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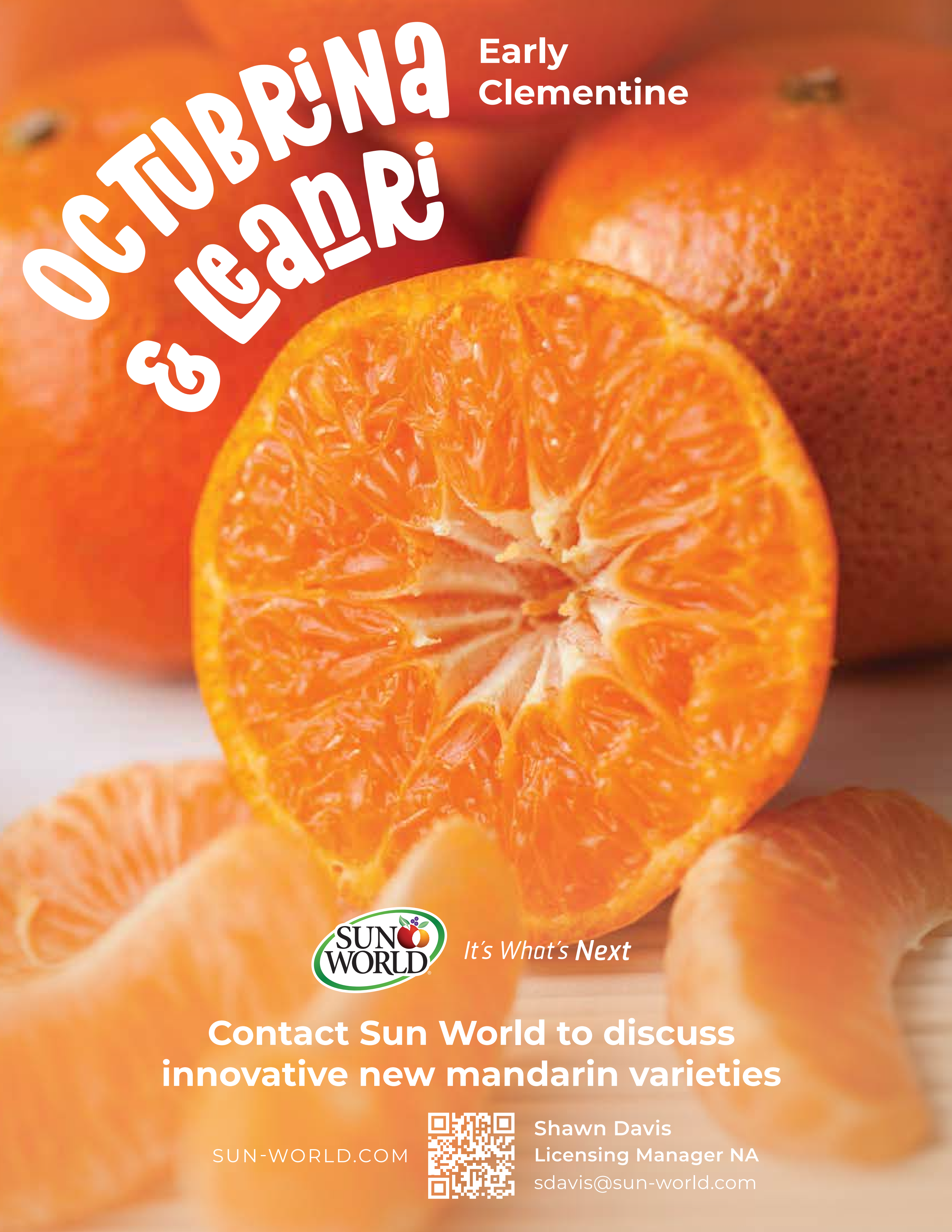
Citrograph

MAGAZINE

FALL 2025

MAKING STRIDES IN POST-HARVEST AND PRODUCTION EFFICIENCY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On the Cover... *The fall 2025 issue of* Citrograph *focuses on post-harvest and production efficiency, and many of the following articles explore developments in these areas. Pictured on the cover is Nanovel's robotic harvester. Having completed successful field trials in Israel, it is on its way to the U.S. to begin California trials in partnership with the Citrus Research Board and commercial orange growers. For more on the robotic harvester, see* From Vision to Reality *by Isaac Mazor and Tal Fogelman on page 26.*

In This Issue

Fall 2025 | Volume 16 • Number 4 The Official Publication of The Citrus Research Board

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

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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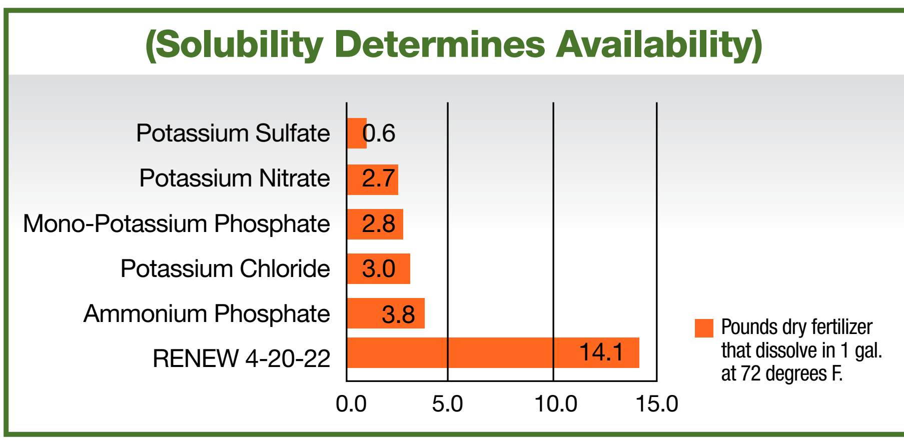

For more information including research results and scientific publications, contact;
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2025 UPCOMING EVENTS

SEPTEMBER 17

CITRUS PEST AND DISEASE PREVENTION COMMITTEE (CPDPC) MEETING

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

SEPTEMBER 23

CITRUS RESEARCH BOARD (CRB) ANNUAL MEETING

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

NOVEMBER 13

CITRUS PEST AND DISEASE PREVENTION COMMITTEE (CPDPC) MEETING

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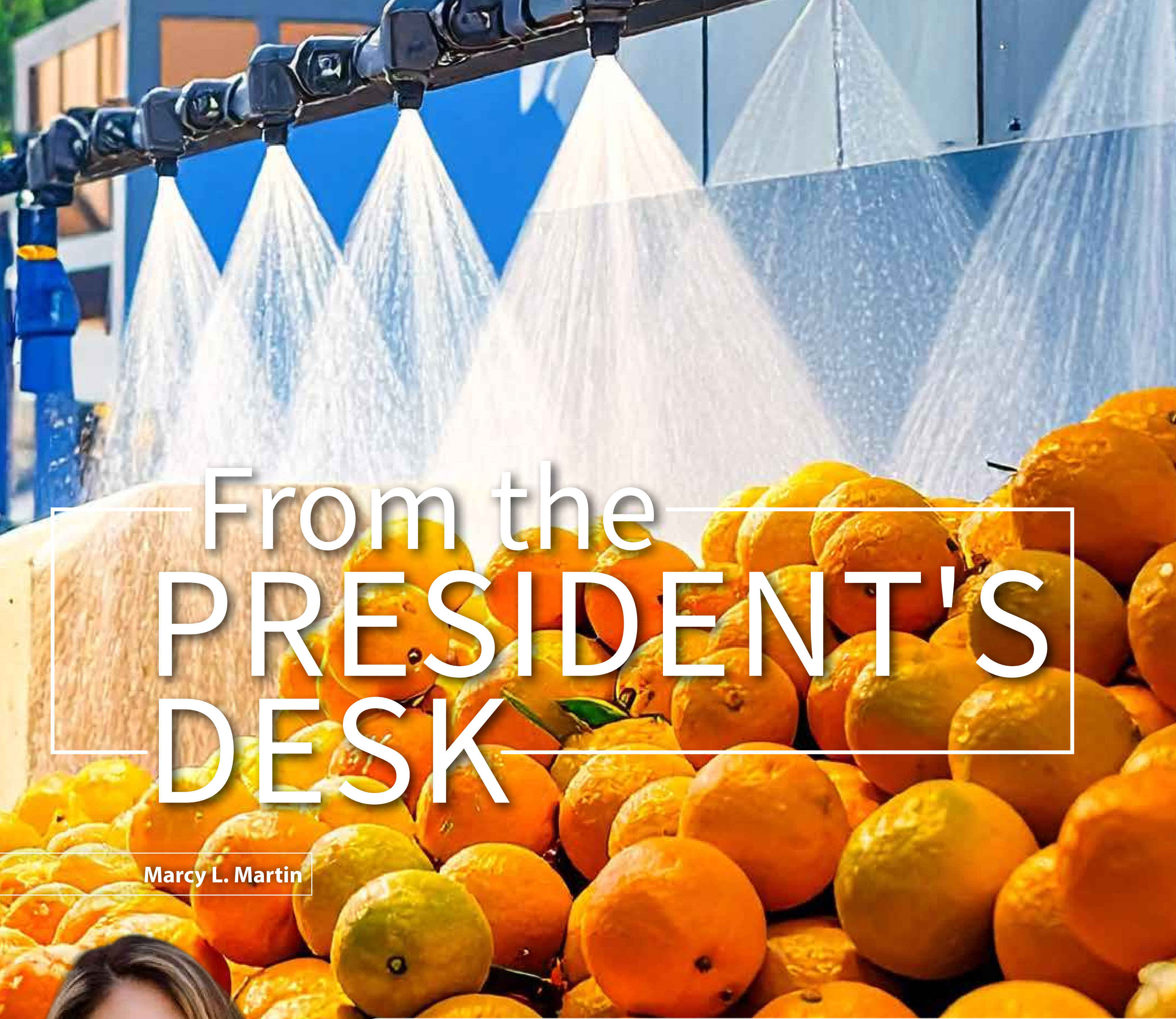

From the PRESIDENT'S DESK

Marcy L. Martin

California's citrus industry was built on a legacy of innovation, stewardship and scientific advancement. As we look at the future, growers and packers alike are facing increasing complexity in both production and post-harvest operations. At the Citrus Research Board (CRB), we remain committed to supporting the industry through science-driven solutions that protect fruit quality, improve efficiencies and safeguard long-term sustainability.

In the field, growers are facing a host of challenges—from water scarcity and regulatory constraints to pest and disease pressure. Our research committees meet quarterly to discuss contemporary issues, review project proposals and determine how our investments will best serve the industry. In this issue of *Citrograph*, you will learn about several CRB-funded projects that are exploring new strategies for more efficient production. One project of interest is Nanovel's robotic harvester, which is currently

Marcy L. Martin

in transit to California to begin trials and make final adjustments before beginning commercial deployment. This machine is designed to adapt to the needs of citrus harvesting with robotic arms that can identify and collect fruit from the trees. Research into mechanical applications that will aid the citrus industry in addressing harvest challenges remains a priority for our organization.

For growers in the Central Valley, lemon pitting continues to be a challenge. The CRB has committed to supporting research toward identifying its cause and subsequent solution. A group of researchers have approached this issue from different viewpoints, including pest interference, environmental influence and pathogen presence, as well as tree health. Researchers continue to collect data into the new season to find added information. A summary of their current findings can be found on page 36. The CRB will continue interfacing with these researchers to ensure the issue is approached from all angles.

Once fruit is harvested from the trees, the challenges do not stop. Jim Adaskaveg, Ph.D., leads the CRB's Pre- and Post-harvest Citrus Disease Management Core Program, where he focuses on a variety of post-harvest issues, such as post-harvest decay, which continues to impact pack-out rates. On page 22, Dr. Adaskaveg and his team provided an update on sour rot research and its occurrence in stored fruit. Their article includes strategies for managing sour rot in the packinghouse as well as fungicide application information. In addition, Dr. Adaskaveg has been working collaboratively with the CRB and the California Citrus Quality Council to address Sweet Orange Scab (SOS) detections and regulatory concerns, which he details on page 68.

The solutions to our industry's challenges will not come from one lab or one field trial. They will come from collaboration—among researchers, growers, packers and regulators. We are proud to serve as a hub for this collaboration by investing in sound science and bringing practical solutions into the hands of those who need them most.

As we finish the 2024-25 fiscal year, we are thankful for the work of our Board Members who dedicate their time to guide the investments we are making on behalf of the California citrus industry. We look forward in the next year to supporting critical research and providing updates through *Citrograph* magazine.

When writing this issue, the CRB received sad news of the passing of Joel Nelson, who served as president of California Citrus Mutual from 1982 to 2019. Joel was a respected leader and tireless advocate for the citrus industry. His contributions left a lasting mark on growers and industry partners across the state and beyond. Our thoughts are with Joel's family. Peace be with them.

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

INDUSTRY VIEWS

Caitlin Stanton

Once fruit is harvested from the tree, it begins its next stage at the packinghouse. This is a quick stop on the way to the consumer for some fruit, while other fruit requires additional storage time. As this issue of* Citrograph *is focused on production and post-harvest, we asked several industry members for different perspectives on their strategies to maintain shelf life and improve storage capabilities.

SCOTT CARLISLE
Villa Park Orchards Association
Strathmore, California

What is your approach to maintaining shelf life?

Harvest approach and fruit handling are the keys to shelf life. When it comes to harvest, block/lot selection is critical. Harvest managers are tasked with constantly reviewing the fruit still on the tree and triaging which blocks/lots to pick when. Everything from size structure to fruit condition is evaluated, so each block/lot is harvested while the fruit is strong before over-maturity. Understanding sales movement and managing export order quantities weekly to avoid burying the packinghouse with domestic fall-out helps with consistent movement and reduces storage time on fruit. Fruit vitality evaluations also help determine which blocks/lots are best suited for this approach to harvesting. When it comes to fruit handling, a dynamic post-harvest approach is necessary. Harvested fruit should be processed within 24 hours of harvest to get the proper fungicides and waxes applied to control post-harvest decay. Following processing, fruit must be kept in coolers to maintain the cold chain in order to maintain shelf life. It must be shipped according to first in, first out (“FIFO”) to ensure the product is not bypassed.

Have you developed any innovative methods for storage?

For the past three seasons, we have used an approach similar to that of lemons. When fruit allows, we color sort fruit, sending packable fruit into the packinghouse and binning out any sorted greens. The binned-out greens are treated with a storage wax with solids of one to three percent depending on the duration for which the fruit is going to be stored. This approach accomplishes two things. It allows us to stop gassing fruit quicker on navels and delay gassing fruit on Valencias when they begin to re-green. The binned-out greens are stored at 48-50ºF for 20-30 days, allowing them to color naturally, just like lemons. Secondly, this approach enables us to continue to harvest at a pace that helps stay ahead of fruit maturity and helps to extend shelf life as fruit is being harvested during a stronger vitality stage. Packinghouse staff must monitor the progression of color on the greens daily, processing fruit according to color rather than date harvested. Greens with the storage wax applied are shipped to the domestic market only to avoid arrival issues.

ARNO ERASMUS
Wonderful Citrus
Delano, California

What is your approach to maintaining shelf life?

Fruit maturity, supply chain efficiency and weather are only some factors that influence shelf life. There are myriad activities and miles of transport between the citrus tree and the consumer where any delays can be detrimental to shelf life. Every hour counts in this race against time, and minor changes can have a big impact.

The moment fruit is picked from the tree, it starts aging. It is still alive and respirating, using oxygen and giving off carbon dioxide, water and heat. However, the slow process of aging and dying has begun because it does not have the supply line from the tree anymore. Our post-harvest actions can either speed those metabolic processes up or slow them down.

Our most effective tool to slow aging is cooling down the fruit. Not only is the aging of the fruit slowed down, but also the growth of pathogens. Floating around in abundant supply are the spores of green mold and sour rot, among others. These pesky fungi cause decay that can result in devastating losses after storage or upon arrival at our customers. The single most effective factor that can stop decay in its microspore tracks is maintaining the cold chain. Sour rot does not grow below 50°F. At this temperature, green mold growth will also slow down significantly and will stop below 40°F. The shorter the time between harvest and initiation of the cold chain, the better. It is here where the difference between great and good is made.

Most of these decay pathogens are citrus-specific and are part of the citrus ecosystem. Green mold and sour rot need a fresh wound to infect successfully. If we could avoid wounds and have an optimal cold chain, we would largely win the shelf-life battle. In addition to these factors, we have fungicide treatments in our arsenal. Many infections are

initiated during the harvest process. The ever-present spores can infect fresh wounds made by clipper cuts, long stems or a bit of rough handling. For the fungicides we use to have optimum curative control, infections should not be older than 24 hours. Here we have that race against time again. We must get the first fungicide treatment on the fruit within 24 hours of harvest.

Get the fruit cold and keep it cold, handle fruit with care and be consistent with treatments. This is the game plan, but it will fall apart completely without good team members to implement it. We can write an entire citrus library full of books on what impacts shelf life, but we have found that instilling enthusiasm for what you do and how your actions can impact the fruit significantly increases the quality of our delivery. This, in turn, impacts shelf-life in a positive way.

Have you developed any innovative methods for storage?

At our Delano campus, we have around 100 storage rooms that can hold anywhere from 500 to 2,000 bins per room, as well as two large shipping facilities with enough space for 9,000 pallets. Storage is all about inventory, and inventory is all about age. Our aim is to maintain the cold chain and first-in-first-out inventory. We went back to basics, with constant monitoring of the fruit. QC performs evaluations of fruit quality and condition on a fixed schedule, and the logistics team performs an audit for accuracy of inventory. Planning and Sales review this information as part of their daily decision-making process. All location and quality data are digitized. Scorecards and dashboards are available very close to real-time. Everything gets scanned or loaded directly into the paperless system. In the near future, we plan to have storage room data like temperature, active fans, humidity and more on a live dashboard to help keep the room parameters within specification.

***Caitlin Stanton is the director of communications for 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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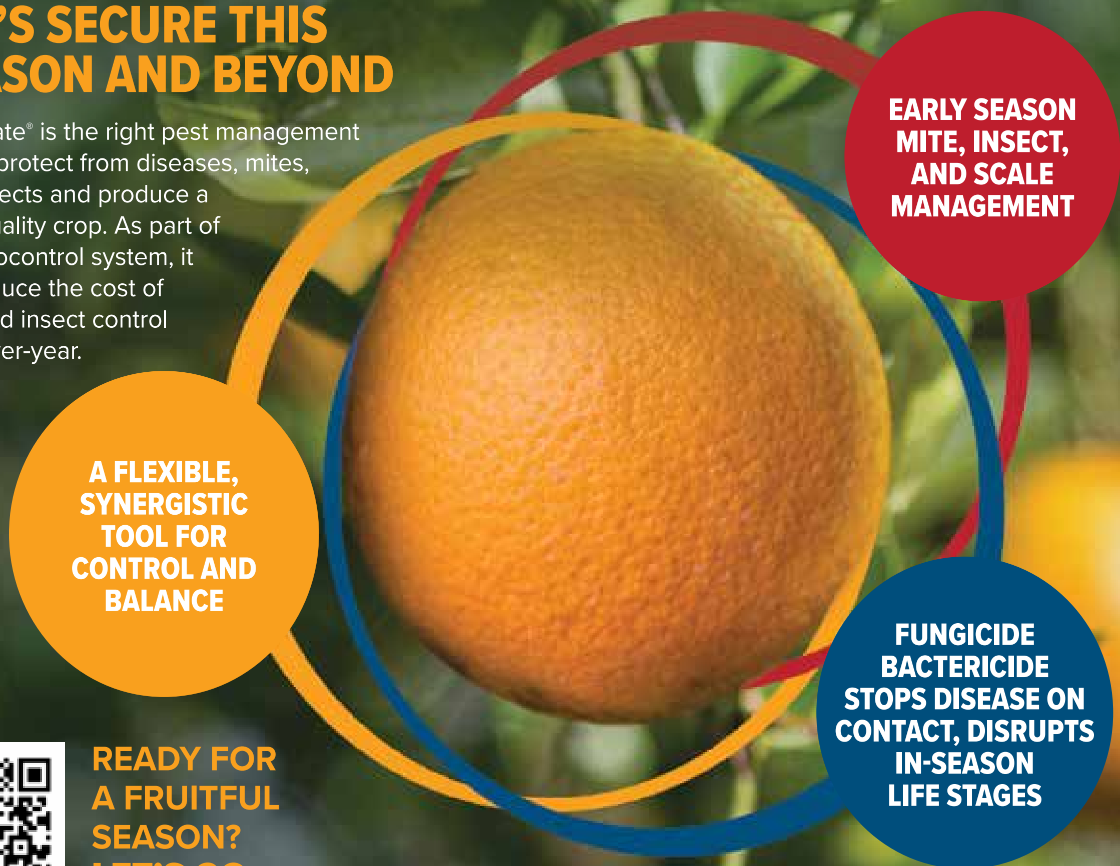

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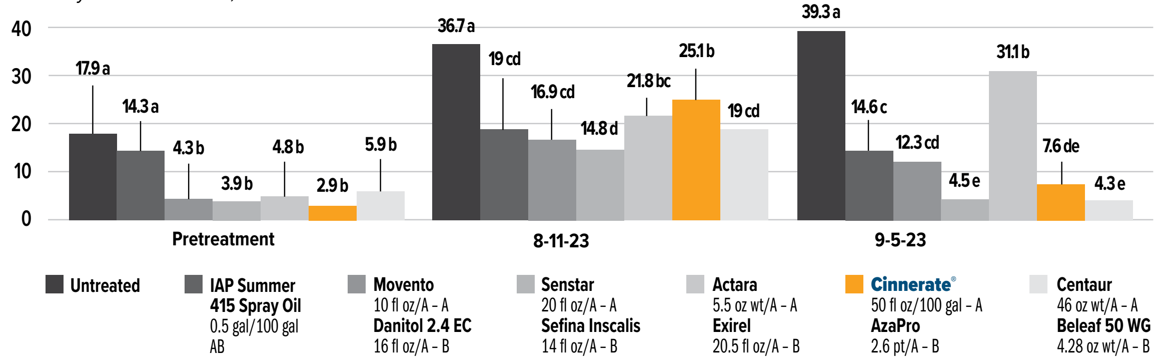
University of CA – Ivanhoe, CA – 2023



Preharvest treatment for fruit disease

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THE HISTORIC ROLE OF CITRUS NURSERIES IN DISEASE PREVENTION

Aaron Dillon

California's multi-billion-dollar citrus industry has benefited from an often-overlooked hero in disease prevention – the citrus nursery industry. For nearly a century, California's citrus nurseries have served as the first barrier against devastating diseases through their commitment to clean stock production.

