WINTER 2014

CITRUS RESEARCH BOARD

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THE OFFICIAL PUBLICATION OF THE CITRUS RESEARCH BOARD

NOTE TO OUR READERS

BY KEN KECK, PRESIDENT CITRUS RESEARCH BOARD

CITRUS RESEARCH BOARD

Welcome to the new *Citrograph*. With this issue, we are taking a fresh approach to the magazine's design. You'll find a contemporary look with more visuals, a new typeface and greater ease of reading. These changes are brought to us through our new graphics, production and advertising sales team, Carolina Evangelo and Eric Cribbs.

We've also made some editorial staff changes. I will serve as executive editor. Ivy Leventhal, a citrus industry veteran, has been named our managing editor. CRB Vice President Dr. MaryLou Polek continues her able job as our chief science editor, and Chad Collin will add to his duties by serving as our associate editor.

With all these changes, I think you will find what has not changed is *Citrograph's* generous number of thorough research reports, giving our readers the latest in progress on the projects funded by California growers.

We hope you enjoy our new look as we continue to evolve to best serve your needs, and we look forward to your feedback.

With appreciation for being a dedicated reader, and for your support of the CRB,

Ken Keck

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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CALENDAR OF EVENTS 2014

March 6
CCM-CRB Citrus Showcase, Visalia Convention Center, Visalia, CA.
For more information, contact California Citrus Mutual at (559) 592-3790.

March 12
CPCPC Board Meeting, Riverside, CA.
For more information, contact CDFA at (916) 403-6652

March 24-26
CRB Research - Review of Projects and CRB Board Meeting, Doubletree Hotel, Ontario, CA.
For information, contact the CRB at (559) 738-0246.

April 3
CRB-UCCE Citrus Postharvest Seminar, Exeter Veterans Memorial Building, Exeter, CA.
For information, contact the CRB at (559) 738-0246.

May 14
CPCPC Board Meeting, Ventura, CA.
For more information, contact CDFA at (916) 403-6652

June 26
CRB Board Meeting, Four Points by Sheraton Ventura Harbor, Ventura, CA.
For information, contact the CRB at (559) 738-0246.

TRIVIA QUESTION

Question:
The most widely-used citrus fruit worldwide is the…
A. Grapefruit B. Lemon C. Lime
D. Orange E. Tangerine

See page 9 for answers.

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COOPERATION AND COLLABORATION

I hope to make "cooperation" and "collaboration" the buzzwords of California's citrus research in 2014 and beyond.

For just more than half a year, I have had the pleasure to lead the Citrus Research Board (CRB), and the evidence is all around that growers and the Board are interested in creating an atmosphere around our pest and disease challenges that inspires everyone to pull together.

The following are recent examples evidencing this trend, along with areas of collaboration to watch for during the course of the new year.

COORDINATION OF CRB AND CCM ON RESEARCH OF FULLER ROSE BEETLE (FRB) CONTROL METHODS

Acutely aware that the grower costs of dealing with FRB for the Korean market could get expensive, California Citrus Mutual (CCM) actively sought entomopathogenic nematode researchers within the University of California (UC) system who might provide a biological control solution. A similar approach is used by pistachio growers in the control of an economically significant pest to their crop, where the nematode actually infects and kills the pest. CCM engaged with members of CRB's board and staff to ensure that the project was properly presented by the researchers for funding consideration in a timely fashion. The project indeed was funded, and the UC researchers are at work. Most importantly, our Korean partners are able to see that the California industry is actively engaged and responsive to their concerns.

CRB AND PRIVATE SECTOR COLLABORATION TO SUPPLY FEDERAL/STATE BIOCONTROL EFFORTS WITH METHODS DEVELOPMENT IN THE REARING AND DEPLOYMENT OF PARASITIC WASPS AGAINST THE ASIAN CITRUS PSYLLID (ACP)

At the moment, there are not enough *Tamarixia radiata* wasps being produced to support the level of field trials needed in both the urban interface as well as other commercial groves. Many questions need answers surrounding the potential role of Integrated Pest Management, specifically biological control, in controlling ACP. As part of a cooperative agreement with the United States Department of Agriculture Animal and Plant Health Inspection Service, Plant Protection and Quarantine, Center for Plant Health Science and Technology (USDA-APHIS-PPQ-CPHST), CRB has solicited and contracted with private sector insectaries to get in the game and help in the production of the parasitic wasps. This three-way collaboration is already working to produce wasps, and the number of private insectaries involved is only expected to grow in 2014.

COORDINATION WITH OTHER STATES (FLORIDA AND TEXAS) ON HLB/ACP PROJECTS, WHICH IS GAINING WITH EVERY FUNDING CYCLE

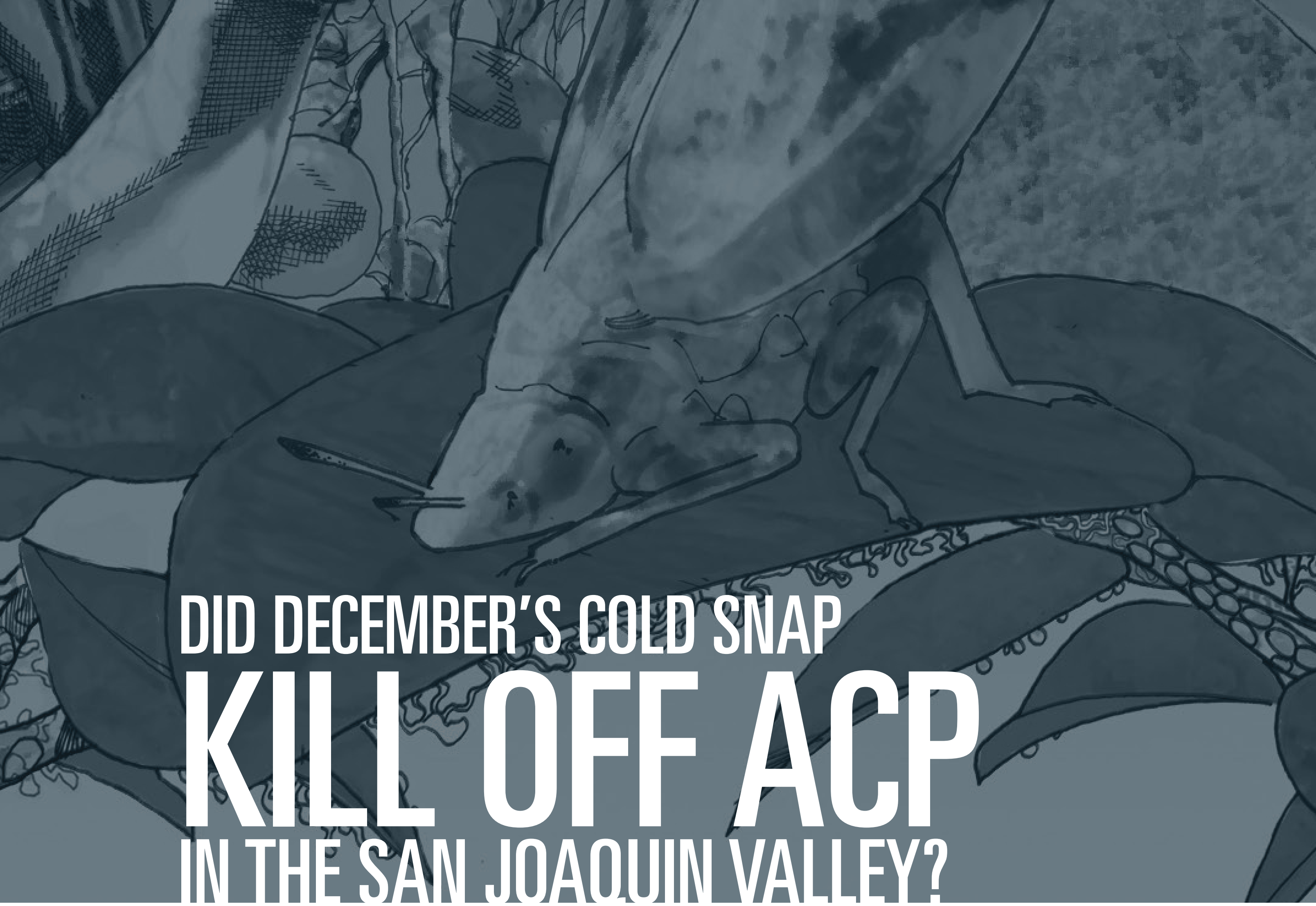
Everyone recognizes the barriers to coordination: the different states are experiencing ACP and HLB at different stages. Why should one state's growers invest in projects that are more relevant to the other state's growers? Not content to get stuck on these impediments, however, the CRB board and staff have been hard at work with other states' research leaders to identify ACP/HLB projects (currently almost $500,000 worth) that have common denominators to all citrus-producing states. The molecular levels of the vector and the disease don't recognize borders. By combining resources, we will continue to create a new collaborative math where 1+1=3.

In closing, the deep level of California citrus grower involvement with their operations shows that they are personally invested in the long haul, and that they have a tremendous will to persevere. The CRB will hold up its end of the bargain by continuing to communicate with and engage the best scientists in the world, asking that they collaborate across disciplines and borders to come up with the near- and long-term solutions that will keep California growers competitive. I am pleased to be leading the CRB forward in fostering this increasingly cooperative approach.

Ken Keck is president of the Citrus Research Board.

TRIVIA ANSWER

B. The lemon is enjoyed across the globe more than any other citrus variety *(Source – www.nutrition-and-you.com). Just a few of the lemon's many uses include household cleaning, beauty treatments, decorating, flavoring, breath freshening, odor destruction and food preservation.*



DID DECEMBER'S COLD SNAP KILL OFF ACP IN THE SAN JOAQUIN VALLEY?

Mark S. Hoddle, Ph.D.

The recent bout of cold weather in the San Joaquin Valley (SJV) from December 4-11, 2013, when low overnight temperatures averaged 26ºF (range 24-30ºF), daytime highs averaged 50ºF (range 48-55ºF) and the daily average temperature was 38ºF (range 36-42ºF) has raised a lot of optimism in the citrus community that this cold snap has killed off Asian citrus psyllid (ACP). At the very least, growers hoped that it put a serious brake on ACP reproduction and spread in areas around Tulare and Dinuba ***(Figure 1)***.

However, it is very unlikely that ACP adults, nymphs or eggs have been "eradicated" by the cold weather. Death by cold for an insect depends not only on the lowest temperature experienced, but also on the duration of exposure to cold. For example, a one-hour exposure to 28ºF is not going to be as severe as being subjected to this temperature for six hours. The longer the exposure to cold temperatures, the more likely the insect is to die, but only **IF** that temperature is at or below its survival threshold. At this time, we don't know what the low lethal temperature is for ACP or how long exposure is needed for to kill adults, nymphs or eggs.

A review of historical weather records for Lahore, Pakistan, which is in the Punjab and part of the native range of ACP, indicates that some of the lowest historical temperatures experienced in this region over November – February are 28-37ºF *(http://www.myweather2.com/City-Town/Pakistan/Lahore/climate-profile.aspx?month=2)*, a bit higher than what the SJV experienced. These data suggest that the ACP, at least in Pakistan, is likely able to tolerate cold temperatures that get close to freezing for short periods. In Japan, field studies for ACP caged on potted citrus indicated that adults began to die over an 11-day period when daily minimum temperatures were constantly 32ºF or lower and punctuated with a single period of more severe cold, 25ºF. Adults needed to be exposed to 21ºF for six hours before 50 percent mortality was observed (Ashihara 2007).

There are at least two other issues to consider with respect to cold temperatures and ACP survival in citrus orchards and the surrounding environment where ACP may be located. If growers are running sprinklers, wind machines or orchard heaters to prevent frost damage to trees, it is possible that these management strategies are also favorable for the survival of ACP as they lessen cold severity, not only for the trees, but for ACP, as well. In urban areas, there is a well-understood phenomenon, "urban heat islands," where temperatures are appreciably warmer because of the day-time heat-absorbing and night-time heat-radiating effects of buildings, sidewalks, walls and roads. These elevated temperatures in urban areas may help ACP survive cold temperatures on backyard citrus.

The cold weather likely did put the brakes on ACP development and reproduction.

Laboratory studies indicate that ACP eggs need temperatures above 48ºF to hatch, and nymphs require temperatures above 50ºF to develop (Liu and Tsai 2000). When temperatures drop below 48-50ºF, development is arrested until this threshold is crossed in a positive direction, that is temperatures increase, and then growth starts again. The 48-50ºF threshold for development is not sufficiently cold enough to kill immature stages of ACP.

Although we don't know precisely how much cold is needed to kill ACP, it is very likely that the cold winter weather in the SJV has not eradicated this pest, but also likely that it has considerably slowed down its development. However, laboratory studies suggest that cold stressed citrus (i.e., plants exposed to 43ºF for six days) may be more attractive to ACP as plant defense chemicals decline and release of attractive volatile chemicals increases (Malik et al., 2012).

Mark S. Hoddle, Ph.D., is in the Department of Entomology at the University of California Riverside.

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Ashihara, W. 2007. Cold hardiness of Asian citrus psyllid, *Diaphorina citri* (Homoptera: Psyllidae). Japanese Journal of Applied Entomology and Zoology 51: 281-287.

Liu, Y.H., and J.H. Tsai. 2000. Effects of temperature on biology and life table parameters of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Homoptera: Psyllidae). Annals of Applied Biology 137: 201-206.

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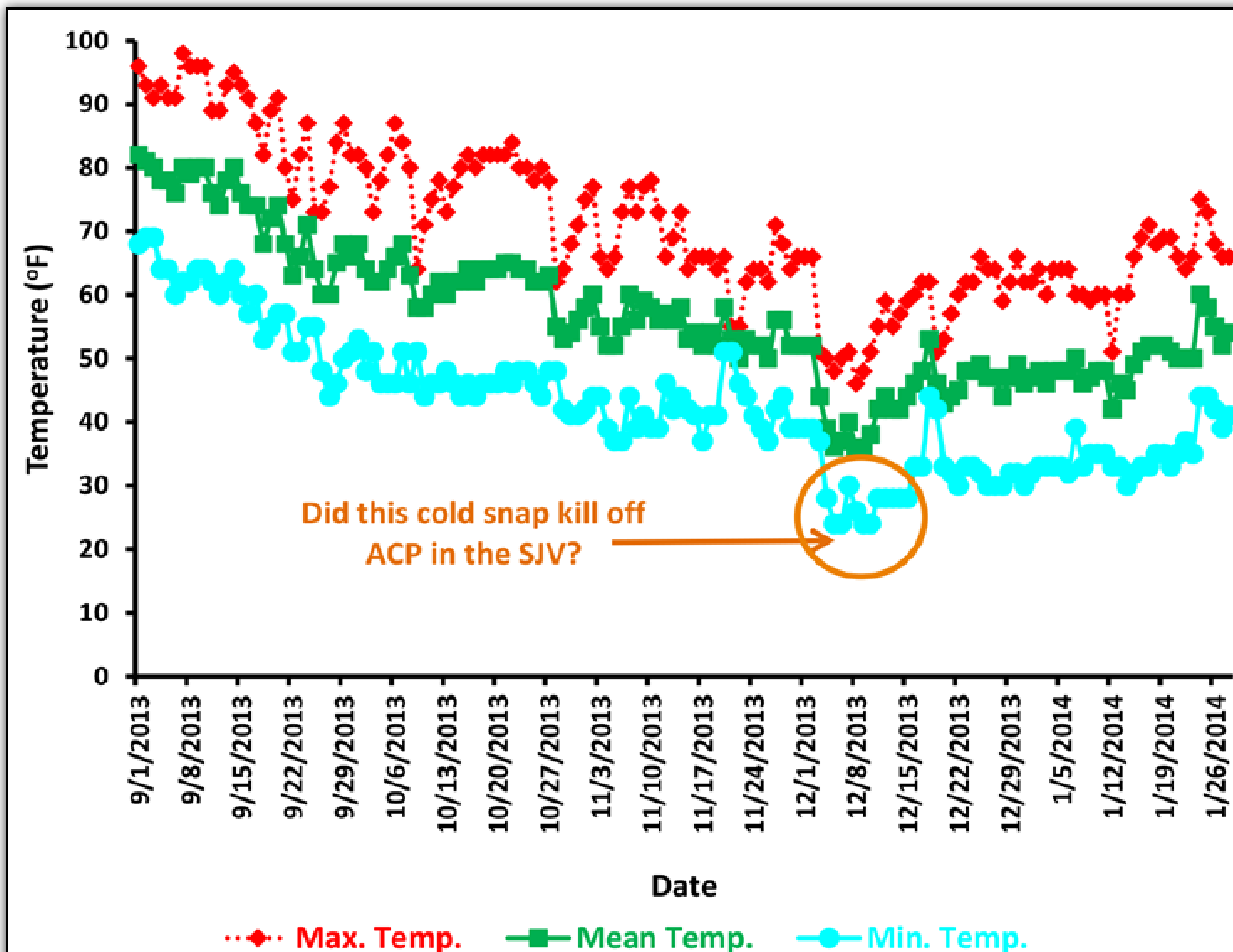

Figure 1. Weather data were sourced from Weather Underground for the Visalia weather station KVIS (http://www.wunderground.com/weather-forecast/US/CA/Tulare.html), and the custom option was used to select data from September 1, 2013, to January 28, 2014. Maximum (Max.), average (Mean), and minimum (Min.) daily temperatures are displayed in the graph.


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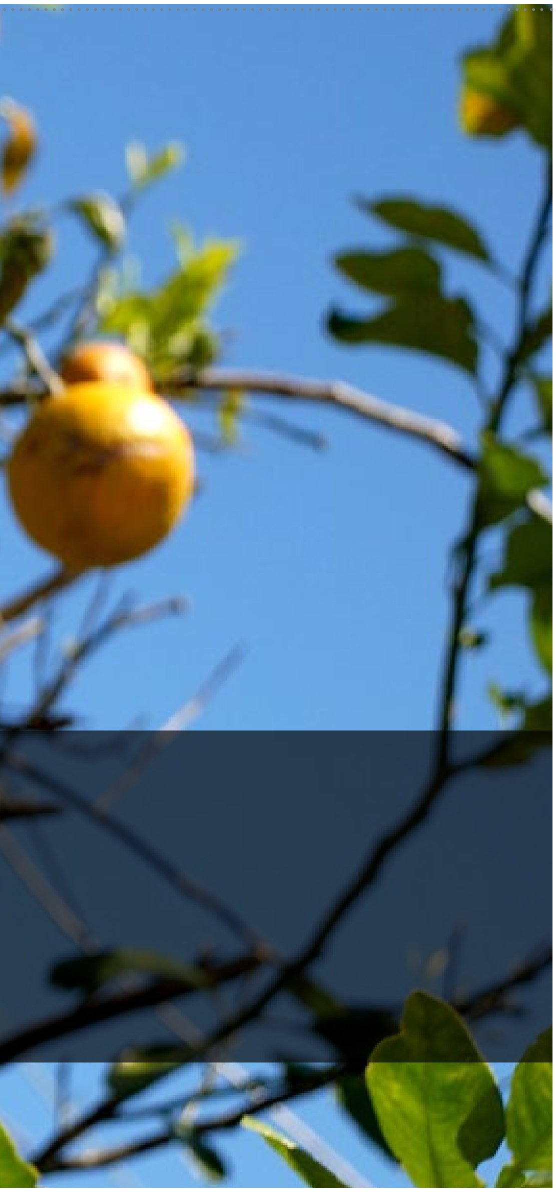
CHAIRMAN'S VIEW *BY ETIENNE RABE, PH.D.*

THE FREEZE OF 2013: THE INDUSTRY WILL ADJUST WITHOUT FORGETTING THE CONSUMER

The effects of the early December cold are now becoming clearer (See "True unfair weather friends", p. 20). It is probably safe to say that it is going to be the biggest event since 1998. Damage varies quite widely between regions and growers, ranches with and without either water and/or wind machines, etc. The financial losses inflicted by the freeze are not just to our growers, but the wider ripple effects on other businesses and operations will doubtless be felt.

For many growers, despite the opportunity they take to purchase insurance against a weather event (usually to recoup cultural costs associated in growing that season's crop), a delay in redevelopment results due to a lack of financial resources, which, in turn, reverberates through many associated industries: nurseries, irrigation companies, etc. By contrast, for some, it may actually hasten the removal of marginally profitable varieties or blocks, e.g., nucellar navels or Valencias. Years like this also serve to "warn" growers to stick to the traditional citrus growing areas of the Valley, where new entrants from stone fruit areas may have started to plant mandarins in other than the traditional citrus growing areas, and damage may have been more severe.



The effects are felt also by the programs funded by the Citrus Research Board (CRB), the California Citrus Pest and Disease Prevention Committee (CPDPC or the HLB committee, as it may be better known) and others who operate on statutory grower levies. The CRB specifically has a certain level of reserves to at least tide it over during events like these. [Note: while we expect the budgeted income to the CRB to be lower due to the freeze event, these reserves will supplement the losses so that most CRB programs and research activities will, hopefully, be able to continue with minimal cuts]. Research entities and researchers have been put on notice regarding the potential for cuts, and they are not unaccustomed to operating with that in mind. As bad as this season's freeze may be, the industry is fortunate that the CRB is in a better financial situation than when the previous freeze event impacted budget levels in 2007.

Related to the issues raised by a damaging freeze, readers may recall that in the previous edition of *Citrograph*, I outlined that "respecting the consumer" through the quality of the product we supply is one of the principles I believe the citrus industry should live by. This is no truer than after freeze events when the industry has to play by certain enforced rules regarding the various levels of frost damage that may be legally shipped. If consumers remain happy with our product, primarily through the restraint we as an industry show, our growers' bottom line far into the future will reflect their satisfaction.

An indication of this self-imposed emphasis on quality is the California Citrus Administrative Committee (CCAC). It was formed for maturity quality control via the counties but very importantly now, due to the freeze, has directed funds for the counties to inspect at a very intensive packinghouse level. This grower-funded effort will better ensure only quality product gets shipped. Again, a healthy reserve has been built up by that committee to address an event like this. However, the CCAC and the counties can only do so much in this regard. It is largely up to the packers/shippers to ensure compliance. Fortunately today, with a number of large brands operating in the industry, each entity more than likely has a vested interest to ensure that its boxed product going to consumers will be beyond reproach.

To ensure we continue to best fulfill our research mandate, your CRB directors went off-site in November for a strategic planning session. The decisions taken at this meeting and the follow-up to be voted on once the outlines have been fleshed out may result in a few changes relative to the Board's operation (see "CRB Unveils New Strategic Priorities" on p. 16). These include how projects are evaluated (in a world where scientific jargon and methodologies are becoming very complex and less easy for us as grower Board members to fathom), Board structure (size, representation, etc.) and committee make up, among other actions to be debated in the coming year.

It should be no surprise that the major emphasis remains on the HLB dangers facing the industry and pre-emptive actions that the California industry can take in this regard. The bulk of CRB funding is then also directed to these types of projects. Where the CRB acts as the entity to supply scientific back-up, the CPDPC is responsible for managing the day-to-day ACP/HLB program. We do not, however, lose sight of the fact that we need to get our fruit in the box to the consumer in sound and safe condition. Thus, CRB-funded projects aimed at post-harvest handling, addressing regulatory issues (ground water pollution concerns, air quality), and so forth also remain on the front burner. I hope you find the various articles in this issue around these critical priority projects timely and engaging.

Our new President, Ken Keck, has now been in the job for just over six months. With the freeze event, HLB, consumer quality challenges and recent strategic session, he is getting a baptism by fire. We are excited about the CRB's role under Ken, both in California and on a wider basis nationally where he represents us.

Until next time.

Etienne Rabe, Ph.D., is chairman of the Citrus Research Board.

MUTUALLY SPEAKING... *BY JOEL NELSEN*

$70 MILLION, AND WHAT DO YOU GET?

Give or take a buck or two, that's what I figure growers have assessed themselves in the past four years for Asian citrus psyllid and huanglongbing (ACP/HLB) related activity. The majority of the dollars were collected via the Citrus Pest and Disease Prevention Committee (CPDPC) with the balance collected by the Citrus Research Board (CRB) and used for varied ACP/HLB research projects. Certainly, it is a wheel barrel full of money, but as a $2 billion industry per annum, that's less than one percent of our industry value in the same time frame.

When Citrus Mutual first broached the idea of this effort, there were several building blocks for the foundation. *One*, we didn't want to go the way of Florida, Brazil and Mexico with ACP and HLB causing tree deaths and production reduction. *Two*, we wanted to find ACP and HLB before they found the commercial industry. *Three*, we questioned whether we could count on sufficient financial support from the state or federal government to address our needs. *Four*, we wanted a say in how the program was going to be run, and thus, we wanted a full partnership.

So, $70 million later, where are we? First, we have made some mistakes. We should never have followed the Florida quarantine model and established 20-mile quarantines. All that did was cede territory to the psyllid. Second, we have been overly restrictive at times, but with growing knowledge about treatment efficacy, we continue to modify. Admittedly modifications can be slow in coming while working with government, but changes that create flexibility without creating vulnerability have been made. We were right on target with our doubts about the state having sufficient revenues to help support the program. USDA's support, while very generous, simply would not have been enough.

