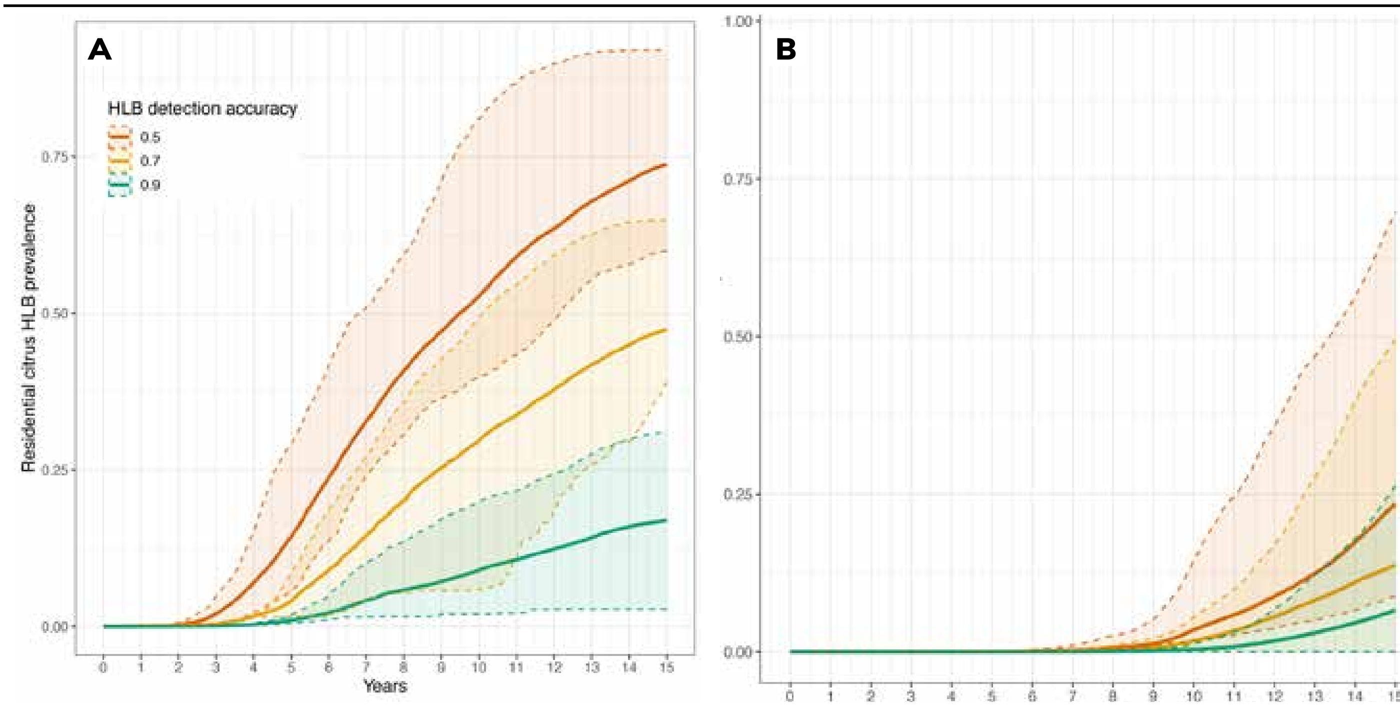

Figure 4. The simulation results of residential (A) and commercial citrus (B) prevalence in the Ventura study area with three different huanglongbing (HLB) detection accuracies.

Even with a lower detection accuracy set at 0.5 (i.e., missing 50 percent of asymptomatic trees), the early stages of the HLB epidemic progress at a considerably slower pace. However, as more undetected CLas-infected trees remain in the landscape, the rate of increased HLB incidence begins to accelerate during the later stages of the epidemic. In contrast, with a high detection accuracy level of 0.9 (i.e., missing only ten percent), the increased incidence of HLB in the residential landscape has been curtailed dramatically. Most notably, the majority of 'buffered' residential citrus trees (those in close proximity to commercial groves) remain uninfected for at least five years. By reducing the vulnerability of residential citrus trees to HLB invasion from nearby sources, the management approach effectively contains the increased incidence of HLB in commercial groves as well, resulting in only a five percent prevalence of HLB at the conclusion of the simulation.

Conclusions and Next Steps

As the HLB spread continues to evolve, it becomes increasingly imperative to identify cost-effective interventions that can curtail its spread effectively while ensuring the sustainability of commercial citrus production. The analytical findings from this project hold considerable potential practical utility for citrus growers and regulatory agencies alike, offering valuable insights for the development and assessment of management strategies within real-world California citrus landscapes. The online interface is a valuable tool, facilitating the rapid proposal and simulated responses across various landscapes including management, epidemiology and social compliance aspects, as well as economic considerations.

The simulation analyses conducted across diverse California citrus landscapes, which encompass various climatological conditions and ACP/HLB pressures, can provide location-specific strategies for combating the ACP/HLB epidemic. We regularly update our analyses with the latest ACP and HLB data, ensuring stakeholders have timely information. We've also explored the relationship between detection accuracy, epidemic stage and control effectiveness, enhancing our understanding of the crucial role early and accurate detection methods play in managing the HLB spread.

CRB Research Project #5300-212

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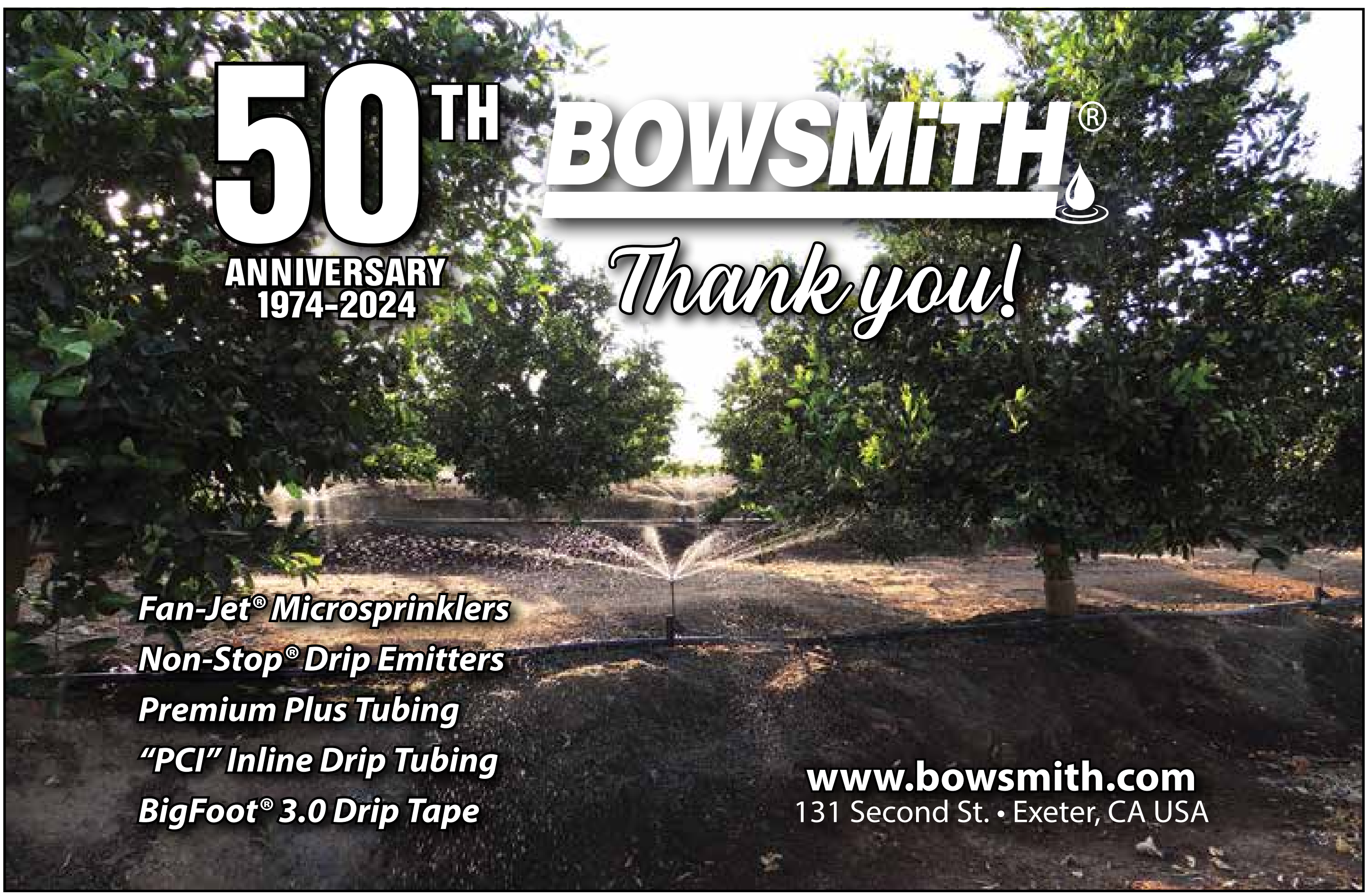