Evolution of Disease Prevention

In the early 1930s, citrus psorosis was discovered to be a graft-transmissible disease of citrus. This led to the first formal citrus nursery stock disease prevention program launched in 1937 called *California's Psorosis Free Program*, a voluntary initiative that established the beginning of systematic disease prevention in California citrus nurseries.

In the late 1930s, tristeza virus emerged in the San Gabriel Valley. This led to the creation of the *Tristeza Suppression and Eradication Program*, which imposed strict quarantine regulations. The California Department of Food and Agriculture designated management zones with specific protocols, preventing movement of infected stock and requiring the use of tristeza-free propagating materials in nurseries. This multi-layered regional approach to managing tristeza has proven very successful and could serve as an effective blueprint for long-term huanglongbing (HLB) management in California. It is interesting to note how the tristeza quarantine boundaries closely mirror today's HLB quarantine boundaries in southern California.

Industry Self-regulation

Perhaps the most significant chapter in the history of California's citrus nurseries began in 2009 when nursery operators gathered in Riverside to learn about the emerging threats of the Asian citrus psyllid (ACP) and HLB. After the conference, the nursery industry agreed to regulate itself. Rather than waiting for governmental mandates and widespread establishment of ACP/HLB in California, the industry worked to create the *Citrus Nursery Stock Pest Cleanliness Program*, California Code of Regulations 3701.

This regulation transformed citrus nursery production by requiring the use of protected structures for propagation sources, comprehensive testing protocols for diseases in mother trees, and strict compliance protocols for nursery operations. As a result, California citrus nurseries have borne substantial costs modernizing their production facilities and transitioning to growing stock inside of protected structures. These investments were made to safeguard not just their businesses but the entire California citrus industry.

Modern Challenges with HLB

Today, with HLB posing an existential threat to California's citrus, the role of citrus nurseries has never been more important.

As HLB quarantine areas expand, residential citrus growers face challenges obtaining new citrus trees. While some controversy exists regarding public access to citrus trees (with concerns that backyard plantings introduce pest problems), the evidence suggests that limiting access to certified clean stock is counterproductive. This approach fails to recognize the reality of consumer behavior. Despite restrictions, homeowners continue to seek out citrus trees for their properties. The question isn't whether people will continue to grow citrus in quarantine areas, but rather if they will obtain those trees through regulated, safe channels or through unregulated, potentially risky ones. When consumers cannot obtain trees from reputable, certified sources, the risk of illegal importation and propagation increases dramatically, potentially introducing the very diseases the industry seeks to prevent.

Since HLB was first detected in California, strict quarantines have limited movement of nursery stock within the HLB quarantine zones. However, as quarantine zones have expanded to include millions of California homeowners, severely limiting access to citrus trees has had unintended consequences. Demand for citrus trees has driven a rise in

Nursery stock from certified growers provides homeowners with healthy trees supporting California's broader disease prevention efforts.

underground markets for uncertified stock, propagation of infected material and even illegal importation from other regions, all of which dramatically increases risk to California citrus.

The Case for Public Access to Clean Stock

The cost to state regulatory officials of policing illegal nursery stock is substantial and ultimately less effective than working with California's certified nurseries. Nurseries have invested millions in screened facilities, testing protocols and compliance measures to ensure disease-free stock.

California's citrus nurseries are a significant resource in the fight against HLB. Their expertise in disease management, commitment to clean stock and direct connection to consumers make them ideal partners in education and outreach. Rather than restricting access to certified trees, the industry should consider leveraging citrus nurseries to distribute not just clean trees, but critical information about disease symptoms, vector identification and best management practices for homeowners. Informed consumers with access to clean stock can become allies in disease prevention.

The reality is that citrus will be planted in California gardens regardless of regulations; the question is simply whether those plantings begin with certified disease-free stock. History has demonstrated repeatedly that prohibition without viable alternatives rarely succeeds.

For decades, nurseries have been committed partners in disease prevention, protecting the entire industry. Their production facilities represent significant investments in California's biosecurity infrastructure. As disease threats evolve, maintaining public access to clean stock through properly certified nurseries remains essential. It is imperative that any citrus tree planted in California begins with the healthiest possible foundation. The long history of successful partnership between the citrus nursery industry, growers and regulatory agencies provides a proven model for addressing the most serious threats facing California citrus.

***Aaron Dillon is the outreach subcommittee chair for the Citrus Pest and Disease Prevention Committee. For additional information, please contact* ad@fourwindsgrowers.com**

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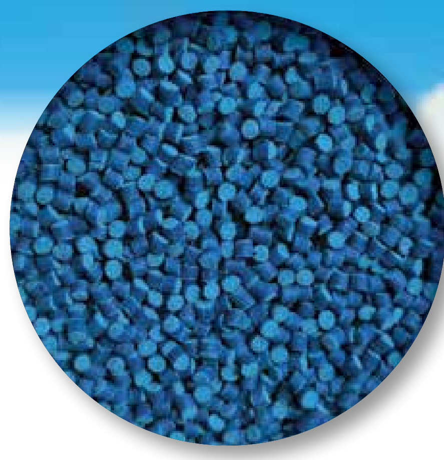

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A Tale of TWO SOUR ROTS in California Lemons

James E. Adaskaveg, Albert M. Nguyen and Helga Förster

For years, there has been a debate as to the cause of sour rot of citrus. Some authors claimed that the originally described pathogen of sour rot, Geotrichum candidum, *was the cause of sour rots of all different kinds of fruit. Several decades later, in the 1970s and 1980s,* G. citri-aurantii *was described as the cause of citrus sour rot based on pathogenicity and sexual mating studies, which defined the two biological species. Thus, the two species were separated by their host crops:* G. citri-aurantii *is found on citrus, and* G. candidum *infects other fruit crops such as tomatoes, melons and stone fruits. In 2020-21, citrus handling practices changed during COVID-19, and there is new evidence that both species can occur on citrus under prolonged storage. So which species is responsible for severe sour rot outbreaks in California citrus packinghouses?*

Introduction

Sour rot is a particularly devastating disease of lemons, grapefruit and mandarins and is an increasingly important decay to manage as the California citrus industry stores fruit for prolonged periods of up to four months prior to marketing. In the orchard, fungal spores (conidia) bound to soil particles are disseminated onto fruit surfaces by irrigation and rain splash, wind or insects. Fruits picked soon after rainfall are highly susceptible to infections. These begin as fruit surface injuries that often occur during harvest and post-harvest handling. Fruits become very soft, leak juices and develop a typical vinegary odor. As bulk-stored infected fruits disintegrate, the leaking juices containing enzymes cause injury to nearby healthy fruit (Adaskaveg et al. 2022). In time, a thin layer of white mycelial growth develops on the fruit surface.

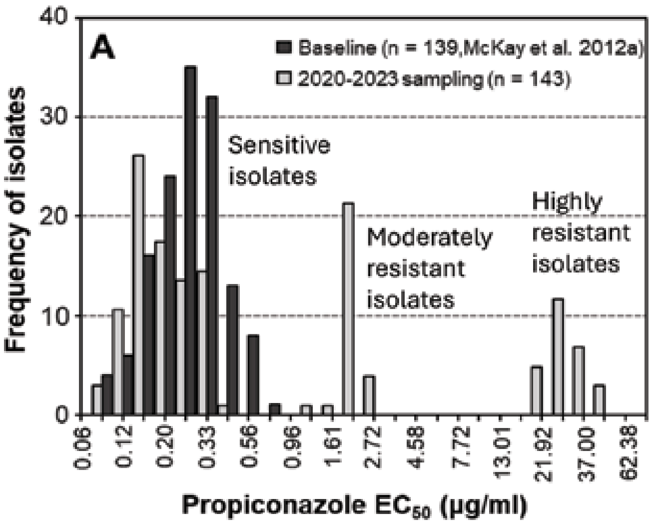
Currently, most researchers agree that there are two fungal species causing sour rot of fruits and vegetables, *Geotrichum candidum* and *G. citri-aurantii*. Both are commonly found in soils but are not restricted to locations of their major host crops. Historically, there have been conflicting views on the taxonomic status of the sour rot pathogen of citrus fruit. In the first report of sour rot, the causal pathogen was named *Oospora citri-aurantii*. The pathogen was re-described in 1955 as a citrus-specific variety, *G. candidum* var. *citri-aurantii*. Subsequently, the sexual stages of both *Geotrichum* species were identified, and the citrus pathogen was sexually incompatible with isolates[1] from non-citrus hosts. Therefore, the citrus pathogen was identified as a separate biological species with the current name *G. citri-aurantii*. Genetically, the two species are distinct and can be differentiated using pathogenicity and classic mating studies. Species-specific PCR primers can delineate the two species (McKay et al. 2012).

Sour Rot Pathogen Detection in California Citrus Packinghouses

High levels of sour rot occurred on propiconazole-treated lemon fruit that were stored for extended periods in some California packinghouses in 2020 and 2021. The COVID-19 shut-down ruined the marketing of lemons from spring through summer of both years, and packinghouses tried to store fruit over these extended periods in case the markets opened up and the demand for fruit would start again. In the meantime, fruit were harvested and treated with storage coatings and propiconazole, a registered post-harvest demethylation inhibiting (DMI) fungicide[2]. Initially, there was no decay; but as months passed, the fruit aged, became senescent and started to decay. With a high incidence of decay, surveys were initiated on fungicide sensitivity of the causal pathogens. Most of the decay was sour rot, and our isolations from diseased fruit in several packinghouses resulted in 157 isolates of *Geotrichum* species. Using species-specific primers and sexual mating assays, 143 (=90.5 percent) were determined to be *G. citri-aurantii*; but to our surprise, 15 (=10.5 percent) isolates were identified as *G. candidum*.

Sensitivity of *Geotrichum* spp. to Propiconazole and Cyproconazole

We then characterized the fungicide sensitivity of our collections from 2020-21. Isolates of *G. citri-aurantii* were either sensitive (EC_{50} 0.06 to 0.34 µg/ml), moderately resistant (EC_{50} 1.20 to 2.34 µg/ml) or highly resistant (EC_{50} >17.68 µg/ml) to propiconazole (**Figure 1A**). Because resistance to propiconazole was detected in *G. citri-aurantii*, concerns for its prolonged use became apparent, and research was initiated on alternative treatments.



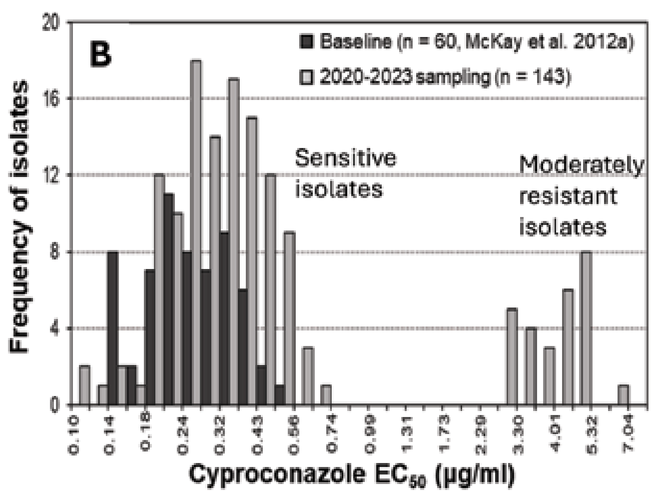


Figure 1. Frequency histograms of effective concentrations (EC_{50} values) of A, propiconazole, and B, cyproconazole to inhibit mycelial growth by 50 percent of *Geotrichum citri-aurantii*. Bar height indicates number of isolates per bin.

The DMI-triazole fungicide cyproconazole currently is registered for field use on some crops and is pending registration for post-harvest use on citrus fruit in the United States. Sour rot isolates resistant to propiconazole were incompletely cross resistant[3] to cyproconazole, meaning that propiconazole-resistant isolates were not necessarily

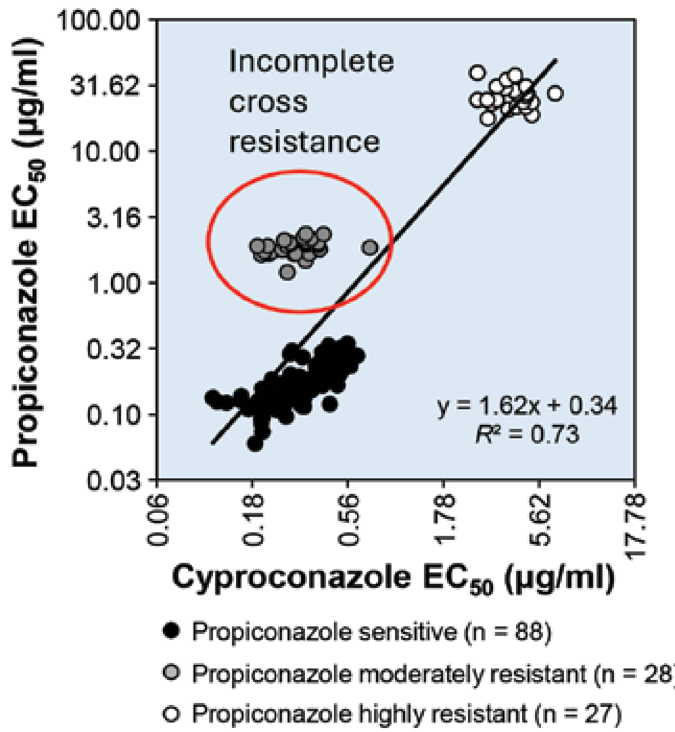


Figure 2. Linear regression of effective concentrations to inhibit mycelial growth by 50 percent (EC_{50} values) by propiconazole on those for inhibition by cyproconazole for 143 isolates of *Geotrichum citri-aurantii*. Each circle represents paired log-transformed EC_{50} values for both fungicides for the isolate tested. Axes are on a logarithmic scale with actual values shown for clarity.

resistant to cyproconazole also, both being DMIs with the same mode of action[4] (**Figure 2**). Thus, *G. citri-aurantii* isolates that were either sensitive or moderately resistant to propiconazole were all sensitive to cyproconazole (EC_{50} 0.11 to 0.63 µg/ml, 0.19 to 0.73 µg/ml, respectively), whereas isolates highly resistant to propiconazole were moderately resistant to cyproconazole (EC_{50} 2.66 to 6.79 µg/ml) (**Figure 1B**). Isolates of *G. candidum* were either sensitive or highly resistant (EC_{50} >9.55) to both fungicides except for one moderately resistant isolate. Isolates of both species were all sensitive to natamycin (EC_{50} <5.01 µg/ml).

Primary *Geotrichum* Species Causing Sour Rot of Citrus

In co-inoculations with 1:1 mixtures of the two *Geotrichum* spp., *G. candidum* was recovered only from the centers of decay lesions and not from the advancing margins. *G. citri-aurantii,* however, was isolated from the centers and from the advancing margins. Healthy juice sacks had a pH of 2.14, while tissue decayed by *G. citri-aurantii* had a pH of 3.1. In laboratory studies, *G. candidum* could not grow below pH 3.0, but both species grew at pH 3.3 to 7.8. Therefore, we concluded that the initial sour rot decay is caused by *G. citri-aurantii*, and *G. candidum* then can colonize the decayed fruit

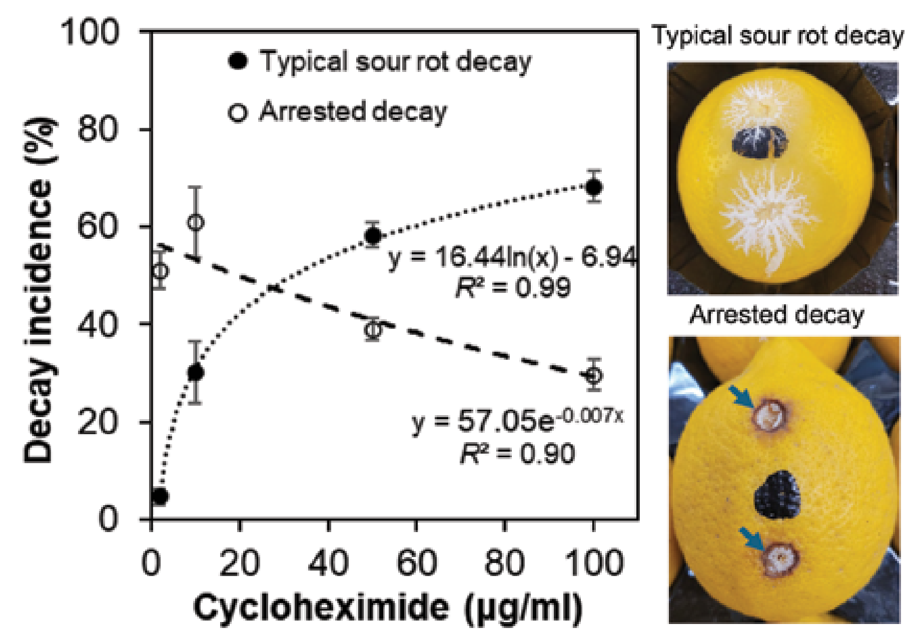


Figure 3. Effect of cycloheximide added to inoculum of *Geotrichum candidum* on the incidence of typical soft sour rot lesions and of firm, arrested decay with brown discoloration. Lemon fruit were wound-inoculated with conidia (5 x 10^5 conidia/ml) in lemon juice amended with 2, 10, 50, or 100 µg/ml cycloheximide and incubated for seven days at 20°C. Decay incidence was $\log_{10}$-transformed for regression analysis, and logarithmic regression equations are shown. Vertical bars indicate standard errors of the means. The blue arrowheads point to the margins of arrested decay.

tissue that has a pH high enough to allow for growth of this secondary species.

In 2020 and 2021 during the worldwide COVID-19 shutdown, fruit also were delayed in harvesting (tree ripened) and were stored for extended periods in packinghouses. We hypothesized that senescent fruit in storage were more susceptible to sour rot caused by both Geotrichum species due to weakened host defenses. To test this, we inoculated lemons with *G. candidum* and supplemented the inoculum with cycloheximide that is a potent inhibitor of protein synthesis and a suppressor of host defense responses. In lemon fruit inoculations with *G. candidum* without the use of cycloheximide, the incidence of typical sour rot symptoms was zero percent, and only small, rather firm lesions of arrested decay[5] developed. Typical soft decay lesions developed at 4.7 percent or 68.2 percent when inoculum was amended with 2 or 100 µg/ml cycloheximide, respectively (**Figure 3**). Thus, as the concentration of cycloheximide increased, the incidence of typical sour rot decay symptoms increased (**Figure 3A**, **B**) and that of 'arrested decay' decreased (**Figure 3A**, **C**). Therefore, the suppression of host defenses allows *G. candidum* that normally is a weak pathogen of citrus to cause typical sour rot decay of lemons. We conclude that *G. candidum* is present in citrus orchards but is a secondary pathogen of weakened, senescent lemons and colonizes fruit that are often first decayed by *G. citri-aurantii*.