The endemic population in Los Angeles continually threatens producers in Ventura and Riverside. Imperial County growers are pressured by hitchhikers from Mexico and Riverside County. The San Joaquin Valley has had increasing detections in the past year, primarily in Tulare County.

With increasing detections and a greater ACP population in urban areas, is the value of the program waning? Is it still worth the eight cents per carton that producers are spending, plus the portion of the CRB assessment? Back up to paragraph two: if those four reasons remain relevant, then the answer is an unequivocal yes.

There are frustrations with government, and there is a constant need to re-evaluate and modify the program, but the basic tenet and success is that ACP and HLB **HAVE NOT** found California's magnificent citrus industry to the point at which destruction of supply or economics for protection have exceeded a tipping point.

This investment is protecting our industry while scientists search for a cure. With the exception of Los Angeles and Riverside, the numbers of ACP are still being counted individually. The term "endemic" does not exist in citrus production areas of California as it does in Florida, Texas, Brazil and Mexico. Last June, growers did their own analysis as asked by CDFA in regards to continuing the program. Nary was a negative word received, and the Secretary never held a referendum. However, that analysis should not be offered as an affirmation of the status quo.

On the contrary, it was analysis that suggests the four building blocks listed above remain the foundation for the future of this industry as it relates to ACP and HLB. The Citrus Pest and Disease Prevention Committee (CPDPC) members are obligated to review and analyze the effort for improvement. They deserve industry support for their time and decisions. That's what item number four is all about.

Joel Nelsen is the president of California Citrus Mutual.

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CRB UNVEILS NEW STRATEGIC PRIORITIES

Staff Report

UPDATED CRB MISSION

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

UPDATED CRB VISION

Over the long-term, CRB helps growers be better growers by partnering with the best problem-solving scientists in the world. In the near-term, CRB will limit the impact of HLB and all markets will be accessible.

Over the past year, changes have been afoot at the Citrus Research Board (CRB). Ken Keck was named president; Etienne Rabe was elected chairman of the board; *Citrograph* became a quarterly magazine with a new editorial and production staff; a new logotype was formally adopted by the board (see magazine cover); and strategic priorities were hashed out and adopted by the CRB Board of Directors.

The priorities were put together during several meetings of the CRB Board, where discussions were held on revamping major processes such as board governance and the proposal evaluation process. The new direction was ratified on January 23, 2014.

CORE COMPETENCIES

The Board defined the CRB's core competencies as funding research to solve problems for citrus growers, providing research-related issue forums and public meetings, and communicating research results back to growers.

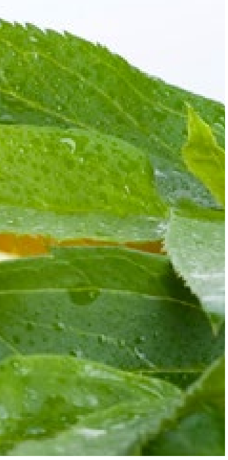
Additionally, the Board identified as yet undeveloped competencies critical to the success of growers: getting research results into the field (implementation) and evaluating research proposals using a more rigorous process.

"To this end," said Keck, "an ad-hoc project evaluation committee has been appointed by

Chairman Rabe. This committee will recommend an improved process of annual scientific proposal review to aid the full Board in its final decision-making and awarding of funds."

STRATEGIC PRIORITIES

One of the most important priorities is to create a *new research proposal award model*. Areas of emphasis include Huanglongbing (HLB), market access, pest management, consumer needs/wants and regulatory issues. Other aspects of the new research award model involve creating an improved decision-making process that prioritizes project proposals in a relative manner, better aligning the process with budgets, recruiting researchers and re-designing the RFP itself.



"Possible changes to be considered include the size of the Board, voting status of members, quorum requirements, geographic representation, members and officer nomination processes, absenteeism policies and electronic attendance at Board meetings."

Establishing and maintaining *communications* is another strategic priority that will serve to best ensure that growers and scientists stay engaged with the CRB. The Board will continue its commitment to publishing *Citrograph* quarterly, and to making research update presentations at grower forums throughout the state's three production areas, as well as indexing all past and current research projects and reports for easy web access.

Growers also will see efforts in early 2015 to create a *single California citrus industry trade show* that includes an expansive research and issues speaker program, with equal partnership between the CRB and California Citrus Mutual.

An important corollary of CRB communications is the need to establish a *deeper and wider network of the best citrus researchers* in the world. So with the goal of engaging these scientists to both submit proposals and to provide peer review of others' proposals, the CRB is not only improving its award process as described above, it will increasingly take actions to communicate directly with this key audience. The newly adopted logo, for example, graphically reflects the kind of cutting-edge molecular science needed to deal with the ACP/HLB disease complex.

Beyond conducting research, *implementation of the results* also is a priority. Possible strategies for implementation may include requiring an up-front analysis of how a particular project's results will make it from the research bench to field deployment.

After the first three strategies have been developed, the final priority, originally slated for exploration in 2015, will be to *seek additional capital*. As of this writing, given the federal response with recent appropriations and farm bill commitments, the seed capital required for quicker implementation of results may begin to arrive just in time.

STRUCTURE

The new plan confirmed the key responsibilities of the Board and the CEO. The Board's purposes are to set direction and establish desired end results, to establish and drive organization values and principles, to delegate execution and to monitor results. Fiduciary responsibility and oversight also are key responsibilities for the Board members, along with ensuring ethical integrity and maintaining accountability.

As President and CEO, Keck is charged with developing and executing the day-to-day steps to achieve the established end results, as well as a staffing plan that supports the achievement of those results. He is also expected to convey those results back to the Board and growers.

BOARD GOVERNANCE

A key discussion during the strategic planning meetings was of Board governance issues that would facilitate better accomplishment of the mission and vision, including exploring ways to reach those who might be interested in serving as Board members and seeking opportunities to use the talents of interested growers.

Keck explained that an additional ad-hoc committee on board governance has been named to address and put forth recommendations to improve the engagement of the entire Board in decision-making. "Possible changes to be considered include the size of the Board, voting status of members, quorum requirements, geographic representation, members and officer nomination processes, absenteeism policies and electronic attendance at Board meetings," he said. "Chairman Rabe is especially interested in recognizing that board members have limited time available to serve, and hopes that by promoting more efficient and fewer meetings, as well as utilizing the talents of all members, member engagement will increase, and better decisions will result."

USDA ESTABLISHES MULTI-AGENCY HLB TASK FORCE

A young HLB-infected citrus tree in Florida after dropping its fruit (dark decayed objects on ground).

Staff Report

A special multi-agency task force was announced December 12, 2013, by the U.S. Department of Agriculture (USDA) to undertake coordinated research efforts to combat huanglongbing (HLB) and Asian citrus psyllids (ACP).

Additional details have been emerging since the news was reported extensively throughout the Central Valley in January. The effort initially was launched with $1 million in funding to support research projects designed to investigate short-term approaches to battling the disease. On January 13, 2014, through its Fiscal Year 2014 Omnibus Spending Bill, the House Committee on Appropriations included an additional $20 million in funding at the request of Congressman David Valadao (CA-21). As of press time, it was unclear if these additional resources would be deployed by the newly created task force directly, or indirectly based on the task force's recommendations.

Participating organizations include USDA's Animal and Plant Health Inspection Service (APHIS), Agricultural Research Service (ARS), and National Institute of Food and Agriculture (NIFA), as well as State departments of agriculture and industry groups (see HLB MAC Group Members sidebar). Ken Keck, president of the Citrus Research Board (CRB) will represent California along with Victoria Hornbaker of the California Department of Food and Agriculture. APHIS will assume the lead and coordinate planning and resource management through its Citrus Health Response Program.

According to the USDA, "These partners are forming an HLB Multi-Agency Coordination (HLB MAC) Group to jointly collaborate on policy decisions, establish priorities, allocate critical resources, and collect, analyze and disseminate information. The HLB MAC Group will help to coordinate and prioritize federal research with industry's efforts to complement and fill research gaps, reduce unnecessary duplication, speed progress and more quickly provide practical tools for citrus growers to use."

The USDA's statement added, "The new framework will maintain and share a common operating picture of HLB response that is accessible across jurisdictions and functional agencies." The Department believes this will greatly improve strategy development, policymaking and resource allocation, ensuring that funds will be invested where they will have maximum impact that is based on the input of all participants.

"As currently understood, the HLB MAC Group will represent the federal government's coordinated one-stop shopping for the citrus-producing states battling ACP/HLB," said Keck. "In addition to a coordinated USDA response, I am eager for the engagement of other federal regulatory agencies, such as EPA, that need to understand that the continued production of citrus in the U.S. hangs in the balance."

Among the first items on the agenda will be collaboration by the participants to define the specific roles and responsibilities of the organizations to maximize their special resources, expertise and perspectives. The federal and state organizations have statutory and regulatory authority that has been crucial in slowing HLB's spread. Additionally, the USDA will launch a new HLB section on its web site.

"USDA listened to the citrus industry's request for more urgency and greater coordination on the response to HLB and is implementing an emergency response structure," said USDA Secretary Tom Vilsack. He added that since he took office in 2009, the federal agency has spent nearly $250 million to research and track the bacterial disease.

"The recent announcement by USDA Secretary Vilsack is welcome news in my opinion," said California Citrus Mutual President Joel Nelsen. "Heretofore, the industries in Florida, Texas and California have been obligated to decipher an alphabet soup of entities that could or would assist for HLB-related research." Nelsen believes that was less than satisfactory because of internal USDA issues, duplication of effort and changing personnel.

He added, "This new organizational structure and the appointment of Dr. Mary Palm to lead the effort gives industry a coordinated and simplified approach for research much like we have with field operations and the Citrus Health Response Plan (CHRP). This need was conveyed to the Department in August and again in October in a meeting with the Secretary.

"With CRB Chair Dr. Etienne Rabe as chair of the national science and technology committee within CHRP and Ken Keck now on the Executive Council along with myself and Sunkist's Mike Wootton, I believe research focus, implementation and, hopefully, results will be streamlined to a greater degree," Nelsen said.

Florida Citrus Mutual Executive Vice President and CEO Michael Sparks agreed. "Florida and California have coordinated and shared research efforts, and now we anticipate additional USDA contributions, which is good news for all citrus producing states," he said.

HLB-infected trees produce green, misshapen, bitter-tasting fruit. While not dangerous to humans or animals, the disease has ruined millions of acres of citrus crops worldwide. Florida and Georgia are entirely under HLB quarantine, as are portions of California, Louisiana, South Carolina, Texas, Puerto Rico and the U.S. Virgin Islands. Although only one confirmed instance of the citrus disease has been found to date in California in a Los Angeles County residential yard, ACP are well established in several citrus-producing counties in California.

By contrast, the majority of Florida's 69 million citrus trees already have been infected. Some estimates range as high as 75 percent of those trees, according to the *Tampa Bay Times*. Grove infection rate is approaching 100 percent. Primarily as a result of HLB, this season's Florida orange crop is on track to be the smallest in 24 years. "Florida is losing the fight against HLB, and all resources are welcomed to find solutions," said Sparks. Senator Bill Nelson of Florida sounded an even more urgent tone when he said, "If we don't find a cure soon, we won't be growing oranges in Florida much longer."

HLB MAC GROUP MEMBERS

Awinash P. Bhatkar	Texas Department of Agriculture
Harold W. Browning	Citrus Research and Development Foundation
G. John Caravetta	Arizona Department of Agriculture
Richard Gaskalla	Florida Department of Agriculture & Consumer Services Division of Plant Industry
Prakash K. Hebbar	USDA/APHIS/PPQ/PHP
Paul Heller	Texas Citrus Producers Board
Victoria Hornbaker	California Department of Food and Agriculture
Ken Keck	Citrus Research Board
Mary E. Palm	USDA/APHIS/PPQ/PHP
Gregory J. Rosenthal	USDA/APHIS/LPA
Gail C. Wisler	USDA/Agricultural Research Service, Horticulture and Sugar
Workabeba (Abby) Yigzaw	USDA/APHIS/LPA

TRUE UNFAIR-WEATHER FRIENDS

MUTUAL MANAGES FREEZE INFO FOR INDUSTRY AND MEDIA

Staff Report

When the temperatures in California's citrus groves threaten to drop to 28 degrees or lower, the staff at California Citrus Mutual (CCM) is ready. During this past December's lengthy seven-day freeze event in the San Joaquin Valley as temperatures plummeted into the low- to mid-20s, growers were not alone in putting in long hours. Mutual immediately implemented their comprehensive assistance and communication program.

Each day, from December 5 – 11, five CCM staffers were at their desks before dawn. By 6:00 a.m., they collectively were phoning 60-70 people throughout the state to determine how long wind machines were running, to assess the extent of the damage and to maintain the integrity of the marketplace. They then met in Mutual's conference room at 7:15 a.m. to compare notes. At precisely 8:00 a.m. each day of the freeze, an updated news release was sent to the media and posted on the Mutual web site.

Several growers were made available daily in each area to speak with reporters. "Emphasis was placed on communicating the human element of the freeze at the local, regional and national levels," said CCM Director of Public Affairs Alyssa Houtby, "and how the family farmer was being hurt." She added that growers also discussed California's environmentally-friendly practice of using wind machines to protect the crops, as opposed to the dated and less effective practice of using smudge pots. "All of our media releases concluded with the need to maintain the integrity of California's reputation," she added.

"Only top-quality fruit will be shipped for consumers to purchase at retail."

However, the key message for the media to share with the public never varied – *only top-quality fruit will be shipped for consumers to purchase at retail.*

CCM's freeze assistance extended beyond keeping the press informed. "Following a negative experience in the late '90s, the industry realized it needed to do a better job to maintain the quality and integrity of the crops," said California Citrus Mutual President Joel Nelsen. He explained that growers willingly imposed an assessment on themselves. "Additionally, between November 15 and March 15, there is a $40,000 budget at Mutual for a weather program, which is separate from the assessment."

In part, funding covers a contracted weather service, called Weather Watch. Growers can phone in to get 10:00 a.m. and 4:00 p.m. updates through an automated system. During critical freeze events, the meteorologist will provide those updates even more frequently.

Mutual's Weather Program provides a broad, accurate perspective of how fruit was affected by variety and area. "We have 18 growers with weather stations in their blocks who volunteer to keep us informed," said Nelsen.

Following the 1998-99 freeze, Citrus Mutual coordinated legislation that created a grower-funded program for frost inspection in affected counties.

Inspectors were kept busy on more than 200,000 acres in the San Joaquin Valley in December during the earliest severe freeze event in more than a quarter-century. The inspectors also checked for damage in the Central Valley's 81 packing houses, where a 48-hour inspection hold was placed on any citrus picked after December 11.

Many of those facilities now have equipment that detects freeze-damaged citrus. The dried-out fruit becomes lighter over time. As the individual pieces travel along sensitive equipment rollers that measure the size and weight of the fruit, individual spoiled pieces are kicked out.

Additional specialized machinery that utilizes an infrared system is now going through the testing and modeling phase at the Lindcove Research and Extension Center, according to LREC Director Beth Grafton-Cardwell.

Mutual reports that there are areas in Kern and Madera counties where the mandarin crops have been completely wiped out, and other areas where the damage is as great as 40-50 percent. Nelsen said, "Losses equate to 4.7 million 40-pound cartons of mandarins and $150 million in lost revenue." At the time of the freeze, only 20 percent of the crop had been harvested.

Houtby added, "The more freeze-tolerant navel crop is estimated to have incurred a 30 percent loss Valley-wide, which equates to 22 million 40-pound cartons and $260 million in lost revenue."

Lemons in the Valley did not fare quite as bad as anticipated. There was an estimated 20 percent loss, which equates to one million 40-pound cartons with a value of $24 million.

"We estimate that Valley citrus producers incurred approximately $441 million in lost revenue due to the freeze," Nelsen said. He noted that a slight price increase might offset some of the loss, but said that the industry was wary of pricing the fruit too high, which could result in increased off-shore demand or switching to alternative fruit.

The cold weather's final blow will be a shorter season for California citrus. Instead of its traditional availability through July, shipments most likely will end in mid-May.

Mother Nature shows no respect for the government. Icicles hang from a CDFA insect trap at Tree Source Nursery in Tulare County during December's big chill. Photo courtesy of Roger Smith, Griffith Farms.

HLB'S TOLL: PLOWING CITRUS GROVES INTO PEACH ORCHARDS

FLORIDA GROWERS BEGINNING TO DIVERSIFY

Staff Report

Tree-ripened Florida peaches arrive from the orchard.

It is a sad fact that nearly 100 percent of Florida citrus groves are now affected by Huanglongbing (HLB). This season's orange crop is predicted to be the lowest since the freeze-ravaged crop of 1989-90.

As fruit drops from the trees at a record rate, the USDA, which originally forecast 125 million orange boxes for the 2013-14 season, now estimates no more than 115 million boxes.* This is nearly 15 percent lower than last season's orange crop, and the final numbers could come in even lower. Grapefruit and mandarin production have been reduced, as well.

Although there are several reasons for the decline, including smaller sizing, researchers on both coasts and in Washington, DC are diligently researching solutions to the critical HLB problem (see "USDA establishes multi-agency HLB task force" on p. 18). In the meantime, groves without aggressive caretaking are quickly dying.

February 10, 2014, USDA Crop Estimate

What to do? Unlike many Golden State growers, most Sunshine State growers have been single-crop focused. That is beginning to change as the Florida citrus industry realizes the need for diversification. Some growers are pushing their HLB-infected trees to make room for a new crop bursting with flavor and favorable returns – peaches. Florida agricultural experts estimate Florida peaches could become a $100 million industry within a decade.
Although Georgia is widely known for the fuzzy fruit, the Peach State actually is in third place behind California and South Carolina. California is the undisputed king, producing more than two billion pounds annually and supplying nearly three-quarters of America's peach production, including half of the fresh crop and almost all of the cling peach processed fruit.

Across the country, University of Florida (UF) scientists launched a warm weather peach-breeding program back in the 1950s. From 1966 to 2004, the effort was led by Dr. Wayne Sherman, who undertook development of innovative new varieties. Since Dr. Sherman's retirement, the research has been conducted under the leadership of Dr. Jose Chaparro.

UF researchers have developed peach hybrids well suited to Florida's warm nights and even warmer days. Dr. Mercy Olmstead, a UF stone fruit specialist, said that breeding involved peaches from North and South America. "The peaches bloom in January and ripen in 70-90 days," she said. "which is perfect for Florida." Traditional peaches may require 800 to 1,000 chill hours (32-45 degrees) to begin blossoming and producing fruit. However, many of the UF peaches developed for Central Florida require only 150 to 200 chill hours or less, according to Olmstead.

With even more choices on the horizon, some of the top newer varieties that are currently driving the market include the UFSun, UFOne and UFBest, which are all patented varieties and must be purchased from entities with the appropriate license, she noted.

Olmstead explained that peaches are available in two different flesh types – melting and non-melting – and that "UF" designates non-melting flesh. She said, "Melting flesh varieties do not ship as well, since they tend to bruise easily, which means they also are harvested much earlier than their peak of ripeness. Since non-melting flesh is a bit firmer, these peaches can stay on the tree longer; and because they are tree-ripened, they develop better flavor and color, along with higher sugar content. The non-melting flesh can

Florida peaches were specifically developed to thrive in warmer temperatures.

"Peaches will never replace citrus," Raley said. "I don't know if it will prove to be a viable option in the long run – we'll be better able to gauge the potential this spring. It's a specialty niche market, but we need to try something different."

handle temperature changes in the post-harvest chain a bit better as well, not succumbing to chill injury like some of the melting flesh varieties." The new varieties are being sold entirely as fresh, since there are almost no eliminations.

Current Florida grower experiments began with 15 acres in 2006. Today, there are an estimated 1,500 acres in the state, which yielded approximately three million pounds last season. That's still a far cry from the 532,000 acres of citrus trees that cover the landscape, but it is a pioneering dent.

Among the approximately 50 Florida peach growers is Lindsay Raley, president of Raley Groves and president and chairman of the board of Dundee Citrus Growers Association (CGA). "I'm testing the waters, although I don't know if I'd be doing this if HLB weren't a factor," explained Raley. "We planted 10 acres three years ago and will plant another 15 acres this spring." By comparison, he grows 1,200 acres of citrus.

Dundee handles the harvesting, packing and marketing for approximately one-third of the state's production and will be the largest peach shipper this year, according to Steven Callaham, executive vice president and CEO of Dundee CGA. In 2010, the CGA established Dundee Stone Fruit Growers Association.

"Our growers have been asking what else is out there that they could farm with a potential market that could be handled by Dundee with our packing and marketing ability," Callaham said.

"Peaches made the most sense for us," he explained. "We've invested capital to put in a peach line; and we have the marketing base through Florida Classic Growers, our wholly-owned marketing and sales organization, to merchandise the product to stores."

Callaham continued, "The new varieties are high-quality eating peaches. They're very high in Brix due to the ability to leave them on the tree till full maturity."

Nonetheless, Raley explained that Florida peaches are more expensive to maintain than citrus. "While oranges run about $2,000 per acre, peaches are roughly $2,500 to $3,500 per acre, depending on tree age. They entail higher labor cost than citrus, requir-

Peach orchards are beginning to dot the central Florida landscape, where citrus trees once thrived.

ing pruning and thinning to ensure the fruit grows plump and healthy." Additionally, citrus trees, which don't require as extensive care, live two to three times longer than peach trees.

Although they may be more expensive to grow, according to Callaham, the advantages of peach crops (beside the fact that HLB does not enter into the equation) are significant. Growers are able to make use of existing orchards and irrigation. Trees bear viable fruit within a year and take two to three years for a completely commercially-viable crop, while orange trees may take five years for their first marketable yield. Peaches also bring in higher returns – about $3.99 per pound at retail versus 99 cents for citrus. Importantly, they fill a timing void for consumers, reaching the marketplace from about March 15 till May 31, well before the competition from Georgia and South Carolina, and thus commanding a premium price. The fruit currently is available only east of the Mississippi River at retail. "Demand is far exceeding supply at this point," Callaham said.

While growers do not need to worry about HLB ravaging their peach orchards, Callaham explained that it is still a new, non-established industry with a huge learning curve, little to no infrastructure, very labor intensive and with unknown market saturation.

"Peaches will never replace citrus," Raley said. "I don't know if it will prove to be a viable option in the long run – we'll be better able to gauge the potential this spring. It's a specialty niche market, but we need to try something different."

Shipments receive a final inspection prior to distribution throughout the southeastern U.S.

State-of-the-art dedicated peach packing lines have been installed at Dundee CGA.

PHOSPHATE STARVATION LINKED TO CITRUS GREENING

Zellwood, FL (January, 2014) -- A research article published in the journal Molecular Plant, (February 2013) reveals that "phosphate starvation" has been found to be a major factor that causes Citrus Greening Disease in HLB infected trees.

The author, Dr. Hailing Jin, Molecular Microbiologist, University of California Riverside, combined her molecular and physiological laboratory research, with field research conducted by Dr. Robert E. Rouse, Citrus Horticulturist, University of Florida Immokalee, FL. The three year field research study, and the inter-related microbiological research study verified each study's results reaching the same conclusion, which is considered rare by most researchers. Dr. Jin's groundbreaking research discovered the small Ribonucleic acid (RNA) molecules that are responsible for HLB. Ten new microRNAs, 76 conserved microRNAs, and many small interfering RNAs were found, some of which induce HLB in citrus because of phosphate starvation.

HLB is one of the most destructive citrus diseases threatening the global citrus industry. This research shows that identified host small RNAs can potentially be used as early dignostic markers for HLB, which could be treated, presumably with oyyanion and Polyoxyanion P solutions, before any visual symptoms occur.

KPHITE® 7LP Systemic Fungicide Bactericide, and ReNew®(3-18-20), manufactured by Plant Food Systems, Zellwood, FL, were the oxyanion and Polyoxyanion P solutions utilized in the research, which resulted in offsetting the effects of HLB. In the field study, yields doubled as the citrus trees recovered.