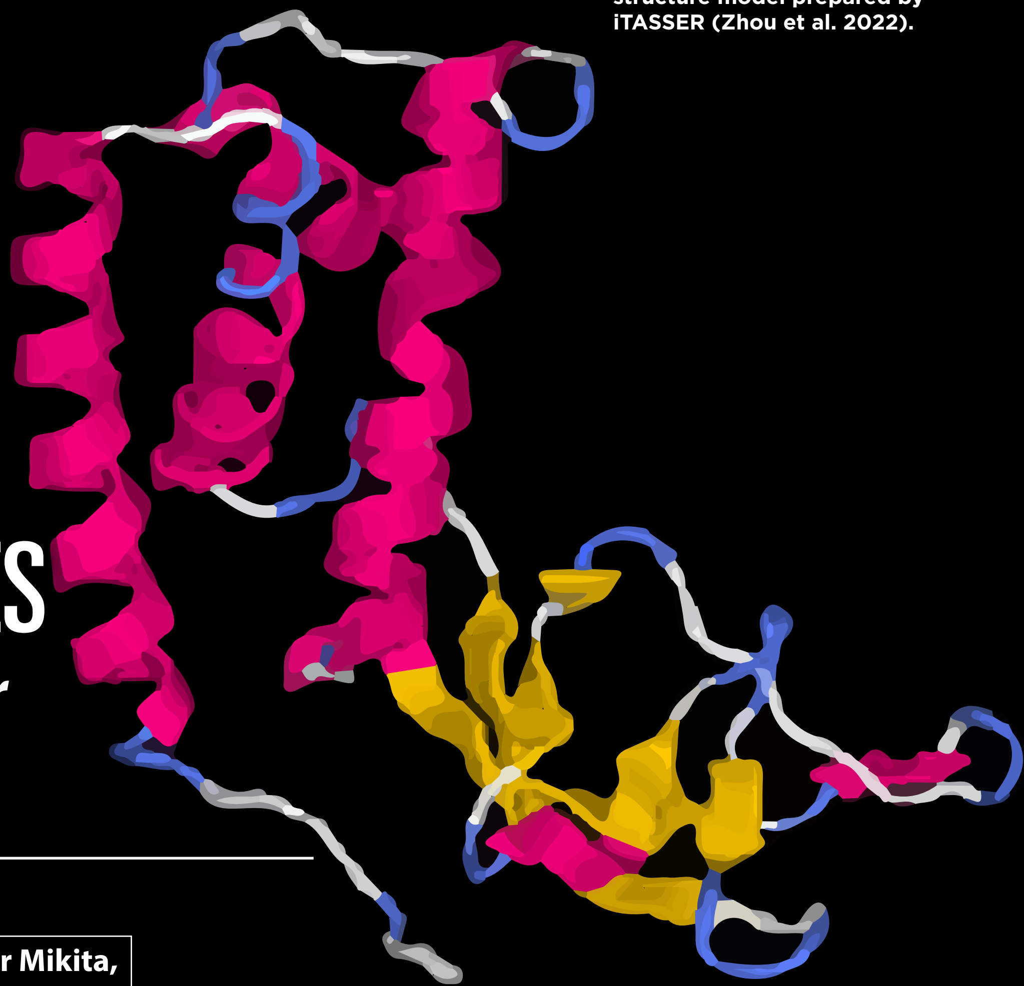

'*Candidatus* Liberibacter asiaticus' hydrolase protein structure model prepared by iTASSER (Zhou et al. 2022).

PHAGE HYDROLASES for *Liberibacter* Control

Janetta Hakovirta, Christopher Mikita, Evan Braswell and Jennie Fagen

Project Summary

*Many bacterial pathogens, including the presumptive causal agent of huanglongbing, '*Candidatus *Liberibacter asiaticus' (CLas), form an extracellular matrix of DNA, polysaccharides and other components called biofilms[1] that protect cells from external threats. Bacteriophages, or viruses that infect bacteria, have evolved to penetrate the biofilms produced by their host bacteria using enzymes (a type of protein), that break down these protective substances. Within the genome of a prophage[2] found in CLas, we identified an enzymatic portion of a common structural bacteriophage protein. We hypothesized that this protein serves to attack bacteria and dissolve the cell's external defenses, such as biofilms. Here, we describe the identification, production and purification of these proteins from California isolates of CLas. Furthermore, we investigated their impact on bacterial growth and biofilm production. Our group successfully produced and purified the two forms of this enzyme found in California, which we named 'A' and 'H.' Both forms of the enzyme significantly impeded the apparent growth of Liberibacter crescens (Lcres) in culture. Initial testing against CLas was not able to reach the needed statistical thresholds due to variable pathogen loads within the insect system utilized in this first step of efficacy testing. Additional evaluation of the protein within the citrus system is needed. Unlike the insect-feeding assay,* in planta *production of the protein provides a more consistent amount of the therapeutic protein to the resident CLas infection.*

Background

In order to infect and replicate in bacteria, bacteriophages have a protein capsule containing a DNA or an RNA genome and other structures that allow them to recognize and attack their hosts. In some instances of the evolutionary arms race between bacteria and bacteriophage, the bacteriophage genome can get incorporated into the bacterial host's genome. One such bacteriophage, SC1, is found in the CLas genome giving us a snapshot view of the components of the phage. Detailed analysis of the bacteriophage genome revealed a tail protein that is responsible for host recognition and attack. This tail protein also has a potential secondary enzymatic function as a hydrolase[3] that could degrade the CLas protective coating, a key component of a biofilm.

CLas has been implicated in forming biofilms at the outer surface of the midgut of the Asian citrus psyllid (ACP; Ammar et al. 2011). These biofilm barriers make it more difficult to access and control CLas. In this study, we hypothesized that a region of the tail protein present in the CLas bacteriophage SC1 is a hydrolase and investigated its activity. The goal of this work is to provide a natural means of CLas control both by minimizing colonization of the pathogen in the psyllid and the plant.

Results

By comparing protein sequences from CLas strains in California, we identified two main groups of the SC1 phage hydrolases (**Figure 1**). CLas strains from regions of Anaheim, San Gabriel and San Bernardino had similar SC1 proteins, which we called group A. CLas strains from Hacienda Heights had SC1 phage hydrolases that were more similar to each other, which we called group H. Both types of hydrolases then were produced in the lab to generate a pure form for further functional analysis.