Management of Sour Rot

To address this issue, disease management efforts should focus on avoiding practices that favor sour rot (e.g., not harvesting tree-ripened fruit and storing fruit for too long). Packinghouse managers are more aware of long-term storage leading to over-ripe fruit and sub-optimal storage conditions (e.g., when juice from decayed fruit carrying inoculum is able to contaminate healthy fruit in stacked bins or when storage rooms are over-filled, which prevents adequate air flow, leading to decreased fruit respiration and increased fruit senescence). Packinghouses also use surface sterilization methods with oxidizing compounds to reduce the "carry-over" inoculum on equipment, and this helps prevent the re-occurrence of sour rot outbreaks.

Suboptimal storage conditions can lead to the selection of propiconazole resistance. To improve sour rot management, a Special Local Need label adjustment was approved in California that allows a single application of propiconazole at a higher rate to lemons going into storage (instead of two applications at a lower rate). This can help manage decay caused by sensitive and moderately resistant isolates. Additionally, we identified two alternative fungicides to propiconazole:

1. cyproconazole, which has a pending registration in the United States and has incomplete cross resistance[3] with propiconazole making it an effective sour rot and green mold treatment; and

2. natamycin with a unique mode of action from other registered fungicides, which is registered for domestic usage with CODEX[6] and has international registrations pending.

These fungicides used alone or in mixtures will provide the tools for packinghouse managers and service company representatives to combat sour rot and fungicide resistance in the future.

CRB Research Project #5400-401

Glossary

[1]**Isolate:** A culture of a microorganism obtained for study such as the "isolate" or culture collected from a decayed fruit.

[2]**Demethylation Inhibitor (DMI) Fungicide:** Class of fungicide (FRAC Code 3) that inhibits the production of ergosterol, an essential component of fungal cell walls required for fungal growth.

[3]**Incomplete cross resistance:** When an organism, like a fungus, develops resistance to one or more fungicides within a class, but not to all members of that class. It implies that the resistance mechanism is not fully transferable across the entire fungicide class, meaning some related fungicides may still be effective.

[4]**Mode of Action (MOA):** The specific cellular process that is repressed by a specific group of fungicides leading to inhibition of fungal growth.

[5]**Arrested decay:** Fruit decay that is initiated but then stops and fails to develop into a typical decay caused by the fungal pathogen.

[6]**CODEX:** A collection of international food standards and pesticide limits developed to protect the health of consumers and promote fair practices in food trade.

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FROM VISION TO REALITY

NANOVEL'S ROBOTIC HARVESTER CONFRONTS THE CITRUS ORCHARD'S TOUGHEST OBSTACLES

Isaac Mazor and Tal Fogelman

Introduction

As labor shortages persist and costs continue to rise, automation in the California citrus industry has become an operational imperative.

As covered in the fall 2024 issue of *Citrograph* (Mazor and Fogelman, 2024), Nanovel has developed a robotic harvester for fresh oranges to address these labor challenges. This update outlines recent progress and explores key technical challenges that must be solved for success in real orchards.

Final Field Trials Before Deployment in California

Before any new harvesting technology can be considered by growers, it must prove itself in the field. Nanovel's harvester is at that stage now.

Recently, Nanovel completed full system integration of its robotic harvester and ran field trials of Valencia oranges in Israeli orchards. These trials focused on evaluating the

The robotic harvester operating at nighttime.

system's fruit detection and picking accuracy in dense canopies, operating continuously throughout the day and maintaining reliability over extended harvesting cycles.

Each trial was designed to evaluate performance across varied conditions, yielding insights into hardware, software and drive reliability. These trials identified several issues — such as intermittent detection failures, motion anomalies and the arms' robustness when facing obstacles — that were systematically analyzed and addressed through targeted improvements, including mechanical redesigns, software tuning and real-time control enhancements.

At the time of writing, the harvester was being prepared for shipment to California, where it will enter field trials in partnership with the Citrus Research Board (CRB) and cooperating commercial orange growers. These trials will validate performance and identify any final refinements before commercial deployment.

Confronting the Core Challenges of Automated Citrus Harvesting

Robotic citrus harvesting is more than identifying fruit and picking it with a mechanical arm. The orchard environment introduces many physical and technical barriers requiring smart, horticulturally informed solutions. Nanovel's system has been designed to address these realities from the ground up.

Here are key challenges and how our system addresses them.

- ***Obstacle-rich Environments and Occlusions:*** Citrus trees have dense, irregular branches and leaves that obscure fruit. Reaching fruit without damaging it—or neighboring fruit—requires millimeter-level accuracy. Nanovel's picking arms use edge-based vision and real-time control to navigate around obstacles while maintaining soft contact with the fruit. Collision-avoidance sensors ensure safe maneuvering throughout the canopy.
- ***Fruit Detection and Visibility in Changing Light Conditions:*** Lighting varies significantly over the course of a day and across seasons, impacting vision-based systems. Nanovel employs proprietary dynamic calibration algorithms to adapt in real time to shifting light and shadow, ensuring consistent fruit detection accuracy throughout the day. For nighttime operation, the system is equipped with integrated lighting that eliminates visibility issues and maintains performance.
- ***Canopy Depth and Access:*** Many fruits are positioned within the tree canopy, making it difficult for robotic arms to reach them effectively. Nanovel's long-reach telescopic arms (up to six feet) are designed to extend into dense foliage. Their slender, flexible design lets them navigate between branches, enabling them to reach fruit that would otherwise remain inaccessible.
- ***Uneven Fruit Distribution and Efficient Arm Utilization:*** The fruit is unevenly distributed, making it hard to keep all arms engaged. Nanovel addresses this with an intelligent central "target bank" that maps fruit positions and dynamically allocates targets to each arm based on reachability. The system's overlapping arm design lets arms dynamically adjust picking zones. This approach helps maximize overall productivity by reducing idle time to improve arm utilization.
- ***Speed and Throughput:*** High productivity is essential to match labor-based picking rates. Nanovel's machine architecture includes high-torque servo motors, parallelized picking arms and robust computing that allows fast, parallel action —essential for commercial use. Importantly, the system prioritizes efficiency over

A robotic arm extending toward fruit.


Harvested fruit gently being transferred into bins via an integrated conveyor system.


Robotic end-effector in action; vacuum sniffer is securing an orange for picking via cutting its stem by blades.


Multiple robotic arms working in parallel to access fruit in the canopy.

completeness—targeting accessible fruit, but not 100 percent of the fruit in each tree. This trade-off reduces idle time and maximizes the harvester's value per hour in commercial operations.

- ***Safety and Fruit Quality:*** The system has safety layers to protect people, machinery and fruit. Visible warning lights alert the operator when the machine is working idle. An anti-collision system was designed to prevent collision of overlapping arms while operating. Gentle gripping, soft-touch end-effectors and real-time feedback loops ensure fruit is picked cleanly and placed without bruising, preserving fresh market quality.

Looking Ahead

The next and most significant phase is now beginning–performance testing in California's commercial groves. These trials–supported by the CRB and growers–will offer insights into current performance. They'll also help define best practices for field logistics, machine supervision and grove compatibility.

Nanovel's system is designed to significantly reduce reliance on manual crews by automating the repetitive and physically demanding task of hand picking. By focusing on the most accessible fruit, the harvester can help ease labor shortages and improve efficiency.

As field testing continues, Nanovel remains committed to working closely with growers to refine and improve the system. The goal is to deliver a practical tool that fits existing operations and supports long-term citrus harvesting sustainability.

CRB Research Project #5400-177

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***Isaac Mazor is the founder and CEO of Nanovel LTD. Tal Fogelman is a California citrus grower and co-founder and head of business development at Nanovel LTD. For more information, please contact* tal@nanovel.ag**

CRB-FUNDED FINAL RESEARCH REPORT

DWARFING OF COMMERCIAL CITRUS VARIETIES USING TsnRNAs

Evaluation of Yield, Size and Tree Care from the 1990s Commercial and University Field Trials

Irene Lavagi-Craddock, Ashraf El-Kereamy, Subhas Hajeri, Carol Lovatt and Georgios Vidalakis

Project Summary

In a landmark field trial initiated in 1998 at the University of California Agriculture and Natural Resources (UC ANR) Lindcove Research and Extension Center (LREC), UC Riverside (UCR), researchers evaluated whether transmissible small nuclear ribonucleic acids (TsnRNAs)[1] could reduce citrus tree size and increase horticultural efficiency. Final measurements for Citrus Research Board (CRB) Project #5100-154—taken after 25 years in the ground—show that TsnRNA-treated trees consistently maintained fruit yields relative to their reduced canopy volume and labor inputs. A parallel evaluation in a 1995 commercial orchard further confirmed that TsnRNA-induced dwarfing delivers practical labor savings and durable size control under non-experimental conditions. These results have significant implications for high-density planting, cost-effective orchard management and long-term citrus production in huanglongbing (HLB)-threatened regions.

Commercial dwarfed citrus trees are desirable in California due to rising land, labor and water costs. Their smaller size supports high-density plantings, Citrus Under Protective Structures (CUPS)[2]—should the California citrus industry elect to move cultivation indoors—and efficient pest and disease management, especially under HLB disease pressure.

A promising approach to achieving dwarf citrus trees involves the use of TsnRNAs, plant interacting regulatory RNA[3] molecules originally identified as viroids[4]. Early CRB-supported studies showed that trees treated with TsnRNA-IIa or TsnRNA-IIIb exhibited canopy volume reductions from 20 to 50 percent, respectively (Semancik et al. 1997).

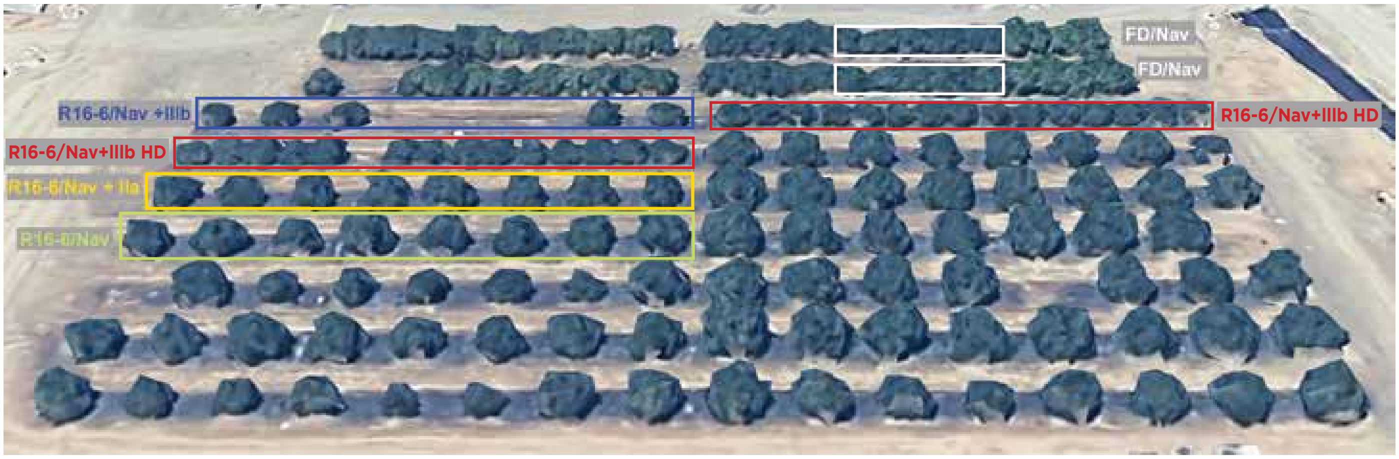

Figure 1: Satellite image (Google Earth) from May 2024 of the 1998 TsnRNA field trial at the University of California Agriculture and Natural Resources Lindcove Research and Extension Center. Trees used for the measurements are highlighted (boxed) as follows; Navel trees on Rich16-6 rootstock ([R16-6/Nav] lime green); Navel trees on Rich16-6 rootstock treated with TsnRNA-IIa ([R16-6/Nav + IIa] yellow); Navel trees on Rich16-6 rootstock treated with TsnRNA-IIIb ([R16-6/Nav + IIIb] purple); Navel trees on Rich16-6 rootstock treated with TsnRNA-IIIb and planted at high density ([R16-6/Nav + IIIb HD] red); Navel trees on Flying Dragon rootstock ([FD/Nav] white). The remaining trees (not highlighted in a box) are of different rootstock/scion combinations treated with various TsnRNA combinations.

Although the California Department of Food and Agriculture approved the use of TsnRNAs for commercial use in 2000 under limited permits, adoption by the citrus industry remained low. Despite the promise of the TsnRNA dwarfing approach, nursery adoption stalled due to regulatory hurdles and the lack of a practical production propagation protocol. Renewed interest from growers in 2014—prompted by a Citrus Clonal Protection Program (CCPP) walk-through at the UC ANR LREC—led to the reexamination of TsnRNAs field trials.

One of the earliest and most comprehensive evaluations of TsnRNAs as citrus dwarfing agents in California is the long-term field trial initiated in 1998 by UCR researchers at LREC (**Figure 1**).

The objective of the LREC trial was to determine whether canopy size reduction through TsnRNA treatments could lead to improved orchard efficiency without compromising fruit yield and quality. 'Parent Washington' navel orange trees grafted on 'Rich 16-6' trifoliate rootstock were either treated with TsnRNA-IIIb or TsnRNA-IIa or did not receive the experimental TsnRNA treatment as non-treated controls (NTCs). Trees were planted at both standard (20 x 22 ft) and high-density (10 x 22 ft) spacing and were never pruned during the study. All trees in the trial were tested to confirm their TsnRNA status and to rule out cross-contamination or other graft-transmissible pathogens. As an additional reference, the block also included unpruned navel ('Parent Washington', 'Frost', 'Atwood' and 'Carter') trees on 'Flying Dragon' rootstock planted in 1984 at high density (6 x 7 ft). 'Flying Dragon' is a slow-growing citrus rootstock that produces naturally smaller trees, thus serving as historical dwarfing controls.

Earlier publications from this trial established the long-term canopy volume reduction effect of TsnRNA-IIIb (Lavagi-Craddock et al. 2018; 2020; and 2022; Vidalakis et al. 2011). However, until now, no data were available on the potential labor savings associated with these size reductions.

To address this knowledge gap, we expanded the study to include time tracking for labor-intensive horticultural operations, such as harvesting, skirting and pest inspections. These data offer growers new insights into the practical benefits of using TsnRNA-treated trees for more efficient and potentially lower-cost citrus production.

Final results for this CRB project collected from the 1998 TsnRNA LREC trial confirm that TsnRNA treatment significantly reduces citrus tree size without proportionally compromising yield and that the effect is long lasting (**Figure 2**). After 25 years, trees treated with TsnRNA-IIIb had only 45 percent (standard spacing) and 41 (high-density spacing) percent of the canopy volume of NTCs. TsnRNA-IIa-treated trees had 82 percent of NTC canopy size, while the 41-year-old navel trees on the 'Flying Dragon' rootstock (FDNav) had 39 percent of the NTC canopy size (**Figure 2**, orange bars).

Despite their reduced size, TsnRNA-treated trees demonstrated consistent yield retention, on a per tree basis, during this study. Trees treated with TsnRNA-IIIb produced 66 percent (standard spacing) and 49 percent (high-density spacing) of the fruit yield compared to the full canopy size NTCs. TsnRNA-IIa trees yielded 84 percent, while FDNav trees achieved only 37 percent of the NTCs reference yield. These yield differences were statistically significant and underscore the efficiency of TsnRNA-IIIb: smaller trees with less vegetative volume still generated meaningful commercial yields (**Figure 2**, yellow bars).

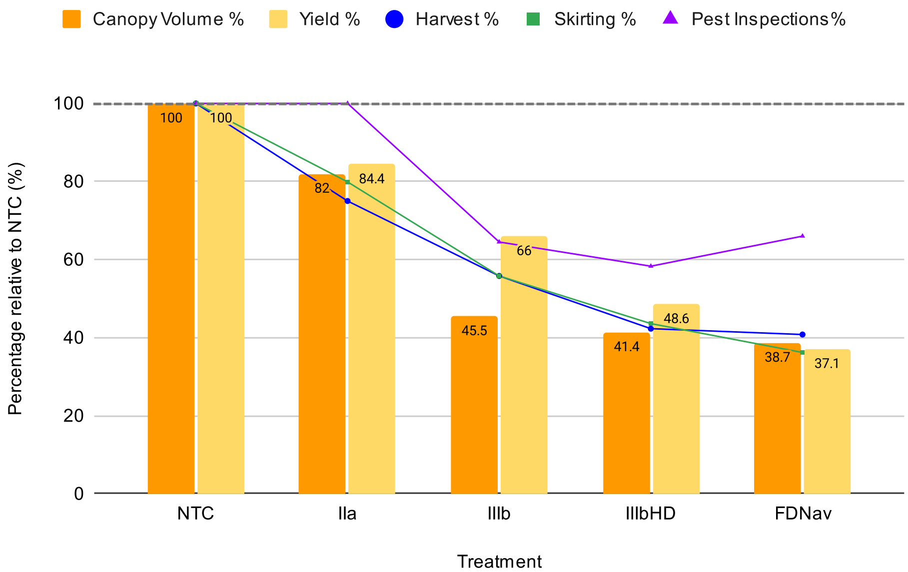

Figure 2. 2020-2024 TsnRNA effects on canopy volume (orange bars), fruit yield (yellow bars) and labor-related measures (lines). Values are expressed as percentages relative to the non-treated control trees (NTC= 100 percent). Average canopy volume and fruit yield (on a per tree basis) over a four-year period (n= 4) are shown as orange and yellow bars, respectively. Line plots represent average harvesting time (blue) during a four-year period (n= 4), two skirting time (green) measurements over a four-year period (n= 2) and four seasonal pest inspection duration (purple) measurements over a two-year period (n= 4). Treatments= non-treated control (NTC), TsnRNA-IIa at standard density (IIa), TsnRNA-IIIb at standard density (IIIb), TsnRNA-IIIb at higher density (IIIbHD) and navel on 'Flying Dragon' (FDNav).

In addition to tree size and yield, we evaluated three key horticultural operations—harvesting, skirting and pest inspections—by tracking the time required to complete each task across treatments. Results showed substantial labor savings for TsnRNA-IIIb-dwarfed trees compared to the full size NTCs:

- **Harvesting time was reduced by 44–58 percent**
- **Skirting time was 44–56 percent lower**
- **Pest inspection time dropped by 36–42 percent**

TsnRNA-IIa trees also showed labor savings, though more modest. Harvesting time was 25 percent lower than NTCs, and skirting time decreased by 20 percent. However, pest inspection duration remained equivalent to controls. FDNav trees had labor reductions similar to TsnRNA-IIIb.

From Trial to Reality: TsnRNA Performance in a Grower's Orchard

To validate findings beyond the LREC experimental setting, a long-term commercial planting was evaluated at a grower-owned site in the Central Valley of California (**Figure 3**).

Veteran citrus grower Tom Mulholland's early experimentation with viroid TsnRNA to dwarf citrus trees began in 1995 with a planting of 'W. Murcott' and 'Cara Cara' trees on trifoliate rootstock (planting density 10 x 20 ft) treated with either TsnRNA-IIIb or TsnRNA-IIa three weeks prior to orchard establishment and planted at a high density of 700 trees per acre, where the orchard thrived for several years. While the initial block was eventually removed due to overcrowding, several rows of 'Cara Cara' remain and continue to display reduced canopy volume nearly 30 years later (**Figure 4**).

Canopy measurements taken in 2024 showed that TsnRNA-IIIb-treated 'Cara Cara' trees in the Mulholland Citrus farm retained just 40 percent of the canopy volume of neighboring untreated controls, while TsnRNA-IIa trees retained 70 percent of the full-size controls. (**Figure 5**). Although no formal yield data were recorded, Mulholland noted that the trees remained productive, yielded high-quality fruit and were significantly easier to manage.