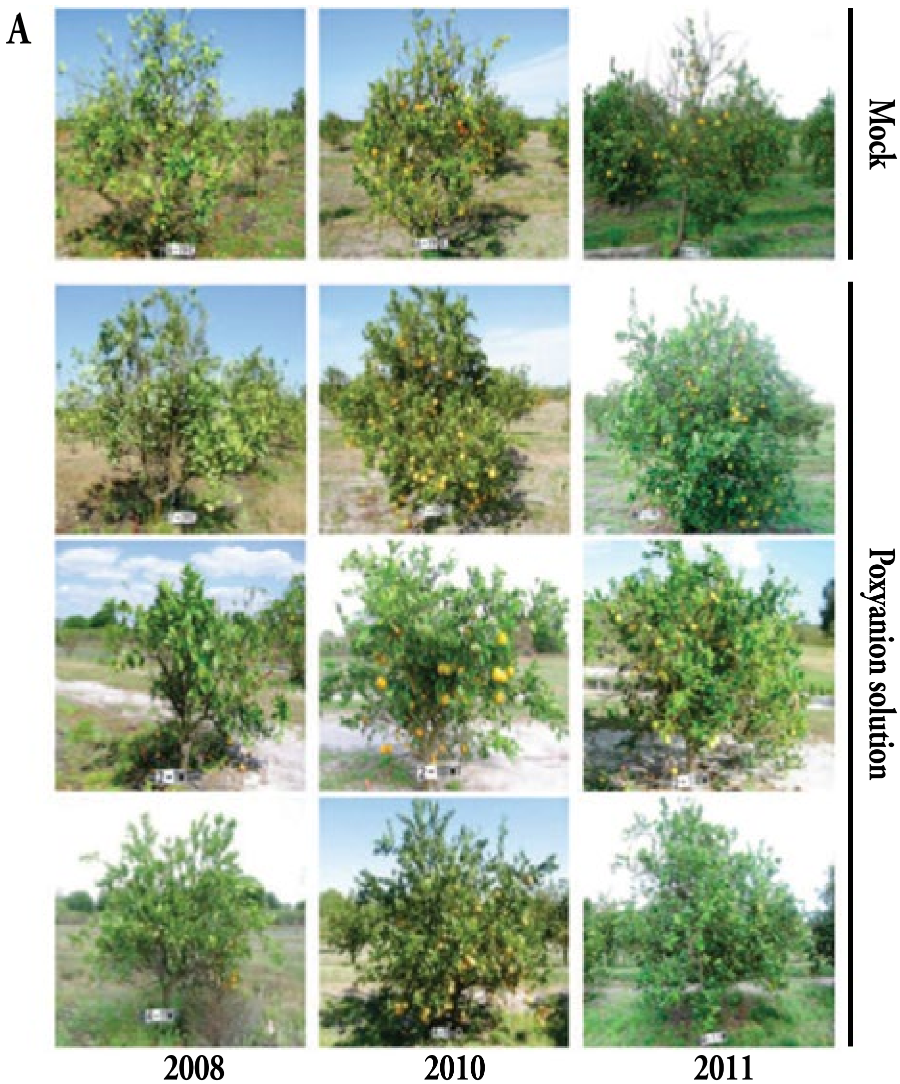
Paul E. Fabry, Vice President of Plant Food Systems, reported similar results. "We have analyzed thousands of citrus leaf tissue samples since the onset of HLB in Florida. We discovered that the large groves down South with the worst greening have the lowest Phosphorus levels, and we shared the samples with the researchers. We are gratified that the growers, who are beating HLB are using the same oxyanion and polyoxyanion P solutions as in the research."

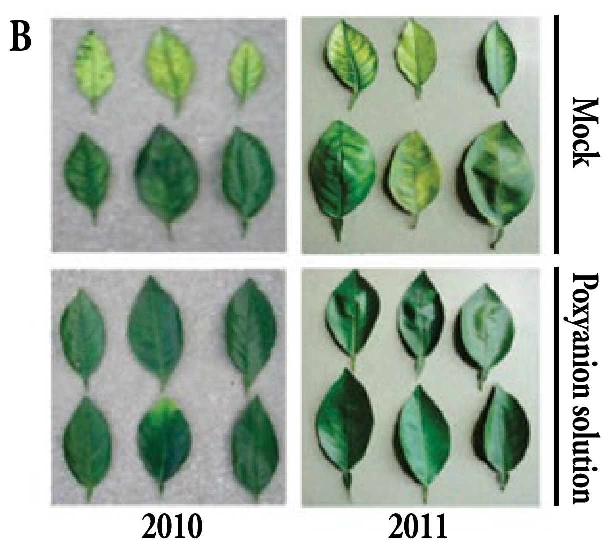
Further support of Dr. Jin's research can be found in a separate and distinct paper "Transcriptome Profiling of Citrus Fruit Response to Huanglongbing Disease", Abhaya M. Dandekar, May 2012. This research identifies Glucose-1-phosphate adenylyltransferase (APL4) was highly down-regulated in symptomatic fruits. The study goes on to support that "Genes encoding ATP synthase gamma and delta chains were also induced in symptomatic fruit, supporting the idea that CaLas may act as an energy parasite by scavenging ATP from its host with a pathogen-specific ATP/ADP translocase.", and "ATP scavenging may be a possible mechanism of pathogenicity, affecting the fruit peduncle, columella, and seed coat."

The study entitled, "Small RNA Profiling Reveals Phosphorus Deficiency as a Contributing Factor in Symptom Expression for Citrus Huanglongbing Disease", the publisher's news release, and other phosphate starvation research can be accessed at www.kphite.com.

Plant Food Systems

Plant Food Systems is an innovative pioneer, leading manufacturer and international distributor of foliar macro and micro nutritional solutions for citrus, and crop health bactericides for all crops under environmental and pathological stress. KPHITE 7LP and ReNew contain patented technology and are themselves the product of groundbreaking molecular research, and contain unique Phosphorus, co-polymeric phosphate and phosphite molecules.





Applying P Solution Alleviated Disease Symptom of HLB and Increased the Fruit Yield of the Diseased Trees. Phosphorus oxyanion solution was applied to Las-infected Las-positive sweet orange trees three times each year for 3 years. Application was synchronized with the initiation of new vegetative flushes in spring (March), summer (June), and fall (September). The treatment was a 3–18–20 liquid fertilizer of P oxyanion solution with 56% mono- and dipotassium salts of P acid (K-Phite, Plant Food Systems, Inc.), plus 3.8 kg of spray-grade potassium nitrate (KNO3) and 18.9 L of 435 citrus spray oil. Control trees received KNO3 and citrus spray oil only. Photos of trees with and without phosphorus oxyanion solution treatment are shown in (A), and photos of random-picked leaves from control and phosphorus oxyanion solution treated trees are shown in (B). Treatments and the year the photos were taken are indicated on the right and bottom, respectively. Pictures were taken around the same time of the year and at the same location with similar camera settings.

For complete results and original research report, contact Mark Brady, Western Marketing Manager, Plant Food Systems (559) 731-1267

AN INTERDISCIPLINARY APPROACH TO COMBAT HLB

RESEARCH IN UC DAVIS' CONTAINED RESEARCH FACILITY

Elizabeth Chin, Darya O. Mishchuk, Jim Bruce, Michelle Cilia, Gitta Coaker, Cristina Davis, Hailing Jin, Wenbo Ma, Glenn Sellar, Cynthia LeVesque, Kris Godfrey, and Carolyn M. Slupsky

This is an introductory article for an ongoing Citrograph report. In subsequent issues, progress on this time-course study will be reported. Readers will be informed of CRB-funded efforts to develop early, pre-symptomatic diagnostic methods for HLB.

An extensive background on the biology of disease development is provided that can be used as a reference by readers. In addition, each participating laboratory briefly describes the diagnostic method they have developed and introduces their research team.

BACKGROUND OF HUANGLONGBING

Huanglongbing (HLB), also known as Citrus Greening Disease, is the most serious disease threatening the citrus industry. The bacterium known as *Candidatus* Liberibacter asiaticus (or CLas, for short) is the suspected causative agent for the disease, which can be transferred to plants through grafting infected material or via feeding by an insect known as the Asian citrus psyllid (ACP). Infection causes reduced fruit yield, poor fruit quality, fruit drop and early tree death. The time between initial CLas infection, detection by nucleic acid-based methods (polymerase chain reaction [PCR]), and symptom manifestation, can take years to occur.

Early detection of CLas in trees is the best prevention for future outbreaks in the state of California. This will enable growers and residents to make informed decisions for disease management and to act quickly to remove infected trees before the ACP vector can spread the disease. Currently, PCR is the only technology approved for regulatory use for detection of infection. This technology relies on a minute amount of CLas to be present in the sample. Since the bacteria are not uniformly distributed throughout the tree, early detection of CLas by PCR is primarily a matter of luck in choosing the correct sample. However, just because PCR cannot detect the bacteria does not mean that CLas is not present, and that ACP cannot feed on the infected tree, acquire, and then spread the bacteria. Because of the long latency period between infection and appearance of symptoms and the inability to detect the pathogen early, CLas is able to spread unnoticed through urban areas and commercial groves. Though some citrus varieties appear to be more tolerant to the disease, all citrus cultivars and clones are susceptible. Fresh citrus production in California is particularly at risk.

HLB was first discovered in Florida in 2005. Just eight short years later, the entire state is under quarantine for the disease. During this period of time, more than $4.5 billion in revenue, representing 30 – 40 percent of all citrus produced in the state, was reported lost to this pathogen, also resulting in more than 8,000 lost jobs.

In California, HLB has been confirmed in a single tree in an urban neighborhood of Los Angeles County in 2012. So far, adjacent trees have not tested positive for the presence of CLas by PCR. At this time, it is unknown how far the bacteria may have spread from this one tree, since no early detection methods have been validated for this pathogen.

ACP insects are spreading from the south of California and have now been confirmed in Tulare County (about 45 miles outside of Fresno). Wherever the ACPs are found, the threat of CLas infection, and thus HLB, is real. There is a desperate need to find early markers of infection to protect citrus growing areas, and more fully understand the pathogen, its insect vector and how it causes devastation to a tree. To address this, the Citrus Research Board,

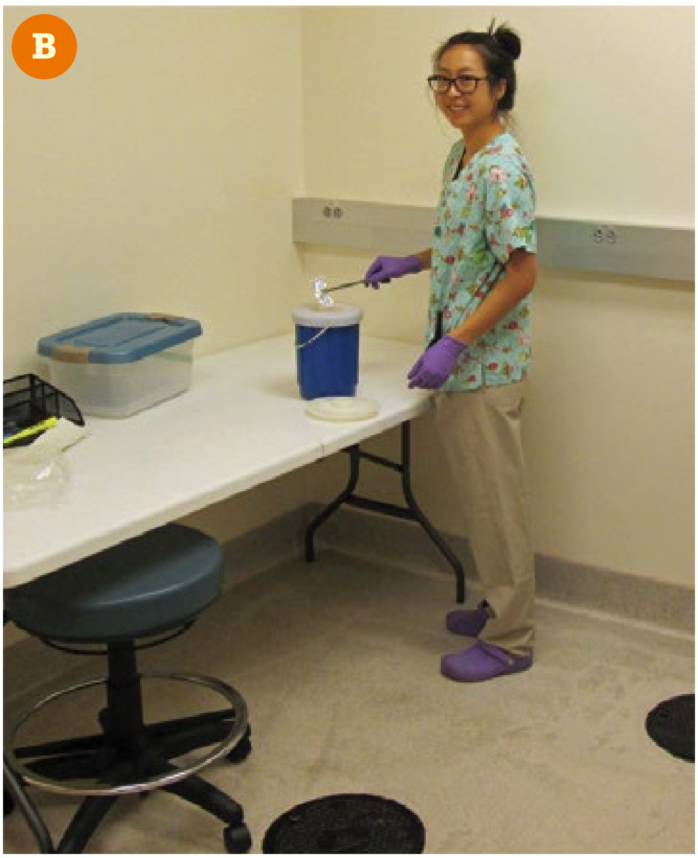


*Figure 1. **A.** The Contained Research Facility (CRF) at the University of California, Davis. The CRF provides a tightly controlled environment to conduct experiments on plants. **B.** Graduate student Elizabeth Chin from the Slupsky lab preparing leaf samples in the CRF. **C.** CRB Board members Richard Bennett and Jeff Steen in the greenhouse during their visit to the facility. **D.** The experimental trees (Parent Washington orange, Tango mandarin and Lisbon lemon) for this CRB-funded project inside the greenhouse. Experimental trees are not infected with CLas at this time.*

together with researchers at UC Davis (Coaker, Davis, and Slupsky), UC Riverside (Ma and Jin), the California Institute of Technology Jet Propulsion Laboratory (Glenn Sellar), the Boyce Thompson Institute for Plant Research on the campus of Cornell University in New York (Cilia) and the University of Washington in Seattle (Bruce), have come together to tackle this important problem in the Contained Research Facility (CRF) at UC Davis.

RESEARCH ON HLB AT UC DAVIS CRF

The CRF at UC Davis has laboratory, greenhouse and growth chamber space that provides a highly controlled and contained research environment ***(Figure 1)***. Kris Godfrey and Tiffanie Simpson manage the facility. Elizabeth Foster is responsible for maintaining the plants, coordinating and helping with sample and data collection, as well as other research needs. Within the facility's greenhouse, the temperature and the amount of light and water the plant receives are regulated. These tightly controlled conditions are ideal for conducting experiments, because the variable environmental factors and subsequent uncertainty in the results that come with sampling field trees are minimized or eliminated.

In the CRF, as many unknowns and variables as possible are removed so that information specific to the bacteria or tree response during the infection and disease development can be detected without confounding factors. Thus, changes to the plant over the course of CLas infection from both graft and ACP inoculation will be studied. Three citrus varieties are being tested – Parent Washington navel orange, Tango mandarin and Lisbon lemon, all of which have different susceptibilities to HLB. Changes in gene, protein and metabolite regulation within the plants, beginning from the moment of inoculation until the development of HLB symptoms, will be examined. Comparison of the earliest time that CLas infection can be detected using these methods will be compared with the gold-standard PCR technique (performed in the CRB diagnostic laboratory by Dr. Cynthia LeVesque, and John Morgan) to determine whether a more accurate early detection method may be found.

If successful, the data obtained will aid the researchers in applying for regulatory approval, the first step in making the method widely available. Furthermore, cooperation amongst the eight collaborators (whose projects are described below) will provide unprecedented information about the bacteria and disease, and potentially result in improved treatment methods.

Each member of the team brings a unique perspective to the study of the disease that, when combined, will provide a systems biology approach to understanding the pathogen and the insect, and the plant's response to them. Systems biology, as implied by its name, is interdisciplinary, combining multiple scientific fields to understand the complex interactions within a biological system, and emergent properties of the system that could not be revealed by studying any individual component in isolation. Our combined effort will provide a more complete understanding of the ACP/CLas/citrus system, and allow for development of early detection methods and a comprehensive management strategy to combat the disease.

PIECES OF A PUZZLE

The unique approach of each researcher in our interdisciplinary team has already provided a wealth of information, some of which has already been published. However, each of these approaches are just pieces of the larger systems biology puzzle ***(Figure 2)*** – Systems biology is the conglomeration of genomic, transcriptomic, proteomic and metabolomics information.

Studying the genome of citrus, the CLas pathogen or the ACP insect provides a blueprint of all the genes that these organisms can produce. Finding genes can help decipher all of the functions that an organism can perform, as the genome is the foundation upon which an organism is built.

However, not all genes are 'turned on' (also referred to as being 'expressed') and allowed to produce a functional product (RNA and/or protein); and if they were, one would not see differentiation between the root system and the trunk of the tree, the leaves or the fruit. Each of these parts of the tree contains the entire DNA of the organism, but not all of the genes encoded in the DNA are expressed in each tissue. The tree's DNA contains the essential information about how to carry out all of the tree's life processes and how to make all the tissue systems, such as the roots that absorb water and nutrients from the soil, the xylem and phloem cells that transport nutrients to all parts of the plant, and, of course, the leaves and fruit. This level of control of gene expression is through a process called epigenetics, where expression of certain genes can be either enhanced or repressed through the addition of methyl groups (a type of chemical group) to specific regions of DNA. Epigenetics determines how a leaf cell knows to be a leaf cell and not a root cell, even though all the cells in the tree have the same DNA. The epigenome can be altered through external influences, such as the amount of water and the presence/absence of micronutrients, soil bacteria or even pathogens like CLas.

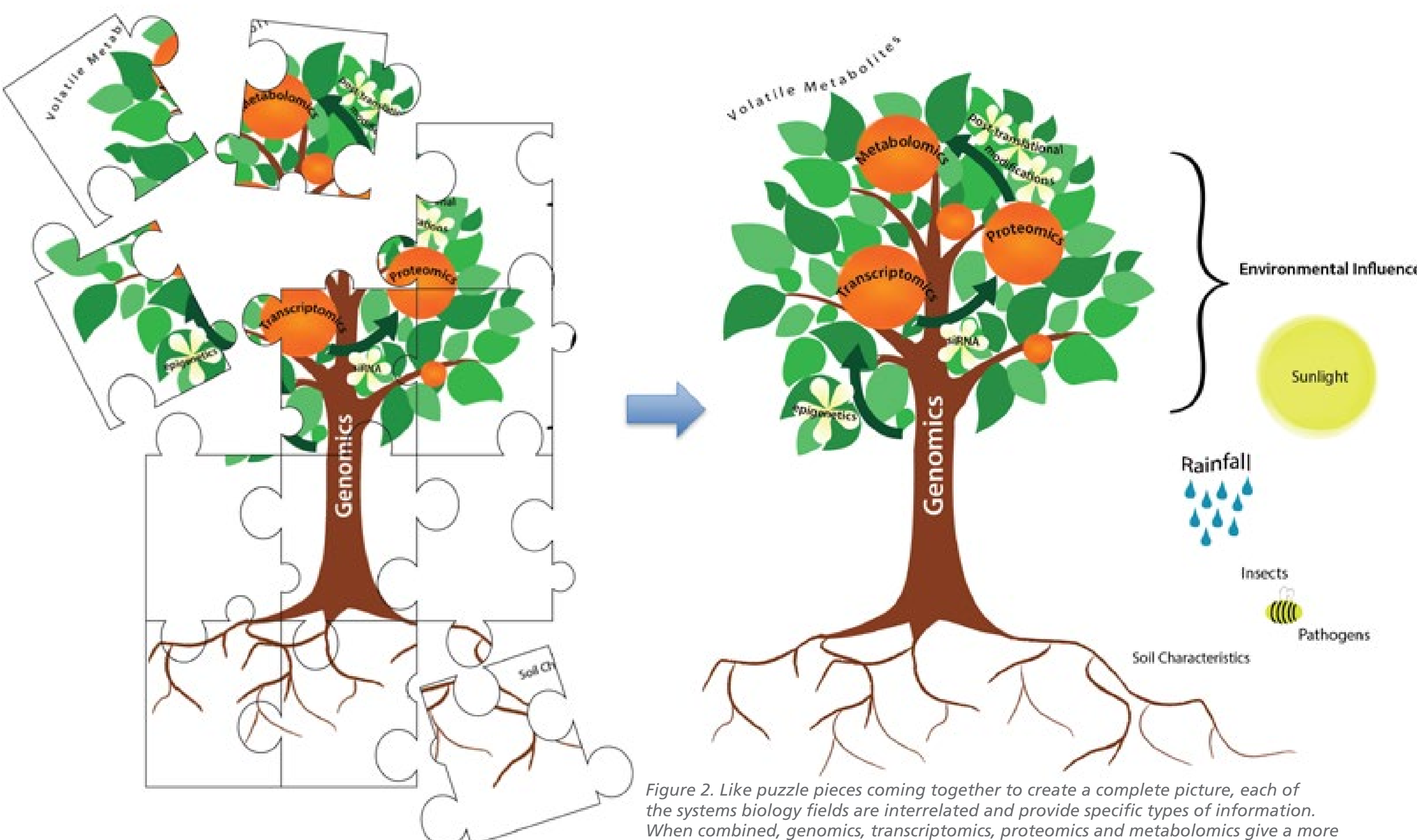


Figure 2. Like puzzle pieces coming together to create a complete picture, each of the systems biology fields are interrelated and provide specific types of information. When combined, genomics, transcriptomics, proteomics and metabolomics give a more comprehensive understanding of the problem than any individual field can provide by itself. A team of researchers from across the U.S. have come together to solve this puzzle for the most serious citrus disease that is now threatening fresh California citrus: HLB.

The first step of gene expression within DNA is a process called transcription. The DNA, or collection of genes, is 'read' and a transcript called messenger RNA (mRNA) is generated. Transcriptomics enables us to know what genes are being expressed and where. It can provide information about changes in the epigenome, and also provide information on the quantity of mRNA being produced from each gene.

Cues from the environment can cause changes in gene expression levels and thus mRNA production. For example, when the temperature rapidly decreases to close to freezing, specific genes will be turned on, and others will be turned off to help the tree cope with the change in the weather. Measuring which genes are turned on or off helps us understand the processes by which the tree is able to recover from exposure to cold, which genes are related to cold tolerance, and how the plant is regulating itself to deal with a change in weather.

After a gene has produced an mRNA molecule, the mRNA provides a template to make a certain protein through a process called **translation**. Proteins are essential for carrying out all functions inside a cell and between cells. They are involved in cell structure, cell signaling, metabolism (such as photosynthesis, respiration, energy transport, synthesis and breakdown of large molecules, etc.), defense against stress and pathogens such as CLas, and even against insects such as the ACP. Proteins function as small molecular machines. Each protein has a highly specific shape that enables it to perform these important cellular processes. Understanding how proteins come together to form functional units is a critical part of proteomics studies.

Protein function can further be regulated through the addition of chemical groups (such as sugars and phosphate) to the protein that will either help or stop it from functioning. This process is called **post-translational modification**, and is essential in helping to define a protein's function or lack thereof. Regulation of protein expression can also occur at the gene level (through epigenetics, as described above), and at the mRNA level through pieces of RNA called small RNAs (sRNAs), which can be divided into two subclasses: micro RNA (miRNA) and small interfering RNA (siRNA). The latter are pieces of RNA that can bind to a specific mRNA transcript and prevent it from being translated into a protein. These sRNAs can be generated by the cell or by other organisms such as bacteria.

Metabolites are essential for the plant to grow and reproduce, as well as defend itself against pathogens, insects and other stresses. They are also responsible for providing sensory aspects of fruit such as taste and smell. These small molecules include things like amino acids (the building blocks of proteins), organic acids (such as citric acid, which is the main organic acid in orange fruit), sugars (such as sucrose, glucose and fructose), lipids (which form the cell membrane that surrounds the cell), various vitamins (such as ascorbic acid), and other molecules. They may be soluble or insoluble in water, and they may or may not be volatile. The sweet smell of citrus blossoms is an example of volatile chemicals produced through citrus metabolism to attract insects to help pollinate the tree. Metabolites are a reflection of the biological processes occurring in the tree. What happens at the metabolite end of things can be traced back to the proteome, the transcriptome, and the genome. If the amount of a metabolite changes, it tells us that there has been a change in the tree's activities.

All of these "-omic" pieces are interrelated, and the study of each contributes to our understanding of the others. Bringing each of these small, but significant, pieces of information together provides a holistic, molecular snapshot of the complete picture. Like puzzle pieces fitting together, genomics, transcriptomics, proteomics and metabolomics are related to one another. Together, they complete a picture that will enable us to create a robust early detection system, and allow development of strategies to combat HLB. Working with all the pieces is the only way to see the complete picture of what happens to a tree during CLas infection.

GLOSSARY

Genomics
Study of the genetic make-up of an organism including DNA sequencing, mapping and gene function.

Epigenetics
Study of changes in gene function that are not the result of changes in the DNA sequence; determines whether a gene should be turned off or turned on.

Transcriptomics
Study of the how an organism's DNA sequence is transcribed into corresponding molecules of RNA.

Transcription and Translation
The process by which an organism's DNA sequence is converted from genes to RNA molecules to amino acids to proteins and other products.

Proteomics
Field of study that examines the structure and function of proteins in a cell.

Metabolomics
Study of metabolites, which are small chemicals or molecules used by proteins involved in plant metabolism.

AN INTERDISCIPLINARY TEAM

To create a more complete picture of how CLas infection affects citrus, eight research groups and two infrastructure teams have come together from across the U.S. to work at the CRF. Each of the research groups is working on different pieces of the puzzle of how CLas affects citrus:

Jin Lab

(UC Riverside, California) - sRNAs (transcriptomics)

Many sRNAs are differentially regulated by pathogen infection. The Jin lab is particularly interested in the sRNAs that are specifically induced or suppressed in CLas-positive plants. Their potential targets are likely involved in the tree responses against CLas. The Jin lab will profile and compare sRNAs and mRNAs from HLB-tolerant and sensitive citrus plants using genome-wide RNA deep sequencing. They aim to identify a group of sRNAs and citrus genes that are specifically induced by CLas-infection, which could potentially be used as early diagnostic markers. Some of the sRNAs and genes are important for natural defense responses against CLas, which will be ultimately used for citrus protection.

Ma Lab

(UC Riverside, California) - Antibody development (proteomics)

The Ma lab is developing antibody detection methods for robust diagnosis of HLB. Using the fully sequenced CLas genome, they predicted 27 CLas proteins that can be secreted outside the bacterial cell and presumably into the phloem of infected citrus. These secreted CLas proteins are called *effectors*. The Ma lab has further identified four CLas effectors that are highly expressed and, therefore, may be present in high abundance in CLas-infected trees. Using these effectors as antigens, the Ma lab has generated antibodies that can specifically bind to the target effectors and detect their presence with high efficiency. These antibodies may allow robust HLB diagnosis because, unlike the bacterium, the effectors are systemically dispersed in the infected trees through the central transport tissues of the plant and thus could be detected away from the infection site(s). CLas effector antibody-based detection may overcome the problems associated with the large variability in the distribution of the pathogen throughout the tree, and holds promise for bacterial detection in a simple, direct and highly specific manner.