Due to the inability to culture CLas under laboratory conditions, we used the close relative *Liberibacter crescens* (*Lcres*), as a proxy in our experiments. While the ability to grow *Lcres* in the laboratory solved some problems, it caused others. CLas forms a biofilm in the gut of its psyllid vector, but *Lcres* does not readily form an independent biofilm in pure culture (Naranjo et al. 2019). The ability of *Lcres* to form a biofilm in the absence of proteins was determined by a crystal violet biofilm assay. The assay did not detect biofilm formation by *Lcres* compared to the control *Klebsiella pneumoniae* (**Figure 2**). Since *Lcres* biofilm was not formed, further testing was done to investigate the effects of the SC1 protein on bacteria themselves. *Lcres* was grown with and without the presence of the purified bacteriophage proteins, and growth was observed by measuring the

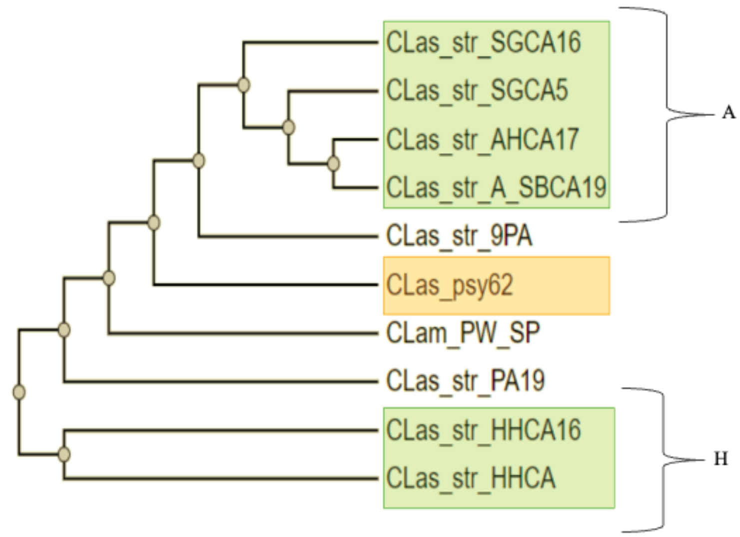

Figure 1. Phylogenetic tree demonstrating similarity of prophage hydrolases. Two protein variants were identified from the SC1 phage present in California *'Candidatus* Liberibacter asiaticus' (CLas) genomes. CLas strains psy62 from Florida, PA19 from Pakistan, 9PA from Brazil and *'Candidatus* Liberibacter americanus' PW_SP from Brazil were included for comparison.

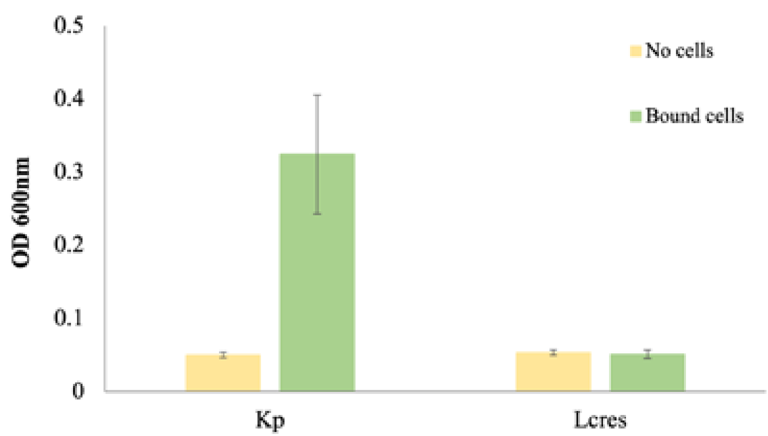

Figure 2. Biofilm formation of *Liberibacter crescens* (Lcres) by crystal violet assay. Higher absorbance (OD 600nm) indicates more surface bound cells (biofilm formation) in comparison to media only (no bacterial cells). As a positive biofilm producer, *Klebsiella pneumoniae* (Kp) was included in the study.

optical density (OD at 600nm) of the cell culture during a four-day period. Cultures grown with Group A and Group H SC1 proteins had lower ODs, than the control (no protein) culture indicating a suppression of *Lcres* growth by the SC1 proteins (**Figure 3**).

However, there are many ways in which SC1 could cause the observed lower absorbance – direct killing of bacterial cells, inhibition of bacterial reproduction or a decreased size of each cell. The SC1 protein has two predicted functional regions: an enzymatic functional region predicted to disrupt biofilms and a structural region predicted to disrupt cell membranes. Either of these regions could limit the measured density of *Lcres* growth by reducing the overall number of bacteria or the apparent size of each individual bacterial cell. Decreased cell size is predicted based on the enzymatic function of the protein, whereas reduced cell number (direct killing) is predicted from the structural portion of the bacteriophage protein. To determine the underlying cause of the lower optical density, we counted the number of viable *Lcres* cells at the end of the treatment period. No significant difference in bacterial cell counts was present between treated and non-treated samples (**Figure 4**); therefore, the decrease in absorbance was not caused by a decrease in cell number indicating that no direct killing occurred.

After our studies demonstrated an impact of the putative bacteriophage hydrolases on *Lcres* in the laboratory, we proceeded to test them on CLas-infected psyllids at the U.S. Department of Agriculture-Animal and Plant Health Inspection Service-Plant Protection and Quarantine-Science & Technology-Insect Management and Molecular Diagnostics Laboratory located in Edinburg, Texas. Psyllids were raised from egg to adulthood on potted CLas-infected citrus trees and then were transferred to an artificial diet with and without hydrolases (Hall et al. 2010). They were allowed to feed for three days. Survival of psyllids after three days was 38-45 percent for all treatments. From the surviving ACP, we estimated the number of CLas in each individual psyllid by using real-time PCR detection of a *nrdB* gene in the CLas genome. Of the survivors, those with detectable CLas infection accounted for only nine percent of the original population. No difference was observed in the amount of CLas within infected psyllids that fed on diet with or without hydrolases (**Figure 5**). Inconsistency in the viability of psyllids, independent of the presence of hydrolase in the diet, made determining the effect of the proteins on CLas in ACP difficult. For better understanding of the effect of the hydrolase, a larger sample size of psyllids is required to offset the low survivorship and CLas infection rate.

Conclusion

Computationally predicted hydrolases in a CLas prophage genome were found to be functional and reduce the optical density of *Lcres* cultures in the laboratory. Further investigations will be conducted to determine the effect of hydrolases on cell properties, such as size, by microscopy. However, no significant effect by the proteins was observed on CLas in infected psyllids under the conditions of this study. Given that the proteins were discovered within CLas and are functional in a close relative species, it is likely the failure to detect activity in psyllids is a consequence of our experimental procedures rather than lack of activity. In either case, further investigations are needed to better understand the effect. However, the work described here sets the stage for identification of other enzymes that may be useful in minimizing the spread of CLas. For longer term development, incorporation of these protein genes in non-disease causing *Citrus tristeza virus* could be evaluated further *in planta*, potentially creating huanglongbing-resistant citrus varieties or psyllids that are incapable of CLas transmission.

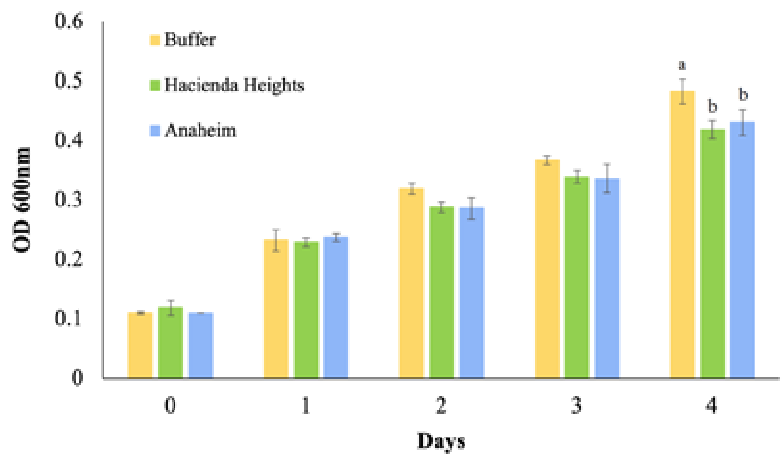

Figure 3. Effect of hydrolase treatments (Hacienda Heights and Anaheim strains) on *Liberibacter crescens* on absorbance (OD 600nm) during a four-day incubation. Significant difference noted for p<0.05. Columns not connected by the same letter are significantly different. Buffer solution (no hydrolase) was included as a control.