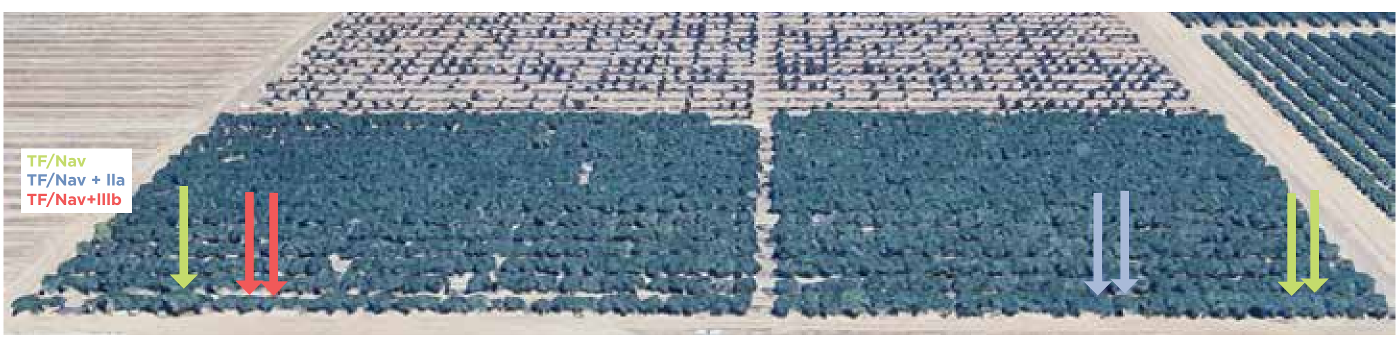

Figure 3: Satellite image (Google Earth) from May 2024 of a commercial orchard located in Orange Cove, California. Examples of trees used for the measurements indicated by the arrows as follows; 'Cara Cara' navel trees on trifoliate rootstock ([TF/Nav] lime green); 'Cara Cara' navel trees on trifoliate rootstock treated with TsnRNA-IIa ([TF/Nav + IIa] light blue); 'Cara Cara' navel trees on trifoliate rootstock treated with TsnRNA-IIIb ([TF/Nav + IIIb] orange).

Figure 4: Long-term reduced canopy volume effect of TsnRNA-IIIb on 29-year-old 'Cara Cara' navel trees on trifoliate rootstock (1995-2024) in a commercial block. TsnRNA-IIIb-treated 'Cara Cara' navel trees (right) compared to the non-treated controls (left).

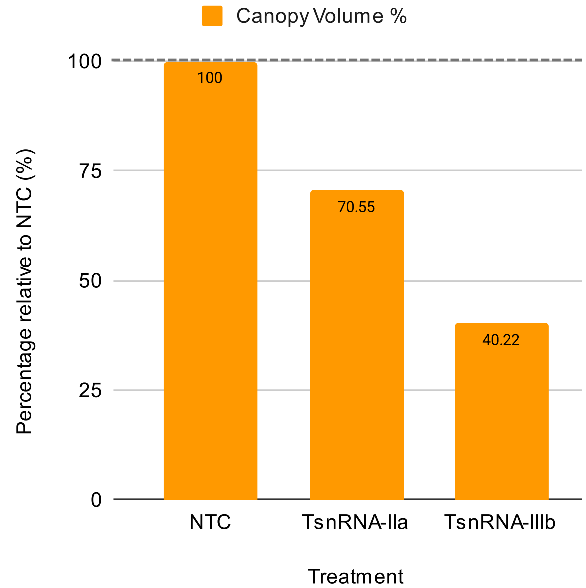

Figure 5: Long-term reduced canopy volume effect of TsnRNA-IIIb in a commercial block. Average canopy volume over a two-year period (n= 2) of non-treated control (NTC) 'Cara Cara' trees set at 100 percent.

To assess any practical benefits of the TsnRNA dwarf trees, pest inspection time also was tracked in the commercial block. Results closely mirrored those from LREC, with a 36 percent time reduction in TsnRNA-IIIb trees and 12 percent in TsnRNA-IIa trees relative to untreated controls (**Table 1**). Polymerase chain reaction testing[5] confirmed the viroid status of the trees and ruled out any cross-contamination between treatments—an important consideration for commercial deployment.

Despite the promise of the TsnRNA dwarfing approach, nursery adoption stalled due to regulatory hurdles and the lack of a practical production propagation protocol. Still, Mulholland remains optimistic. He sees renewed potential for TsnRNA-induced dwarfing as a pathway toward higher-density, lower-maintenance citrus orchards that sustain both productivity and fruit quality over the long term.

"Even after 25 years, the remaining 'Cara Cara' trees still express that dwarfing essence. They've produced well, stayed small and made harvest easier. If we can refine the approach using targeted viroid TsnRNA inoculations, I believe there's still great potential for this practice in California citrus."

— Tom Mulholland, citrus grower, nurseryman and innovator

Table 1: Pest inspection time in 29-year-old (1995-2024) 'Cara Cara' navel trees on trifoliate rootstock treated with TsnRNA-IIa, TsnRNA-IIIb or non-treated control (NTC) trees in a commercial block. Average values of the duration of four seasonal pest inspection duration measurements over a two-year period (n= 4).

TREATMENT NAME	Pest Inspection Time (%)
NTC	100
TsnRNA-IIa	87.78
TsnRNA-IIIb	63.12

Mulholland also expressed interest in hosting future high density TsnRNA dwarfing trials (though not at 700 trees per acre, but rather at 10 x 18 ft spacing), with 'W. Murcott', 'Washington Navel', Valencia and lemon trees, reflecting a belief that this technology is not only scientifically credible but commercially relevant.

Economic Perspective and Practical Significance for Growers

Both research and real-world data confirm that TsnRNA-IIIb delivers durable size control and reduced labor needs without severely compromising yield. For growers exploring high-density planting, preparing for CUPS or looking to cut input costs, this approach offers a viable path forward.

While direct economic figures were not part of this study, the documented percentage-based savings provided by this study—particularly for labor-intensive tasks such as harvesting, skirting and pest inspections—enable growers to estimate their own return on investment based on their specific labor costs.

Dwarf trees are central to the development of high-density systems, which support precision horticultural practices such as efficient spraying, mechanized harvesting and optimized water use. While other fruit crops have long benefited from dwarfing rootstocks, true citrus dwarfing rootstocks only recently have become available and remain largely untested under California conditions (Bowman et al. 2016; Webster 2002). Until those options are validated in our state, TsnRNAs remain a scalable, regulatory-approved alternative worth further exploration.

CRB Research Project #5100-154

Glossary

[1]**Transmissible small nuclear ribonucleic acid:** A plant cell regulatory ribonucleic acid molecule originally classified as a viroid, which reduces citrus tree size by altering vegetative development.

[2]**Citrus Under Protective Structures:** Screenhouse-like coverings used to protect citrus trees from pests and to reduce disease pressure.

[3]**RNA:** Ribonucleic acid, a nucleic acid present in all living cells. RNAs are transcribed from DNA and help convert genetic information into proteins. Some viruses and all viroids use RNA as their genetic material.

[4]**Viroid:** A small, circular RNA molecule that infects plants. Unlike viruses, viroids do not encode proteins.

[5]**Polymerase chain reaction testing:** A laboratory method for detecting specific genetic material. Used in this study to confirm the presence of TsnRNAs.

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CRB-FUNDED PROGRESS REPORT

Update on Lemon Pitting in the Central Valley

Ashraf El-kereamy, Tariq Pervaiz, Alaaeldin Rezk, Bharani Manoharan, Sandipa Gautam, James Adaskaveg and Mary Lu Arpaia

Project Summary

Lemon pitting continues to pose a serious challenge for citrus growers in California's Central Valley. First observed several years ago, this disorder varies in severity from year to year and from orchard to orchard, but when present, can significantly impact fruit quality and marketability. In April 2023, the Citrus Research Board (CRB) launched a multi-disciplinary research initiative to investigate the underlying causes and explore potential mitigation strategies. In 2024, we continued to collect data on microclimate, insect activity, pathogen presence, tree health and fruit quality. Sticky trap analysis showed no correlation between damage severity and any specific flying insect subspecies. Similarly, multiple laboratories conducted pathogen testing on symptomatic fruit and foliage, and there was no evidence of known causal pathogens. Comparative analysis of the 2023 and 2024 harvests revealed notable differences in damage levels, with significantly higher pitting seen in 2023. Several field treatments were implemented in 2024 to reduce lemon pitting. Among the treatments tested, Vapor Gard®, Parka®, silicon and one of the phosphorus applications showed the most promising results in reducing fruit damage. It is worth mentioning that the water treatment resulted in a lower percentage of lemon pitting, suggesting that these results are preliminary and need to be confirmed in another season. Grower-reported data and project observations shared by researchers from both seasons were subjected to an exploratory data analysis by an independent firm (EcoData Technology LLC) contracted by the CRB. The analysis suggested possible associations that are being investigated further this season.

Introduction

In 2023, the CRB launched a comprehensive research project to identify the factors associated with lemon pitting and provide growers with guidelines to effectively manage and control this condition in the Central Valley of California. Twelve growers generously agreed to participate, providing detailed records of their pesticide and fertilization practices over the past three seasons. To monitor environmental conditions, localized weather stations were installed and operating at each of the 12 participating orchards by December 2023. These stations recorded microclimate parameters, such as air temperature, relative humidity and wind speed on an hourly basis. Each orchard also has been surveyed for insect populations and fruit morphology to document the presence, severity and timing of pitting damage.

Figure 1. Lemon pitting begins early in the season, typically during the first week of May (A), and becomes visible as rind damage on the fruit at harvest (B). ***Photo by Ashraf El-kereamy.***

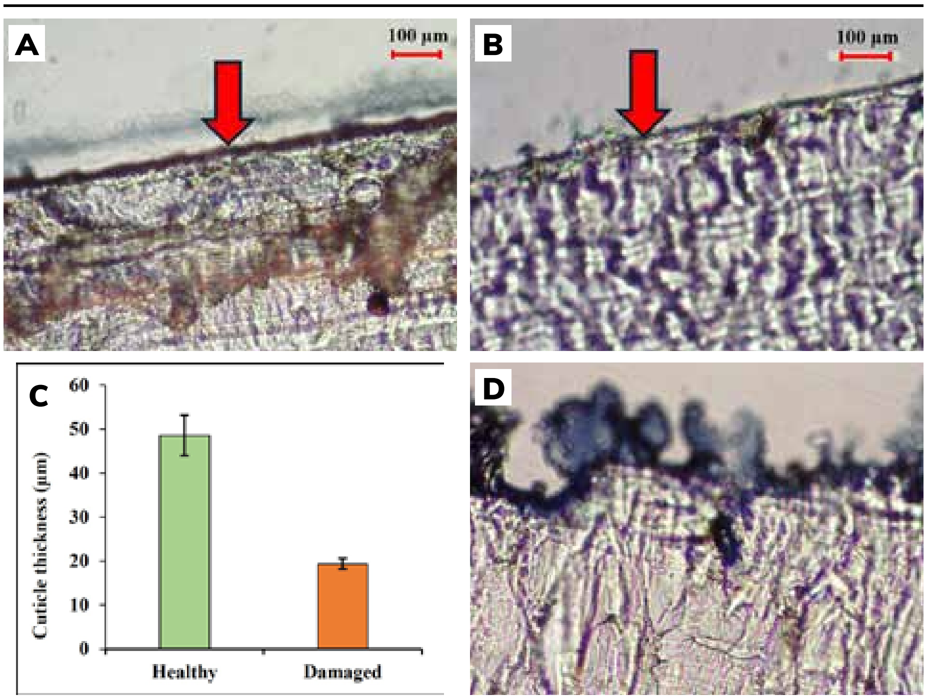


Figure 2. Sudan Black staining of (A) healthy and (B) damaged fruit. Panel C presents data on cuticle thickness. Values are the average of 12 readings from three different fruit. Panel D shows the rupture of the cuticle layers under a light microscope at 20x magnification.

As outlined in a previous *Citrograph* article (El-kereamy et al. 2024), several hypotheses are under investigation:

- insect damage,
- physiological stressors (such as nutrient deficiencies or water imbalance),
- disruptions to the fruit cuticle layer or
- interactions among these factors.

The research team continues to analyze field data to understand how these variables may contribute to lemon pitting, with the goal of developing effective mitigation strategies for growers.

Current Results

Microscopic Analysis of Rind Tissue

Microscopic comparisons between healthy and damaged rind tissue at harvest revealed that the damage is superficial, limited to the waxy cuticle layer with no observable injury to the underlying flavedo or albedo tissues. Histological analysis further showed that the cuticle layer on damaged fruit is noticeably thinner than that of healthy fruit (**Figure 2**). Additional investigations currently are underway to measure cuticle thickness across different fruit developmental stages

Variation of Lemon Pitting Between 2023 and 2024

During the 2023 and 2024 harvests, we randomly collected 800 fruit from each ranch and assessed the level of damage using the scale previously developed (**Figure 3**). A comparison of damage levels showed that the damage was significantly lower in the 2024 season than in the 2023 season (**Figure 4**). For example, the average percentage of fruit exhibiting significant pitting (Grade 4) was two percent in 2024 compared to 11 percent during the 2023 season.

Unfortunately, as the project began mid-season, we do not have microclimate data for ranch sites during the early part of the 2023 season when

Figure 3. The grading scale used for the damage evaluation on Lisbon lemons at harvest. ***Photo by Ashraf El-kereamy***

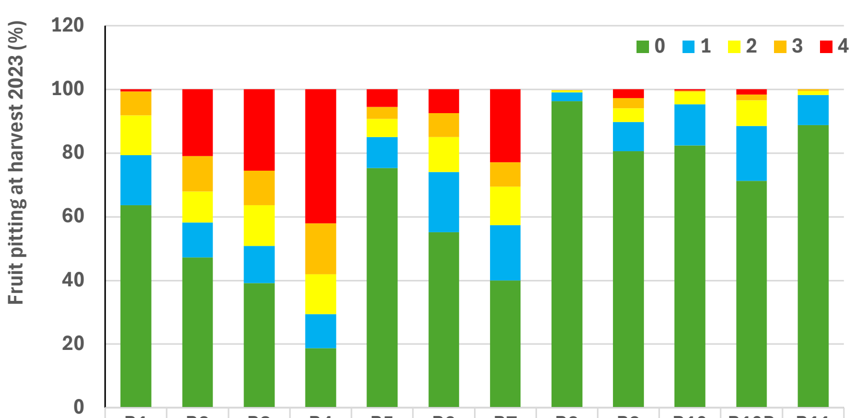
damage was first seen. However, we continue to monitor weather conditions across all 12 ranches and plan to analyze the correlation between environmental variables and seasonal variation in pitting severity.

Treatment Strategy for Mitigating Lemon Pitting in 2024

In the 2024 season, we implemented a series of targeted field treatments with the goal of reducing the incidence and severity of lemon pitting. While the cause of lemon pitting is still being investigated, our approach was guided by the hypothesis that environmental stress, particularly high temperatures and rapid changes in humidity, was exacerbating lemon pitting during critical fruit development stages. To address this, we designed treatments in three main categories: stress mitigation strategies, nutritional supplementation and hormonal treatments.

1. Stress Mitigation Treatments

We selected anti-transpirant and cuticle protective films as treatments to reduce the potential physiological stress experienced by fruit during the early stages of development when pitting is first observed. Silicon also was included here due to its known role in strengthening epidermal tissues and enhancing plant tolerance to both abiotic and biotic stress.



	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R10B	R11
4	1	21	26	42	6	8	23	0	3	0	2	0
3	8	11	11	16	4	8	8	0	3	0	2	1
2	13	10	13	13	6	11	12	1	4	4	8	1
1	16	11	12	11	10	19	17	3	9	13	17	10
0	64	47	39	19	75	55	40	96	81	82	71	89

Figure 4. Percentage of healthy and damaged fruit in the 12 groves during the 2023 and 2024 season harvests.

2. Nutritional Supplementation

Based on data from the 2023 season, orchards with higher levels of lemon pitting often were characterized by lower leaf phosphorus concentrations. Since phosphorus plays a central role in energy transfer, membrane stability and plant stress responses, we included foliar and soil-applied phosphorus in the treatment plan.

3. Hormonal Treatments

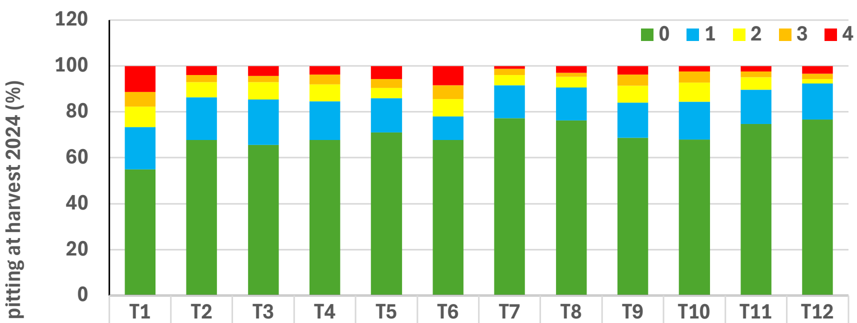
We explored the use of gibberellic acid (GA_3, ProGibb®), a plant growth regulator commonly used in citrus to improve rind quality. This application was inspired by its successful use in reducing rind breakdown in oranges and aimed to test its effectiveness in preventing or reducing lemon pitting. Treatments were applied to representative blocks at three of the twelve sites (Ranch 2, 3 and 4). Each treatment was replicated and monitored for efficacy through visual assessment and fruit evaluation at harvest. A list of treatments, including rates, timing and mode of application, is provided in **Table 1**.

Preliminary Results from 2024 Treatment Trials

Data from the 2024 treatment trials demonstrated a reduction in lemon pitting with the application of several stress-mitigation and nutrient-based treatments (**Figure 5**). Vapor Gard®, Parka®, Mainstay-Si™ and the high-phosphorus fertilizer P-58™ (10-58-0) showed measurable benefits in decreasing the severity and incidence

Table 1. Field treatments carried out to reduce the incidence of lemon peel pitting during the 2024 season. Applications started right after petal fall during the first week of May and were repeated every three weeks when multiple applications were made.

	TREATMENTS	RATE	APPLICATION	# OF APPLICATIONS
T1	Untreated control	N/A	N/A	N/A
T2	Untreated water	200 gallon/acre	Foliar	3
T3	AGphite 57 (0-30-27)	2 qt/acre	Foliar	3
T4	Tracite 8-30-2	2 qt/acre	Foliar	3
T5	High Phos™ (8-25-3)	2 qt/acre	Soil	3
T6	ProGibb®	10 ppm	Foliar	1
T7	Parka®	1%	Foliar	3
T8	Vapor Gard®	1%	Foliar	3
T9	Haven®	1%	Foliar	3
T10	Silicon 7%	2 qt/acre	Soil	3
T11	Mainstay-Si™	2 qt/acre	Foliar	2
T12	P-58™ (10-58-00)	3 lb/acre	Foliar	2



	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
4	11	4	4	4	6	8	1	3	4	2	2	3
3	6	3	3	4	4	6	3	2	5	5	3	2
2	9	7	8	7	4	8	4	5	7	8	5	2
1	18	19	20	17	15	10	14	14	15	16	15	16
0	55	68	66	68	71	68	77	76	69	68	75	77

Figure 5. Percentage of healthy and damaged fruit during the 2024 season harvest following 12 treatments conducted at Ranch 2, Ranch 3 and Ranch 4. Values are the average of the results obtained from the three orchards.

of pitting. Other treatments did not show any effect on lemon pitting including ProGibb®, AGphite 57 (0-30-2), Tracite® (8-30-2), High Phos™ (8-25-3), Haven and Silicon 7%.

While these treatments reduced severity and incidence of damage, none of them completely eliminated lemon pitting. It is also worth mentioning that the 2024 season was characterized by relatively low overall pitting pressure. Building on the insights gained from 2024, these treatments along with additional treatments derived from a detailed analysis of environmental, nutritional and management data across the 12 orchards have been selected for further testing during the 2025 season. Ongoing trials will include Parka, Vapor Gard, Mainstay-Si, Agri-Mek® (Insecticide and Acaricide), Priaxor® (Fungicide), and Murate of Potash (0-0-49.5). It is expected that the 2025 trial will validate the 2024 season findings and strengthen recommendations for managing lemon pitting effectively.

Preliminary Exploratory Data Analysis

As lemon pitting may be influenced by several factors, the collected data were provided to a third party (EcoData Technology LLC) for exploratory data analysis. This type of analysis is well suited to uncover patterns and relationships between multiple data sets and can help focus research efforts toward those factors most likely involved. Based on the information shared, a preliminary analysis found a few correlations for further investigation. Ranches with the most lemon pitting incidence were significantly drier and warmer than other ranches based on weather station data. Lemon pitting incidence was correlated with a higher level of potassium in leaves based on leaf nutrient analysis from 2023, and a similar pattern was observed for 2024. Fruit height, width and weight were significantly higher on ranches with high lemon pitting incidence; however, rind and juice traits were not significantly different, with one exception. Rind thickness was higher in more damaged fruit. Ranch inputs also were analyzed. However, there was low replication; therefore, further work is needed in this area. Based on these preliminary findings, we anticipate this information will assist with trials and project activities in 2025.