Coaker Lab

(UC Davis, California) - Proteomics

The Coaker lab is using a proteomics-based approach to determine the expression pattern and citrus targets of the four CLas proteins that were identified by the Ma lab as being highly expressed in CLas-infected citrus trees. In other bacteria, effectors are required for pathogen virulence and can facilitate nutrient acquisition, insect feeding and suppression of defense responses. Her group will focus their efforts on investigating the expression patterns and citrus targets of these four unique CLas effectors. They hypothesize that these effectors are important for bacterial survival or HLB symptom development by targeting important citrus proteins to manipulate their host. They are also using mass spectrometry, a technique that enables protein identification, to isolate citrus proteins that are targeted by each CLas effector in different plant tissues. Successful completion of these experiments will facilitate HLB detection strategies, as well as provide a set of pathogen targets that can be manipulated to protect citrus against HLB.

Bruce Lab

(University of Washington, Washington) - Advanced Proteomics Technology Development

The Bruce lab is focused on development and application of advanced mass spectrometry technology for biological, biophysical and biochemical studies. This lab specializes in using technology called Fourier transform ion cyclotron resonance mass spectrometry for proteomics, biological, and biomedical applications. They will apply their cutting-edge Protein Interaction Reporter Technology and other technologies currently under development to gain insights into the protein interactions that regulate CLas transmission in collaboration with the Cilia lab.

Cilia Lab

(USDA - Agricultural Research Service, Boyce Thompson Institute for Plant Research, Cornell University, New York) - Vector-Host-Pathogen Proteomics

The Cilia lab is interested in understanding how proteins orchestrate the process of CLas transmission by ACP vectors and the spread of CLas inside of infected citrus trees. These processes involve changes in expression and protein interactions among ACP, citrus and CLas proteins. The Cilia lab is partnered with the Bruce lab to use advanced mass spectrometry technology developed in the Bruce lab for studying these complex processes. The Cilia lab will perform functional assays to understand which proteins and protein interactions are critical for CLas infection and transmission. Practical applications of the Cilia-Bruce collaboration include the development of early infection biomarkers for detection of HLB in citrus trees and the identification of novel molecular targets in the ACP for CLas transmission control.

Davis Lab

(UC Davis, California) - Volatile Metabolomics

The end products of some biochemical pathways are volatile organic compounds (VOCs) that radiate into the environment. The Davis lab measures these VOC metabolites in the canopy of citrus trees, and uses those "odors" to non-invasively diagnose a tree infected with a specific pathogen. In this CRF experiment, the Davis lab will use a suite of hardware and software approaches to monitor VOCs pre- and post-infection. First, they will use a mobile gas chromatograph differential mobility spectrometer (GC/DMS) to sample and analyze the VOCs emanating from the citrus leaves. This is a field-portable instrument that has previously been used to detect VOCs associated with citrus greening. Secondly, they will use an active sampling device to concentrate the VOCs onto a sorbent trap for later analysis on a mass spectrometer system for chemical identification. This experiment will help to determine when the VOC signature of a tree changes post-infection, and will provide valuable data regarding VOCs as early diagnostic markers for field detection of citrus greening.

Slupsky Lab
(UC Davis, California) - Metabolomics

The Slupsky lab is measuring the metabolome of citrus infected with CLas using a technique known as nuclear magnetic resonance (NMR). This technology is similar to that of an MRI (magnetic resonance imaging). NMR allows for each molecule's identity and concentration in a single sample to be determined at the same time. This group already has identified unique changes in citrus fruit that occur with infection. The changes have provided information showing that CLas is able to "turn-off" the production of metabolites used for plant defense. These results partially explain why CLas is so good at surviving in the tree and causing so much damage while evading plant defense. The Slupsky lab has begun to expand measurement from fruit to leaves and other tissues in citrus. Her work will provide valuable information on metabolic responses of the tree to infection, helping to understand why the pathogen is so difficult to detect early, and will be useful for validating early diagnostic metabolite markers of CLas infection.

Sellar Lab
(JPL, CalTech, Pasadena, California) - Optical Imaging

Inspection and detection are the first lines of defense against the spread of HLB. The interdisciplinary team at NASA's Jet Propulsion Laboratory (operated by the California Institute of Technology) is using optical data from NASA's "Airborne Visible and Infrared Imaging Spectrometer" ("AVIRIS") collected from an aircraft to produce maps of the distribution of citrus trees in urban areas to help guide inspection efforts.

***

Together, this interdisciplinary team is working to provide growers and the scientific community with as much information as possible about the disease itself, the bacteria that cause it, how the tree responds to infection and how it is transmitted. By putting together the pieces of this puzzle, our team will help to generate the 'big picture,' so that we can develop early detection methods and a targeted approach to fully combat HLB.

Individuals working on the project:

Jin Lab:
Dr. Hailing Jin (PI)
Dr. Rishi Aryal

Ma Lab:
Dr. Wenbo Ma (PI)
Dr. Jinxia Shi
Dr. Deborah Pagliaccia
Dr. Yongli Qiao

Coaker Lab:
Dr. Gitta Coaker (PI)
Gang Yu

Bruce Lab:
Dr. Jim Bruce (PI)
Juan Chavez

Cilia Lab:
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Infrastructure Provided by:

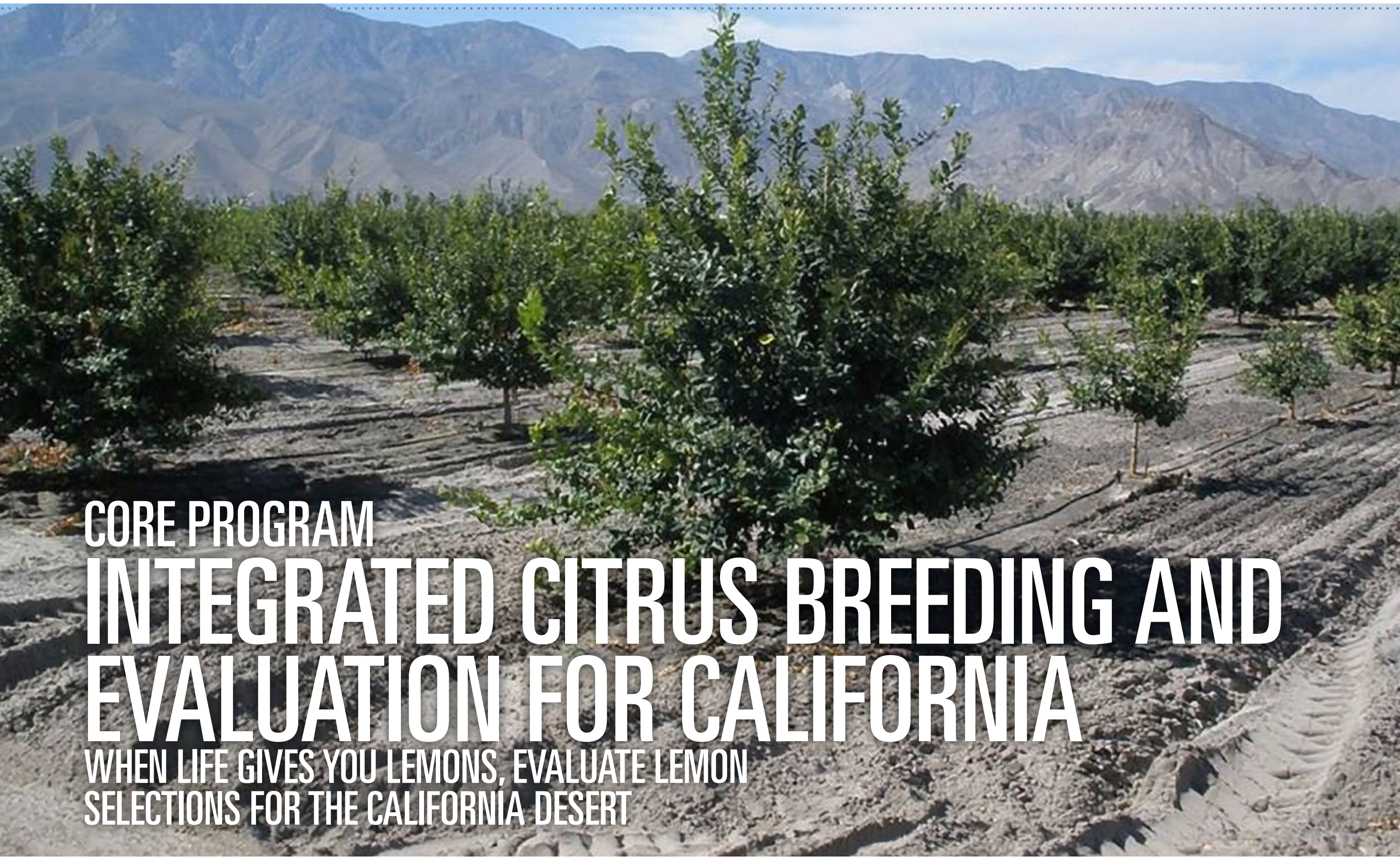
UC Davis Contained Research Facility:
Kristine Godfrey, Director
Tiffanie Simpson, Assistant Manager/ Containment officer
Elizabeth (Lyzz) Foster, Junior Specialist

CRB Jerry DiMittman Lab:
Cynthia LeVesque, Director
John Morgan

CORE PROGRAM

INTEGRATED CITRUS BREEDING AND EVALUATION FOR CALIFORNIA

WHEN LIFE GIVES YOU LEMONS, EVALUATE LEMON SELECTIONS FOR THE CALIFORNIA DESERT



Appearance of trees in October 2008

Glenn C. Wright, Vince Samons and Tracy L. Kahn

California leads the nation in acreage and production of lemons. Lemons are grown predominantly in the coastal and desert regions of the state with a small amount of acreage in the San Joaquin Valley, thus taking advantage of the range of climatic regions in the state and providing the market with year-round production. Lemon trees tend to produce flowers and marketable fruit throughout the year in coastal California due to the cool summers and mild winters. Most lemon production in the coastal region occurs in late spring and early summer, when prices for lemons are highest. Lemon trees grown in the desert and interior regions of California have a shorter season, with a higher percentage of the crop produced during the fall and winter.

Yet the first lemons of any season in California are grown in the desert. Desert lemons occupy an important early-season market niche, which could be lost to international competitors, such as Chile and Mexico. Choosing lemon varieties that produce well, occupy important market niches and meet changing market needs are crucial to address global competition. As an important component of the California fresh fruit industry, desert lemons are also an important source of fruit for packinghouses located in other areas of the state.

During the 2005-2006 season, we received funding from the Citrus Research Board to initiate a replicated evaluation trial entitled: "Evaluation of Lemon Selections for the Desert" (CRB Project 5200-127). The selection of varieties for this replicated trial was based on past initial screening of a large number of lemon selections in Riverside by Tracy Kahn, as part of her CRB project to evaluate citrus varieties for trueness-to-type and commercial potential. Additional varieties were selected for this trial based on lemon selection trials conducted by Glenn Wright in Yuma, Arizona, funded by the Arizona Citrus Research Council.

During the 2011-2012 season, this project became incorporated into a larger core CRB project with the goal of developing and evaluating new citrus scion and rootstock varieties suitable for California entitled "Integrated Citrus Breeding and Evaluation for California." Before this project was initiated, there had been no lemon variety trials in the California desert which could be used to evaluate yield and packout. The trial was designed to be maintained for ten years. Information obtained from this trial will help to further define performance of these selected varieties in the California desert and suggest new varieties for future multi-location replicated trials for other lemon production regions. The major objective of our trial is to provide the industry with information on the tree growth, yield, packout,and fruit quality characteristics for selected lemon varieties in the California desert.

Based on our prior research results, we chose several new lemon selections that we found to have promising characteristics in Riverside and Yuma and included some standard commercial lemon selections currently produced in California as controls. A list and short description of each of the 12 new lemon selections and commercial standards, Allen Eureka and Limoneira 8A Lisbon included in this study follows. Also included is the Citrus Clonal Protection Program (CCPP) Variety Index (VI) numbers used by citrus nurseries to order budwood.

1. **Allen Eureka** (V.I. 227) was one of the two standards in this trial and the most commonly planted nucellar Eureka selection for California and Arizona. Trees have a spreading growth habit that leads to a lower cold tolerance than Lisbon. Allen Eureka is popular in coastal California due to its propensity to set fruit over a wide range of the season.

2. **Corona Foothills** is a limb sport selection of the Villafranca variety. This selection is the most commonly planted lemon in Arizona, but not at all common in California. Although there are currently registered bud sources of Corona Foothills in California at one nursery (Young's Nursery, Thermal, California), last year, Willits and Newcomb Nursery of Arvin, California, sent budwood of this variety to the CCPP. It is currently in quarantine, so there will be a VI source from the CCPP in the future. Corona Foothills has characteristics intermediate between Eureka and Lisbon; generally considered to be precocious, yielding early fruit that has a smooth peel.

3. **Femminello Santa Teresa** originated in Italy as a selection of Femminello Comune that produces fruit elliptical to oblong in shape with a neck. This selection is resistant to the Mal Secco disease found in Italy, and purportedly produces a greater percentage of its fruit in the late summer as compared to other Femminello selections. Initial evaluations of this cultivar in Riverside were promising. There is a registered bud source of this variety at Willits and Newcomb nursery, but no CCPP bud source. We will request budwood of this registered source be sent to the CCPP so there will be a VI source from the CCPP.

4. **Interdonato** (V.I. 667) originated in Italy, and is considered a lemon x citron hybrid. This variety is resistant to the Mal Secco disease and is acknowledged to be the earliest of the Italian varieties. This variety was not previously tested in California or Arizona.

5. **Limoneira 8A Lisbon** (V.I. 380) is the other commercial standard for this trial and the most commonly planted Lisbon selection in California and Arizona. This variety is considered to be vigorous and produce early season fruit.

6. **Limonero Fino 49** (V.I. 480) is the earliest yielding lemon variety and chief winter lemon of Spain. Limonero Fino 49 trees are thorny and highly productive, with spherical, smooth fruit. First introduced into California in 1987, this variety was not evaluated in California but performed well in Arizona trials.

7. **Limonero Fino 95** (V.I. 674) is very similar to the Limonero Fino 49 according to the Spaniards, but harvest is about two weeks earlier, and productivity is lower. It was imported into California in 2003 and had not been previously tested in Arizona or California.

8. **Messina** (V.I. 661) was imported from Spain to California in 2002, but is likely to be very similar to the Italian selection Femminello Messina. Purportedly resistant to the Mal Secco disease, Messina is extremely early bearing, but yields poorly.

9. **Seedless** (V.I. 492) was received as a cutting from Lasscocks Nursery, South Australia, in 1939, but did not become a registered variety until 1985. This lemon was formerly known as Seedless Lisbon, but is now believed to not be a Lisbon-type lemon at all. It has since been given the name "Seedless Lemon". Consumer preference of seedlessness and data from evaluations in Riverside confirming the low seediness of this cultivar are the reasons that this variety was included in this trial.

10. **Variegated Pink Eureka** (V.I. 486) originated as a limb sport of a conventional Eureka lemon, and was introduced into the Citrus Variety Collection in 1931. This variety has unique fruit and foliage. Leaves are variegated green and white, while fruit is variegated green and cream, turning to yellow with pink oil glands and a pink blush at maturity. Variegated Pink Eureka produces fruit from late winter to early summer and is less vigorous than a conventional Eureka.

11. **Walker Lisbon** (V.I. 415) is a vigorous selection of Lisbon lemon from Ventura County. Prior to 1949, it was referred to as a Lisbon selection of lesser importance in the Citrus Industry Volume I (Hodgson 1967), but has performed well in Arizona trials. Based on observations of trees growing at the Lindcove Research and Extension Center by Glenn Wright and Keith Mori, Walker Lisbon appeared to be precocious and early yielding.

12. **Yen Ben Lisbon** (V.I. 586) is a Lisbon lemon sport selection that originated in Queensland, Australia, in the 1930s. This variety is known to be precocious and a heavy yielder, but produces small fruit.

Trees of 11 of the selections were planted in May 2006 on a 3.2 acre site at the Coachella Valley Agricultural Research Station (CVARS), near Thermal, California. Trees of the twelfth selection, Limonero Fino Largo (95), were planted in April 2007. In total, the trial has about 20 trees each of 12 selections budded to *Citrus macrophylla* rootstock in five groupings of four trees per selection. The spacing for the trial is 24 ft. by 24 ft., and the experimental design is randomized complete block. Irrigation is provided by microsprinklers. Trees are fertilized with a combination of fertilizers applied via fertigation and as foliar sprays. We collected leaves for nutrient analysis in August 2011. Nitrogen, manganese and zinc levels were found to be low, while all other nutrients were in the optimum to high range. Fertilization practices were adjusted in response to these findings.

This article describes our initial results for this trial since planting in 2006 through the 2011-2012 season. Below are results comparing the 12 lemon selections for freeze tolerance, tree growth based on canopy volume, yield and earliness. All data were analyzed statistically using univariate and multivariate analysis of variance. Means separations were analyzed using Duncans Multiple Range Test at $\alpha=0.05$. Results of experimental and commercial fruit packout, exterior and interior quality, and fruit storage life will be presented in subsequent issues of *Citrograph*.

FREEZE TOLERANCE

There is a long history of natural experiments in many areas of research from public health to agriculture to economics. Natural experiments occur when the experimental conditions are dictated by nature and are out of the control of the researcher. They are especially useful whenever controlled experimentation would be difficult and when there is a large change in the treatment or response as when a freeze occurs.

In January 2007, an arctic low pressure system plunged extremely far west, delivering record cold temperatures in the western states and resulting in damage to citrus crops throughout California. Temperatures at CVARS dropped to 23ºF, according to Vince Samons, station superintendent. Data from the nearby CIMIS station located at Mecca, California, showed a minimum temperature of 26.7ºF on January 13, followed by lows of 23.4ºF, 27.5ºF, 30.6ºF and 24.4ºF on the four subsequent nights. During January 13-17, nighttime minimum temperatures were at or below 28ºF for four to nine hours, and nighttime minimum temperatures were above the freezing point only beginning on January 18.

We were immediately aware that there was significant damage to some of the trees, and it appeared as if there was a selection response. By early February, it became clear that there was a noticeable difference between the selections in tree response to the freeze, as well as a spatial difference in the field. Trees on the south edge of the field were more damaged than those planted further north, and trees in the lower part of the field were more damaged than those trees on higher ground.

On February 20, 2007, Glenn Wright and his technician, Marco Peña, returned to the site to rate the trees according to the damage they had sustained from the freeze. Although the rating was somewhat subjective, they developed a rating scale to be as objective as possible. The scale was from 0 to 5, with 0 being complete scion death and 5 having no damage, as shown in ***Table 1***. They found that there were no trees that had complete scion death, but 49 trees had a rating of "1." Some of those trees were killed to within a half-inch of the bud union, but Wright and Peña did notice small, new shoots growing from above the bud union on many of these highly-damaged trees. Following the rating of these trees, they pruned off all the dead scaffolds, leaving only the trunk. Many trees had ratings of "2," "3" and "4," and there were also 54 trees with a rating of "5." Wright and Peña assumed that all the trees with ratings above "1" would refoliate quickly.

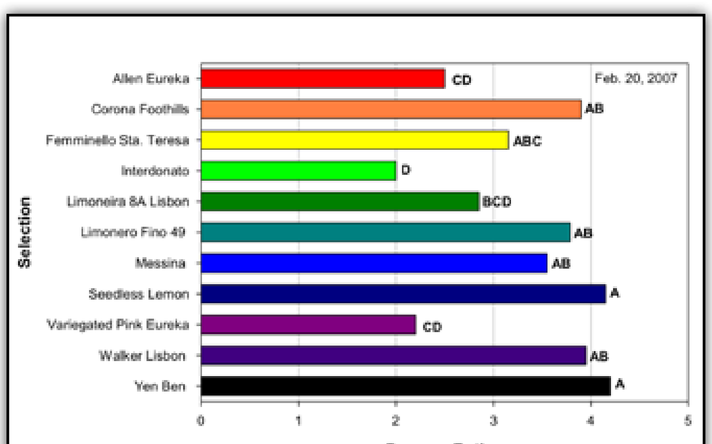


Figure 1. Freeze damage ratings of 11 lemon selections on 2-20-07. Selections with bars not sharing similar letters are significantly different.

After analyzing the ratings for all of 11 of the 12 selections in this trial, and removing the effect of field position, we found that Corona Foothills, Femminello Santa Teresa, Limonero Fino 49, Seedless Lemon, Walker Lisbon and Yen Ben Lisbon sustained the least damage, while we were surprised to note that the Allen Eureka and Limoneira 8A Lisbon had moderate damage ***(Figure 1)***. Interdonato and Variegated Pink Eureka were the most heavily damaged ***(Figure 1)***.

On March 20, 2007, Wright and Peña returned to the experimental site to again inspect and rate the trees. They found that of the 49 trees previously rated as "1," most had new shoots growing from the undamaged part of the trunk. Several trees had multiple shoots growing from the trunk. Where this was the case, they selected the two or three most vigorous shoots, and removed the rest. Trees of this type were rated again with a "1." Five trees, though still alive, had no shoot growth from the trunk; these trees were rated with a "0.5." No trees were rated "2," four trees had ratings of "3," and all the rest were either "4" or "5." Therefore, the rating system was modified slightly as shown in ***Table 1***.

Table 1. 3/20/07 Damage Ratings

Value	Description
0	Complete scion death
0.5	All scaffold limbs dead, all or part of trunk alive, No shoot growth.
1	All scaffold limbs dead, all or part of trunk alive, may have shoots
2	All or most of leaves dead, trunk alive, all or most of scaffolds alive
3	15 - 50% of the leaves alive, little or no scaffold damage
4	50 - 85% of leaves alive, no scaffold damage
5	Less than 15% damage to leaves, no scaffold damage

Results of the March 20, 2007, rating for tree damage are shown in ***(Figure 2)***. All the selections showed improvement since February 20, 2007, with Corona Foothills, Femminello Santa Teresa, Limonero Fino 49, Seedless Lemon, Walker Lisbon and Yen Ben Lisbon having the best ratings; Allen Eureka, Limoneira 8A Lisbon and Interdonato with still moderate ratings; and Variegated Pink Eureka as the lowest-rated selection. Compared with the others, the performance of Allen Eureka and Limoneira 8A Lisbon was surprisingly poor.

Peña traveled to the site in late April 2007 to make further observations. Each tree that was rated as "3," "4" or "5" was a "5" on the rating system described in ***Table 1***, so no further ratings were necessary. For those trees still rated as "1," all but the strongest shoot was removed, then tied to the trunk, thus giving the new growth some support and re-establishing the trunk. Once the shoot reached about 30 inches, it was cut back to re-establish the head of the tree. Sixteen of these trees rated "0.5" or "1" were too weak, so they were replaced on May 30, 2007, with new trees produced by Young's Nursery.

By the following fall, it was apparent that only one of the trees affected by the freeze died outright. However, a few trees were killed back to a point below the bud union or were completely defoliated and forced to regrow shoots that eventually died. Ultimately, eight of the 240 trees in the experimental plot died as a result of the freeze, and we had to reestablish trunks and main scaffold limbs of some of the trees. Since some of the trees lost substantial canopy, and apical dominance was lost, most trees had latent buds that emerged from the trunk and scaffold. In many cases, several of these buds would emerge from the same location. It was necessary to select those new shoots that were strongest and oriented in the correct direction and remove the rest. Despite considerable time spent pruning some of the trees to regain proper form, approximately six and a half years later, the effects of this "natural experiment" are no longer apparent and we have moved on to collecting data on tree growth, yield, packout and fruit quality characteristics for selected lemon varieties in the California desert.

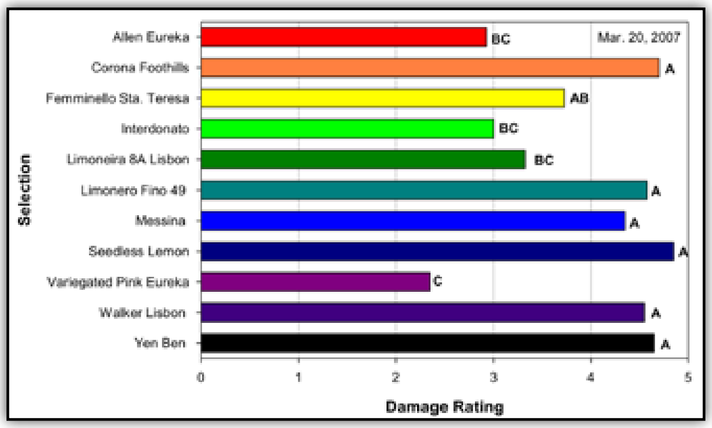


Figure 2. Freeze damage ratings of 11 lemon selections on 3-20-07. Selections with bars not sharing similar letters are significantly different.

TREE CANOPY VOLUME AND YIELD EFFICIENCY

We collected the first data on canopy volume on October 25, 2007, 15 months after the trees of the first 11 selections were planted and six months after trees of the twelfth selection, Limonero Fino 95, were planted. To determine the canopy volume, we first evaluated whether the existing canopy of each tree was cylindrical or spherical. If cylindrical, we collected the height and diameter of each tree, and calculated the volume using the formula: Volume = $\pi r2$ x height. If spherical, we collected the diameter of each tree, and calculated the volume using the formula: Volume = 4/3 $\pi r3$. Trees of Corona Foothills had the greatest canopy volume, but were not significantly higher than Walker Lisbon and Seedless lemon or Yen Ben or Limonero Fino 49 ***(Figure 3)***. Trees of Messina, Femminello Santa Teresa and Limoneira 8A Lisbon had intermediate canopy volumes; and Allen Eureka, Interdonato and Variegated Pink Eureka had the smallest canopy volumes. Trees of Limonero Fino 95 were one year younger than the other selections and were not included in the statistical analysis.