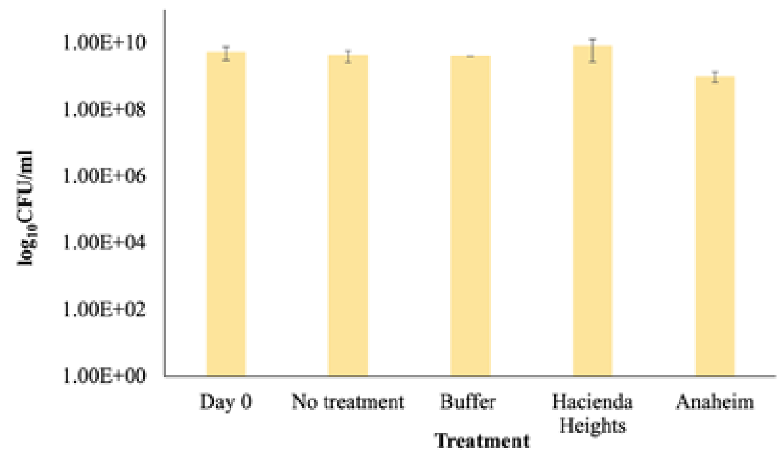

Figure 4. Effect of hydrolase treatments (Hacienda Heights and Anaheim strains) on viability of *Liberibacter crescens* colony forming units (CFU) after incubation for four days (d4) compared to day zero (d0). No treatment and buffer solution (no hydrolase) were included as controls.

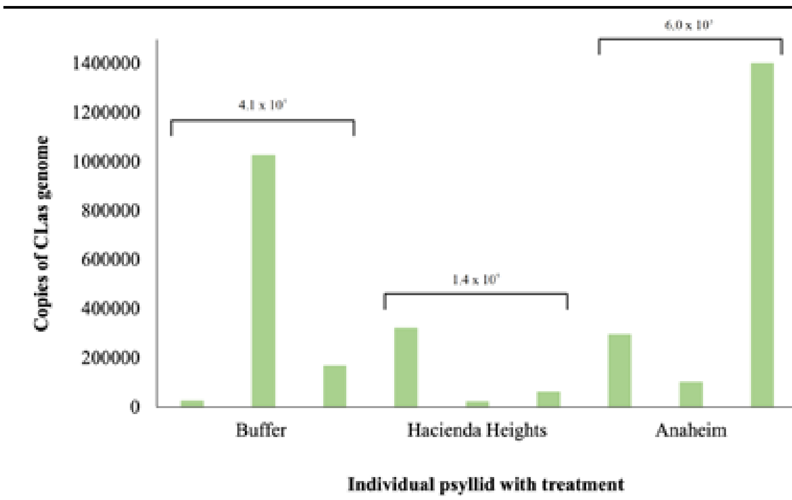

Figure 5. Effect of hydrolases (Hacienda Heights and Anaheim strains) on '*Candidatus* Liberibacter asiaticus' (CLas) infected Asian citrus psyllids. CLas genome copy number determined by detection of the nrdB gene by quantitative PCR. Average copy number is indicated for each treatment. Buffer solution (no hydrolase) included as a control.

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Glossary

[1]**Biofilm:** Bacteria adhering to a surface or aggregated together surrounded by a self-producing extracellular matrix of DNA, polysaccharides and other components.

[2]**Prophage:** Inserted genetic material from a bacteriophage into a bacterial genome.

[3]**Hydrolase:** An enzyme that uses water to break down chemical bonds.

References

Ammar, E.D.; Shatters, R.G.; Hall, D.G. 2011. Localization of *'Candidatus* Liberibacter asiaticus,' associated with citrus huanglongbing disease, in its psyllid vector using fluorescence in situ hybridization. *Journal of Phytopathology* 159(11-12):726-734.

Hall, D.G.; Shatters, R.G.; Carpenter, J.E.; et al. 2010. Research toward an artificial diet for adult Asian citrus psyllid. *Annals of the Entomological Society of America* 103(4):611-617.

Naranjo, E.; Merfa, M.V.; Ferreira, V.; et al. 2019. *Liberibacter crescens* biofilm formation *in vitro*: Establishment of a model system for pathogenic *'Candidatus* Liberibacter spp.' *Scientific reports* 9:5150. *https://doi.org/10.1038/s41598-019-41495-5*

Zhou, X.; Zheng, W.; Li, Y.; et al. 2022. I-TASSER-MTD: a deep-learning-based platform for multi-domain protein structure and function prediction. *Nature Protocols* 17:2326-2353.

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The citrus yellow vein-associated virus field trial established at University of California, Riverside Agricultural Operations in Riverside, California.

BIOLOGY AND TRANSMISSIBILITY OF CYVaV-LIKE RNA

Arunabha Mitra, Sohrab Bodaghi, Kiran R. Gadhave, Sydney Helm Rodriguez, Raymond K. Yokomi, Abigail M. Frolli, Ashraf El-Kereamy and Georgios Vidalakis

Project Summary

*A novel citrus-infecting virus-like RNA named citrus yellow vein-associated virus (CYVaV) was identified in association with the 1957-reported citrus yellow vein disease (CYVD). CYVaV has traits making it suitable for developing a virus expression vector[1] that potentially could be used to protect citrus plants against economically important citrus pathogens and pests. The primary objective of this Citrus Research Board (CRB)-funded project was to study the impacts of CYVaV on commercially popular citrus varieties and the potential transmissibility of CYVaV via pollen or aphids. A field trial was established in 2020 at the University of California, Riverside comprising 12 replicated commercial citrus scion/rootstock combinations graft-inoculated with CYVaV. The inoculated and non-inoculated control trees were monitored periodically for symptoms and screened for the presence of CYVaV, which was detected in 32 percent of the inoculated trees after three years and three graft-inoculation events. In the infected trees, primarily lemon and mandarin types, CYVaV induced mild or no canopy symptoms and was detectable by molecular testing in stems, leaves, roots and in association with pollen. One in-field hand-pollination experiment provided no evidence of CYVaV pollen transmissibility. In one aphid transmission greenhouse experiment, CYVaV was detected in cotton aphids (*Aphis gossypii*) feeding on infected trees; however, no evidence of aphid transmissibility was found. The first fruit harvest, in spring 2023, showed no evidence of negative CYVaV effects on fruit production or quality.*

Background

In 2021, a novel plant-associated RNA named citrus yellow vein-associated virus (CYVaV) was described (Kwon et al. 2021), not to be confused with *Citrus yellow vein clearing virus* (CYVCV), which was recently discovered in central California. CYVaV had its origins in the yellow vein disease reported from four limequat trees in California (Weathers 1957). The properties of CYVaV – a small genome and being phloem-limited with sustained and systemic colonization of citrus tissues – make it suitable as a potential virus expression vector (Simon et al. 2020). Development and commercial deployment of this or any forthcoming CYVaV-based technologies hinges on comprehensive field-based evaluations of CYVaV to ensure that it is citrus-safe and does not pose risk of accidental release and spread into the environment or to other hosts from inoculated plants.