Conclusions of the Current Findings

Lemon pitting remains a significant issue for citrus growers in California's Central Valley. Data from the 2024 season showed reduced pitting severity compared to 2023, and survey efforts across all the potential factors continue in the current season to identify the possible link between environmental conditions and pitting damage. Targeted field treatments aimed at reducing stress and improving nutritional status to limit lemon pitting appear promising, and work in 2025 is building on the initial results from the 2024 treatments. Continued monitoring and data analysis will be critical in developing science-based recommendations to help growers mitigate lemon pitting and protect fruit quality in future seasons.

CRB Project #5400-171

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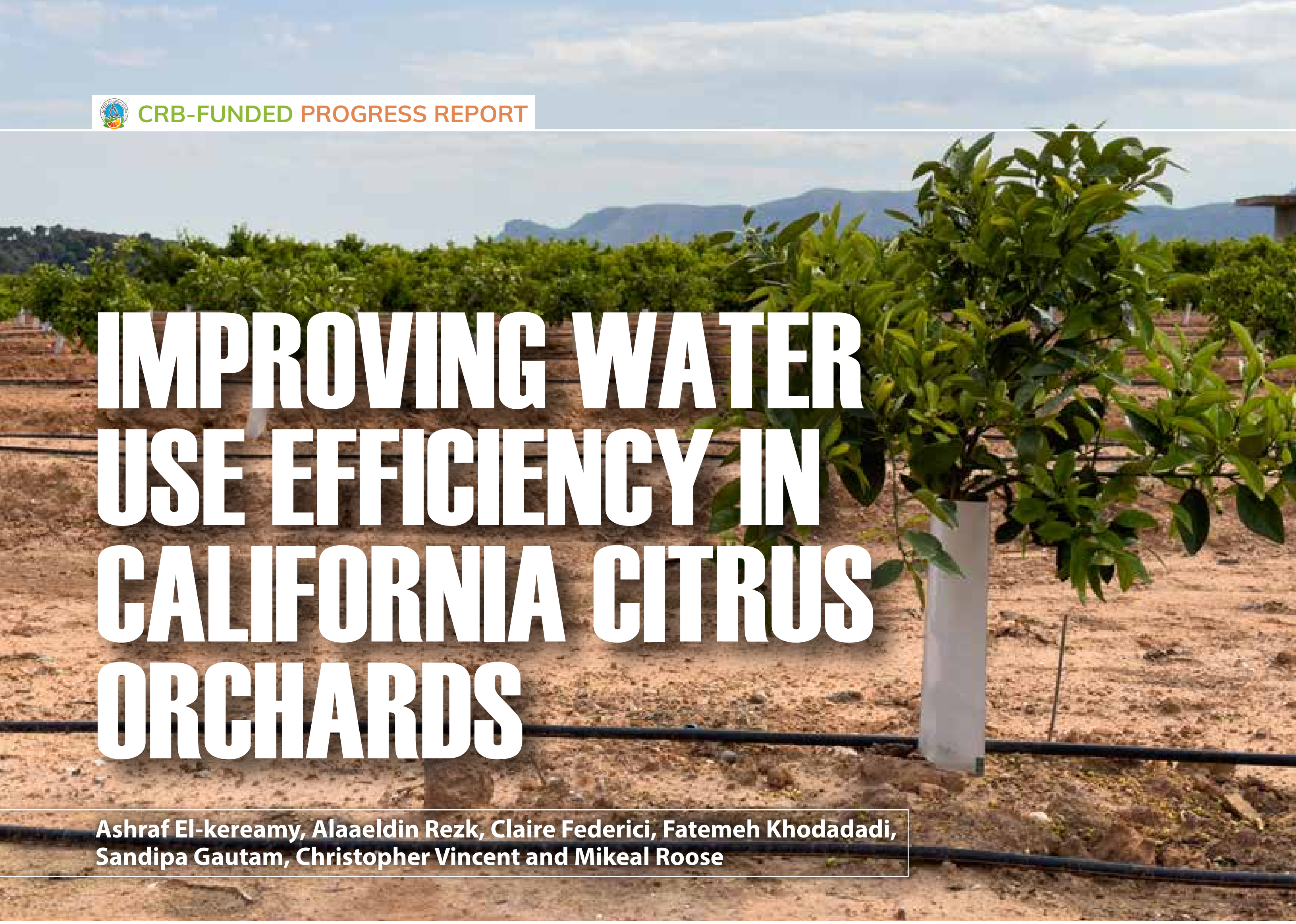

IMPROVING WATER USE EFFICIENCY IN CALIFORNIA CITRUS ORCHARDS

Ashraf El-kereamy, Alaaeldin Rezk, Claire Federici, Fatemeh Khodadadi, Sandipa Gautam, Christopher Vincent and Mikeal Roose

Project Summary

In this project, we are testing various strategies to improve citrus water use efficiency. By evaluating practical strategies for irrigating with limited water, this project will support citrus growers in maintaining orchard yield and fruit quality despite ongoing water constraints. The project is evaluating the tolerance of 'Nules' Clementine grafted onto various rootstocks under water-limited conditions. In addition, it is testing a range of irrigation technologies and cultural practices in comparison to Fan-Jet irrigation on 'Parent Washington' Navel orange and 'Tango' mandarin trees. These technologies and practices include synthetic and wood-based ground mulching, screen shade covers, drip irrigation, subsurface irrigation and alternate drying of the root zone and are intended to support citrus production under water restrictions while maintaining orchard yield and fruit quality. All treatments were established, and data collection was started at Lindcove Research and Extension Center (LREC) during the 2024 season. We determined the initial cost of each irrigation system and cultural practice; however, we must wait to calculate the amount of water saved to provide the growers with a full analysis.

Introduction

In addition to the drought conditions that California has been experiencing for years now, citrus growers will be required to reduce their water use to comply with the Sustainable Groundwater Management Act (SGMA). Improving water use efficiency in groves can help California's citrus growers sustain the state's high-value agricultural productivity now and in the future. Fan-jet irrigation has been the primary method used for citrus orchard irrigation as it offers targeted watering, reduces surface run-off and can help mitigate winter frost damage. However, this method is more susceptible to water and nutrient loss due to factors such as wind drift and evaporation, particularly in hot and dry weather conditions. Various cultural practices could be used to improve water use in the citrus orchards, either by reducing water loss or by improving the efficiency of tree water use. While existing studies offer valuable insights into improving water use efficiency, there remains a need for practical irrigation information that enables citrus growers to maintain orchard yield and fruit quality under limited water conditions. By evaluating such strategies, this project can support growers in navigating ongoing water constraints with informed, field-validated solutions.

Project Goals

1. Determine the effect of various rootstocks on the response of 'Nules' Clementine mandarin to water limitation.
2. Determine the effect of various cultural practices on Parent Washington Navel orange and 'Tango' mandarin water use.
3. Determine the cost of each irrigation system and the water saving.

Current Results

This first objective is to test drought tolerance of various citrus rootstocks (both released and experimental) using mature trees. Rootstocks differ in many traits likely to affect water use efficiency, including rooting depth, diameter of feeder roots, the number and size of the xylem and phloem cells that transport water and also adaptive stress responses such as proline accumulation. There are many studies of drought tolerance in rootstock seedlings but few studies of responses of older, grafted field trees; and seedling responses sometimes differ from those of mature, grafted trees. Testing trees in an existing trial allows information to be obtained more quickly than if a new trial were to be planted, grown to maturity and then tested for drought response. The study plot is a 12-year-old trial (Roose and Federici, 2023) of 'Nules' Clementine on 22 rootstocks (**Table 1**). Although some of the experimental rootstocks now appear unlikely to be released, if they show drought tolerance, they could be useful as breeding parents.

Table 1. Rootstocks used in the drought tolerance study, whether currently released or still experimental, the number of trees on each rootstock in the trial when drought treatments were imposed in September 2024, and comments on whether the rootstock is used for commercial citrus production anywhere in the world. For experimental rootstocks, the comment indicates whether current information indicates it is likely to be released.

ROOTSTOCK	RELEASE STATUS	TOTAL TREES	COMMERCIAL USE OR POTENTIAL
African Shaddock x 'Rubidoux' trifoliate	Released	12	Limited by CTV susceptibility
'Australian trifoliate #22'	Experimental	12	Good (standard in Australia)
'Bitters' (C22) (Sunki x Swingle trifoliate)	Released	12	New, some use
'Brazilian' Sour Orange	Released	12	Used, but limited by CTV
'C146' (Sunki x Swingle trifoliate)	Released	12	New, little use yet in CA
'C35' citrange	Released	12	Commercial Use
'Carrizo' citrange	Released	12	Commercial Use
'Cleopatra' mandarin	Released	12	Commercial Use
'Furr' (C57) (Sunki x Swingle trifoliate)	Released	12	Little use so far but new
Macrophylla	Released	12	Commercial Use
'Pomeroy' trifoliate	Released	12	Commercial Use
'Rich 16-6' trifoliate	Released	12	Commercial Use
'Rubidoux' trifoliate	Released	12	Commercial Use
Rangpur x 'Marks' trifoliate	Experimental	12	Release possible
Rangpur x Shekwasha	Experimental	11	Release unlikely
Rangpur x 'Swingle' trifoliate	Experimental	12	Release unlikely
Shekwasha x 'English' trifoliate	Experimental	12	Release possible
'Schaub' rough lemon	Released	12	Commercial Use
'Sun Chu Sha Kat' mandarin	Released	12	Not promising, poor yield
'Swingle' citrumelo	Released	12	Commercial Use
'Tosu' sour orange hybrid	Released	6	Not promising
'Volkameriana'	Released	11	Commercial Use

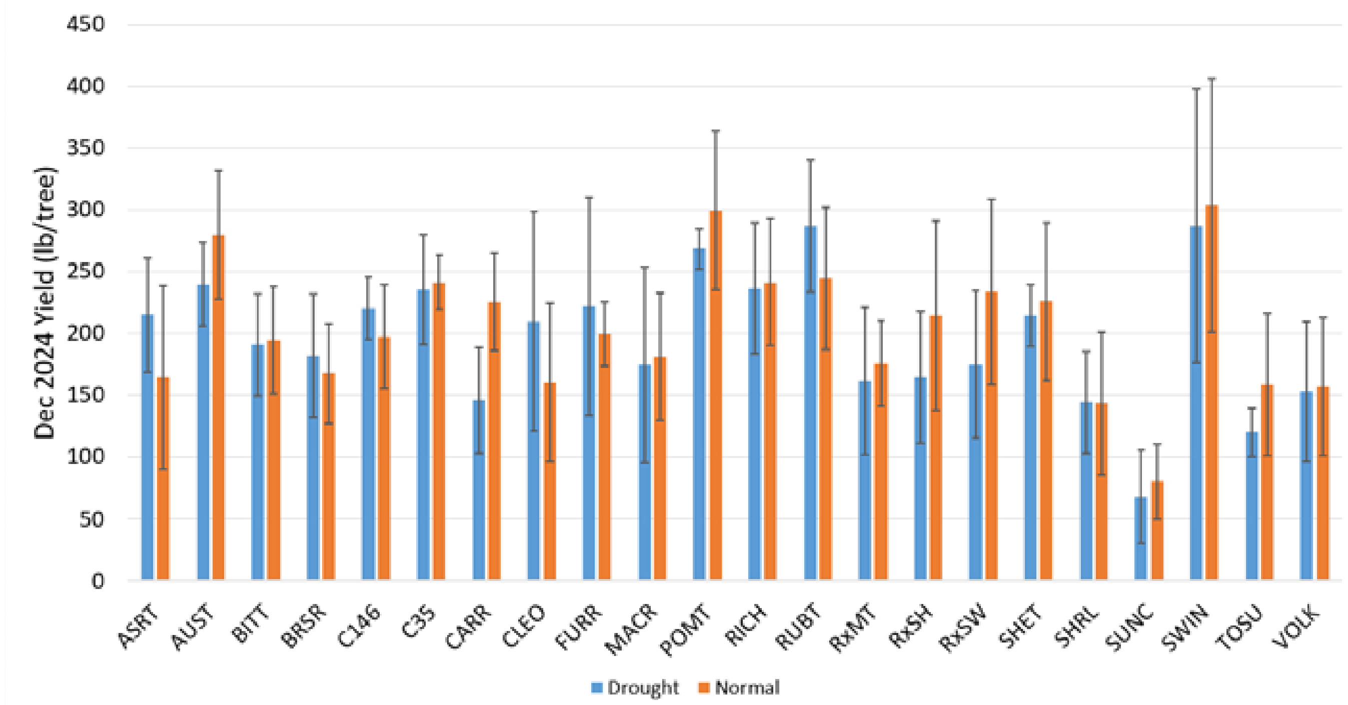

Figure 2. December 2024 yield of trees on 22 rootstocks in drought tolerance experiment. Rootstock abbreviations follow order in Table 1. Differences in drought response among rootstocks are not statistically significant. Error bars are standard deviations.

The drought regime selected was based on experiments conducted in Morocco with Clementines, which showed that a late season (about three months prior to harvest) reduction in irrigation to 75 percent of normal had only slight effects on yield and fruit size (El-Otmani et al., 2020). The 'Nules' trial at LREC is composed of 12 complete blocks – one tree on each rootstock, and each block consisting of two rows on separate irrigation lines. The block is irrigated with fan jets with normal irrigation runs of eight hours, adjusting the frequency of irrigation to apply water at 100 percent of predicted evapotranspiration (ET). We imposed drought treatments on six replications by reducing the duration of each irrigation from eight hours to six hours. For the deficit irrigation plots, a mild (91 percent of ET) regime was initiated September 1 to acclimate the trees, and the 75 percent of the ET treatment was imposed September 15 to November 30. Initially, flowmeters were installed to measure water application to all 12 irrigation lines to normal plots and four drought treatment plots. They were added to the other eight drought treatment lines in April 2025. Flowmeter readings for the reduced irrigation treatment period in 2024 indicate that the drought treatment plots received about 70 percent of the water applied to the normally irrigated plots, but there was high variation in water volume among the normal plots. In 2025, we improved system uniformity by placing new pressure regulators on each line and replacing some flowmeters.

The late December 2024 harvest showed about five percent lower yield in the drought treatment, a difference that was not statistically significant. Overall, we did not detect significant differences among rootstocks in yield (lb/tree) response to drought, although the effect approached significance ($p=0.15$). **Figure 2** shows the yield responses of the 22 rootstocks in this trial, with Carrizo, Tosu and some unreleased hybrids appearing most susceptible. Analysis of packline data from each tree showed that irrigation had a significant effect on only one trait – percent of fruit in size classes undersize + small (here called "percentage small"). We will assess whether these effects occur in other years since crop load and other factors can influence the percentage of small fruit.

This experiment also will explore the use of dendrometers to help evaluate plant water status and growth responses by measuring and reporting out real time changes in trunk diameter. This technology may contribute to more precise irrigation methods; and in an experiment with different rootstocks, dendrometers may be useful in identifying the most susceptible rootstocks as those that show stress sooner or delayed recovery. Dendrometers from ePlant Inc. were installed on four diverse rootstocks ('Volk', 'Carrizo', 'C35 'and 'Rich 16-6') in four replications of both treatments in July 2024. **Figure 3** shows dendrometer data for three trees of 'Nules' Clementine on Rich 16-6 trifoliate from September 1 to December 31. The lower part of the figure shows dendrometer readings over time, a measure of cumulative trunk growth. Note daily fluctuations. The upper part of the figure shows daily changes in trunk diameter at the dendrometer location. Stress is evident in the large fluctuations in daily trunk growth for the drought-stressed trees in comparison to the tree with normal irrigation (black line). The trees show stress with trunk shrinkage, then recover when irrigated.

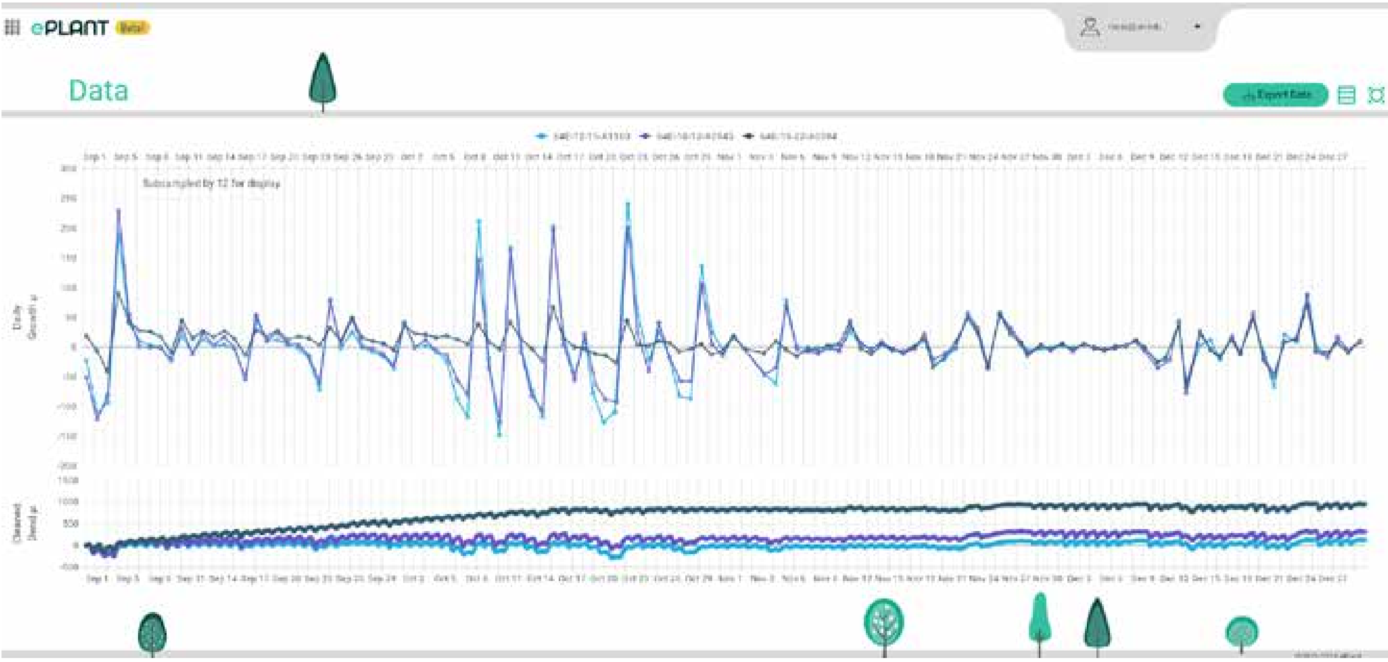

Figure 3. Plot of dendrometer data for three trees of 'Nules' on Rich 16-6. The two droughted trees are shown as blue and purple lines, while the tree with normal irrigation is a black line. Daily trunk growth becomes negative when trees are stressed, and then positive when they recover after irrigation.

In the second objective, field work began to evaluate the efficacy of various cultural practices on water saving while maintaining orchard yield and fruit quality. This objective includes four main goals.

- **Goal 1.** Study the effect of different synthetic and organic mulching materials. Synthetic white woven plastic mulch was installed on both sides of the row, leaving approximately three feet in the center to allow irrigation water to reach the soil (**Figure 4A**). Wood mulch obtained from removed citrus trees at LREC was applied in a three-inch layer to cover the entire space between the rows (**Figure 4B**).
- **Goal 2.** Test the efficacy of drip irrigation and alternate partial root-zone irrigation. This treatment was performed by irrigating 100 percent of the crop evapotranspiration (ETc), using drip irrigation on one half of the root zone, while the other half was allowed to dry. Another set of trees was irrigated using a single line placed in the middle of the row, supplying 100 percent of ETc.