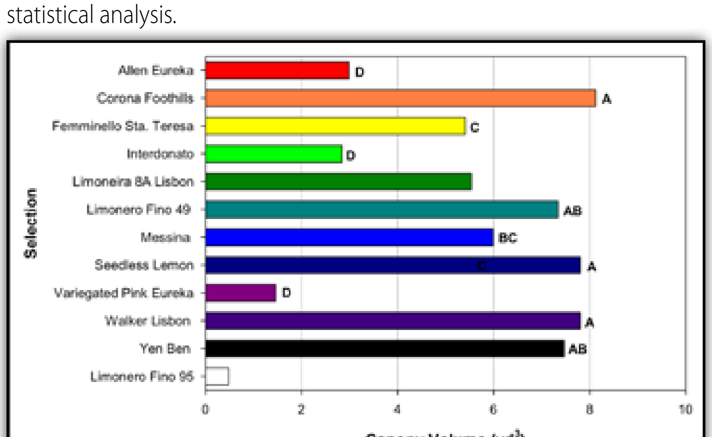


Figure 3. Canopy volume of 11 lemon selections on 10-25-07. Selections with bars not sharing similar letters are significantly different.

For the second collection of canopy volume measurements taken on March 1, 2010, due to increased growth of trees and changes in shape, volumes were calculated based on the assumption that the tree canopy was generally spherical ***(Figure 4)***. Messina had the largest canopy, while Corona Foothills, Seedless Lemon, Walker Lisbon and Yen Ben were statistically similar. A grouping of intermediate-sized trees included Allen Eureka, Femminello Santa Teresa, Limoneira 8A Lisbon, and Limonero Fino 49. Selections with the smallest trees included Interdonato and Variegated Pink Eureka. We noted that there were indications of nitrogen deficiency on the Interdonato selection, and this deficiency was not found on any of the other selections under test. We applied additional fertilizer to counter this problem. Also, we noted that the small size of the Variegated Pink Eureka canopy was due to the adverse effects of sunburn on the leaves.

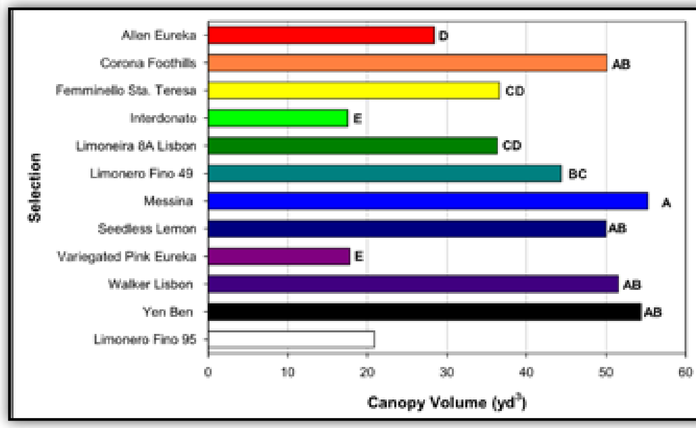


Figure 4. Canopy volume of 11 lemon selections on 3-1-10. Selections with bars not sharing similar letters are significantly different.

Using data collected on canopy volume, yield efficiency for the November 23, 2009, harvest was calculated for each of the selections ***(Figure 5)***. Yield efficiency is a measure of the productivity of the tree per volume of canopy, and it varied greatly among the selections. Because of a small canopy and intermediate yield, Interdonato had the greatest yield efficiency of over 5 lbs. of fruit per cubic yard, followed by Femminello Santa Teresa and Walker Lisbon with between 4 and 5 lbs. per yd3. Selections with intermediate yield efficiency included Allen Eureka, Corona Foothills and Limonero Fino 49 with 2.5 to 3.5 lbs. per yd3, while selections with intermediate to low yield efficiency (1 to 2.5 lbs. per yd3) included Limoneira 8A Lisbon and Yen Ben. Messina, Seedless Lemon and Variegated Pink Eureka had the lowest yield efficiency of less than one pound of fruit per cubic yard of canopy. No further measurements of canopy volume or yield efficiency were taken because as trees get larger, they become similar in size due to topping and hedging operations.

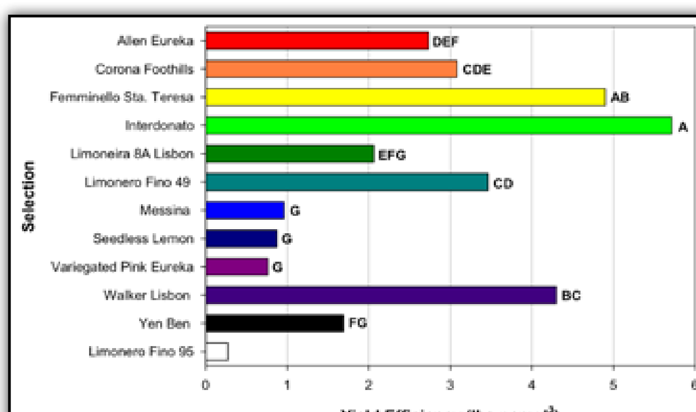


Figure 5. Yield efficiency of 12 lemon selections on 11-23-09. Selections with bars not sharing similar letters are significantly different.

YIELDS AND EARLINESS

The young trees had sufficient fruit production during the 2008-2009 season despite the freeze in January 2007 to conduct the first harvest on January 13, 2009. For this first harvest, members of our research groups strip-picked the fruit from each tree into boxes. Maintaining the fruit from each tree separately, we processed the entire yield through an automated, portable, single-line electronic eye sorter (manufactured by Aweta-Autoline, Inc., Reedley, California). This sorter provides fruit weight, fruit diameter, exterior peel blemish (fruit grade, expressed as fancy, choice and juice grade), peel color and fruit shape for each fruit.

Yield for all trees was low, which is typical of first-year yields for young lemon trees ***(Figure 6)***. There was no yield for the Limonero Fino 95, as this selection was planted one year later than the other selections. While all the yields were small due to the young tree age, both Walker Lisbon and Femminello Santa Teresa had considerably greater yields than the other selections and appeared to be precocious, a trait that would be valuable if it were to persist.

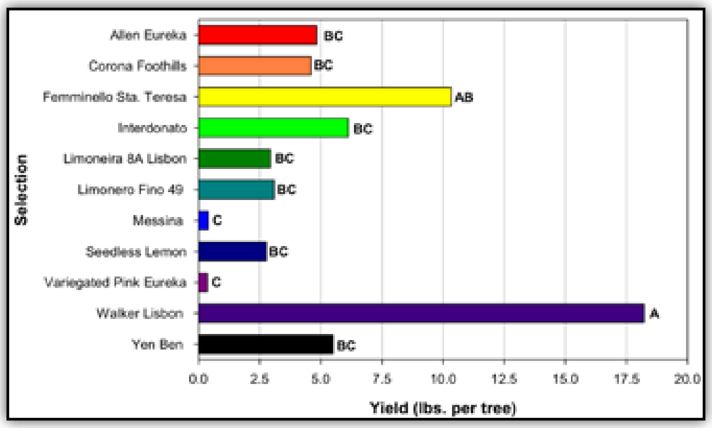


Figure 6. Yield of 11 lemon selections on 1-13-09. Selections with bars not sharing similar letters are significantly different.

The second annual harvest occurred November 23, 2009. We harvested the fruit in the same manner as before. Yields ranged from 158 lbs. per tree for Walker Lisbon to 11 lbs. per tree for the Variegated Pink Eureka ***(Figure 7)***. Walker, Femminello Santa Teresa, Corona Foothills and Limonero Fino 49 all had yields surpassing 100 lbs. per tree. The yields for these four selections make them precocious and clearly superior to the others tested for the 2009-10 season. Allen Eureka, Interdonato, Limoneira 8A Lisbon and Yen Ben all had yields between 50 and 100 lbs. per tree.

Messina, Seedless Lisbon and Variegated Pink Eureka all had yields of less than 50 lbs. per tree. The Limonero Fino 95 trees, which are one year younger than the others, had 5 lbs. of fruit per tree. This yield is similar to the previous yield for the other selections.

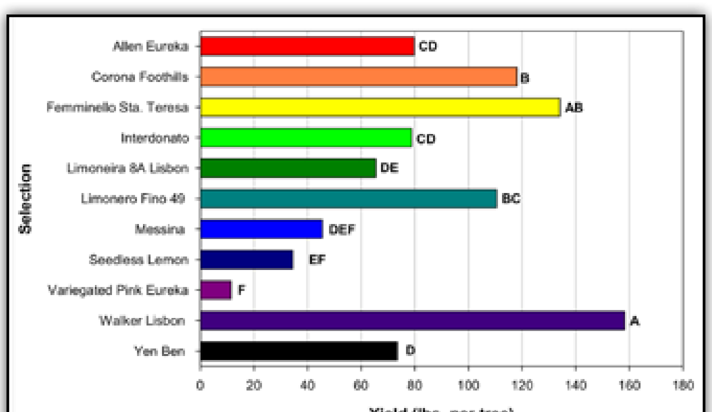


Figure 7. Yield of 11 lemon selections on 11-23-09. Selections with bars not sharing similar letters are significantly different.

Due to the large fruit load during the 2010-11 and third harvest season, we decided that it would be appropriate to conduct two harvests. The first harvest was November 2, 2010, and the second took place on January 7, 2011. Harvest dates were determined by officials at Richard Bagdasarian Inc. (RBI) based on market conditions. For the first harvest, fruit was ring picked with a #10 ring by pickers from Coachella Valley Citrus (CVC). For the second harvest, the pickers stripped the remaining fruit from the trees. We collected yield from each group of four trees by counting the numbers of whole and fractional picking sacks harvested from each group.

For the first harvest, Corona Foothills, Limonero Fino 49 and Walker Lisbon had the greatest yields, ranging from 200 to 250 lbs. per tree, while Seedless Lemon, Variegated Pink-Fleshed Eureka and Yen Ben had the least yield of less than 75 lbs. per tree ***(Figure 8)***. All the other selections had intermediate yields ranging from 120 to 160 lbs. per tree.

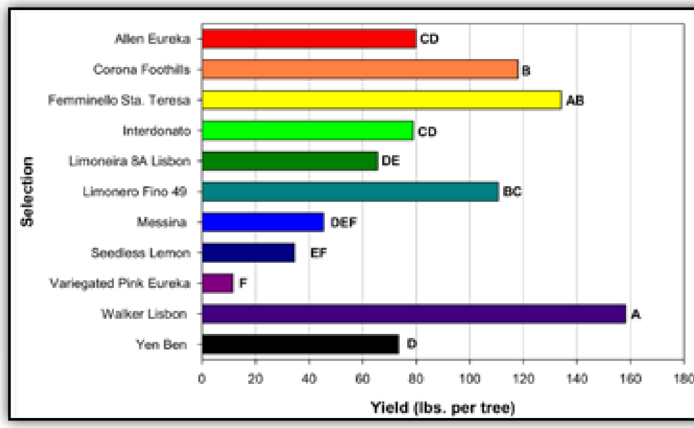


Figure 8. Yield of 12 lemon selections on 11-02-10. Selections with bars not sharing similar letters are significantly different.

For the second harvest of the 2010-11 season, Yen Ben, Walker Lisbon Limoneira 8A and Femminello Santa Teresa had the greatest yields ***(Figure 9)***, ranging from 250 to 270 lbs. per tree. Allen Eureka, Corona Foothills, Limonero Fino 49 and Seedless Lemon had yields ranging from 150 to 250 lbs. per tree. All other selections had yields less than 75 lbs. per tree.

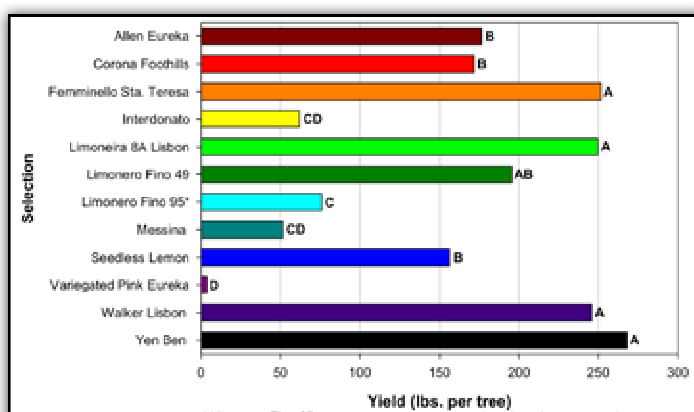


Figure 9. Yield of 12 lemon selections on 1-7-11. Selections with bars not sharing similar letters are significantly different.

Total yields for 2010-11 are shown in ***(Figure 10)***. Walker Lisbon had the greatest yield, about 450 lbs. per tree, for the season, but was not significantly greater than Corona Foothills, Femminello Santa Teresa and Limonero Fino 49, all of which had yields between 400 and 450 lbs. Limoneira 8A Lisbon yields, at about 375 lbs., were not significantly less than the aforementioned three selections, but significantly less than Walker Lisbon. Allen Eureka and Yen Ben had total yields of about 300 lbs. per tree, not significantly different than Limoneira 8A Lisbon. Yields of the remaining selections, Interdonato, Limonero Fino 95 (one year younger trees), Messina and Seedless Lemon, just surpassed 200 lbs. per tree, while Variegated Pink Eureka brought up the rear at about 60 lbs. per tree.

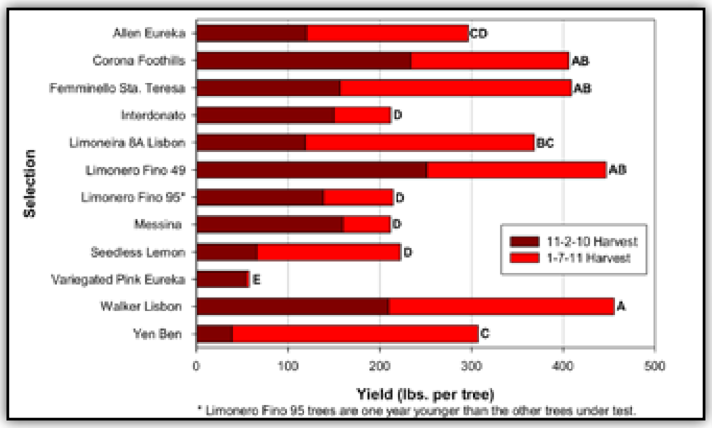


Figure 10. Total yield of 12 lemon selections for the 2010-11 season. Selections with bars not sharing similar letters are significantly different

If one were to assign the total yield of the standard Limoneira 8A Lisbon with a value of 100 percent, then the yields of the other selections, in descending order would be: Walker Lisbon - 124 percent; Limonero Fino 49 - 121 percent; Femminello Santa Teresa – 111 percent; Corona Foothills – 110 percent; Yen Ben – 83 percent; Allen Eureka – 80 percent; Seedless Lemon – 60 percent; Limonero Fino 95 – 58 percent; Interdonato – 57 percent; Messina – 57 percent; and Variegated Pink Eureka – 16 percent.

When lemon harvest begins for the season, large fruit are at a premium. Those that are larger are harvested earlier than those that are smaller. Earliness varied widely among the selections ***(Figure 11)***. The Variegated Pink Eureka had the earliest fruit for 2010-11, as virtually all its crop was picked at the first harvest. This may be because this selection is highly sought after in the markets, and fruit size is of lesser importance than for the other selections. For the others, Messina Interdonato, Limonero Fino 95, Corona Foothills and Limonero Fino 49 had more than 50 percent of the total yield picked at the first harvest. Walker Lisbon, Allen Eureka,

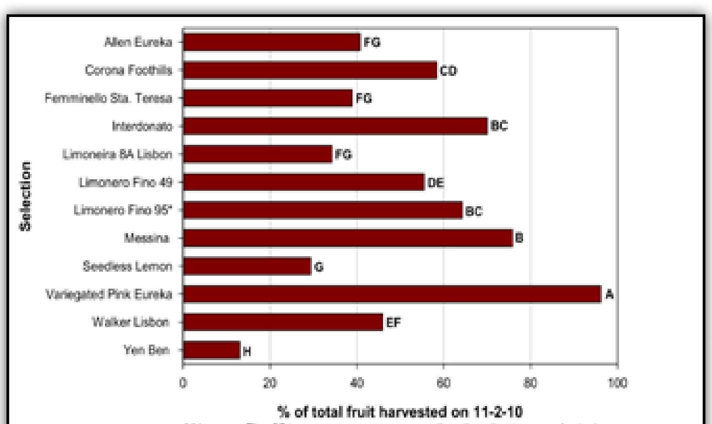


Figure 11. Earliness of 12 lemon selections for the 2010-11 season. Selections with bars not sharing similar letters are significantly different

Femminello Santa Teresa, Limoneira 8A Lisbon, Seedless Lemon and Yen Ben had less than 50 percent picked at the first harvest. Notably, the Yen Ben had the least fruit picked at the first harvest. For the desert grower, earliness is of prime importance, as lemon prices are greatest in the late summer and fall. They fall as the season progresses, typically reaching a low-point in February. Yen Ben might have more utility for growers in other areas of the state where early fruit size might not be as important.

For the fourth harvest season, 2011-12, we again harvested the trees using commercial pickers from RBI. The first harvest was on October 11, 2011, and the second was on December 16, 2011. For the first harvest, fruit was picked using a #9 ring, and the range of yields was similar to those of 2010-11, ranging from less than 50 to about 250 lbs. per tree ***(Figure 12)***. Walker Lisbon, Corona Foothills, Limonero Fino 49 and Femminello Santa Teresa had the greatest yields of at least 200 lbs. per tree; while Seedless, Yen Ben and Variegated Pink Eureka had the least yield of less than 75 lbs. per tree. All the other selections had yields ranging from about 120 to 160 lbs. per tree. For this harvest, yields for the four best performers ranged from 185 percent to 220 percent of that of the Limoneira 8A Lisbon standard.

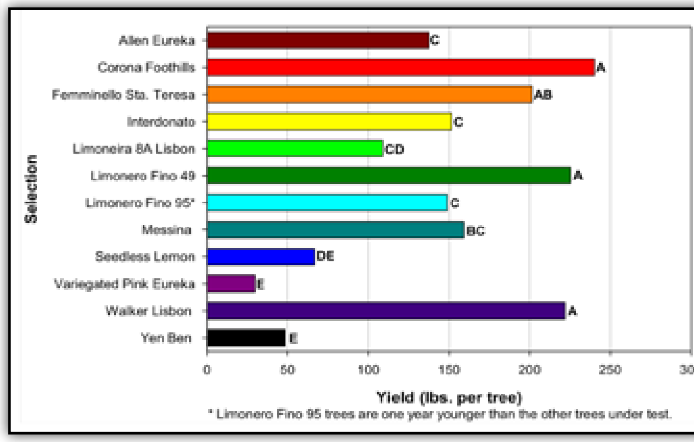


Figure 12. Yield of 12 lemon selections on 10-11-11. Selections with bars not sharing similar letters are significantly different.

For the second harvest of 2011-12, yield of Yen Ben was the greatest, surpassing 250 lbs. per tree. This is because fruit of this selection is small, and was not suitable for harvesting on the first harvest date. No other selection surpassed 150 lbs. per tree, while Variegated Pink Eureka, Limonero Fino 95 and Messina produced about 50 lbs. or less of lemons ***(Figure 13)***.

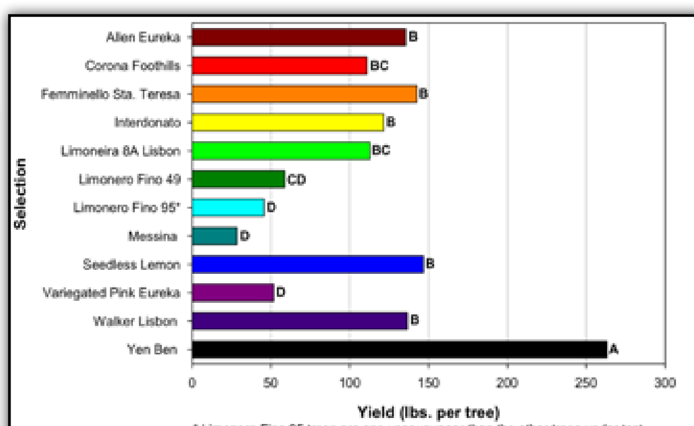


Figure 13. Yield of 12 lemon selections on 12-16-11. Selections with bars not sharing similar letters are significantly different.

Greatest yields for the entire 2011-12 season included Walker Lisbon, Corona Foothills, Femminello Santa Teresa Limonero Fino 49 and Yen Ben, while the lowest yielding selections were Variegated Pink Eureka, Limonero Fino 95 and Messina ***(Figure 14)***. These results are similar to those of the previous year. Again, if one were to assign the total yield of the standard Limoneira 8A Lisbon a value of 100 percent, then the yields of the other selections, in descending order, would be: Walker Lisbon – 162 percent; Corona Foothills – 158 percent; Femminello Santa Teresa – 155 percent; Yen Ben – 140 percent; Limonero Fino 49 – 128 percent; Allen Eureka – 123 percent; Interdonato – 123 percent; Seedless Lemon – 96 percent; Limonero Fino 95 – 88 percent; Messina – 85 percent; and Variegated Pink Eureka – 37 percent.

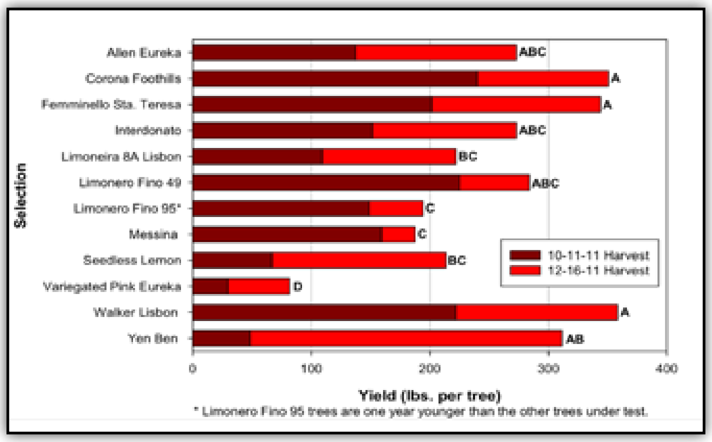


Figure 14. Total yield of 12 lemon selections for the 2011-12 season. Selections with bars not sharing similar letters are significantly different

Messina, Limonero Fino 49, Limonero Fino 95 and Corona Foothills all produced at least 66 percent of their total yield in the 2011-12 first harvest, as compared with only 50 percent first harvest yield for Limoneira 8A Lisbon ***(Figure 15)***. In contrast, Yen Ben produced 85 percent of its yield at the second harvest.

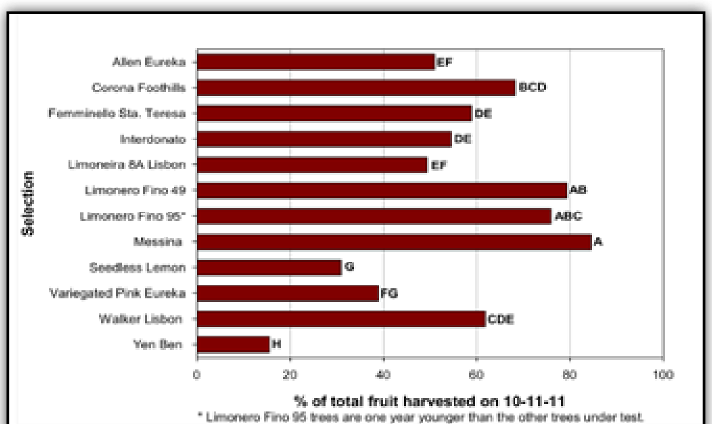


Figure 15. Earliness of 12 lemon selections for the 2011-12 season. Selections with bars not sharing similar letters are significantly different

Walker Lisbon, Corona Foothills, Limonero Fino 49 and Femminello Santa Teresa had the greatest cumulative yields for the 2009-10 through 2011-12 seasons ***(Figure 16)***. Seedless Lemon, Messina, Limonero Fino 95 and Variegated Pink Eureka had the least cumulative yield for the four harvest years reported here. Once again, if one were to assign the total yield of the standard Limoneira 8A Lisbon a value of 100 percent, then the yields of the other selections, in descending order, would be: Walker Lisbon – 148 percent; Femminello Santa Teresa – 134 percent; Corona Foothills – 133 percent; Limonero Fino 49 – 128 percent; Yen Ben – 105 percent; Allen Eureka – 99 percent; Interdonato – 87 percent; Seedless Lemon – 72 percent; Messina – 68 percent; Limonero Fino 95 – 63 percent; and Variegated Pink Eureka – 23 percent.