This CRB-funded project continued the field-based evaluations of CYVaV initiated under a previous CRB grant (#5300-207; Simon et al. 2023). Continuing these evaluations, the field trees (inoculated and non-inoculated controls) were monitored continuously for emergence of the characteristic yellow vein leaf symptoms and periodically screened for CYVaV by reverse transcription quantitative polymerase chain reaction (RT-qPCR)[2] to evaluate infection rate over time. To gauge CYVaV-driven effects on commercial citrus growth and productivity, we routinely collected tree height measurements and in spring 2023 harvested the first fruit crop to assess yield and fruit quality. Additionally, to investigate that any future CYVaV-based expression vectors cannot "escape" into the environment from treated trees by natural modes, such as pollen or aphids, we conducted pollen and aphid transmission bioassays with the wild type (WT) CYVaV.

Results

I. CYVaV field infection rate in commercial citrus

Among the graft-inoculated trees, CYVaV was not detected by RT-qPCR or symptom expression in 68 percent (48 of 71) of the trees after three rounds of graft inoculations performed in 2020, 2021 and 2022 (**Table 1**). In four CYVaV infected trees, characteristic leaf symptoms began emerging

Table 1. Summary of Citrus yellow vein-associated virus (CYVaV) incidence screening of the University of California, Riverside CYVaV field trial at the six-month, one-, two- and three-year pgi timepoints for the 12 scion/rootstock (S/R) combinations. Results include graft inoculum survival (after May 2022 repeat inoculations), CYVaV infection rate (%) and symptom rate (%). Note: N=6 except for S/R ID 12 where N=5.

S/R ID	SCION	ROOTSTOCK	INOCULUM SURVIVAL[a]	6 MONTHS PGI (JUNE 2021)	1 year pgi (November 2021)		2 years pgi (November 2022)		3 years pgi (November 2023)	
				CYVAV INFECTION RATE (%)	CYVAV INFECTION RATE (%)	CYVAV SYMPTOM RATE (%)	CYVAV INFECTION RATE (%)	CYVAV SYMPTOM RATE (%)	CYVAV INFECTION RATE (%)	CYVAV SYMPTOM RATE (%)
1	Limoneira 8A Lisbon lemon	Rubidoux Trifoliate	6/6	83	66.7	33.3	66.7	33.3	66.7	33.3
2	Limoneira 8A Lisbon lemon	Macrophylla	6/6	33	50	17	66.7	17	66.7	17
3	Parent Washington navel	Carrizo Citrange	5/6	17	0	0	0	0	0	0
4	'Shiranui' mandarin	Carrizo Citrange	6/6	33	0	0	0	0	0	0
5	Limoneira 8A Lisbon lemon	Carrizo Citrange	6/6	83	100	50	100	33.3	100	33.3
6	Cara Cara navel	Carrizo Citrange	5/6	17	0	0	0	0	0	0
7	'Shiranui' mandarin	Rubidoux Trifoliate	6/6	0	17	0	17	0	50	0
8	Cara Cara navel	Rubidoux Trifoliate	6/6	0	0	0	0	0	0	0
9	'Tango' mandarin	Rubidoux Trifoliate	6/6	67	17	33.3	33.3	66.7	50	83.3
10	Miho Wase satsuma	Carrizo Citrange	5/6	0	0	0	0	0	0	0
11	'Tango' mandarin	Carrizo Citrange	6/6	33.3	0	0	0	17	0	0
12	Parent Washington navel	Rubidoux Trifoliate	5/5	0	0	0	0	0	0	0

[a]*Inoculum survival determined based on visual inspection of the graft inoculation sites. Scores are cumulative of three rounds of graft inoculations performed in 2020, 2021 and 2022.*

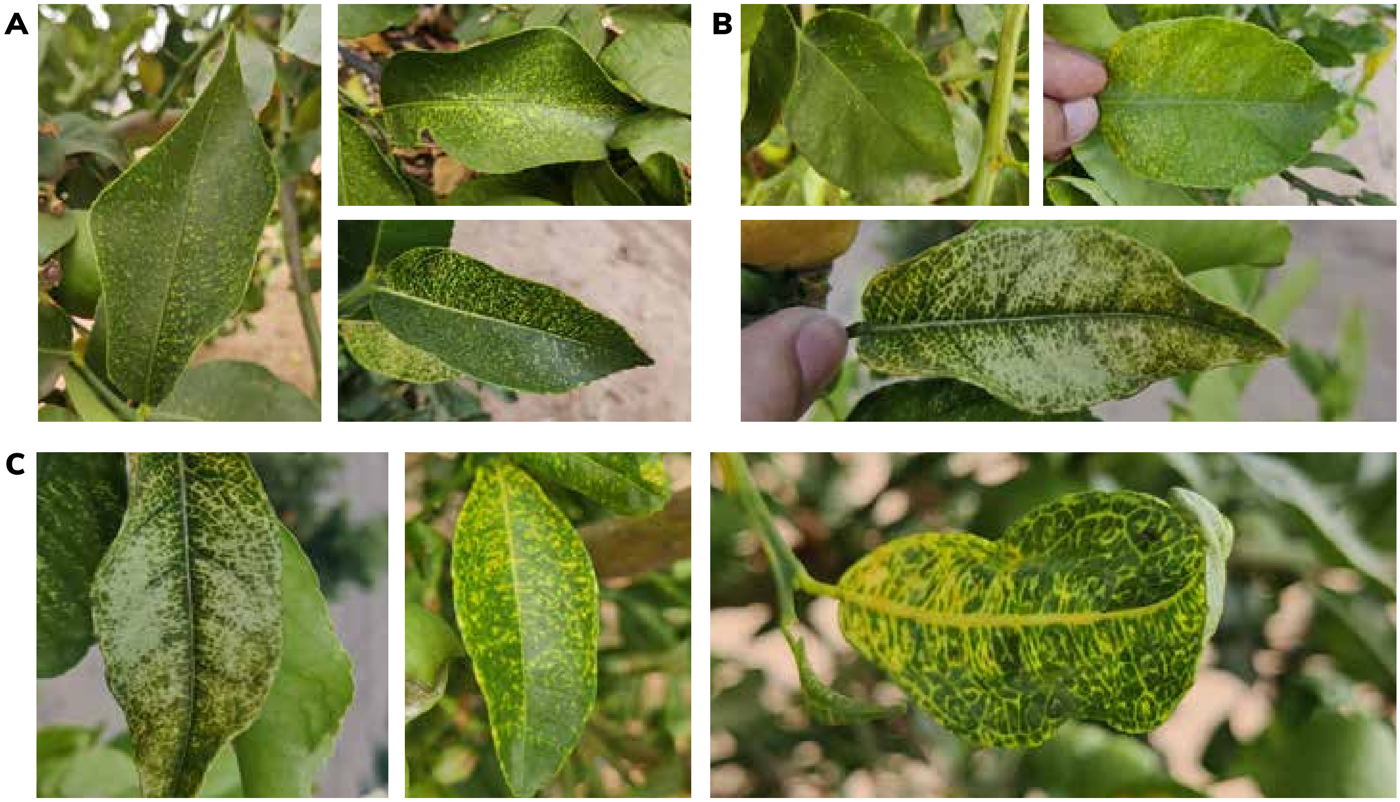

Figure 1. Characteristic yellow vein leaf symptoms of citrus yellow vein-associated virus infection in graft-inoculated trees of (A) Limoneira 8A Lisbon lemon, (B) Perrine lemon/lime hybrid and (C) 'Tango' mandarin.

approximately six months post-graft-inoculation (pgi). Based on visual observation only, the number of symptomatic trees graft-inoculated with CYVaV gradually increased to a total of 13 by November 2023 (three years pgi). Distinct yellow vein leaf symptoms primarily were observed on Limoneira 8A Lisbon lemon, 'Tango' mandarin and a (single, non-replicated) Perrine lemon/lime hybrid tree (**Figure 1**). At the same time, RT-qPCR screening detected three additional CYVaV-positive 'Shiranui' mandarin trees, but none of them showed CYVaV symptoms. Sentinel citron indicator trees planted adjacent to the CYVaV field trial so far have been non-symptomatic, and RT-qPCR testing will be performed at the trial conclusion to test for potential cryptic (i.e., non-symptomatic) natural CYVaV transmission.