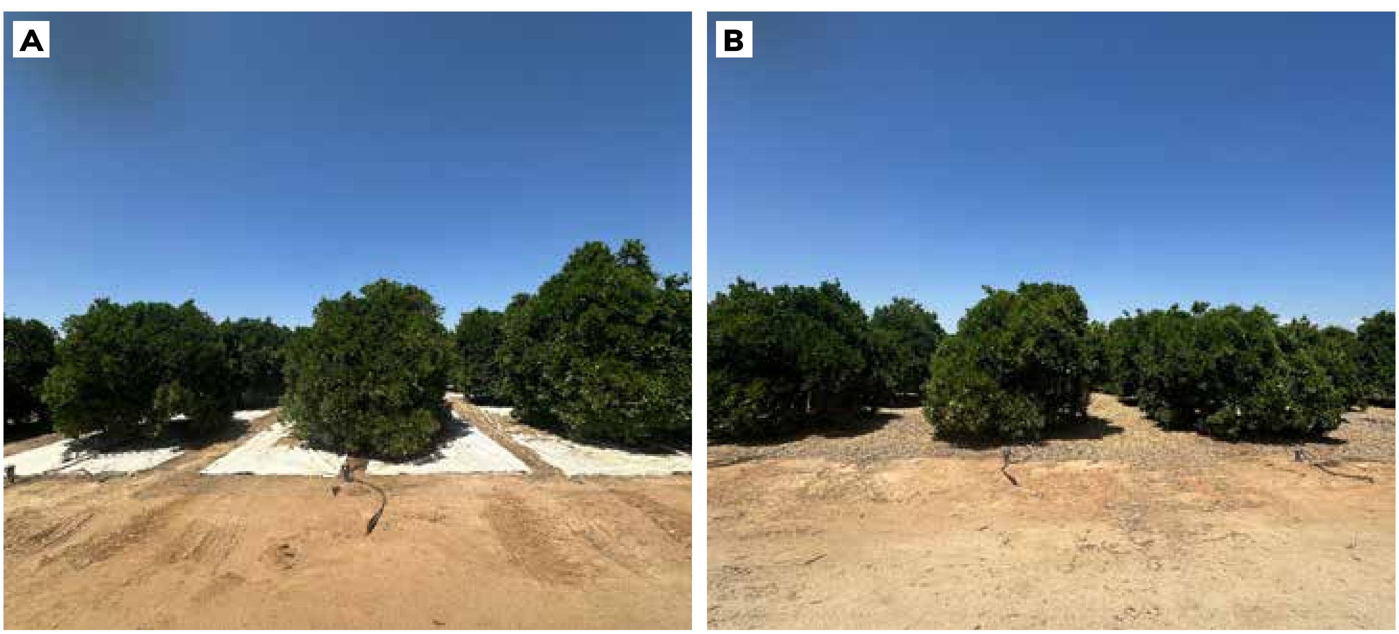

Figure 4: (A) Synthetic and (B) wood mulch applied at the experimental site of Parent Washington Navel orange.
Photo by Ashraf El-kereamy

Figure 5: Shade netting installed at the experimental site of Parent Washington Navel orange. *Photo by Ashraf El-kereamy*

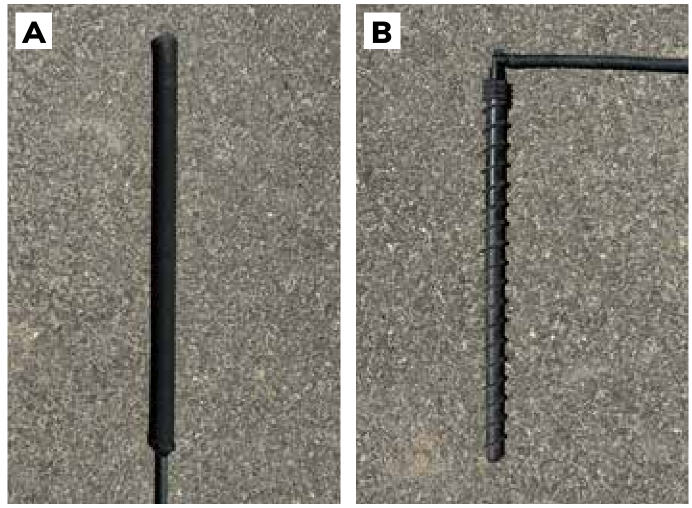

Figure 6: Deep Root (A) and DripScrew (B) irrigation technologies evaluated in the project.

- **Goal 3.** Determine the effect of shade netting. Twenty percent shade netting was installed using concrete poles with a height of 17 feet above the ground (**Figure 5**).
- **Goal 4.** Determine the efficiency of the Deep Root and DripScrew irrigation systems (**Figure 6**). Following manufacturer recommendations, 12-inch Deep Root units were installed at a depth of 15 inches, three inches below the soil surface; DripScrews were installed at a depth of 9.5 inches, flush with the soil surface.

These treatments (**Table 2**) were applied in mid-June 2024 to 30-year-old Parent Washington navel oranges and 12-year-old 'Tango' mandarins grafted on Carrizo rootstock at LREC (**Figure 7**). Each treatment was applied to four rows of 12 trees, with data collected from the middle two rows. Irrigation scheduling started the second half of June 2024 and was determined based on real-time soil moisture data, which guided timing and frequency. When moisture dropped below a defined threshold, irrigation was triggered to replenish the root zone. The amount of water used by each irrigation technology was recorded using a digital water flowmeter connected to each treatment; however, the total water use varied significantly across treatments during the subsequent optimization period following installation, as the new systems required ongoing monitoring and adjustment. At the end of the season, we harvested the fruit and measured total yield and fruit quality. The data showed no significant differences among treatments, and we need to confirm our findings during 2025 season. We are continuing to monitor the trees, and all measurements will be assessed again this season.

Table 2: Irrigation technologies evaluated under Objective 2 of the project.

TREATMENTS
Fan Jet
Fan Jet + Wood chip mulch
Fan Jet + Plastic synthetic mulch
Fan Jet + 20% Shade netting
Drip irrigation, single line
Alternate root zone drying
Deep Root irrigation (2 - 12-inch units per tree)
DripScrew irrigation (4 - 9.5-inch units per tree)

For the third objective, we determined the initial cost of each irrigation system and cultural practice; however, we are still working on determining the amount of water saved to provide growers with meaningful data on installation costs and return on investment.

Conclusions

During the 2024 season, significant progress was made toward evaluating strategies for improving citrus water use efficiency under deficit irrigation and alternative cultural practices. For Objective 1, baseline data from the 'Nules' Clementine rootstock trial confirmed no pre-treatment differences between control and drought-stressed trees. Drought treatments began in September 2024, and future harvests are expected to reveal rootstock-specific differences in yield, fruit quality and stress response. Continuous monitoring using dendrometers and long-term evaluation over three years will provide robust insight into rootstock performance under water-limited conditions. For Objective 2, the installation and optimization of multiple water-saving treatments, including mulches, shade netting, alternate root-zone drying and deep/drip irrigation systems, were completed at the LREC site. Although yield and fruit quality differences were not observed in the first season, likely due to system calibration and variability in water delivery, these practices are now in place for more consistent evaluation moving forward. Continued monitoring and data collection in the upcoming seasons will further clarify the effectiveness

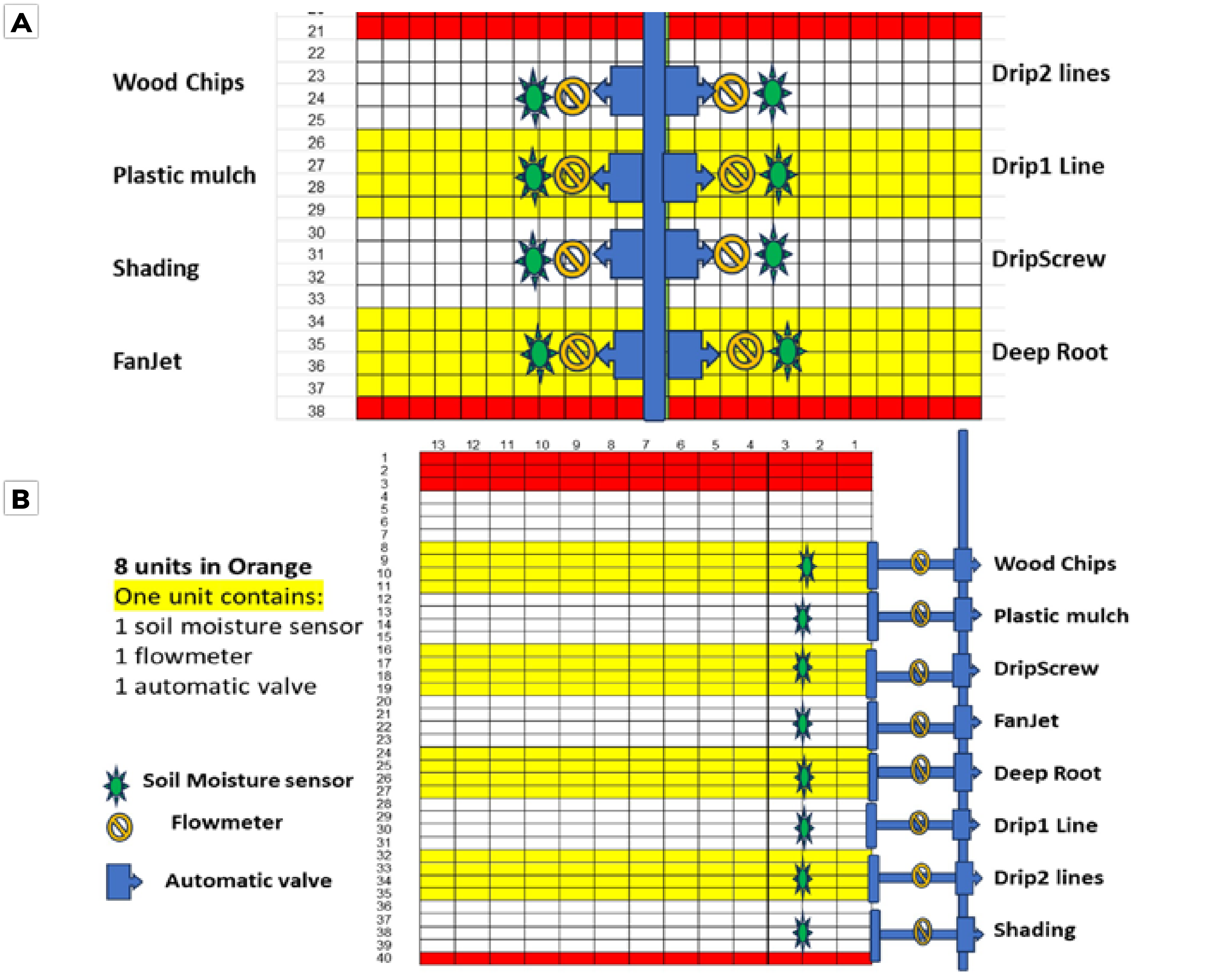

Figure 7. Layout of the various irrigation treatments in (A) 'Tango' mandarin and (B) Parent Washington Navel orange grown at Lindcove Research and Extension Center.

of these strategies in improving water efficiency without compromising fruit production or quality. This information will be shared with growers through extension articles, seminars and field days, helping California growers maintain orchard yield and fruit quality while irrigating with a limited water supply.

CRB Project #5400-175

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DEVELOPING NOVEL END EFFECTORS FOR HARVESTING CITRUS FRUIT

Melissa Tanner, Marc Campbell, Makayla Campbell and John Tanner

Project Summary

Labor is a significant production cost for specialty crops. Fresh citrus producers are increasingly interested in automation, particularly for the picking process, which constitutes most of the labor involved. The first step in automation is the development of a robotic end effector[1] designed to handle citrus fruit with care by gently grasping, cutting and placing it without damage.

We determined key design criteria for the end effector:

- *a slim profile to minimize tree damage,*
- *adaptability to different fruit shapes and*
- *the ability to work quickly and efficiently.*

A design should use the minimum possible number of actuators to minimize complexity and keep manufacturing costs low.

Two versions of the end effector were developed and tested: one with a stem-gripping mechanism and another with soft, flexible fingers for fruit gripping. The stem-gripper performs well in firmly attached fruit and is agnostic to fruit size. The soft-fingered gripper offers a firm hold for ripe fruit, though it requires precise adjustments for fruit size and cutting height. Both designs contain a cutting mechanism to sever the stem just above the calyx button.

The prototypes demonstrated the potential of automation in citrus harvesting, with the stem gripper showing promise for reaching delicate fruit at various angles, while the soft-fingered gripper is better suited for ripe fruit. These designs could significantly reduce labor dependency in the citrus industry, paving the way for a more sustainable future.

Introduction

The upward trend of harvest labor costs and a shortage of available workers have challenged California growers (Daniels 2019). The country is cracking down on worker eligibility status for migrant workers, who constitute most of the agricultural labor force; and labor laws continue to escalate minimum hourly rates (Rubin 2024). Humans also are limited to how many hours per day and per week they can work, in addition to needing vacation, holiday, sick and weekend time off. Robots don't have these limitations.

In a traditional manual process, lemon harvesters spend most of their time picking (the remainder is walking their fruit from the tree to a collection station). Offloading this task to the proposed autonomous picking robot would reduce harvesting labor and reduce labor costs by millions of dollars across the entire California citrus industry.

Our long-term goal is to automate the picking portion of citrus harvesting so that growers can maintain production levels with less available labor. As the first step in this effort to develop an autonomous harvest robot, we proposed to design and prototype a robotic end effector specialized for harvesting citrus fruit. The objective was to develop a robotic end effector that could grasp and hold citrus fruit, cut the stem to separate the fruit from the tree and deposit the fruit at a target location. Such an end effector must handle the fruit gently to avoid causing damage like bruising or oleocellosis. It should cut the fruit very close to the calyx button, since one protruding stem can damage a lot of other fruit during post-harvest processing.

Design Specifications

We talked with growers to define a set of requirements and design criteria for the gripper. In addition to handling the fruit gently and cutting the stem close to the fruit, we determined that a gripper should have a slim profile to reach through leaves and branches with minimal damage to the tree. It must be able to function over the wide range of citrus fruit shapes and sizes.

Ideally, it should be easy to swap between fruit by swapping grippers or adjusting the end effector. To be cost effective, a gripper must work quickly in concert with a computer vision and control system to identify and reach the fruit. We placed a high priority on grippers that could reach and cut fruit from any angle, requiring less computational load on the vision system. In addition, we tried to minimize the number of actuators required. This simplifies the design, making it lighter and more reliable while keeping manufacturing costs low.

A final product meant for the field must be weather-resistant, robust and easily maintained. However, these were not our primary concerns in designing a prototype.

Project Results

We proposed a "grip-clip-grip" style end-effector, as pictured in **Figure 1**. In this architecture, a pair of pliers-like pincers grips the stem slightly above the fruit. Above it (tree-ward) on the stem, a blade cuts the fruit from the tree. The robot then can carry the fruit away from the tree to place it gently in a collection location. At this point, a blade positioned below the grippers clips the fruit from the stem. The grippers then can discard the leftover piece of stem they are holding.

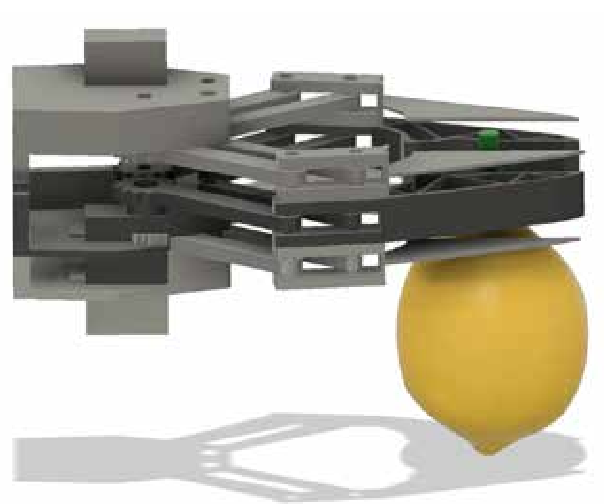

Figure 1: Computer-aided design rendering of a proposed stem-gripper.

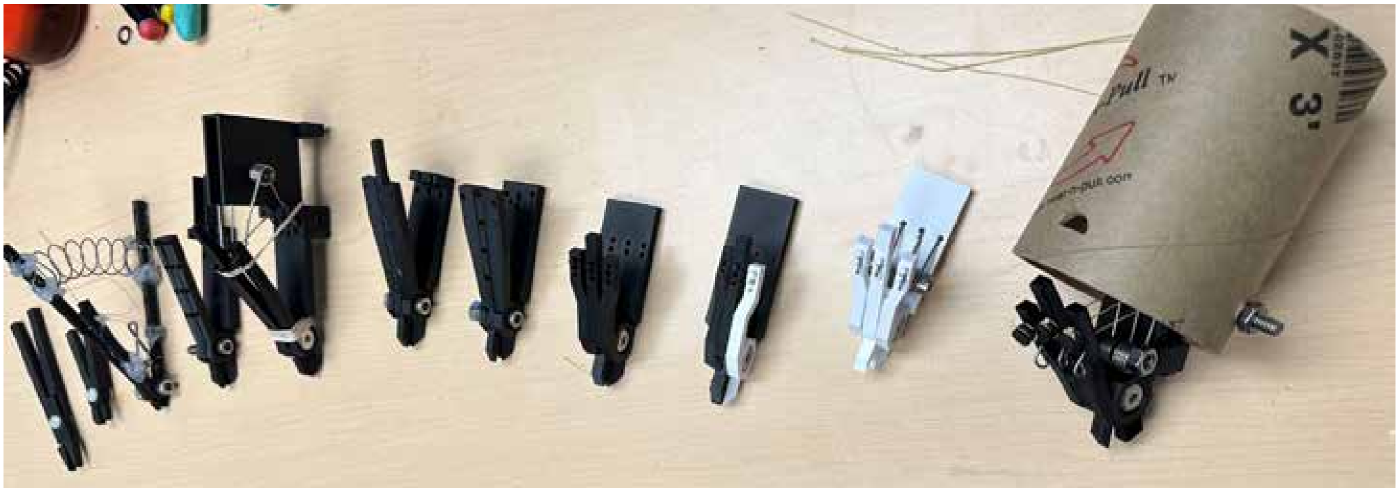

Figure 2: Evolution of stem gripper prototypes.

Figure 2 shows the progression of stem-gripping prototypes. We experimented with anvil-style and bypass cutters, ultimately settling on bypass cutters because these allowed us to simplify the number of moving parts in our design. The design shown in **Figure 3** involved a pair of jaws in the middle, B and C. On the top (treeward) side, cutting blade A moves past jaw B, enacting a bypass cut. On the bottom (fruitward) side, cutting blade D moves past jaw B, enacting another bypass cut. The metal prototypes shown here were 3D-printed using an external laser sintering service. They are actuated by Kevlar cord so that we could experiment with the amount of pressure needed to actuate the device.

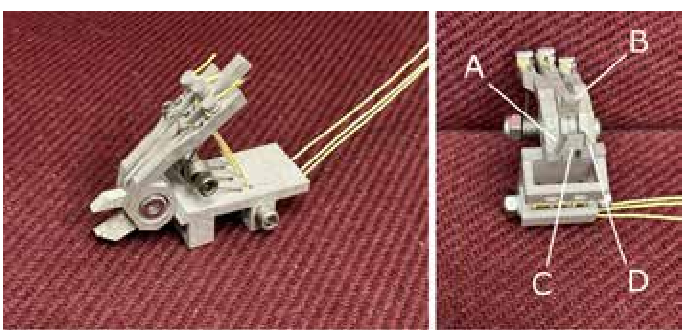

Figure 3: Side (left) and front (right) views of a corded triple-actuated stem gripper. (A) top cutting blade, (B and C) pair of jaws and (D) bottom cutting blade.

We soon realized that the turnaround times on the metal 3D printing service were too long, hampering our iterative design process. We switched to a different approach involving embedding utility blades in 3D printed plastic. Note that this design choice is only to improve prototyping; a production model could easily be 3D-printed or even cast in metal.

In testing the stem gripper, we realized that a pair of blades with adequate sensing were sufficient to grasp the stem when not completely closed. This allowed us to simplify the design and lighten the end effector by reducing the number of actuators to two. The newer version, shown in **Figure 4**,

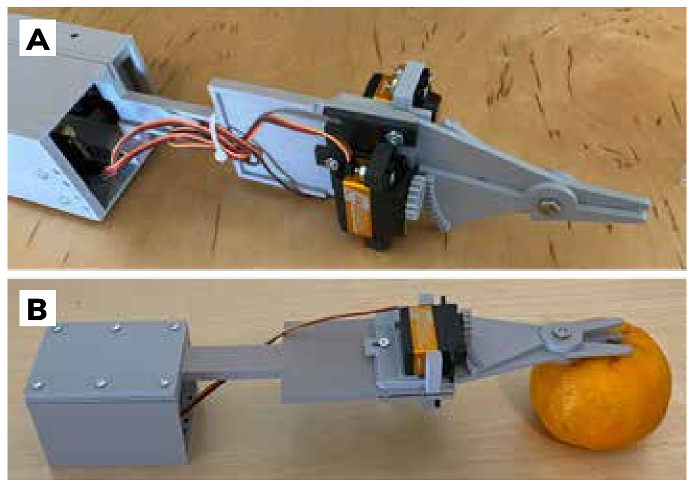

Figure 4: (A) Side and (B) top views of the dual-actuated stem gripper.

has two bypass cutting blades above and below a stationary central jaw. Partially closing the bottom blade against the jaw secures the fruit. Fully closing the top blade severs the stem from the tree; and after moving the fruit to a target location, the bottom blade can be closed the rest of the way to fully sever the fruit from its stem.