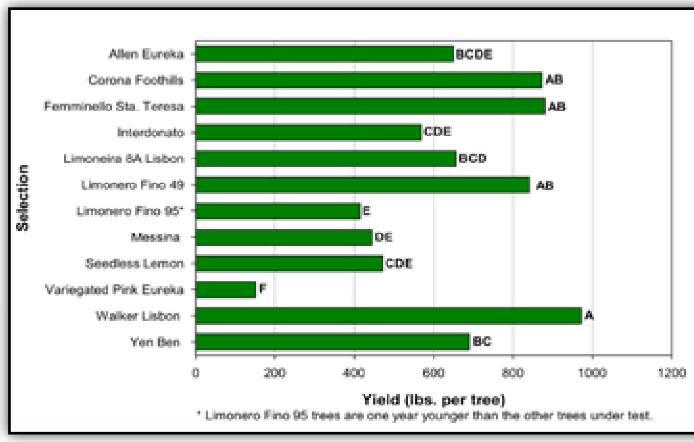


Figure 16. Cumulative yield of 12 lemon selections for the 2009-10 through 2011-12 seasons. Selections with bars not sharing similar letters are significantly different

SUMMARY OF THE ATTRIBUTES OF EACH SELECTION

Allen Eureka was in the middle-of-the-pack relative to annual and cumulative yield and was not particularly early in maturity. Trees of Allen Eureka were surprisingly susceptible to freeze damage.

Corona Foothills had the best first harvest yield per season when fruit was harvested twice and next-to-best total yield with excellent cumulative yield. Fruit of this selection was relatively early in maturity and tolerated the freeze better than most of the other selections.

Femminello Santa Teresa had a good first and second harvest yield when fruit was harvested twice with excellent total yield and cumulative yield. Fruit of this variety were not as early in maturity as some of the others, but relatively tolerant of freeze damage.

Interdonato was middle-of-the-pack relative to yield per harvest, total yield and cumulative yield. This variety was not particularly early in maturity, nor particularly tolerant of freeze damage, likely due to its citron parentage.

Limoneira 8A Lisbon had a comparatively poor first harvest yield, second harvest yield, total yield and cumulative yield, especially considering that it is a standard. Limoneira 8A fruit was not particularly early in maturity and was surprisingly susceptible to freeze damage.

Limonero Fino 49 had excellent first harvest yield, but unremarkable second harvest yield, excellent total yield and cumulative yield. After Messina, this selection was the earliest of the selections and tolerated the freeze better than most of the other selections.

Limonero Fino 95 trees were one year younger than the others. This selection was middle-of-the-pack in terms of first harvest yield, unremarkable second harvest yield, and relatively poor total yield and cumulative yield. Limonero Fino 95 had excellent fruit earliness. Tolerance to freezing temperatures for this selection is unknown.

Messina had a relatively poor first harvest yield, second harvest yield, total yield and cumulative yield. Messina was the earliest in maturity of all the selections because fruit size is large. We estimate that fruit of Messina could be harvested as early as late July in the desert. Messina was quite tolerant of the freeze.

Seedless had a poor first harvest yield, and an unremarkable second harvest yield, total yield and cumulative yield. This selection was not early in maturity but had a relatively good tolerance to freezing.

Variegated Pink Eureka had poor first harvest yield, second harvest yield, poor total yield and cumulative yield. This selection was not early in maturity and had poor tolerance to freezing temperatures.

Walker Lisbon had excellent first harvest yield and best total yield. This selection had the best cumulative yield to date. Fruit were relatively early in maturity and trees demonstrated tolerance to freezing temperatures.

Yen Ben had poor first harvest yield, and excellent second harvest yield, good total yield and cumulative yield. This variety does not appear to perform as an early selection in the California desert, because the fruit size is small. Among all the selections, this was the most tolerant to the freeze.

THE FUTURE

This replicated lemon trial for the desert is designed to be maintained for at least 10 years so that yield and quality of production can be evaluated. Information obtained from this trial will help to further define performance of these selected varieties in the California desert.

As part of the Integrated Citrus Breeding and Evaluation for California Core Program, we have initiated plans to add lemon selections to our multi-location replicated trials based on the results of this desert lemon trial. These multi-location trials, which currently include trees of selected Satsuma and Clementine varieties and selections from the UCR breeding program, were designed to systematically evaluate the most promising new scion cultivars. The five field plot locations represent the main citrus production zones in California (Exeter, Riverside, Arvin, Santa Paula and Thermal). The first sets of multi-location replicated trials were planted in the summer of 2012 at four locations appropriate for mandarins (Exeter, Riverside, Arvin and Santa Paula). The main purpose of these is to compare selected mandarin varieties against three releases from the UCR breeding program including Daisy SL, Fairchild LS and Nova LS. These multi-replicated scion trials will make it possible for us to evaluate small numbers of varieties on three established rootstocks for tree performance, including tree health and size, fruit quality, yield and other packout data. Our intention is to collect additional data on post-harvest and consumer acceptance in the later years of the 10-year trial.

References

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Acknowledgements

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Glenn Wright is an Associate Professor and Tree Fruit Specialist at the University of Arizona – Yuma Agriculture Center. Vince Samons is a Principal Superintendent of Agricultural Operations at the University of California, Riverside. Tracy Kahn is a Principal Museum Scientist in the Department of Botany and Plant Sciences, and curator of the University of California, Riverside Citrus Variety Collection.

Project 5200-201

UPDATE ON THE CITRUS CLONAL PROTECTION PROGRAM
OCTOBER 2008–SEPTEMBER 2013

Georgios Vidalakis, Ph.D.

The potential problems resulting from the introduction of pests into a country, state or local geographic area where these pests or pathogens are absent cannot be overemphasized. Likewise, the need for pathogen-tested citrus propagative materials is recognized as basic to the establishment and maintenance of a viable, sustainable and competitive citrus industry. The presence of graft-transmissible pathogens such as viruses, viroids or bacteria in citrus propagative materials can be deleterious to tree survival and fruit production. These pathogens can be easily distributed by way of infected plants, budwood or vectors to areas free of them, where they become a potential hazard to existing and future plantings.

Realizing that the principal means of spread of most citrus viral diseases in California occurred through propagation using infected nursery stock, a comprehensive program was initiated to produce, maintain and distribute budwood tested negative for graft-transmissible diseases. This article reports on the Citrus Clonal Protection Program (CCPP) activities and exciting, recent updates.

FUNDING THE CCPP

The CCPP was established more than 50 years ago under the name Citrus Variety Improvement Program. Today, it stands as a cooperative program with the University of California, Riverside (UCR), the California Department of Food and Agriculture (CDFA), the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS) and the California citrus industry represented by the California Citrus Nursery Board (CCNB) and the Citrus Research Board (CRB).

Since 2009, the CCPP has been a part of the National Clean Plant Network (NCPN) for specialty crops. The CCPP has been a core program of the CRB since the establishment of the marketing order in 1968. As such, the CRB has been the main funding source for core day-to-day CCPP activities. USDA-NCPN funding has supplemented CCPP activities in the past few years, including funding for facilities and equipment upgrades. The CDFA and the CCNB have been providing CCPP with funding to execute the citrus nursery registration program for budwood and seed tree sources. UCR is supporting CCPP with infrastructure and facilities and is covering all related indirect costs.

Finally, in an effort to support and enhance the CCPP future, UCR established the "Citrus Clonal Protection Foundation" and welcomes all donations *(for your tax deductible gift, visit http://cnas.ucr.edu/supporting_cnas/, click on "Make a donation now!" and type "clonal" in the search box).*

The purpose of the CCPP is to provide a safe mechanism for the introduction of citrus varieties from any citrus-growing area of the world into California for research, variety improvement, or use by the commercial industry of the state. This mechanism includes disease diagnosis and pathogen elimination, followed by maintenance and distribution of true to type, primary citrus propagative material of the important fruit and rootstock varieties.

OBJECTIVES

The CCPP is the basic element of a long-term, multi-level program focusing on avoiding or restricting the spread of graft-transmissible pathogens of citrus in support of a profitable, competitive and sustainable citrus industry. The CCPP has two primary objectives:

1. Provide a safe mechanism for the introduction of citrus germplasm into California from any citrus-growing area of the world for purposes of research, variety improvement, or for use by the California commercial industry.
2. Maintain primary sources of disease-tested and true-to-type sources of citrus propagative material of all important fruit and rootstock varieties in protected foundation blocks for budwood distribution to the California citrus industry and citrus researchers.

The CCPP fulfills its primary objectives by performing the following activities/functions:

1. Introduction of propagative materials of citrus varieties in the state of California
2. Testing of the introduced propagative materials for graft-transmissible diseases/pathogens
3. Elimination of any disease causing pathogens from the propagative material
4. Maintenance and continuous disease testing of the established trees sources for citrus propagative materials
5. Distribution of true-to-type citrus propagative material for the needs of the California citrus industry and researchers
6. Extension of citrus pathogens and varieties related information for the education of the industry and the public

1. INTRODUCTION OF PROPAGATIVE MATERIALS OF CITRUS VARIETIES IN THE STATE OF CALIFORNIA.

Between October 2008 and September 2013, the CCPP received 58 citrus introductions (IPPN 438-495) at its renovated Rubidoux Quarantine Facilities ***(Figure 1)***. Sources of 49 introductions (24 public domain and 25 proprietary) were successfully established, and disease testing and therapy procedures were initiated.

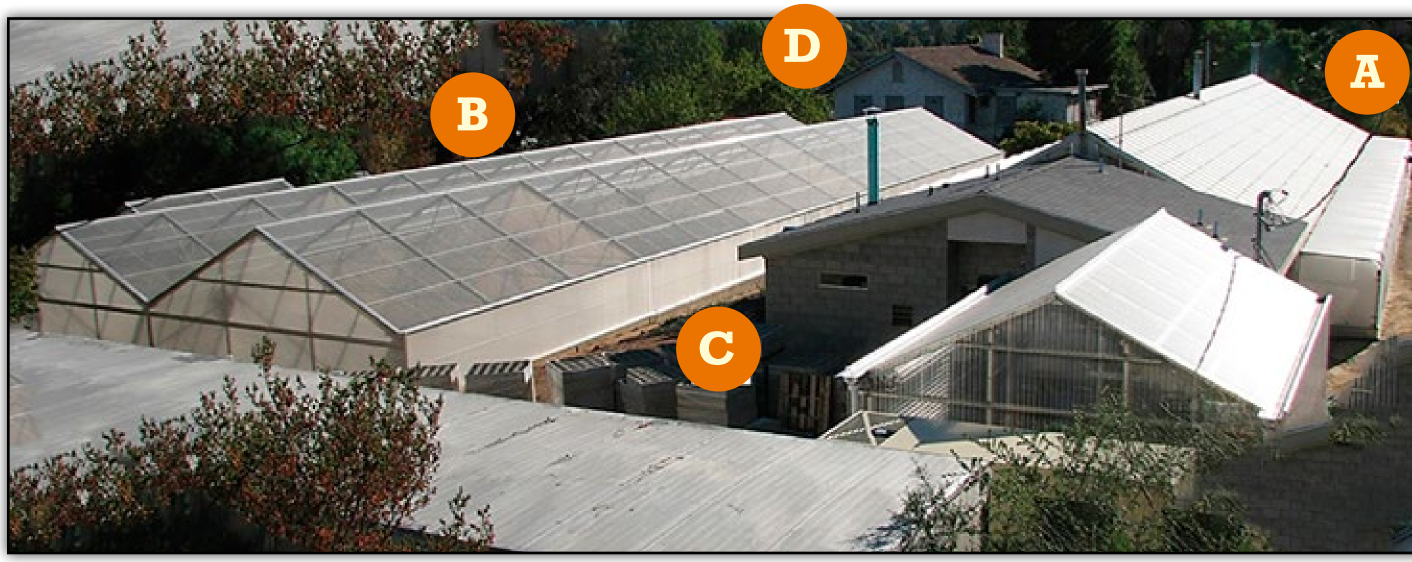

Figure 1. Panoramic view of the CCPP Rubidoux Quarantine Facilities at Riverside, California. Quarantine 5,000 sq. ft. glasshouse (A) and 9,000 sq. ft. screenhouse (B) for biological indexing and maintenance of citrus germplasm, respectively. The original structures were built between 1959-62 and were fully renovated between 2010-12. The 750 sq. ft. Delfino Family Plant Laboratory (C) for citrus therapy and diagnostics was constructed in 2010-11. The original Citrus Experiment Station (1907), the precursor of UC Riverside, houses the CCPP offices (D) since October 2013.

2 AND 3. DISEASE TESTING AND PATHOGEN ELIMINATION.

Also between October 2008 and September 2013, the CCPP performed 65 Variety Index tests of public domain and proprietary varieties (VI 777-841), as well as citrus experimental materials (e.g. citrus breeding program, positive disease controls). As a result, 50 citrus introductions became available to the citrus industry and researchers. The public domain citrus varieties ***(Table 1)*** have been deposited in the Lindcove Research and Extension Center (LREC) CCPP Foundation Block ***(Figure 2)***. For budwood availability, licensed varieties and orders, visit *http://ccpp.ucr.edu/budwood/budwood.php*.

Figure 2. Panoramic view of the CCPP Foundation Block Operations in the Lindcove Research and Extension Center, Exeter, California. The first 40,000 sq. ft. screenhouse (A) was constructed in1998-99, the second 30,000 sq. ft. screenhouse (B) was completed in 2010, and the 5,700 sq. ft. positive pressure greenhouse (C) was completed in 2011.

Table 1. Public domain citrus varieties completed VI index between October 2008 and September 2013

VI	Variety	Origin
777	Primasole	IVIA, Spain
778	Fukumoto navel IVIA 364	IVIA, Spain
779	Miyamoto Satsuma	IVIA, Spain
780	Nichigan Ichi Go Satsuma	IVIA, Spain
781	Iwasaki Satsuma	IVIA, Spain
785	Kinnow LS Mandarin	CPB, UCR
792	Bitters C-22 trifoliate hybrid	CPB, UCR
793	Carpenter C-54 trifoliate hybrid	CPB, UCR
794	Furr C-57 trifoliate hybrid	CPB, UCR
795	Rush navel	Porterville, CA
797	Ueno Satsuma IVIA 406	IVIA, Spain
798	Maltaise Demi Sanguine Blood Orange IVIA 393	IVIA, Spain
799	Louisiana Early Satsuma (LA Early)	LSU, Louisiana
803	Femminello Siracusano White Flower Lemon	Italy
804	Kao Nun Pummelo	Thailand
805	Fukushu Kumquat	USDA, Riverside
806	C. sunki	USDA, Riverside
825	Early St. Ann Satsuma	LSU, Louisiana
835	Santa Teresa Lemon (W&N)	Bakersfield, CA
836	Fukushu Kumquat (W&N)	Bakersfield, CA

Table 1. The VI testing is the most comprehensive index that a citrus introduction undergoes during quarantine in CCPP. The VI Index includes graft-inoculation of a number of different citrus species/plant indicators maintained under controlled environmental conditions that will express disease symptoms if a pathogen is present.

IVIA: Instituto Valenciano de Investigaciones Agrarias, CPB: Citrus Breeding Program-University California Riverside, LSU: Louisiana State University, and USDA: United States Department of Agriculture-National Clonal Germplasm Repository for Citrus and Dates.

Table 2. Citrus indicators and growing conditions used in the CCPP VI index

CITRUS SPECIES	COMMON NAME [1]	TEST CONDITIONS: NO. OF INOCULATED PLANTS	TEST CONDITIONS: TEMPERATURE (°F)
Citrus aurantifolia	West Indian/Mexican Lime	8	~75
C. sinensis	Madam Vinus or Pineapple Sweet Orange	4	~75
C. reticulata x C. sinensis	Dweet Tangor	4	~75
C. aurantium	Standard Sour Orange	4	~75
C. excelsa	*Citrus excelsa*	4	~75
C. medica	'Etrog' Citron[2]	2	~75
C. sinensis x Poncirus trifoliata propagated on *C. jambhiri* rootstock	Rusk Citrange propagated on Rough lemon rootstock	2	~75
Fortunella sp. propagated on Citrange (*C. sinensis x Poncirus trifoliata*) rootstock	Kumquat propagated on Citrange rootstock	2	~75
C. medica propagated on *C. jambhiri* rootstock	Etrog Citron[2] propagated on Rough lemon rootstock	2	~90

[1] All indicators are seedlings unless otherwise indicated
[2] 'Etrog' Citron Arizona 861-S-1

Figure 3. Various symptomatologies of citrus indicators expressed under specific environmental conditions during VI index at the CCPP Rubidoux Quarantine Facilities. Healthy citrus (A), severe leaf epinasty and stunting (B), mid vein necrosis and leaf bending (C), leaf cupping (D), shock dieback (E), and lethal yellows (F).

Table 3. Laboratory tests used in the CCPP VI index

TEST NAME	ACRONYM	TYPE	TISSUE TESTED
Enzyme Linked Immunosorbent Assay	ELISA	Immunological	Source plant
Reverse Transcription-Quantitative Polymerase Chain Reaction	RT-qPCR	Molecular	
Polymerase Chain Reaction	PCR	Molecular	
Double-stranded RNA	dsRNA	Molecular	
Culture and Microscopic Observation	N/A	Biological	
Sequential Polyacrylamide Gel Electrophoresis	sPAGE	Molecular	'Etrog' Citron[1]
Hybridization	N/A	Molecular	
Reverse Transcription-Quantitative Polymerase Chain Reaction	RT-qPCR	Molecular	Kumquat[2]

[1] 'Etrog' Citron Arizona 861-S-1 propagated on Rough lemon rootstock grown at ~90°F for the bioamplification of citrus viroids.
[2] Kumquat propagated on Citrange rootstock grown at ~75°F for the bioamplification of Citrus leaf blotch virus.

Additional laboratory tests, such as sPAGE, RT-qPCR, and microscopic observation of cultures are also a part of the VI index ***(Table 3 and Figure 4)***. These tests are performed at the third facility of the CCPP, the Citrus Diagnostic Laboratory on the University of California, Riverside Campus, located four miles east of the Rubidoux Quarantine Facilities and 260 miles south of the Foundation and Evaluation Block Operations at the LREC.

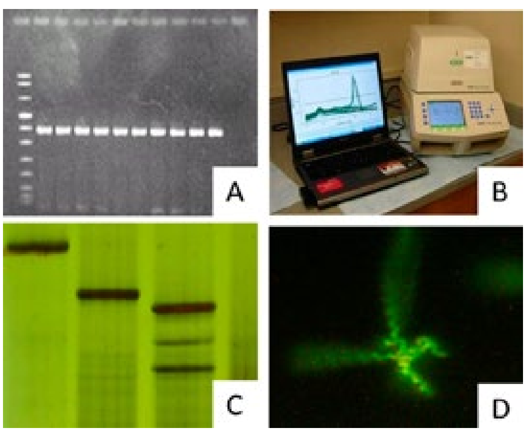

*Figure 4. Examples of laboratory tests used in the VI index at the CCPP Citrus Diagnostic Laboratory on the University of California, Riverside Campus. **(A)** Polymerase chain reaction (PCR) products visualized in gel electrophoresis **(B)** Real time quantitative PCR equipment; results are visualized real time on a computer. No gel electrophoresis is required. **(C)** Sequential polyacrylamide gel electrophoresis for universal detection of subviral pathogens such as viroids. **(D)** Microscopic observation of Spiroplasma citri, the causal agent of citrus stubborn disease.*

4. MAINTENANCE AND CONTINUOUS DISEASE TESTING OF THE ESTABLISHED TREES SOURCES FOR CITRUS PROPAGATIVE MATERIALS.

When new citrus introductions have successfully completed the VI index and are released from the State and Federal quarantines, they are then moved to the CCPP Foundation Block at LREC for budwood distribution and horticultural evaluation. The movement of citrus germplasm from the Riverside Quarantine Facilities to the LREC Foundation Block is done in accordance with the CDFA and USDA regulations for diseases and pest quarantines (Permit No. QC 1353: Permit For The Intrastate Movement Of ACP/CTV/GWSS Host Propagative Material/Plants).

The CCPP Foundation Block trees are carefully evaluated several times during each year for trueness-to-type, fruit quality and overall health and growth characteristics. All CCPP trees at LREC are tested annually for life for tristeza. Trees registered for budwood distribution receive additional tests as required by the CDFA regulations (Section 3701, Citrus Nursery Stock Pest Cleanliness Program) ***(Table 4)***.

Table 4. Testing protocol for CCPP Foundation Block trees registered for budwood distribution

DISEASE[1]	PATHOGEN	TEST	FREQUENCY OF TEST: Prior to 2010	FREQUENCY OF TEST: After 2010
Tristeza, Quick Decline, Stem pitting, and Seedling yellows	*Citrus tristeza virus* (CTV)	Mexican lime[2], ELISA[3, 4], and/or RT-qPCR[11]	Three to four times per year.	
Exocortis, Cachexia, and various citrus growth abnormalities and symptomatologies related to citrus viroids.	*Citrus exocortis viroid* (CEVd) *Hop stunt viroid* (HSVd) *Citrus bent leaf viroid* (CBLVd) *Citrus dwarfing viroid* (CDVd) *Citrus bark cracking viroid* (CBCVd) *Citrus viroid V* (CVd-V) *Citrus viroid VI* (CVd-VI)	Etrog citron[5]/RL[6, 7] sPAGE[8, 9] and/or IH[10]	Every three years	
Psorosis A & B (syn. ring spot)	*Citrus psorosis virus* (CPsV)	Sweet orange or Dweet tangor	Every six years	Every five years
Huanglongbing	*Candidatus* Liberibacter asiaticus	RT-qPCR[11] and observation of source plant	N/A	Annually

[1] For the diseases listed here, the CCPP has positive controls in planta and/or in vitro to perform complete and definitive testing.
[2] Biological indexing is performed on seedlings of the indicated species at cool temperatures (~75°F) unless otherwise described.
[3] Enzyme Linked Immunosorbent Assay.
[4] All laboratory testing is performed on the source plant unless otherwise described.
[5] Etrog citron Arizona 861-S-1.
[6] Rough lemon rootstock.
[7] Indicator is growing under warm conditions (~90°F).
[8] Sequential Polyacrylamide Gel Electrophoresis.
[9] sPAGE is performed on Etrog citron Arizona 861-S-1 tissue grown under warm conditions.
[10] Imprint Hybridization
[11] Reverse Transcription-Quantitative Polymerase Chain Reaction.

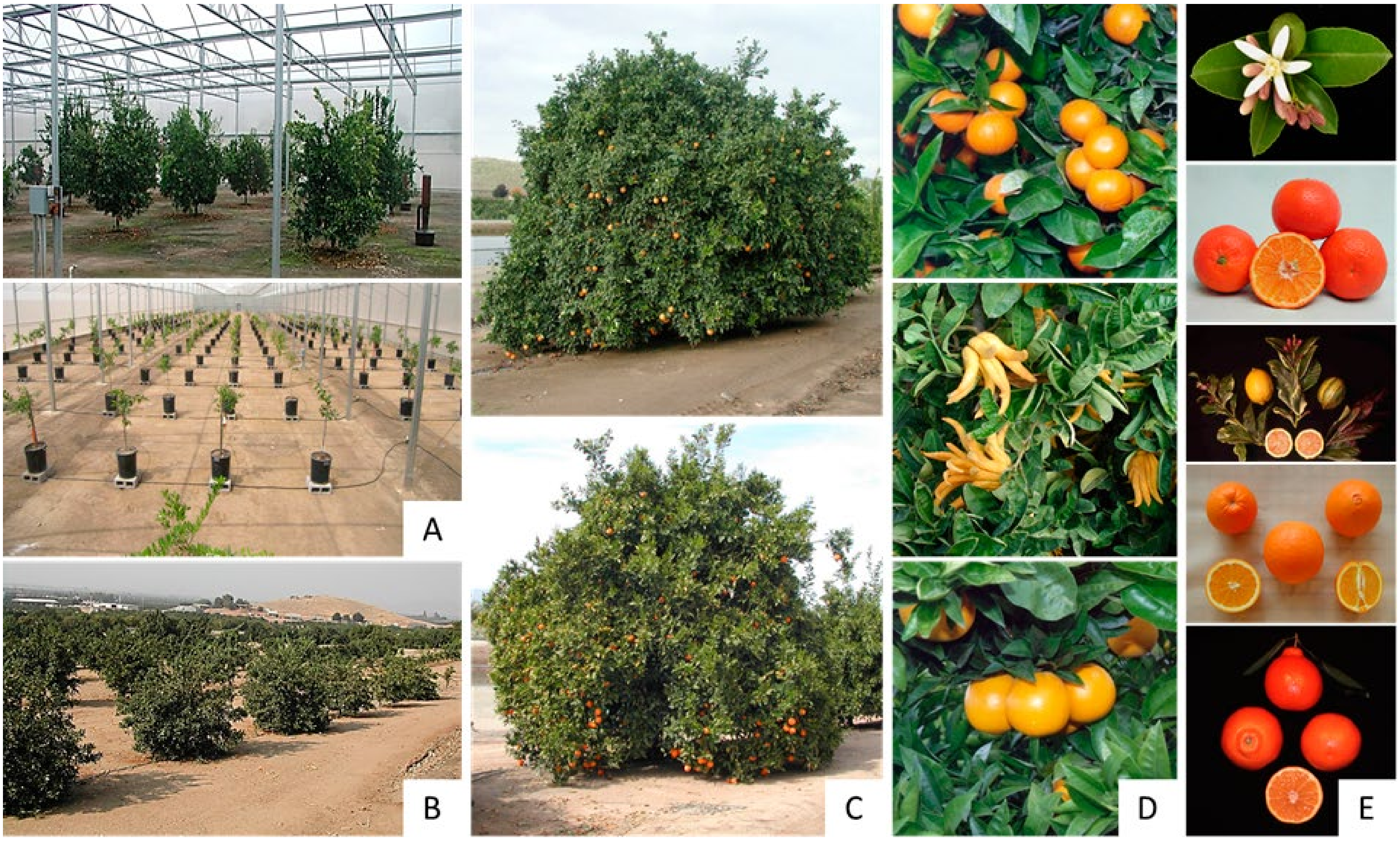

Figure 5. Plant materials at the CCPP Foundation Block Operations at Lindcove. Budwood from the in-ground and container-planted budwood source trees of the Protected Foundation Block (A) is used to propagate field trees for the Foundation and Evaluation Block (B). Individual trees are carefully examined for trueness-to-type, including overall health and growth (C) fruit set/crop (D), and laboratory fruit evaluation (E).