The main takeaways regarding the field CYVaV infection rates in relation to inoculum survival, repeated graft-inoculations, RT-qPCR results and CYVaV symptom expression are:

1. CYVaV infection among the tested S/R combinations appears to be non-uniform and

2. Limoneira 8A Lisbon lemon, 'Tango' and 'Shiranui' mandarin were the most field-compatible citrus cultivars for WT CYVaV colonization.

II. CYVaV-driven impacts on the growth and productivity of commercial citrus

Beginning in October 2022, the field trees showed some height differences. In the case of Limoneira 8A Lisbon lemon (S/R IDs 2 and 5), there was a noticeable difference emerging between inoculated and non-inoculated control trees – inoculated trees were shorter than the control trees (**Figure 2**). An opposite trend occurred for S/R IDs 1 (Limoneira 8A Lisbon lemon) and 11 ('Tango' mandarin) (**Figure 2**). It is notable that by the three-year pgi symptom survey and RT-qPCR screening, for S/R IDs 1 and 2, four of six inoculated trees were CYVaV-positive; for S/R ID 5, all inoculated trees were CYVaV-positive; for S/R ID 11, two of six inoculated trees were CYVaV-positive (**Table 1**). Therefore, these emerging height differences could not be attributed solely to CYVaV but could be also due to S/R-specific growth rates.

The first fruit crop from the field trial was produced in spring 2023. Fruit was harvested from all trees, and yield data (fruit counts, yield weights) were recorded. For each of the 12 S/R combinations, samples of ten fruit per CYVaV treatment (inoculated and control, **Figure 3**) were subjected to an array of standardized citrus qualitative analyses and measurements at the Lindcove Research and Extension Center (LREC) that included rind thickness, fruit firmness, juice weight, juice volume, juice sugar content and juice titratable acid content. Results of these preliminary analyses indicated no fruit yield (**Figure 4**) or quality differences; however, further analyses of future harvests is required to assess any CYVaV effects.

III. CYVaV transmissibility

In the foundational studies by the University of California, Riverside's L. G. Weathers, citrus yellow vein disease was reported to be readily graft-transmissible with limited

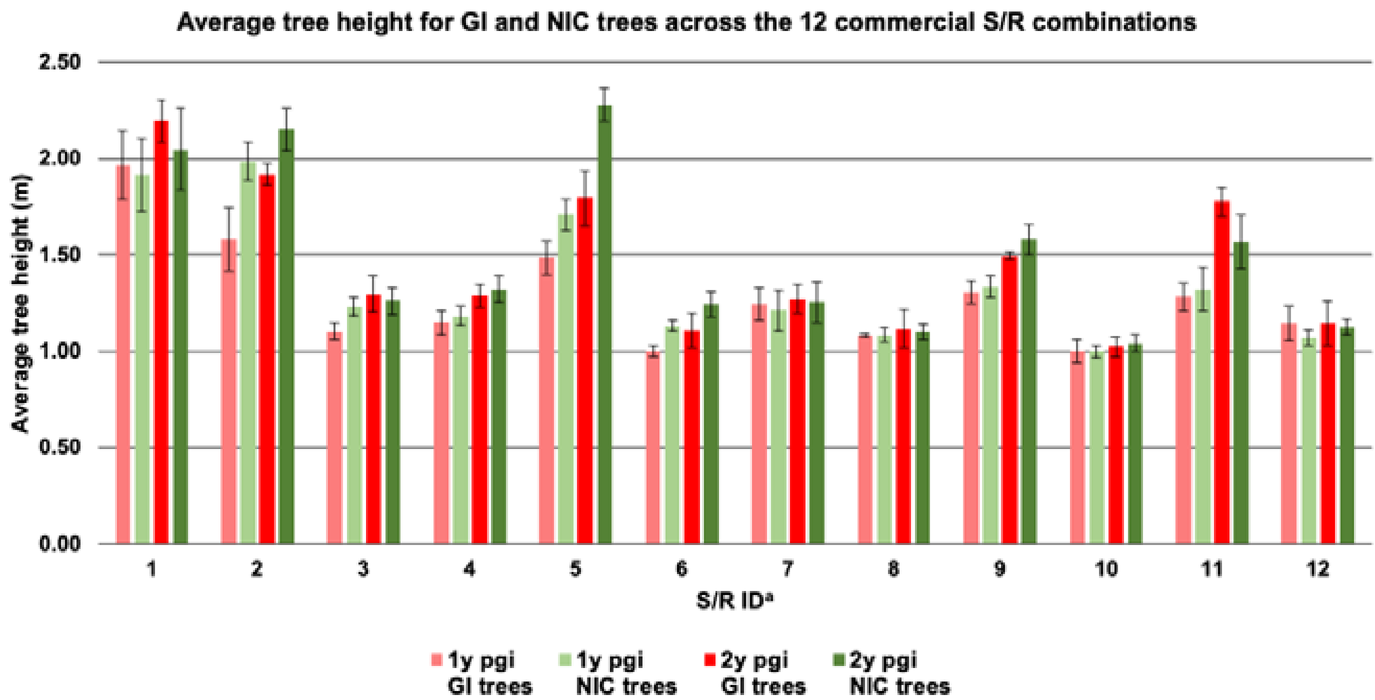

Figure 2. Average tree height in meters (m) of graft-inoculated (GI) and non-inoculated control (NIC) trees across the 12 replicated commercial scion/rootstock (S/R) combinations. Tree height data included from February 2022 and April 2023 (approximately one- and two-years post-graft-inoculation, respectively). Vertical lines on top of the colored bars represent standard error.

[a]Scion/rootstock (S/R) variety combinations. Refer to Table 1.

S/R ID 5 **S/R ID 11**

GI TREE FRUIT

NIC TREE FRUIT

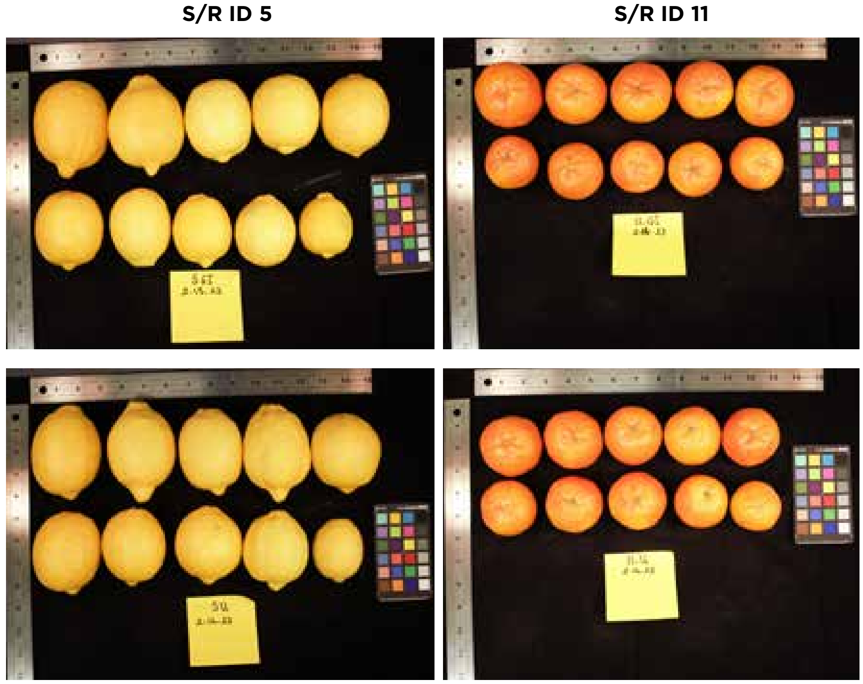

Figure 3. Fruit samples for qualitative analyses from the 12 scion/rootstock (S/R) combinations (pooled samples for graft-inoculated [GI] CYVaV-infected and non-inoculated control [NIC] trees per S/R combination). Each sample consisted of ten fruit as shown in the photographs. Rulers included for size reference and a color checker card for rind color reference.

presence in California citrus plantings and no evidence of natural spread (Weathers 1957 and 1960). To investigate potential mechanisms of natural spread (or lack thereof) of the CYVaV, pollen and aphid transmission bioassays were designed and performed during this project.