One potential downside of this approach is that it depends on a solid connection between stem and fruit. However, as citrus fruit ripens, the stem weakens in preparation to abscise at its junction with the calyx button. This is particularly common with fruit that is picked ripe such as grapefruit. To address this concern, we also developed an alternative end effector design that gently holds the fruit while cutting the stem.

This fruit-gripping end effector is based around the design of a flexible cable-driven finger. **Figure 5** shows the principle behind the design, in which a cord threaded through one side of a flexible finger can be pulled tight, forcing the finger to curl. Mirroring this concept creates a finger that can flex in either direction.

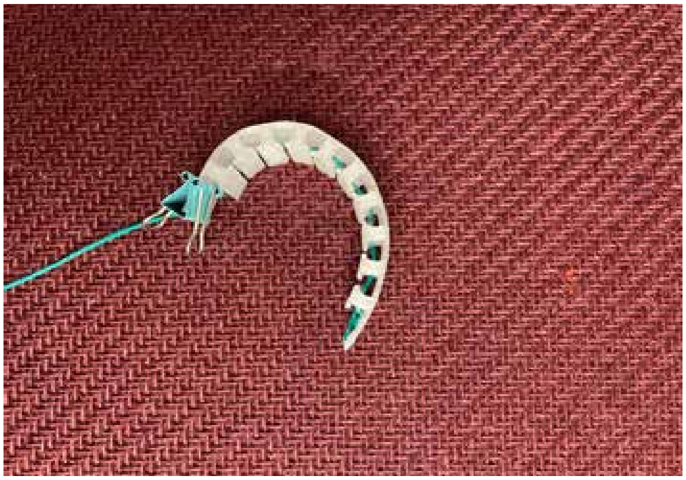

Figure 5: Demonstration of tendon-like finger actuation.

We combined three of these fingers to make a soft gripper. **Figure 6** shows a close-up of the resulting compliant gripper in action. The fingers were 3D-printed in thermoplastic polyurethane (TPU) and each threaded with two Kevlar cords. We combined the three "opener" cables to the same spool and the three "closer" cables to another spool. Thus, we only needed two actuators to completely open and close the hand.

Figure 6: Close-up image of tendon-driven flexible fingers.

For added grip surface friction and to better protect the fruit, we added a silicone cover to the fingers. This cover could be removed for cleaning and tailored to provide the desired level of cushion. **Figure 7** shows the end effector with and without its silicone covers.

The flexible fruit-gripping fingers can be paired with any stem-cutting device, and we briefly experimented with a "crochet hook" style cutter as shown in **Figure 7**. However, this hook-shaped cutter works best when it approaches the stem from the side, while the gripper works best with a straightforward approach. With this in mind, we decided to use a cutting mechanism based on our initial, stem-gripping design. The end effector would approach the fruit with clippers and fingers open. It lightly closes the fingers around the fruit, with the clippers around the stem. It then slides down the stem until the clippers contact the top of the fruit, holding the stem taut and providing a close cut. The fingers can be closed tighter for a firmer grip before the clippers cut the fruit from its stem. Once the end effector has reached a collection point, it can open to gently place the fruit in that target location.

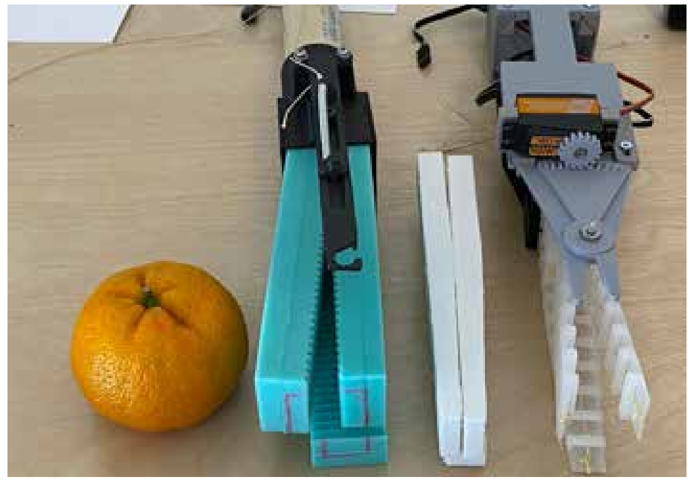

Figure 7: Left: A soft-fingered fruit gripper with a "crochet hook" style cutter and silicone-covered fingers. Center: the silicone finger coverings. Right: Bare flexible fingers and a scissors-like stem cutter. Mandarin fruit included on far-left side for scale.

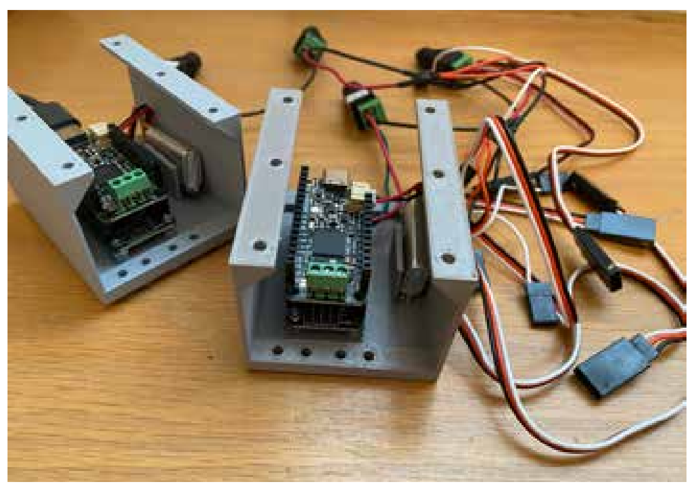

Figure 8: The electronics boxes used for end effector prototypes.

We also developed the electronics necessary to actuate these end effectors. **Figure 8** shows two electronics control boxes, designed to be interchangeable between the two designs.

Both the stem-gripping and fruit-gripping designs were tested in citrus fruit trees near our office. The stem-gripping design required some precision to operate to ensure that the stem ended up between the open cutting blades. The operator must slide the cutters down the stem until contacting the fruit, which is relatively easy for a human but would require computer vision or force sensing for a robot to do autonomously. However, the slim profile means that this cutter can reach almost any fruit on the tree at any angle. The fruit gripper was easier to operate, since the caging grasp tended to force the fruit into position so that the stem was in the cutter. However, it was more dependent on accurately sizing the fingers and cutter height to the target fruit. Its larger profile also was more disruptive, touching more leaves as it went.

Conclusion

For this project, we designed multiple end effectors for fruit collection and handling. We iterated and improved on those designs until we successfully prototyped two designs for harvesting citrus fruit: a stem gripper and a soft-fingered fruit gripper. The stem gripper has the advantage of being agnostic to fruit size and can harvest the fruit without touching it. The soft-fingered fruit gripper provides a firm grasp for ripe fruit that may readily abscise. These designs have been selected to achieve the desired motion with a minimum of actuators.

Developing these end effectors was a key step in the process of automating citrus harvesting. The automation of citrus harvesting could significantly cut labor costs, enhance efficiency and ensure the sustainability of the industry in the face of increasing pressures. As these technologies advance, they offer a promising path toward a more cost-effective and reliable future for citrus farming.

CRB Research Project #5400-174

Glossary

[1]**End effector:** A device at the end of a robotic arm designed to interact with the environment.

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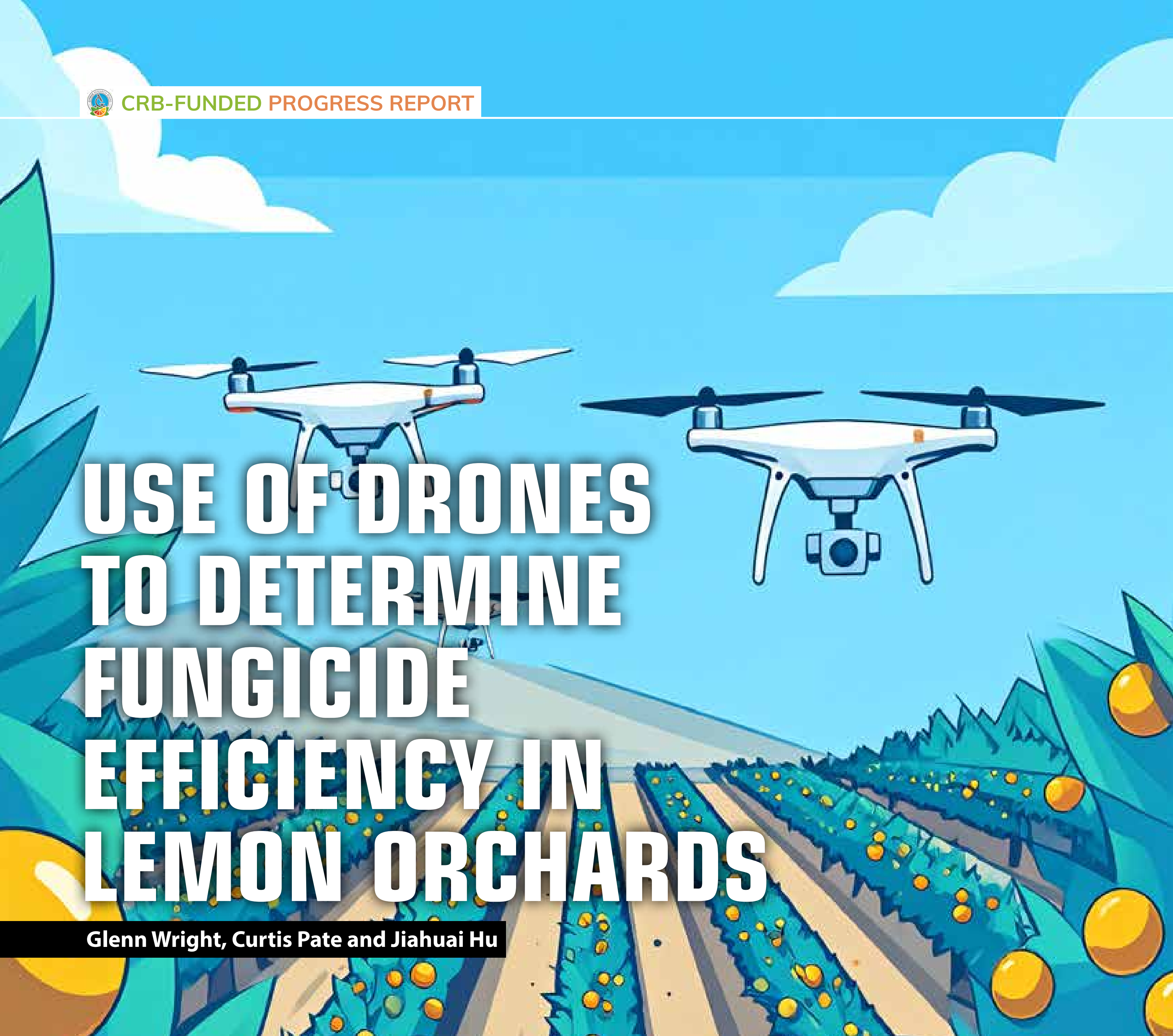

USE OF DRONES TO DETERMINE FUNGICIDE EFFICIENCY IN LEMON ORCHARDS

Glenn Wright, Curtis Pate and Jiahuai Hu

Project Summary

We are finding an increasing incidence of wood rot fungal diseases in desert lemons. The current recommendation is to prune out all infected wood and remove it from the orchard since there are no fungicides registered for these diseases. However, after laboratory and small plot tests in Arizona, we have identified nine candidate fungicides that reduce the incidence of the diseases. To determine the efficiency of the fungicides in commercial orchards, we are using drone technology to collect before- and after-application images across visible and non-visible wavelengths for several thousand trees. These images are geolocated and combined to form a vegetation index for each tree. After ground truthing and categorization of the trees based on their index, preliminary results showed that several of the fungicides led to improved tree health. Successful selection of efficacious fungicides and their application by growers is expected to lead to improved orchard health, production and returns.

Figure 1. Lemon tree in Imperial County with Brown Wood Rot. ***Photo taken by Glenn Wright***

Wood rot fungal diseases increasingly are a problem in citrus trees older than 15 years old in the California desert (Hu and Wright 2021). The fungal causal agents are *Fomitopsis meliae* and *Hypoxylon macrocarpum*, which cause brown and white wood rot (BWR and WWR), respectively. Both rots produce wind-borne spores that infest citrus trees, producing hyphae that consume the wood or bark, leading to tree collapse and death (**Figure 1**). Of these, BWR is the most prevalent and destructive. In 2022, we conducted a survey to determine the fungi associated with branch canker, dieback and wood rot disease of citrus in the desert (Wright et al. 2023). We found *Fomitopsis meliae, Coniophora eremophila* and four other fungal species. About 53 percent of the survey samples contained *F. meliae*.

For this project, which began in spring 2024, we conducted commercial orchard trials of fungicides at three Imperial County lemon orchard sites. Fungicides were labeled for bearing citrus, with active ingredients that were shown to be effective in the Hu laboratory (Hu, *unpublished data*) and at the Yuma Agriculture Center (Wright 2024). Fungicides applied in June 2024 and scheduled for May or June 2025 by airblast sprayer include Quadris Top®(15.4 fl. oz. per acre), Headline®(15 fl. oz. per acre), Priaxor® (11 fl. oz. per acre), Pristine® (18.5 fl. oz. per acre), Tilt® (7 fl. oz. per acre), Ceyva® (5 fl. oz. per acre), Serifel® (10 fl. oz. per acre), Serenade® (3 qt. per acre) and Bio-Tam® (2 lbs. per acre).

There are 945 trees at Site A, 2,052 trees at Site B and 2,088 trees at Site C. Given that there are nine treatments plus an untreated control, each treatment is applied to ten percent of the trees at each site. Prior to the applications in May 2024, we selected three symptomatic branches per treatment per site for isolation of branch canker/wood rot fungi. We found *Fomitopsis meliae* (39 percent) and *Coniophora eremophil*a (36 percent), both brown wood rot fungi; *Neoscytalidium dimidiatum* (8 percent), the causal agent of Sooty Canker; and *Eutypella microtheca* (17 percent) a branch canker, at each of the three sites.

To determine the effectiveness of the fungicide applications, we used a drone to visually survey every treated and untreated tree at each site before and after application. Drone flights were made in May 2024 (prior to fungicide applications in May and June 2024) and October 2024 (to assess product efficacy). The drone (**Figure 2**) is a Draganfly™ Commander 3XL (*https://draganfly.com/*). It is a large four-prop drone with a flight time of 50 minutes for 35 acres on one set of batteries. The multispectral[1] sensor attached to the drone is a MicaSense Altum-PT™, which can sense blue, green, red, red-edge, near-infrared and infrared/thermal wavelengths with panchromatic sharpening[2].

Figure 2. The Draganfly™ Commander 3XL drone. ***Photo taken by Curtis Pate***

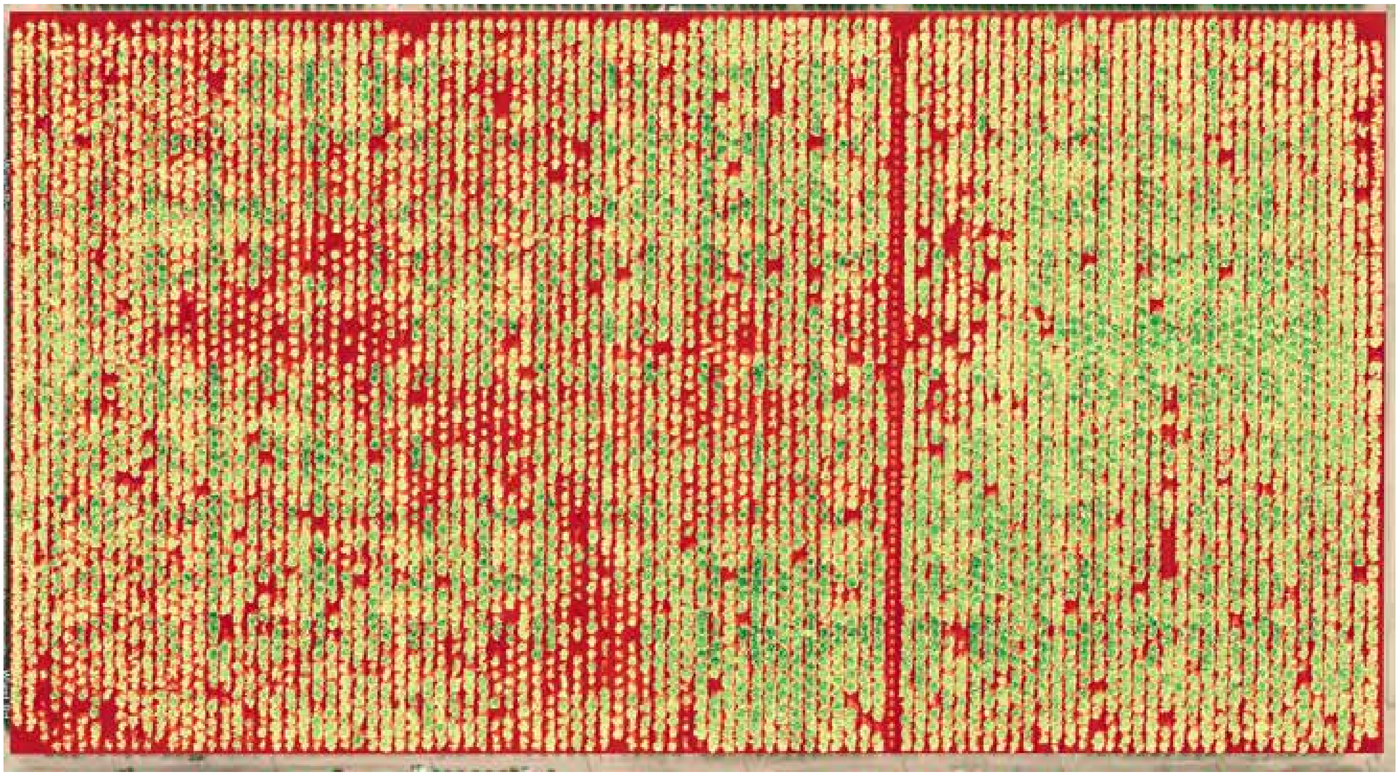

Figure 3. Normalized Difference Red Edge (NDRE) Color Codes for Site C.

The sensor captures each of the wavelengths every 0.5 seconds. This allows the construction of vegetation indices, such as the Normalized Difference Vegetation Index (NDVI[3]), Normalized Difference Red Edge (NDRE[4]), Green Leaf Index (GLI [5]) and thermal imaging, which are useful in assessing crop health (Boiarskii and Hasegawa 2019; Eng et al. 2019; Solvi AB 2023). The panchromatic sharpening permits the drone to fly at a ceiling of 400 feet with about 1.5 cm per pixel[6] resolution. Four pixels joined together provide enough resolution to allow a citrus leaf or fruit to be discerned from another leaf or fruit.

For the experiment, we chose to evaluate the NDRE rather than NDVI, as NDRE is less likely to saturate (give a value of 1.0) than the other indices. It allows for observation deeper into a dense canopy, which is better for permanent crops, and it shows plant stress (Boiarskii and Hasegawa 2019). NDRE values range from -1 to 1, with higher values corresponding to healthier trees. We also chose to evaluate GLI, expressed as percent canopy cover. GLI is another way to look at tree health. These two indices (NDRE and GLI) can show different characteristics because they show different spectrums and potentially may unveil different information about the disease or tree health.

For each 35-acre block, about 4,000 to 5,000 images were generated during the flight, geolocated[7] and "stitched" together with 70 percent overlap using a data pre-processing program. Next, using post-processing software, we adjusted the plot size to the spacing of one tree (for example, 20 x 24 ft). Each tree-space-sized plot was then matched with the average NDRE and GLI value for that area

Table 1. Average vegetation indices indicating tree health among ten treatments at Site B and Site C. Green Leaf Index (GLI) was not collected in May.

	SITE B			Site C		
	May 2024	October 2024		May 2024	October 2024	
Treatment	**NDRE**	**NDRE**	**GLI (%)**	**NDRE**	**NDRE**	**GLI (%)**
Quadris Top	0.2348	0.3915 ab	70.90	0.2531	0.4014 ab	53.36
Headline	0.2162	0.3800 c	65.53	0.2489	0.3891 d	49.57
Priaxor	0.2306	0.3823 bc	69.86	0.2625	0.4081 a	54.23
Pristine	0.2276	0.3847 bc	67.57	0.2595	0.3958 bcd	52.08
Tilt	0.2255	0.3951 a	71.10	0.2503	0.3921 cd	51.89
Cevya	0.2334	0.3661 d	62.84	0.2407	0.3937 cd	49.00
Serifel	0.2305	0.3920 ab	69.44	0.2613	0.4088 a	54.79
Serenade	0.2308	0.3822 bc	67.36	0.2540	0.3897 cd	51.49
BioTam	0.2206	0.3830 bc	66.14	0.2580	0.3969 bc	56.68
Control	0.2256	0.3690 d	66.22	0.2535	0.3935 cd	55.07

Table 2. Percentage of trees in each treatment classified as dying (red, NDRE less than 0.1), declining (orange, NDRE between 0.1 and 0.35) and healthy (green, NDRE greater than 0.35) at Site B.