In 2008, the overall number of tristeza positive trees at LREC significantly increased, including eight trees in the CCPP Foundation-Evaluation Block. Subsequently, after almost 50 years of budwood distribution from the outdoor foundation block, the CCPP Protected Foundation Block became the primary source of budwood.

In response, the Citrus Research Board invested heavily in upgrades for the CCPP Protected Foundation Block operations. The size of the Protected Foundation Blocks has nearly doubled, and a positive pressure greenhouse complex has been constructed ***(Figure 2)***. Because of these expansions, the number of budwood source and trueness-to-type evaluation trees has steadily increased ***(Figure 5)***.

Conversely, the number of CCPP personnel has been reduced with the retirement of three experienced CCPP and LREC staff, namely John Bash, Jim Diaz and Raul Gonzales, whom we thank for many years of service.

In 2012, and after coordinated actions of the industry and the University of California Agricultural Natural Resources, a new CCPP position was created specifically to meet the needs of the expanded CCPP Foundation Operations at the LREC. This position was filled by Dr. Rock Christiano, an experienced scientist with a strong hands-on citrus background from his studies and family in his native Brazil. This position has transformed the CCPP Foundation Block services with the on-site management and continuing improvement of the foundation block protocols, as well as the increased frequency of budwood distribution. Rock Christiano at Lindcove and Greg Greer at Riverside manage the day-to-day budwood distribution and quarantine operations of the CCPP.

5. Distribution of True-to-Type Citrus Propagative Material for the Needs of the California Citrus Industry and Researchers.

Since 1961, when the first tree was planted in the outdoor foundation block, budwood distributions or cuts, have been performed regularly. Budwood cuts have been performed typically in January, June and September following the citrus flush patterns in the San Joaquin Valley and the business-propagation cycles of the citrus nurseries.

In recent years, however, the threats from serious citrus pests and pathogens (e.g. Asian citrus psyllid and Huanglongbing) have transformed citrus nursery operations in California. Since May 2010, mandatory CDFA regulations require that budwood source plants and increase blocks must be grown under protective structures (Section 3701, Citrus Nursery Stock Pest Cleanliness Program).

In addition, USDA and CDFA quarantines prohibit the movement of citrus nursery stock unless it is produced within protective structures and moved under a certificate of compliance (CFR §301.76 Animal and Plant Health Inspection Service 7 Citrus Can-

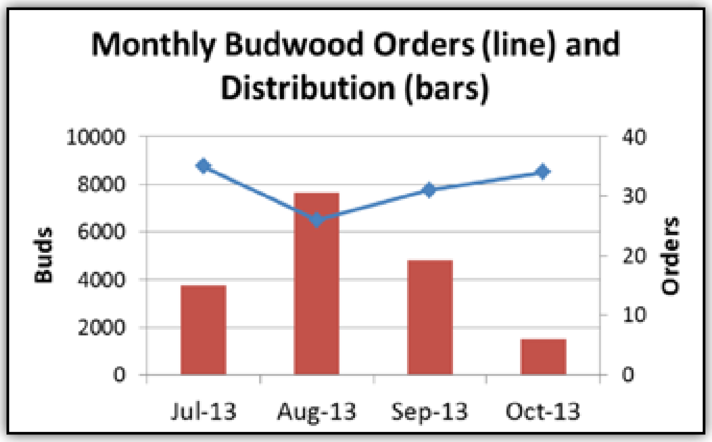

Figure 6. Preliminary data of the first four months of the CCPP monthly budwood distribution scheme.

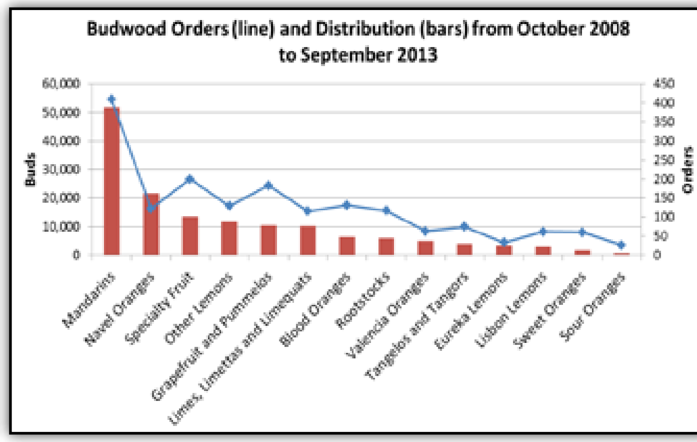

Figure 7. Orders (line) per budwood category and distribution (bars) from October 2008 to September 2013.

ker, Citrus Greening, and Asian Citrus Psyllid; Interstate Movement of Regulated Nursery Stock). This transition to citrus nursery production in protective structures has affected the budwood demand dynamics. Therefore, since July 2013, the CCPP is not offering budwood three times a year, but monthly *(http://ccpp.ucr.edu/budwood/cutdates.php)* ***(Figure 6)***.

Between October 2008 and September 2013, the CCPP executed 581 orders and distributed 149,095 buds of 290 different varieties. Mandarins represented approximately 35 percent of the budwood demand. Navel oranges, lemons and specialty varieties represented 14 percent, 12 percent, and 9 percent of the distributed budwood, respectively ***(Figure 7)***. The top five varieties from selected budwood categories with the highest demand are presented in ***(Figure 8)***.

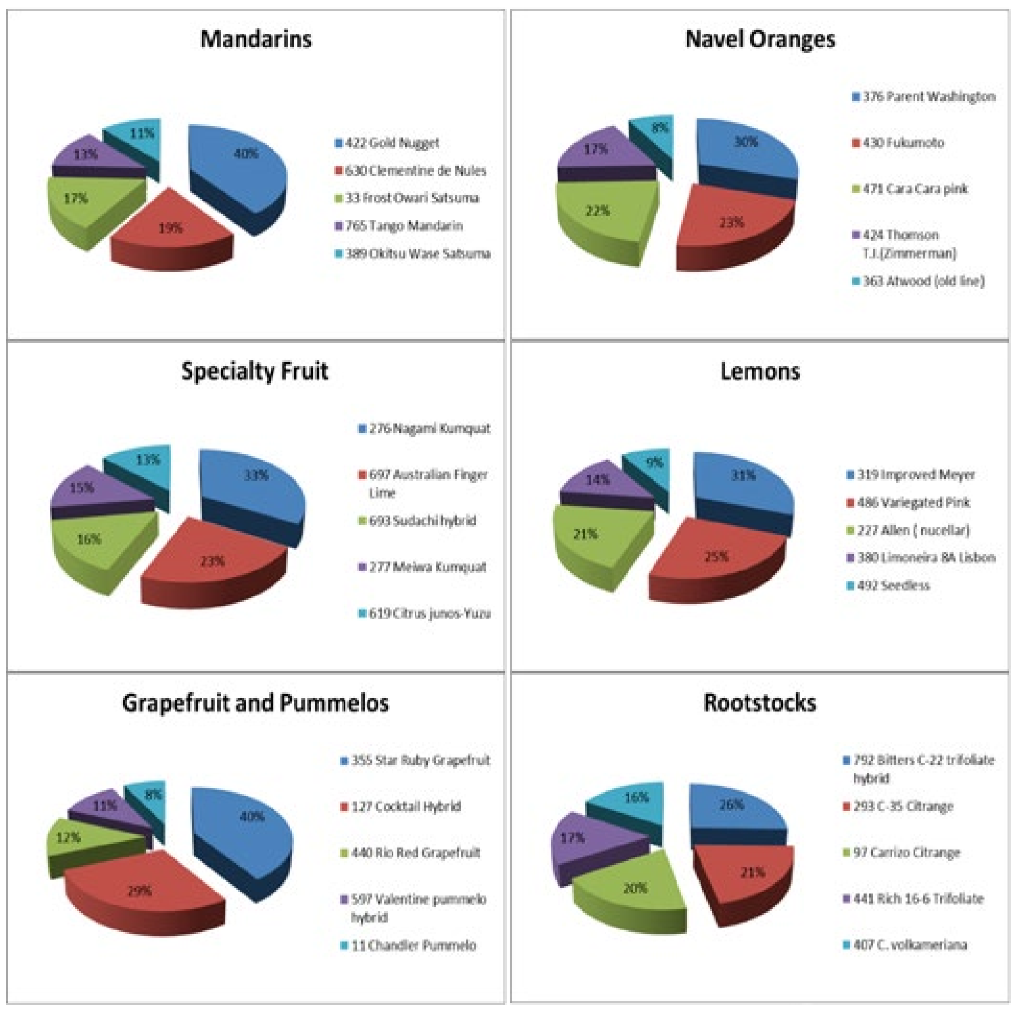

Figure 8. Top five varieties of different budwood categories with highest demand from October 2008 to September 2013.

Figure 9. CCPP annual Foundation Block Field Day or Walkthrough (A) and CRB booth at the World Ag Expo with CCPP Foundation Block fruit display (B).

6. EXTENSION OF CITRUS PATHOGENS AND VARIETIES RELATED INFORMATION FOR THE EDUCATION OF THE INDUSTRY AND THE PUBLIC.

The CCPP collects fruit evaluation data from field propagations during two- to three-week intervals just prior to and during fruit maturity. All fruit evaluation data are presented in the CCPP web site at: *http://ccpp.ucr.edu/variety/index.html*.

The CCPP has hosted annual Foundation Block Field Days, which have been very well attended. During this field day, the entire foundation collection, cultivar bank and experimental plots have been available for viewing and fruit tasting. Fruit from CCPP trees also has been made available for the annual Lindcove fruit display for growers and homeowners, the World Ag Expo, the Citrus Mutual Citrus Showcase, and other events of this type ***(Figure 9)***.

The CCPP is dedicated to helping maintain California in the forefront of high-quality citrus nursery and fruit production. The continued availability of pathogen-tested propagation materials from the CCPP is essential for the continued protection and viability of California's citrus industry. We at the CCPP wish to thank the CRB for its continuous support.

CRB Project 6100

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IMIDACLOPRID REPORT

Frank Byrne, Elizabeth Grafton-Cardwell and Joseph Morse

Imidacloprid is an important insecticide used for the control of Asian citrus psyllid (ACP) in both commercial (nursery and field citrus) and residential settings. It is a systemic neonicotinoid insecticide that is applied to the soil or through irrigation systems and taken up through the roots, where it is subsequently distributed throughout the tree via its water conducting system. We have conducted extensive research on the impacts of soil type and irrigation on the uptake of imidacloprid. In addition, we have evaluated different treatment timings and application rates so that uptake can be optimized to protect the trees during flush periods, when psyllids are most attracted to the young leaf tissue for feeding and oviposition. A third area of study, and the focus of this report, revolves around the impacts of imidacloprid on honeybees. Samples of nectar were collected from multiple sites within California citrus-growing areas, and residues of imidacloprid and two key metabolites were quantified. These data have been submitted to the California Department of Pesticide Regulation to assist them in their review of the safety of neonicotinoid insecticides to honeybees.

BACKGROUND

In recent years, there has been considerable controversy surrounding the likely impacts of imidacloprid, and other neonicotinoids, on non-target organisms. In particular, neonicotinoids are being investigated as a potential contributing factor to the decline in honeybee populations.

In 2008, the California Department of Pesticide Regulation (DPR) received an adverse effects disclosure pursuant to the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) section 6(a)(2) and Food and Agricultural Code section 12825.5 regarding imidacloprid. The disclosure included 12 residue and two combination residue studies involving imidacloprid use on a number of ornamental plants. The DPR's evaluation of these data determined that (1) imidacloprid was toxic to honeybees, (2) residues as high as 4 mg kg-1 were measured in the leaves and blossoms of some plants, and (3) stable residues of imidacloprid could persist within plants for more than 500 days.

Based on these conclusions, the DPR, in conjunction with the United States Environmental Protection Agency (EPA), initiated a re-evaluation of imidacloprid and other neonicotinoids. Re-evaluation is a tool utilized by the DPR that requires pesticide registrants to submit information and/or data to determine the nature and extent of a hazard and strategies that could be used to mitigate those hazards. One of the principal concerns of the DPR was that minimal data were available to determine impacts to honeybees from imidacloprid treatments on various crops.

Imidacloprid is highly mobile within vascular plants and has prolonged persistence following application as a seed treatment, soil drench or trunk injection. Because of its systemic distribution, it has the potential to enter nectar and pollen tissues within flowering plants, thereby raising concerns that it is a potential threat to pollinators and other beneficial insects that use those resources as food. In citrus, the close timing between the development of the spring flush and bloom poses an interesting question regarding the likelihood that imidacloprid residues could enter nectar and pollen producing tissues, even if applications were made well in advance of bloom. An additional question arises as to what extent residues from applications targeting the fall foliar flush on citrus could persist through to the following spring bloom.

We recently published a paper in the *Pest Management Science* journal dealing with the likely exposure limits of honeybees foraging on commercial trees treated with systemic imidacloprid [Byrne et al., 2013: Determination of exposure levels of honey bees foraging on flowers of mature citrus trees previously treated with imidacloprid. Pest Management Science doi: 10.1002/ps.3596]. We presented data comparing imidacloprid residues in nectar sampled from mature citrus trees treated in spring versus fall. In addition, we conducted controlled experiments where honeybees were confined to foraging on treated citrus trees within netted tunnel enclosures. Imidacloprid residues were compared in nectar sampled from the flowers, the honeybees as they returned from foraging trips, and from freshly stored nectar within their hives.

Similar experiments were conducted with honeybees foraging under normal open-field conditions. We showed that imidacloprid, imidacloprid olefin and 5-hydroxy imidacloprid were detected in nectar and pollen sampled from the flowers of citrus trees treated with imidacloprid up to 232 days prior to bloom. In the tunnel studies, imidacloprid residues in nectar extracted from flowers and from bee crops were similar (<10 ppb) and below the established no-observed-effects limit; however, the residue levels were about threefold higher in nectar sampled from comb. Concentrations of imidacloprid in nectar were higher in trees treated with higher application rates.

The data from these studies were presented to the DPR as part of their data call-in, and following their review, they requested additional residue studies for citrus trees growing in different locations within California. With guidance from the DPR, we chose sites with different soil types and collected samples from trees that had been treated in successive years with imidacloprid to determine whether residues could accumulate with repeated use. The data from those additional residue studies are presented and discussed in this report.

No Observable Effect Level (NOEL)

The term "No Observable Effect Level", or NOEL, is used by the EPA to define a concentration or dose of insecticide where no effect is observed in test animals. A 20 ppb NOEL for imidacloprid against honeybees was originally derived from a 39-day feeding study in which honeybees were fed different imidacloprid concentrations in 50 percent sucrose solutions. At 20 ppb, the highest concentration tested during that study, the authors concluded that there were no adverse impacts of imidacloprid on the following tested parameters:

- **Mortality**
- **Feeding activity**
- **Wax/comb production**
- **Breeding performance**
- **Colony vitality**

Given that the relative density of a 50 percent sucrose solution is about 1.2, this equates to an adjusted NOEL of 24 ppb imidacloprid to which the data in this report can be compared because they are based on volumetric (ng ml-1) rather than gravimetric (ng g-1) determinations. It is also important to note that the original NOEL was derived for residues of imidacloprid alone. Given the toxic nature of some key imidacloprid metabolites (see separate text box), it is better to use the combined residues of imidacloprid, 5-OH imidacloprid and imidacloprid olefin to define the NOEL, rather than imidacloprid residues.

Total Residues

The active ingredient in Admire Pro is imidacloprid. Imidacloprid is highly toxic to many insect pests, which is why it is so effective against many of the important agricultural pests in California. When imidacloprid is inside the plant, it is metabolized by plant enzymes. Most of the metabolites are less toxic than imidacloprid or may have no toxicity at all. However, two metabolites that retain toxicity and contribute to the overall toxic effect of the imidacloprid application are the 5-hydroxy (5-OH) and olefin derivatives of imidacloprid. Therefore, when we evaluate the non-target impacts of imidacloprid applications, it is not enough to just measure imidacloprid residues. We also must consider the role that the metabolites might play in contributing to non-target effects. For this reason, when we conducted our residue studies, we quantified three components within the nectar – imidacloprid, 5-hydroxy imidacloprid and imidacloprid olefin.

MULTI-YEAR TREATMENTS WITH IMIDACLOPRID

Many growers rely on imidacloprid treatments for pest management and use the insecticide annually. The objective of this component of the study was to determine if residues of imidacloprid and its important metabolites could persist and/or accumulate in nectar in situations where the insecticide was used on the same trees in successive years.

As a requirement of the DPR re-evaluation process, we were asked to choose sites that reflected the variety of soil types where citrus is grown in California. The DPR requested a minimum of two nectar samples from each site (although they requested just two samples from each site, we provided additional samples from multiple groves at each site to augment the residue analysis database). To accumulate sufficient nectar for the residue analysis, it was necessary to combine the nectar from multiple flowers. Thus, each sample tested for imidacloprid residues comprised nectar from a minimum of five flowers.

TULARE COUNTY – PORTERVILLE CLAY

Nectar samples were hand-collected in April 2011 from trees at six commercial orchards in Tulare County that were treated with the full label rate of imidacloprid. Five of the sites had been treated annually during May, June or July of 2008-2010 as part of the area-wide control program for glassy-winged sharpshooter. A sixth site was not treated in 2008, but was treated in both 2009 and 2010 as part of the same program. The soil type at each site was clay. During April 2011, total residues for the six sites never ex-

Table 1. Results of imidacloprid, 5-OH imidacloprid and imidacloprid olefin residue analysis of citrus nectar collect from multiple citrus blocks in Tulare County during April 2011. Please note that Site 1 was not treated in 2008.

Site	Citrus Variety	Tree Age	Treatment Dates			Residues in April 2011 (ppb)				
			2008	2009	2010	Sample Number	Imidacloprid	5-OH	Olefin	Total Residues
1	Tangelos	25	Not treated	June 16	May 21	1	0.72	0.08	0.17	**0.97**
						2	1.07	0.09	0.12	**1.28**
2	Navel oranges	46	May 22	June 29	June 18	1	2.34	0.35	0.43	**3.13**
						2	3.31	0.28	0.62	**4.21**
3	Navel oranges	20	July 8	June 18	June 9	1	1.77	0.17	0.33	**2.26**
						2	0.34	0.24	0.10	**0.68**
4	Navel oranges	20	May 17	June 18	June 6	1	0.11	0.08	0.10	**0.29**
						2	0.05	0.16	0.10	**0.31**
5	Navel oranges	16	May 21	June 29	June 15	1	1.58	0.09	0.18	**1.84**
						2	1.62	0.12	0.33	**2.07**
6	Valencia oranges	41	July 3	June 26	June 16	1	1.20	0.13	0.22	**1.54**
						2	1.39	0.17	0.27	**1.83**
						Means	**1.16**	**0.15**	**0.23**	**1.54**

ceeded 5 ppb, and the overall mean was 1.54 ppb. These values are well below the 24 ppb NOEL for honeybees. All sample data are provided in *Table 1*.

TEMECULA VALLEY, SAN DIEGO COUNTY – FALLBROOK ROCKY SANDY LOAM

Nectar samples were hand-collected in April 2010 and April 2011 from trees at six commercial orchards in Temecula in which the trees had been treated each year in May, June or July during 2008-2010 as part of the area-wide control program for glassy-winged sharpshooter. The soil type at each site was a sandy loam. In 2010, samples were taken from trees that were randomly selected for sampling at each site, and the overall mean total residues (after two years of treatments) was 5.15 ppb. During April 2011, total residues for the six sites never exceeded 6 ppb and the overall mean was 2.57 ppb (after three years of treatments). The values from both years are well below the 24 ppb NOEL. We attribute the differences in 2010 and 2011 data to normal variability, possibly due to better imidacloprid uptake following the 2009 application. All sample data for the 2011 collections are provided in ***Table 2*.**

Table 2. Results of imidacloprid, 5-OH imidacloprid and imidacloprid olefin residue analysis of citrus nectar collected from multiple citrus blocks in Temecula Valley, Riverside County during April 2010 and April 2011 after two and three years of treatments, respectively. The individual sample readings (two from each site) for April 2011 are presented, with mean values for April 2010 samples (from the same sites) included for comparison.

Site	Citrus Variety	Tree Age (yrs)	Treatment Dates			Residues in April 2011 (ppb)				
			2008	2009	2010	Sample Number	Imidacloprid	5-OH	Olefin	Total Residues
1	Star Ruby grapefruit	20-25	June 7	May 27	May 22	1	0.97	0.15	0.34	**1.46**
						2	2.27	0.41	0.69	**3.37**
2	Star Ruby grapefruit	20-25	June 9	April 13	June 11	1	1.29	0.14	0.35	**1.78**
						2	1.58	0.16	0.41	**2.15**
3	Valencia orange	20-25	June 6	April 7	June 12	1	1.00	0.21	0.36	**1.57**
						2	1.14	0.24	0.31	**1.69**
4	Star Ruby grapefruit	20-25	June 9	April 21	May 17	1	3.21	0.72	1.60	**5.52**
						2	3.48	0.81	1.62	**5.91**
5	Star Ruby grapefruit	20-25	June 7	April 10	June 2	1	0.72	0.10	0.19	**1.02**
						2	1.04	0.18	0.40	**1.62**
6	Star Ruby grapefruit	10.00	June 16	April 17	May 14	1	1.65	0.23	0.43	**2.30**
						2	1.77	0.31	0.53	**2.60**
					2011 Means		**1.67**	**0.3**	**0.6**	**2.57**
					2010 Means		**3.17**	**0.51**	**1.48**	**5.15**

Table 3. Results of imidacloprid, 5-OH imidacloprid and imidacloprid olefin residue analysis of citrus nectar collected from a lemon block in Ventura County during April 2011. Trees within the block were selected to compare treatments at three different timings during the 2010 season. No tree was treated more than once during the season.

Sample Number	Treatment Date	Imidacloprid	5-OH	Olefin	Total Residues
T1_1	May 20, 2010	0.42	0.08	0.41	**0.91**
T1_2		0.48	0.20	0.17	**0.85**
T2_1	July 12, 2010	0.33	0.17	0.18	**0.68**
T3_1	Sept 16, 2010	0.44	0.26	0.14	**0.84**
T3_2		0.28	0.15	0.10	**0.53**

VENTURA COUNTY – MOCHO LOAM

Nectar samples were hand-collected from lemons in April 2011 that were treated at different timings (May, July and September) during 2010 with the full label rate of imidacloprid. Different sets of trees were treated on May 20, July 12 and September 16. During April 2011, two composite samples were taken from trees treated in May (Timing 1) and September (Timing 3), and one composite sample was taken from trees treated in July (Timing 2). No total residues measurement exceeded 1 ppb. All sample data are provided in ***Table 3*****.**

LINDCOVE RESEARCH AND EXTENSION CENTER (LREC), TULARE COUNTY - SAN JOAQUIN LOAM

In April 2010 and May 2011, nectar samples were hand-collected from imidacloprid-treated trees in five blocks within the LREC near Exeter, Tulare County. The soil type at the LREC is very uniform and is classified as a loam. During May 2011, one sample collected from the Red Valencia block (Block 5 of ***Table 4*** had a total residue of 23.95 ppb, which is equal to the 24 ppb NOEL. A second sample taken from within the same block had a total residue of 5.43 ppb. Overall, the grand total residues mean for all blocks at the LREC was 4.55 ppb. This value is at least half of that measured from the same blocks during April 2010 (after two successive years of treatments). All sample data for the 2011 collections are provided in ***Table 4***. The mean values from the April 2010 collections are appended at the bottom of the table for comparison.