IIIa. Pollen transmissibility

During spring 2022, pollen samples were collected from CYVaV-infected and uninoculated field trees (Limoneira 8A Lisbon lemon and 'Shiranui' mandarin) and pooled together as "CYVaV-positive" and "CYVaV-negative" pollen inoculum, respectively. Pollen from CYVaV-infected trees tested CYVaV-positive by RT-qPCR, indicating CYVaV association with the pollen (i.e., presence on surface or within pollen grains), whereas pollen collected from uninoculated trees tested CYVaV-negative. These pollen samples were used for hand-pollinating 229 flowers of several trees across the two above-mentioned scion varieties in the field trial between April-June

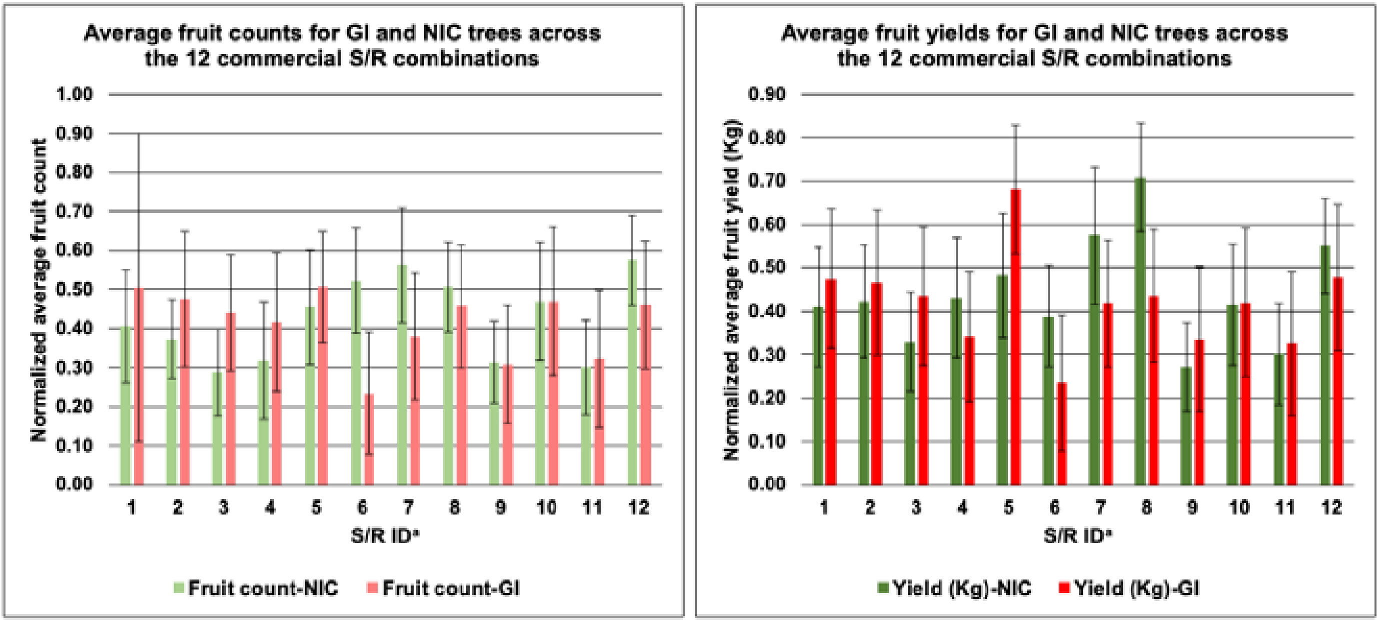

Figure 4. Summary of fruit harvest from the citrus yellow vein-associated virus field trial. Left: Bar graph illustrating the average number of fruit for non-inoculated control (NIC) and graft-inoculated (GI) trees per scion/rootstock (S/R) combination. Right: Bar graph illustrating the average fruit yield (kilograms, kg) for NIC and GI trees per S/R combination. In both cases, the data was normalized to account for variations in total number of trees per treatment and S/R combination. Vertical lines on top of the colored bars represent standard error.

[a]*Scion/rootstock (S/R) variety combinations. Refer to Table 1.*

2022 (**Table 2**). These two varieties were chosen because of abundant flowering in the field during that time of the year. Hand-pollinations were conducted across three treatments: CYVaV-positive pollen on healthy flower stigma (CYVaV on H), healthy pollen on CYVaV-positive flower stigma (H on CYVaV) and healthy pollen on healthy flower stigma (H on H).

By winter 2023, 25 of 229 (11 percent) of hand-pollinations set fruit and matured (**Table 2**). These fruit were harvested between January-February 2023 and tested for CYVaV by RT-qPCR. The 16 fruit from the treatment "CYVaV on H" tested CYVaV-negative, indicating lack of CYVaV transmission from the infected pollen to the healthy/untreated flower and the resulting fruit. The two fruit from the treatment "H on CYVaV" tested CYVaV-positive. This was expected, as CYVaV typically has systemic presence within infected trees. The seven fruit from the treatment "H on H" tested CYVaV-negative.

Table 2. Summary of the citrus yellow vein-associated virus pollen transmission bioassay performed in 2022.

SCION	ROOTSTOCK	TREATMENT[a]	POLLINATED FLOWER (PF) COUNTS	HARVESTED FRUIT SET COUNT	PF SURVIVAL RATE (%)
Limoneira 8A Lisbon lemon	Carrizo citrange	CYVaV on H	13	5	38.5
Limoneira 8A Lisbon lemon	Macrophylla	CYVaV on H	58	11	19.2
Limoneira 8A Lisbon lemon	Rubidoux trifoliate	CYVaV on H	21	0	0
'Shiranui' mandarin	Carrizo citrange	CYVaV on H	9	0	0
'Shiranui' mandarin	Rubidoux trifoliate	CYVaV on H	8	0	0
Limoneira 8A Lisbon lemon	Rubidoux trifoliate	H on CYVaV	18	0	0
Limoneira 8A Lisbon lemon	Carrizo citrange	H on CYVaV	8	0	0
Limoneira 8A Lisbon lemon	Macrophylla	H on CYVaV	12	2	16.7
Limoneira 8A Lisbon lemon	Carrizo citrange	H on H	11	3	27.3
Limoneira 8A Lisbon lemon	Macrophylla	H on H	31	0	0
Limoneira 8A Lisbon lemon	Rubidoux trifoliate	H on H	23	2	4.8
'Shiranui' mandarin	Carrizo citrange	H on H	7	2	28.6
'Shiranui' mandarin	Rubidoux trifoliate	H on H	10	0	0

[a]*CYVaV on H: CYVaV-positive pollen on healthy flower stigma; H on CYVaV: healthy pollen on CYVaV-positive flower stigma; H on H: healthy pollen on healthy flower stigma.*

The low fruit set success rate of the hand-pollinations in 2022 is attributable to variable pollen and agroclimatic conditions, as well as the natural propensity of fruit/flower drop in citrus. The hand-pollination experiments need to be repeated to obtain additional data on CYVaV pollen transmissibility.