	May 2024	October 2024		May 2024	October 2024	
Treatment	**%Red**	**%Orange**	**%Green**	**%Red**	**%Orange**	**%Green**
Quadris Top	0.9	99.1	0	0	19.7	80.3
Headline	0.9	99.1	0	0	26.3	72.8
Priaxor	1.7	98.3	0	0	26.4	73.6
Pristine	0.4	99.6	0	0	27.2	72.8
Tilt	1.3	98.7	0	0	22.8	77.2
Cevya	1.3	98.7	0	0	33.3	65.8
Serifel	0.9	99.1	0	0	22.5	77.5
Serenade	0.9	99.1	0	0	24.6	74.6
BioTam	1.1	100.0	0	0	24.6	75.4
Control	0.9	98.9	0	0	30.7	69.3

Table 3. Percentage of trees in each treatment classified as dying (red, NDRE less than 0.1), declining (orange, NDRE between 0.1 and 0.35) and healthy (green, NDRE greater than 0.35) at Site C.

	May 2024	October 2024		May 2024	October 2024	
Treatment	**% Red**	**%Orange**	**%Green**	**%Red**	**%Orange**	**%Green**
Quadris Top	0	97.8	2.2	0	9.8	89.0
Headline	0	97.8	2.2	0	19.3	79.9
Priaxor	0	97.8	2.2	0	9.1	90.2
Pristine	0	98.7	1.3	0	12.5	87.1
Tilt	0	98.7	1.3	0	14.8	84.5
Cevya	0	96.9	3.1	0	15.9	83.0
Serifel	0	99.1	0.9	0	8.0	91.7
Serenade	0	99.1	0.9	0	15.9	83.3
BioTam	0	99.6	0.4	0	12.9	85.2
Control	0	98.7	1.3	0	14.8	84.7

and was assigned a "tree health" color of red, orange or green, corresponding to the NDRE. We classified trees as dying (red, NDRE less than 0.1), declining (orange, NDRE between 0.1 and 0.35) and healthy (green, NDRE greater than 0.35) (**Figure 3**).

Ground truthing is necessary to confirm the results gained from the sensors. We went to each of the three orchard sites after both the May and the October flights. We pre-selected and viewed about 12 trees from each of the three 'tree health' categories and determined if the NDRE and GLI categorization (GLI only for the second flight) were correct based only on the severity of the wood rots. We wanted to determine if there was some reason for tree decline other than BWR, such as a faulty irrigation system, herbicide damage or non-diseased broken limbs that would lead to a low NDRE and GLI value. Based on our inspection, we determined that the NDRE and GLI values were indicative of BWR rather than other causes. Drone imagery is a great tool to optimize time in the field to make one's visits to the site more efficient; but at this point, it must be paired with ground truthing so that one can be confident in the results.

We evaluated the data from the drone flights in May 2024 (prior to experiment initiation) and October 2024 at Site B and Site C. We did not fly to Site A because the owners decided to stop farming the experimental block. Instead, we moved the experiment to an adjacent block and flagged it in October. GLI and NDRE for each treatment per site are summarized in **Table 1**. There were significant differences in NDRE among treatments. Tilt, Priaxor and Quadris Top had significantly higher NDRE indices than the untreated control at both sites. The percentage of healthy trees was much higher after treatment than prior to treatment as shown in **Tables 2** and **3**.

So far, we are encouraged with the results, but the 2024-25 season treatments are not yet completed, and we expect to continue during 2025-26. Evidence of successful control will provide California citrus growers with fungicide tools to control these diseases and improve tree health and returns.

CRB Research Project #5400-176

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Glossary

[1]**Multispectral:** Operating in or involving several regions of the electromagnetic spectrum.

[2]**Panchromatic sharpening:** The combination of a high-resolution panchromatic (sensitive to light of all colors in the visible spectrum) image with a lower resolution multispectral image to create a single high-resolution color image. This increases image quality and spatial resolution.

[3]**NDVI - Normalized Difference Vegetation Index:** A value that quantifies vegetation, ranging from -1 to +1, that is calculated using a combination of visible red light and near-infrared (NIR) light detected with remote sensors.

[4]**NDRE - Normalized Difference Red Edge:** A value that quantifies vegetation, ranging from -1 to +1, that is calculated using a combination of visible red light and red edge light detected with remote sensors.

[5]**GLI:** A value that quantifies vegetation, ranging from -1 to +1 that is calculated using visible (red, green and blue) light detected with remote sensors.

[6]**Pixel:** The smallest unit in a digital display.

[7]**Geolocated:** Identifying the location of an object (in this case an image) with latitudinal and longitudinal coordinates.

Common harborage sites for Listeria are where there is standing water and sheltered crevasses.

PROACTIVE LISTERIA CONTROL IN CITRUS PACKINGHOUSES

Still Relevant Lessons from Seminal Packing Facility Study

James R. Cranney, Jr. and Trevor Suslow

As food safety standards tighten, customer expectations are also rising, and California's citrus industry finds itself at a critical crossroads. While fresh citrus fruits have not been linked to Listeria recalls or outbreaks, recent high-profile incidents in other temperate and sub-tropical tree fruits have served as a wake-up call. A two-year research project in 2017-18 led by Trevor Suslow, Ph.D., formerly of the University of California, Davis, still holds significant relevance in providing a comprehensive look at the real-world risks citrus packers face and the solutions available to mitigate those risks.

Funded by the Center for Produce Safety and cooperatively supported by several citrus industry partners, this study offers the first thorough assessment of *Listeria* *monocytogenes* and its nonpathogenic environmental indicator cousins (*Listeria* spp.) in California's fresh citrus packinghouses. The message is clear: the risk potential is real; tools exist to mitigate the risk and proactive action is the key to protecting both public health and brand integrity.

Listeria in Citrus: the Silent Threat

Listeria monocytogenes is a dangerous foodborne pathogen responsible for fewer total illnesses but a much greater number of serious consequences – around 255 deaths and 1,455 hospitalizations annually in the U.S., as compared to other key pathogens, such as Salmonella. While not currently linked to citrus-related illness, it thrives in cold, damp environments; but it also can persist in dry and warm conditions from season to season in the kind of environment found in produce packing facilities and produce handling equipment.

Suslow's team swabbed more than 1,200 sites across seven California citrus packinghouses to identify where Listeria hides, how it spreads and whether current and emerging cleaning and sanitizing practices in use were reliable mitigations.

The findings were enlightening. Roughly 31 percent of all samples tested positive for *Listeria* spp. While fewer food contact surfaces were tested, they still showed relatively low contamination rates of just 5.2 percent positive, and none were positive for *L. monocytogenes*. However, other areas like floors, drains and bin stations were common safe harbors for *Listeria* spp. and *L. monocytogenes establishment*. Intensive cultural re-working of indicator *Listeria* spp. rapid-test-positives demonstrated that, in a substantial number of cases, *L. monocytogenes* was present in the background, as well, though initially negative by molecular testing, and culturable. The point is not to rely on expensive and lengthy confirmation tests for *L. monocytogenes* as a routine but to react to *Listeria* spp. detection since the pathogenic species is likely to be lurking there, too.

Listeria is often found in standing water, especially around drains where it can be transported to other locations in a packinghouse by forklifts or foot traffic.

High-Risk Zones: Where Listeria Lives

The study identified clear "hot spots" for Listeria, most notably in and around bin handling areas:

- ***Bin drying areas:*** 81 percent positive
- ***Fruit dumping stations:*** 58 percent positive
- ***Bin washers:*** 51 percent positive

Other problematic zones included UV inspection rooms, cold storage areas and final pack-out zones. These areas often are associated with high traffic, frequent water use and persistent organic debris, which all are conditions that promote Listeria survival and biofilm formation.

The study also found that *Listeria* prevalence increased during wet weather, particularly in zones with poor drainage or standing water. This suggests that moisture management and facility design are just as important as chemical disinfection when it comes to controlling *Listeria* and its spread.

The Best Option for Sanitizing

While cleaning and sanitizing did reduce Listeria rates, not all disinfectants performed equally under real-world conditions.

RelyOn™ emerged as the most effective sanitizer, achieving complete elimination of *Listeria* in many trials — even on well-established biofilms. By contrast, Peroxyacetic Acid (PAA) and Safe Zone™ showed mixed results. In some facilities, these sanitizers were less effective, with Listeria persisting even after treatment. Chlorine-based products had limited impact, particularly against older biofilms and on porous surfaces.

This variability underscores the need for packinghouses to verify sanitizer efficacy in their specific environments, considering not just the product, but also contact time, application method, surface material and getting ahead of biofilm formation. Biofilm formation is recognized as a barrier to effective sanitation, so investing in a dedicated deep-cleaning phase to remove it pays off in the effectiveness of routine cleaning and sanitation.

Phased and Prioritized Infrastructure Improvements Mitigate Risk

Outdated infrastructure was a recurring theme throughout the study. Legacy facilities and equipment often had design flaws that made thorough cleaning nearly impossible. Exposed bolt connections, areas around threaded levelers, hollow equipment parts, poorly sloped or cracked and exposed aggregate in flooring, water-saturated insulation and water pooling zones were all difficult areas to thoroughly clean. Another common harborage site is the "temporary" fix site that is never permanently fixed. These are often "quick fixes" that keep processes working in a daily operation, which are never permanently addressed. These sites often are active harborage points for Listeria.

To address this, the study emphasized the importance of sanitary design principles, including:

- seamless, sloped flooring to prevent standing water;
- hermetically sealed hollow parts;
- easy-to-access surfaces, free from overlaps, for scrubbing and sanitizing;
- facilitated disassembly design for periodic deep cleaning; and
- clear separation of raw and finished product zones.

Facilities built before these principles were common may need retrofitting or workflow changes to reduce cross-contamination risk. However, progress can be made in reducing risk by identifying changes to be implemented in the short, medium and long term and following a phased approach for improvements.

Building a Culture of Clean

Even the best chemicals and equipment are useless without trained, motivated personnel. The study found that inconsistencies in sanitation practices, under-trained crews and lack of accountability were major contributors to Listeria persistence over time in the same locations. In an extension of this project, genetic sub-typing of numerous *L. monocytogenes* isolates from different geographically diverse packinghouse facilities showed three important findings.

- Indistinguishable or highly related dominant *L. monocytogenes* types were found within and across two seasons in the same facility. This shows that there is a repetitive source or resident population that needs to be controlled.
- Indistinguishable or highly related dominant *L. monocytogenes* types were found in geographically separated facilities. This means that Listeria is being introduced among the packinghouses in the study by common sources such as bins, trucks or personnel, so measures should be taken to eliminate introduction from these sources.
- Of the subset of 80 isolates submitted for Whole Genome Sequencing (WGS), none matched clinical isolates in the public database. This means that, so far, there are no known illness from isolates found in packinghouses associated with this study.

Suslow's team emphasized the need for:

- Sanitation Standard Operating Procedures,
- Environmental Monitoring Programs (EMPs) to verify SSOP effectiveness and identify trends,
- regular staff training and performance-based retraining and
- visual inspections by someone *other* than the sanitation supervisor.

One key insight? What "looks clean" isn't always "microbiologically clean." Adenosine triphosphate (ATP) testing and Listeria-specific swabbing should be part of every facility's verification toolkit.

The Industry Path Forward

California's citrus industry now has a robust, science-based baseline for Listeria risk and control. Here are the key takeaways for citrus packers and processors.

- ***Know your zones.*** Focus cleaning and monitoring efforts on bin areas, cold rooms and all sorting and packing lines, especially where process water is used or collects.
- ***Invest in sanitation infrastructure.*** Good design pays dividends in both food safety and long-term efficiency.
- ***Don't trust your eyes.*** Use swabs and indicator tests to validate your cleaning procedures.
- ***Stay ahead of the storm.*** Expect higher Listeria levels during rainy seasons and adjust your environmental monitoring accordingly.
- ***Choose sanitizers carefully.*** RelyOn showed the strongest results in this study, but other combinations of cleaners and sanitizers can be effective. Verification by in-house trials is essential.
- ***Most importantly, recognize that food safety is not a one-time fix.*** It's a continuous process of monitoring, improvement, keeping up with new science and technology developments and teamwork across departments.

Conclusion

The good news? *Listeria monocytogenes* has not yet caused a primary fresh citrus-supplier related recall or outbreak. The better news? With the findings of this study, the industry now has the tools to keep it that way. By focusing on facility design, strategic sanitation and science-based monitoring, citrus packinghouses not only can meet evolving regulatory standards but also strengthen consumer trust in the safety and quality of their products.

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Sweet Orange Scab Update

James E. Adaskaveg and Helga Förster

Lack of scientific confirmation remains a core issue surrounding Sweet Orange Scab (SOS) detections in California, which negatively impacts growers who are subject to SOS quarantines. An SOS diagnosis currently is based on molecular protocols designed by the United States Department of Agriculture-Animal and Plant Health Inspection Service (USDA-APHIS) and followed by the California Department of Food and Agriculture (CDFA). We continue to work collaboratively with the Citrus Research Board and the California Citrus Quality Council in addressing regulatory concerns with federal and state agencies in the ongoing Sweet Orange Scab (SOS) detections. Our lab received samples from the CDFA that were identified by the CDFA as positive for SOS. However, we could not confirm 40 of 42 fruit samples sent to us by the CDFA as positives using the official protocol, and the other two samples were not positively identified using other independent methods (e.g., culturing, observation of acervuli, other genes, etc.). The following facts were presented to federal and state regulators:

- fruit exhibited atypical symptoms that resembled wind scar,
- *Elsinoë australis* was not successfully cultured from all 156 fruit from three orchards identified by the CDFA as positive and from 42 fruit samples sent to us by the CDFA,
- no spores and/or other structures of *E. australis* were found on all fruit samples received and
- there is a demonstrable lack of specificity of DNA primers developed to detect *E. australis*, but these primers are still used by APHIS and CDFA.

Regulators counter that the internal transcribed spacer (ITS) region of *E. australis* DNA is unique, in high copy number and, when amplified and sequenced, matches the type specimen[1] of the fungal pathogen. The goal of this SOS update is to demonstrate the variability of ITS sequences in the genus *Elsinoë* and to suggest that a more rigorous diagnostic protocol is needed to avoid misidentification of other fungal species as *E. australis*.

SOS and Sequencing of DNA

The ITS-derived Eaut-5 primer pair was developed by Hyun et al. (2007) and is being used in the APHIS protocol for determining the presence of *E. australis*. Our amplifications using this primer pair produced a DNA fragment (amplicon[2]) of expected length in only two of the 42 fruit samples provided by the CDFA, which we subsequently sequenced. In our analysis, the highest matches of these two CDFA samples were with *E. australis* and *E. punicae*. The DNA sequence of our reference culture of *E. australis* aligned 100 percent with the sequence of *E. australis* from Kunta et al. (2014) deposited in GenBank. The alignment of sequences in **Figure 1** shows that the two CDFA samples are 100 percent identical with *E. punicae* (one of the two sequences was submitted by the research team of a worldwide-respected fungal taxonomist), and there is a single mismatch with an *E. australis* culture we obtained from Florida (highlighted in green). Therefore, amplicons of the two CDFA samples cannot be assigned to *E. australis*. Only a single base pair in the ITS region differentiates this *E. australis* sequence from *E. punicae*. For comparison, three additional sequences of *E. australis* (EausCBS314.32, which is the type specimen of the species, EausCBS229.64 and EausCBS230.64) from the publication by Fan et al. (2017) also were included (**Figure 1**). These three sequences were 100 percent identical to the one of *E. punicae*, but not to our *E. australis* reference. This indicates that closely related species of *Elsinoë* cannot be separated based on their ITS sequences alone, and additional genetic loci need to be characterized. Three sequences of *E. australis* from Kunta et al. (2013) also were included. Interestingly, these were not identical – one of them matched *E. punicae*, whereas the other two matched the *E. australis* type specimen.

The genus *Elsinoë* is poorly studied with more than 75 species described. The ITS region is very similar in multiple species including the type specimen of *E. australis* (CBS314.32) and *E. punicae*. In a recent publication by Elliott et al. (2023), the ITS region of *E. australis* was sequenced using primers ElsB_fw, *Elsinoe* pr and Els B_rv, and sequences were identical to *E. genipae-americanae, E. violae, E. anacardii* and *E. semecarpi*. Ahmed et al. (2019, 2020) used five loci (ITS, translation elongation factor 1-alpha, glyceraldehyde-3-phosphate dehydrogenase, actin and 28S rRNA) and performed multiple sequence alignments. The genes were amplified using quadruplex qPCR and conventional PCR methods. The results indicated that *E. fawcettii, E. australis, Phyllosticta citricarpa* and *P. angolensis* could be distinguished from other species such as *E. veneta, E. centrolobi, E. eucalypticola* and other pathogens of citrus. Unfortunately, they did not include *E. genipae-americanae*, *E. violae*, *E. anacardii* and *E. semecarpi* as Elliott et al. (2023) did or *E. punicae* as Fan et al. (2017) did. Thus, multiple closely related *Elsinoë* species that overlap based on ITS analysis were not included in either study. It is important to note that these primers also can amplify other fungal species with a similar product size. A *Meira* sp., a yeast that we found on citrus fruit, also produced bands of similar size as for *E. australis* using primer pairs Eaut-1 and Eaut-3 although additional bands were also present.

Considering that currently more than 75 species of *Elsinoë* have been described based on sequence analyses of four loci, some of which are very closely related, additional evidence would be needed to assign the affected fruit submitted by the CDFA as SOS. Additionally, the four other *E. australis*-specific primer pairs that were designed by Hyun et al. (2007)

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EpunicaeDAR77387              aacgtcaggagcaccggtgttaacctctgccgtgaa------------------------
EpunicaeCPC19968Crous         aacgtcaggagcaccggtgttaacctctgccgtgaa------------------------
EaustCBS229.64                aacgtcaggagcaccggtgttaacctctgccgtgaa------------------------
EausCBS230.64                 aacgtcaggagcaccggtgttaacctctgccgtgaa------------------------
EausCBS314.32 type specimen   aacgtcaggagcaccggtgttaacctctgccgtgaa------------------------
EaustralisFL                  aacgtcaggagcaccggtgttaacctccgccgtgaaacctttcaaactatttttctaagg
EausKuntaS3829C               aacgtcaggagcaccggtgttaacctccgccgtgaaacctttcaaactatttttctaagg
KuntaKC211046.1               aacgtcaggagcaccggtgttaacctccgccgtgaaacctttcaaactatttttctaagg
CDFA 1                        aacgtcaggagcaccggtgttaacctctgccgtgaaacctttcaaactatttttctaagg
CDFA 2                        aacgtcaggagcaccggtgttaacctctgccgtgaaacctttcaaactatttttctaagg
KuntaKC211044                 aacgtcaggagcaccggtgttaacctctgccgtgaaacctttcaaactatttttctaagg
                              *************************** ********
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Figure 1. Sequence alignment of Eaut-5 amplicons of our *Elsinoë australis* reference (sixth line from top), CDFA samples and of ITS regions deposited in GenBank of *E. punicae* (including one by Fan et al. 2017), three *E. australis* CBS accessions including the type specimen and three *E. australis* references of Kunta et al. A single mismatch is highlighted in green.

did not amplify samples from CDFA. Based on our findings and the ambiguity of using ITS-derived primers, as well as our inability to culture the pathogen, it is unlikely that *E. australis* is the cause of the atypical symptoms on the fruit from California orchards. Kunta et al. (2013) found citrus fruit in the western region of Texas with atypical wind scar-like symptoms that amplified with the Eaut-5 primers, and cultures of *E. australis* were obtained that identified these symptoms as SOS. APHIS assumes that this also must be the case in California. Unfortunately, we could not confirm this and, therefore, additional evidence is needed to determine if SOS is present in California.

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Glossary

[1]**Type specimen:** The specimen from which the description and name of a new species is based.

[2]**Amplicon:** DNA fragment that results from amplification, usually through methods like polymerase chain reaction (PCR). It is essentially a replicated piece of a specific DNA sequence, and the term is often used interchangeably with 'PCR product.'

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