IIIb. Aphid transmissibility

In April 2023, in collaboration with Raymond Yokomi, Ph.D. (United States Department of Agriculture-Agricultural Research Service), we conducted a CYVaV/citrus vein enation virus (CVEV) aphid transmission bioassay with the cotton aphid (*Aphis gossypii*) and four virus treatments: CYVaV alone, CVEV alone, CYVaV and CVEV mixed infection and healthy virus-free control (**Figure 5**). Experiments included CVEV because it has been hypothesized to enhance graft transmission and facilitate aphid transmission of CYVaV (Weathers 1960 and 1961, Simon et al. 2023). Prior to initiating the bioassay, all source plants used for the experimental treatments were RT-qPCR-tested to confirm their virus status. Aphid acquisition access period (AAP) and inoculation access period (IAP) of 48 hours each were tested. Citron and *Citrus macrophylla* were used as test plants – ten citron and four *C. macrophylla* test plants per treatment.

Aphid samples were collected at the end of the 48-hour AAP to independently test for virus uptake from the source plants. After IAP completion, all test plants were sprayed to remove aphids, monitored for virus-like symptoms and RT-qPCR-tested for CYVaV and CVEV at six-weeks and four-months post-IAP. At six-weeks post-IAP, all test plants tested negative for both viruses. At four-months post-IAP, one of the 14 test plants from the mixed infection treatment tested CVEV-positive, indicating an aphid-mediated CVEV transmission event at a transmission rate of 7.14 percent. All test plants tested CYVaV-negative at both timepoints. CYVaV was consistently detected in aphids feeding on the CYVaV single- and mixed-infected source plants, indicating that CYVaV was taken up by the aphids during AAP phloem-feeding. The aphid transmission experiments need to be repeated to obtain additional data on CYVaV aphid transmissibility.

Conclusions

- CYVaV was detected only in 32 percent of the inoculated trees after three years and three graft-inoculation events.
- Under field conditions, CYVaV requires extended periods of time following graft-inoculation to establish infection.
- Limoneira 8A Lisbon lemon, 'Tango' and 'Shiranui' mandarin were the most field-compatible citrus cultivars for CYVaV infection among those tested.
- The emerging tree height differences in CYVaV-inoculated and non-inoculated trees will continue to be monitored but cannot be attributed solely to the virus-like RNA at this time.
- Although CYVaV was associated with pollen collected from infected field trees and detected in cotton aphids feeding on infected source plants, we did not record any incidence of pollen- or aphid-mediated CYVaV transmission in the respective bioassays conducted.

The initial experiments of this project, particularly of CYVaV transmissibility (via grafting, pollen and aphids), need to be

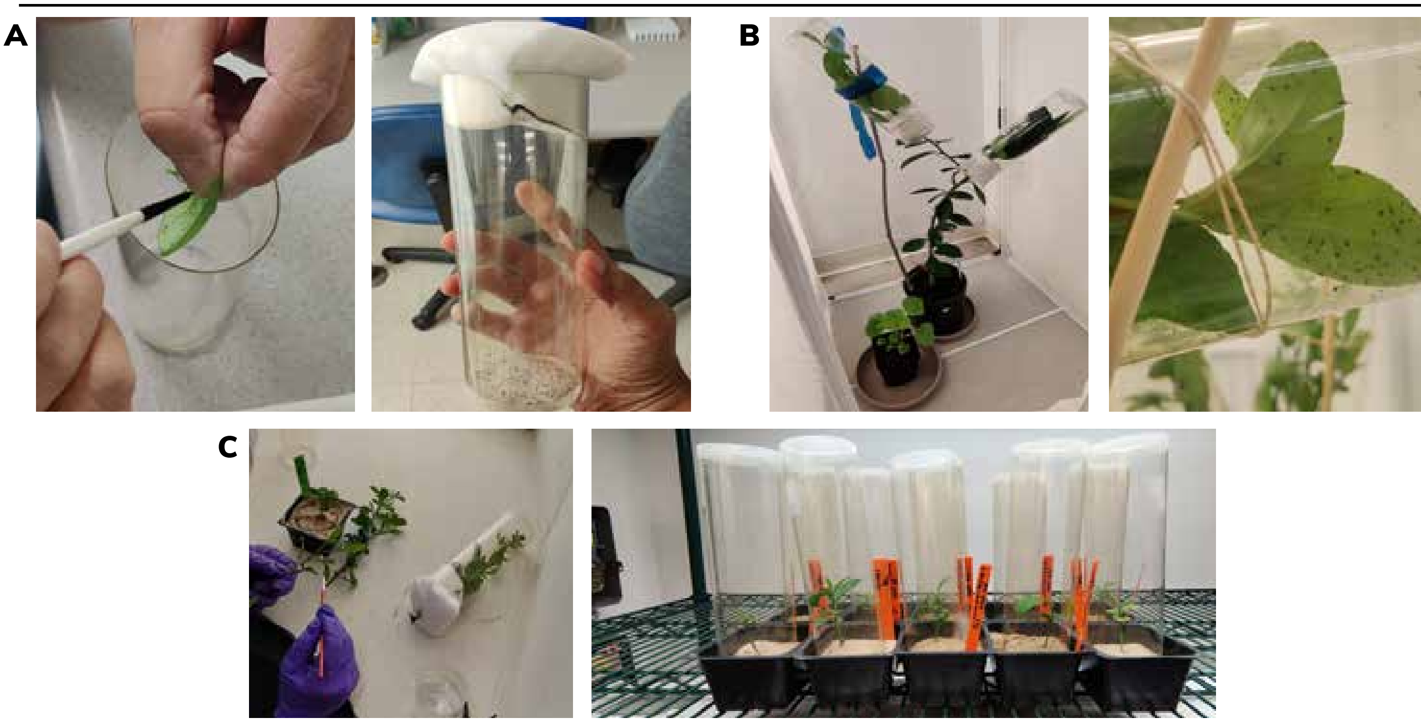

Figure 5. Citrus yellow vein-associated virus and citrus vein enation virus aphid transmission bioassay. A: Transferring cotton aphids (*Aphis gossypii*) into plastic cylinder cages with foam plugs. B: Placement of the aphid-containing cages onto branches of the source citrus plants having young flush for aphid acquisition access period (AAP) feeding. C: Transferring aphids post-AAP from the virus source plants onto citrus receptor plants with a camel-hair brush, followed by enclosing the receptor plants with individual plastic cages for inoculation access period incubation.

repeated, the results statistically validated and published in peer-reviewed technical journals, so they can be referenced in regulatory processes. This is critical so that any future CYVaV-based expression vector system can be field-evaluated before their potential use by the California citrus growers.

CRB Research Project # 5300-214

Glossary

[1]**Virus expression vector:** Lab-generated circular DNA sequence designed to express a particular gene of interest in a host cell system. This is different from insect vectors.

[2]**Reverse transcription quantitative polymerase chain reaction (RT-qPCR):** Lab-based diagnostic test that starts with a sample of RNA, converts it into complementary DNA, and then measures how much of a specific DNA target sequence is present.

References

Kwon, S-J; Bodaghi, S.; Dang, T.; et al. 2021. Complete nucleotide sequence, genome organization, and comparative genomic analyses of citrus yellow vein associated virus (CYVaV). *Frontiers in Microbiology* 12:683130. *https://doi.org/10.3389/fmicb.2021.683130*

Simon, A.; Vidalakis, G.; Kwon, S. J.; et al. 2020. Plant vectors, compositions and uses relating thereto. International patent publication number WO 2020/102210 A1. Issued May 22, 2020.

Simon, A.; Nelson, D.; Brown, J.; et al. 2023. A novel virus-like agent for use against citrus pathogens. *Citrograph* 14(2):44-50.

Weathers, L.G. 1957. A vein-yellowing disease of citrus caused by a graft-transmissible virus. *Plant Disease Reporter* 42:741-742.

Weathers, L.G. 1960. Yellow-vein disease of citrus and studies of interactions between yellow-vein and other viruses of citrus. *Virology* 11(4):753-764.

Weathers, L.G. 1961. Responses of citrus to concurrent infection with two or more unrelated viruses. *International Organization of Citrus Virologists Conference Proceedings* (1957-2010) (Vol. 2, No. 2).

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