UNIVERSITY OF CALIFORNIA RIVERSIDE, RIVERSIDE COUNTY – ARLINGTON LOAM

Nectar samples were hand-collected from Valencia oranges that were treated in October 2010 with the full label rate of imidacloprid. The soil type at the UCR site is a loam. During April 2011, 16 composite samples were taken from randomly selected trees throughout the block. The highest reading was 13.88 ppb, and the overall total residues mean was 3.68 ppb. These values are well below the 24 ppb NOEL. All sample data are provided in ***Table 5*****.**

Table 4. Results of imidacloprid, 5-OH imidacloprid and imidacloprid olefin residue analysis of citrus nectar collected from multiple citrus blocks at Lindcove Research and Extension Center during April 2010 and May 2011 after two and three years of treatments, respectively. The individual sample readings (two from each site) for May 2011 are presented, with mean values for April 2010 samples (from the same sites) included for comparison.

Block	Citrus Variety	Tree Age	Treatment Dates			Residues in May 2011 (ppb)				
			2008	2009	2010	Sample Number	Imidacloprid	5-OH	Olefin	Total Residues
1	Atwood navel	40	Sept 18	Sept 10	Sept 13	1	1.95	0.21	0.66	**2.82**
						2	4.01	0.63	1.78	**6.42**
2	Atwood navel	24	Sept 17	Sept 10	Sept 10	1	1.22	0.20	0.36	**1.78**
						2	1.27	0.16	0.34	**1.77**
3	Caracara navel	13	Sept 18	Sept 16	Sept 9	1	0.29	0.08	0.15	**0.52**
						2	0.87	0.13	0.21	**1.21**
4	Parent navel	13	Sept 18	Sept 16	Sept 9	1	1.72	0.15	0.24	**2.10**
						2	2.80	0.22	0.50	**3.52**
5	Red Valencia	7	Sept 17	Sept 14	Sept 13	1	16.87	3.88	3.20	**23.95**
						2	3.96	0.41	1.06	**5.43**
						2011 Means	**3.22**	**0.56**	**0.77**	**4.55**
						2010 Means	**6.51**	**1.80**	**2.86**	**11.16**

Table 5. Results of imidacloprid, 5-OH imidacloprid and imidacloprid olefin residue analysis of citrus nectar collected from Valencia orange trees at Agricultural Operations, University of California Riverside (Riverside County) during April 2011. Trees were treated in October 2010.

Sample Number	Imidacloprid	5-OH	Olefin	Total Residues
UCR_1	3.583	0.396	0.434	**4.41**
UCR_2	3.162	0.458	0.297	**3.92**
UCR_3	1.645	0.010	0.163	**1.82**
UCR_4	2.442	0.385	0.177	**3.00**
UCR_5	2.578	0.394	0.409	**3.38**
UCR_6	1.221	0.010	0.153	**1.38**
UCR_7	0.682	0.010	0.136	**0.83**
UCR_8	2.163	0.259	0.250	**2.67**
UCR_9	1.855	0.177	0.145	**2.18**
UCR_10	6.267	0.893	0.992	**8.15**
UCR_11	3.303	0.332	1.101	**4.74**
UCR_12	11.055	1.699	1.127	**13.88**
UCR_13	3.153	0.440	0.560	**4.15**
UCR_14	1.029	0.220	0.147	**1.40**
UCR_15	1.467	0.010	0.164	**1.64**
UCR_16	1.092	0.13	0.136	**1.36**
Means	**2.92**	**0.36**	**0.40**	**3.68**

HEMET, RIVERSIDE COUNTY – METZ LOAMY SAND

At a grapefruit grove in Hemet, trees were treated in successive years (each time during the Fall) with the full label rate of Admire Pro at 14 fluid ounces per acre. ***Table 6*** shows a comparison of the total residues arising from the 2008/09 applications (nectar collected in April 2010) and the 2008-09-10 applications (nectar collected in April 2011). Total residues for trees treated in three successive years with the 1X label rate of imidacloprid were 24.40 ppb, which is equal to the NOEL. Significantly, the 2010 application did not result in a measureable increase in the total residue levels relative to those that were present after the 2008/2009 applications.

Currently, imidacloprid treatments are not permitted during bloom or when honeybees are actively foraging within the grove. The data reported here are measurements of residues in nectar sampled from trees treated at least six months before bloom. The data clearly show that residues at or above the NOEL are a rare event.

During our monitoring program, there were two sets of data in which the NOEL was reached, both from trees that were treated during late August, September or October. At Lindcove ***Table 2***, one of the ten samples had a reading close to the NOEL, while the remaining nine samples, including a second sample from within the same block that registered the high reading, were four-fold lower. In Hemet ***Table 6***, the data were means for up to 10 samples, indicating that the residues were generally high within those trees.

Table 6. Residues of imidacloprid, 5-hydroxy imidacloprid and imidacloprid-olefin in nectar collected from grapefruit trees in Hemet during April 2010 and April 2011 after two and three years of treatment, respectively. Each value represents the mean for 7-10 samples for each treatment.

Treatment Dates	Nectar Collections	Imidacloprid	5-OH	Olefin	Total Residues
10-3-08/8-25-09	April 2010	16.07	5.05	2.72	23.84
10-3-08/8-25-09/10-12-10	April 2011	19.68	3.13	1.59	24.40

PUTTING THE DATA INTO CONTEXT

Imidacloprid is critical to the management of the Asian citrus psyllid in California citrus, and its use will likely increase as the number of insect detections on commercial citrus increases. The correct timing of treatments will be essential to provide protection to the youngest flush on the trees, which are important for psyllid feeding and development. But the timing of treatments may also impact the levels of imidacloprid in nectar, even if treatments are applied several months in advance of the bloom.

Importantly, we have shown that imidacloprid residues do not accumulate from year to year, even when toxic imidacloprid metabolites are factored into residue measurements. This result means that successive years of applications do not appear to have an additive effect on the total residues. Rather, it is the timing of the most recent application that will govern what residues are present in the nectar. It is very difficult to quantify the relationship between the proximity of treatment timing in relation to bloom and the likelihood that NOEL levels of imidacloprid will occur in the nectar. The soil type has a major influence on this, with lighter, sandier soils permitting greater uptake and, therefore, increasing the risk to the nectar.

Our data suggest that Fall treatments of imidacloprid, particularly those on sandier soils (as in Hemet), present a greater risk of the nectar having residues at NOEL levels during the subsequent bloom. As the soil changes towards a more loamy texture, there is less likelihood that the NOEL levels will be reached, but there may be instances when this occurs.

To minimize the risk of residues reaching the NOEL, treatments should be made after bloom and before the fall, preferably during the summer months of June-August (see the data for Ventura and Temecula). This is opportune treatment timing for controlling ACP during fall flush periods. If applications of imidacloprid are made between June and August, then growers will benefit from better targeting of ACP populations during the fall flush (and during the summer months, due to more favorable uptake conditions) and will also lessen the risk of treatment impacts on honeybees during the following spring.

Acknowledgements

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Dr. Frank Byrne is an Associate Researcher in the Department of Entomology at UC Riverside; Dr Elizabeth Grafton-Cardwell is an IPM Specialist and Research Entomologist in the Department of Entomology at UC Riverside and is the Director of the Lindcove Research and Extension Center. Dr. Joseph Morse is a Professor of Entomology in the Department of Entomology at UC Riverside.

CRB PROJECT 5500-179

UNTIL THEY FIND A CURE... WE CAN PROVIDE A CONTROL

BEFORE – OCT 2006 | AFTER – APR 2012

The grove above was diagnosed with HLB in 2006. Our team worked directly with the grower to develop a program for his groves to strengthen and protect his trees. It combined K-PHITE 7LP and other Plant Food Systems nutritionals, along with psyllid control. The photo on the right is the same row in the same grove six years later – healthy and productive.

Plant Food systems

KPHITE® 7LP

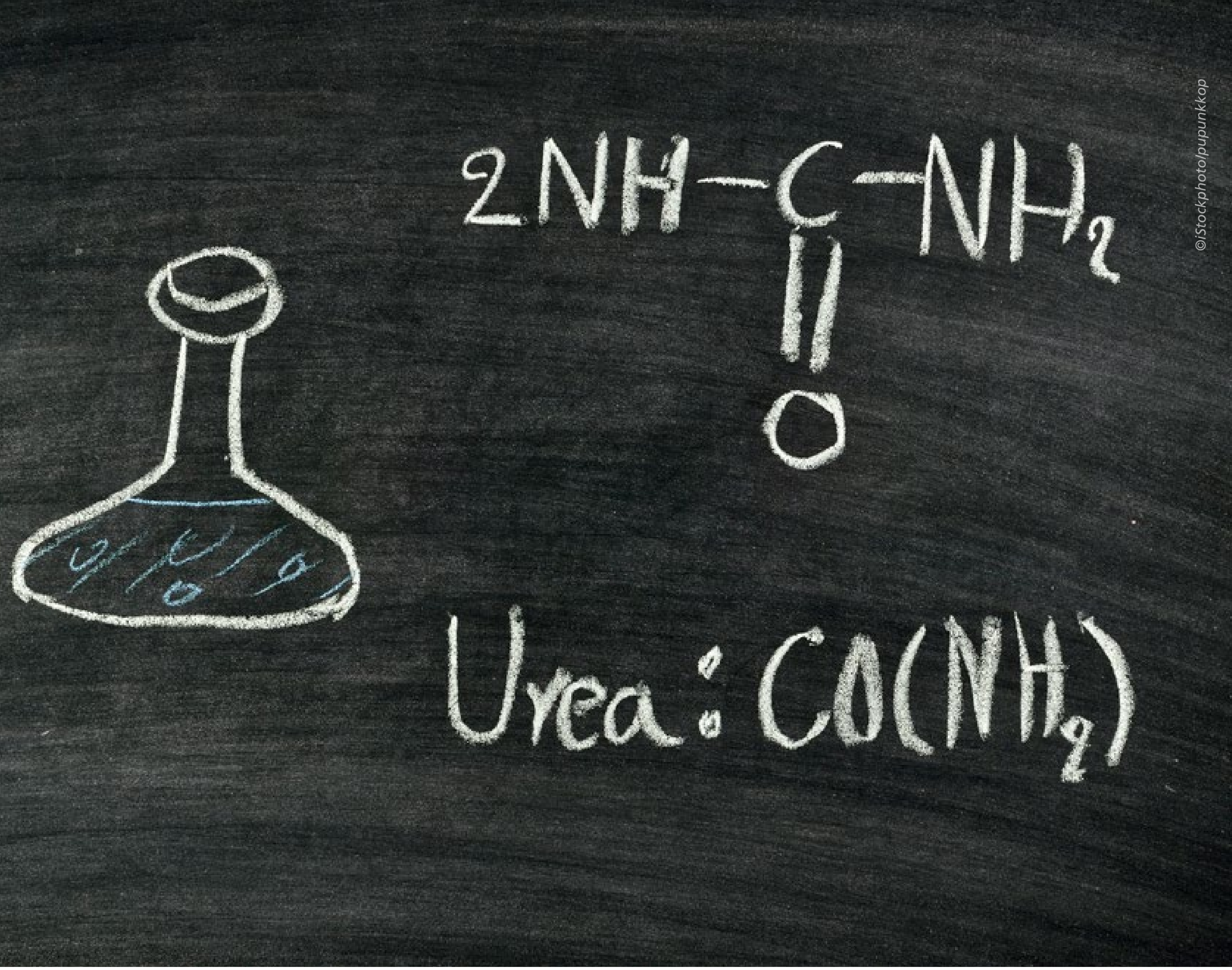
SYSTEMIC FUNGICIDE BACTERICIDE

Finding a cure is a long term effort at best. And when and if a cure is eventually found, its implementation may take years to actually execute. In the meantime, it does not make sense to go totally unprotected when your company or your family's livelihood is at stake. K-PHITE 7LP Systemic Fungicide/Bactericide in combination with superior nutritional programs has shown excellent control that is supported by years of positive University of Florida Research. Trees that are symptomatic have actually been returned to asymptomatic producing quality fruit on vigorously growing trees.

An early preinfection program with K-PHITE 7LP will also provide valuable results in the control of Phytopthora Gummosis, Phytopthora Root Rot, Pythium, Dry Root Rot, Brown Rot, and Foaming Canker. K-PHITE 7LP is an EPA and California registered biopesticide providing a strong and effective management tool for today's citrus grower while being sensitive and eco-friendly to the environment.

While we completely support the future possibilities of research, ***UNTIL THEY FIND A CURE... WE CAN PROVIDE A CONTROL.***

To find out more about K-PHITE 7LP and K-PHITE 7LP Programs, contact your California Plant Food Systems Representative directly: Mark Brady – Western Marketing Manager 559 731-1267.



UREA – CITRACULTURE'S AMAZING LITTLE MOLECULE

UREA CAN INCREASE CITRUS FLOWERING, YIELDS, FRUIT SIZE AND GROWER INCOME WHEN APPLIED AT SPECIFIC STAGES OF CITRUS TREE PHENOLOGY

Carol J. Lovatt

EXECUTIVE SUMMARY

Timing the application of foliar fertilizers to key stages of tree phenology (periods of intense growth and development, such as flowering, fruit set, exponential fruit growth, vegetative shoot growth and root flushes) when demand for the nutrient is likely to be high, especially when soil conditions are likely to compromise nutrient uptake by roots, has proven successful for increasing yield, fruit size and grower income even when the tree is not nutrient deficient by standard leaf analysis.

Foliar applications of urea (≤ 0.25% biuret) timed to specific stages of citrus tree phenology provide excellent examples of the efficacy of this approach. Winter pre-bloom foliar-applied urea was documented to increase total yield of both navel and Valencia oranges (Citrus sinensis), with an accompanying increase in the yield of commercially valuable-size fruit for navel and juice total soluble solids for Valencia. Adaptation of this treatment to Nules Clementine mandarin (C. reticulata) increased the yield of commercially valuable-size fruit in four separate experiments. A later foliar application of urea to navel orange trees when fruit were at maximum peel thickness increased the total number of fruit per tree and the number of commercially valuable larger-size fruit.

Replacing soil-applied nitrogen fertilizer with foliar-applied urea contributes to fertilizer best management practices and reduces the potential for nitrogen to negatively impact the environment and human health. When properly timed, foliar-applied urea is a cost-effective production management tool for increasing yield of commercially valuable-size fruit and grower profit.

THE BENEFITS OF FOLIAR-APPLIED UREA TO THE ENVIRONMENT, HUMAN HEALTH AND CITRUS PRODUCTION

The benefits of foliar-applied fertilizers in protecting the environment are well known. Specifically, foliar-applied urea reduces the potential for nitrogen (N) accumulation in the soil, run-off water, surface water (streams, lakes and the ocean), and groundwater (drinking water supply), where it can contribute to salinity, eutrophication and nitrate contamination, all of which have a negative impact on the environment and also human health. With less water of good quality available to leach accumulating fertilizer salts from orchard soils, replacing soil-applied nitrogen fertilizers, at least in part, with foliar-applied urea in citrus best management practices will contribute to the sustainability of the industry.

Citrus growers in California have an excellent reputation as stewards of the land. Many adopted the use of foliar-applied urea to partially replace soil-applied nitrogen fertilizer in the late 1970s based on the results of the pioneering research of Embleton and Jones (1974), which demonstrated that maximum nutritionally-attainable yields for sweet orange cultivars annually required between 0.99 and 1.32 lb nitrogen per tree, independent of fertilization method. Foliar-applied urea is a rapid and efficient way to supply nitrogen to citrus trees during periods of high nutrient demand (e.g., flowering, fruit set, exponential fruit growth), or when soil conditions (cold wet soils in spring, hot dry soils in summer, salinity, pH) during the growing season render soil nitrogen, and hence soil-applied nitrogen fertilizers, less available to the tree. Even a transient or incipient nitrogen deficiency needs to be corrected quickly.

The longer the nitrogen status of the tree remains at the low end or below the optimal range during key stages of tree phenology, the greater the negative effects on yield, fruit size, quality and next year's bloom. Foliar urea fertilization provides the nitrogen required for photosynthesis and other important metabolic functions to prevent reductions in carbon fixation, DNA, RNA and amino acid synthesis, hormone metabolism and plant productivity.
For citrus trees grown in California, urea is taken up by young leaves at one-half to two-thirds full expansion, mature leaves and other organs in sufficient quantities to elicit a physiological response. According to PureGro, n.d. of Sacramento, California, the uptake of urea is rapid, with 50 percent of foliar-applied urea moving into

leaf tissues in only 30 minutes to two hours after application, and 96 percent by the end of 12 to 24 hours. Urea-N is phloem mobile and thus can be translocated to all parts of the tree, including the feeder roots. It is no wonder that foliar fertilization can be 5 to 30 times more efficient than soil fertilization in achieving the same yield, depending on the nutrient, crop and soil in which the tree is growing. Thus, foliar fertilization can save growers money over soil fertilization, because the amount of a nutrient applied to the foliage is significantly lower than the amount that must be applied to the soil to achieve the same yield.

The cost of foliar fertilization is frequently associated with application cost. To mitigate this, the goal has been to identify the role that essential nutrients play in the physiology of a tree crop and then to apply that nutrient as a foliar fertilizer at the appropriate time in the phenology of the tree, i.e., a time when the demand for the nutrient is likely to be high in order to stimulate a specific physiological process that increases yield, fruit size or fruit quality. With this knowledge, foliar application of a fertilizer can result in a net increase in grower income, even when the tree is not deficient in the nutrient by standard leaf analysis.

THE EFFECT OF UREA ON CITRUS TREE PHYSIOLOGY

When applied to the foliage, urea is broken down by the enzyme urease into one molecule of carbon dioxide and two molecules of ammonia:

$$(NH_2)_2CO + H_2O \Rightarrow CO_2 + 2NH_3.$$

The increasing concentration of ammonia quickly activates the citrus tree's metabolic pathways for ammonia detoxification, which includes the accelerated synthesis of the amino acid arginine, which is subsequently used in the synthesis of polyamines. Polyamines are known to regulate many physiological processes, among them flowering, fruit set and fruit size.

Our research provided clear evidence that increasing citrus tree (leaf) ammonia concentrations with foliar-applied urea was accompanied by the accumulation of both arginine and polyamines and also an increase in inflorescence number, flower number, fruit set and fruit size. Flowering in citrus is initiated by low temperature (59-64°F day and 50-55°F night) and moderate water-deficit stress, both of which cause ammonia accumulation in citrus leaves. Thus, foliar-applied urea is used to artificially raise the leaf ammonia content of citrus trees, trigger arginine and polyamine biosynthesis and increase flowering.

Whereas our research on the role of urea in citrus flowering was conducted with five-year-old trees under controlled, optimal temperature conditions, the capacity of foliar-applied urea to increase flower number was confirmed in a commercial Valencia orange grove in Florida by Gene Albrigo. Valencia trees treated with a winter prebloom foliar application of urea produced 45 percent more flowers than untreated control trees.

PROPER TIMES TO APPLY UREA TO CITRUS FOLIAGE

The most effective time to apply urea as a winter pre-bloom spray is around early January in Southern California. In most years, December 15 to February 15 seems to be appropriate for Clementine mandarins and sweet oranges. Later applications (March and April) are better than those that are too early (October and November). This is because once the opportunity to increase flower number has passed, late applications of foliar urea fertilizer can still increase the retention of reproductive organs by improving their nitrogen status and contribute to increasing fruit set and fruit size. However, as time progresses from flowering through early fruit set to June drop, the contribution that foliar-applied urea can make to increased yield has diminished. This is due to the steady increase of flowers and fruit abscision; yield potential also has steadily declined.

When used as a winter pre-bloom foliar spray, urea (46% N, ≤ 0.25% biuret) is applied as a single spray most frequently between January 1 and February 15 for navels and December 15 and January 15 for Clementine mandarins at the rate of 50 lbs. in 200 gallons of water per acre. Lower volumes can be used as long as tree coverage is good, but high volumes (500-700 gallons per acre) show greater incidence of tip burn due to pooling of the urea spray at the leaf tip and increased potential for ammonia toxicity. The summer application of urea is designed to extend the cell division stage of fruit development to achieve an increase in fruit size without increasing yield. Fortunately, in citrus, the end of the cell division stage of fruit development is associated with maximum thickness of the peel. We experimentally determined that in California (Irvine to Madera), maximum peel thickness occurs between approximately June 11 and July 26 for navel, Valencia and Clementine mandarin. In an alternate bearing orchard, maximum peel thickness occurs earlier within this period in high-yield, on-crop years, because peels are thinner than in the low-yield, off-crop years.

When applied in the summer at maximum peel thickness, urea (46% N, ≤ 0.25% biuret) is applied as a single spray typically between July 1 and July 26 for navels or between June 15 and July 7 for Clementine mandarins at the rate of 50 lbs. in 200 gallons of water per acre. Applications of urea that are too early (May and June for Clementine mandarin and navel orange, respectively) increase fruit retention and thus are less effective in increasing fruit size.

Urea applied to the foliage of citrus should not contain more than 0.25% biuret, a compound formed from two urea molecules during the manufacture of urea. Biuret is toxic to citrus, causing leaf chlorosis and tip burn. Urea applications should be made to give good canopy coverage, much like applying a pesticide or plant growth regulator. Solutions should be between pH 5.5 and 6.5. Applications of urea should be made taking into consideration the potential maximum daytime temperature. Winter applications should be made toward midday when it is warm enough for the tree to be metabolically active. If temperatures are projected to exceed 100ºF, summer applications should be made early

High yielding mandarin trees

morning when the air temperature is below 80ºF or early evening as temperatures start to drop. Applications of foliar-applied urea should not be repeated at less than two-week intervals; 30- day intervals are recommended by Unocal Agriproducts.

POTENTIAL YIELD INCREASES WITH A WINTER PRE-BLOOM FOLIAR APPLICATION OF UREA TO CITRUS SWEET ORANGES

A single winter pre-bloom foliar application of urea (54 lbs./acre, 46% N, ≤ 0.25% biuret) significantly increased yield in all three years of the research compared to control trees, which received five times more urea applied to the soil. Urea applied in January or February produced a three-year cumulative net increase of 181 lbs./tree and 143 lbs./tree, respectively. Based on a standard planting density of 96 trees per acre, the foliar-applied urea treatments would have resulted in a net increase of 17,355 and 13,757 lbs. per acre for the three years of the research, respectively. For both application times, with the increase in total yield, there was an increase in the yield of fruit of packing carton sizes 88 and 72 (fruit transverse diameter 2.7-3.1 inches). At the time of this research, the emphasis was on total yield and not on large size fruit. Hence, the yield of fruit of packing carton size 56 (3.2-3.5 inches in diameter) was not determined.

The capacity of a winter (December 25-31) pre-bloom foliar application of urea (54-60 lbs./acre, 46% N, ≤ 0.25% biuret) to increase total yield per tree was tested with Valencia juice orange trees in Florida by Albrigo. The treatment resulted in a four-year cumulative net increase of 14,247 lbs. of fruit per acre and a four-year cumulative net increase in juice total soluble solids of 1,183 lbs. per acre.

CLEMENTINE MANDARINS

To adapt the winter pre-bloom foliar application of urea to Nules Clementine mandarin, orchards in two different growing areas of the state, Grapevine (35° N, 119° W) and Fresno (36° N and 119° W), were treated with urea (50 lbs./acre, 46% N, ≤ 0.25% biuret in 200 gallons of water/acre) applied monthly from November through February to the foliage of different sets of trees. For Nules Clementine mandarin trees in either orchard, foliar-applied urea did not increase total yield per tree in any year of the study or as two-year cumulative total yield compared to untreated control trees. In contrast, in both orchards, the winter pre-bloom foliar application of urea significantly increased the yield of commercially valuable-size fruit in pounds and number of fruit per tree.

In Grapevine, at the southern end of the San Joaquin Valley, the most effective application time was mid-December. Fruit yield of jumbo size (fruit transverse diameter 2.54-2.79 inches) was significantly increased in both years of the experiment and thus significantly increased the two-year cumulative yield of jumbo-size fruit as both pounds and number of fruit per tree compared to the

California grows picture perfect fruit for the fresh fruit market

control trees. The December application of urea also increased the two-year cumulative yield of mammoth-size fruit (fruit diameter >2.79-3.05 inches) compared to all treatments, except the January foliar application of urea. At planting densities of 242 trees per acre (12 x 15 feet) to 538 trees per acre (9 x 9 feet), the December foliar application of urea would have resulted in a two-year cumulative net increase in yield of jumbo-size fruit (2.54-2.79 inches in diameter) of 5,593 and 12,426 lbs./acre for the two planting densities, respectively.

In Fresno, 140 miles north of Grapevine, it was the early January (January 9) winter pre-bloom foliar application of urea that significantly increased the two-year cumulative yield of commercially-valuable fruit of packing carton size 28 (fruit diameter 2.32-2.40 inches) as both pounds and number of fruit per tree compared to the untreated control trees. These results confirm that for Clementine mandarin, the winter pre-bloom foliar application of urea increases the yield of commercially valuable-size fruit with no effect on total yield. At planting densities of 242 to 538 trees per acre, the two-year cumulative net increase of only 16.5 lbs. (66 fruit) of commercially valuable larger-size fruit per tree would have resulted in a net increase of 3,467 lbs. (15,972 fruit) to 7,710 lbs. (35,508 fruit) per acre.

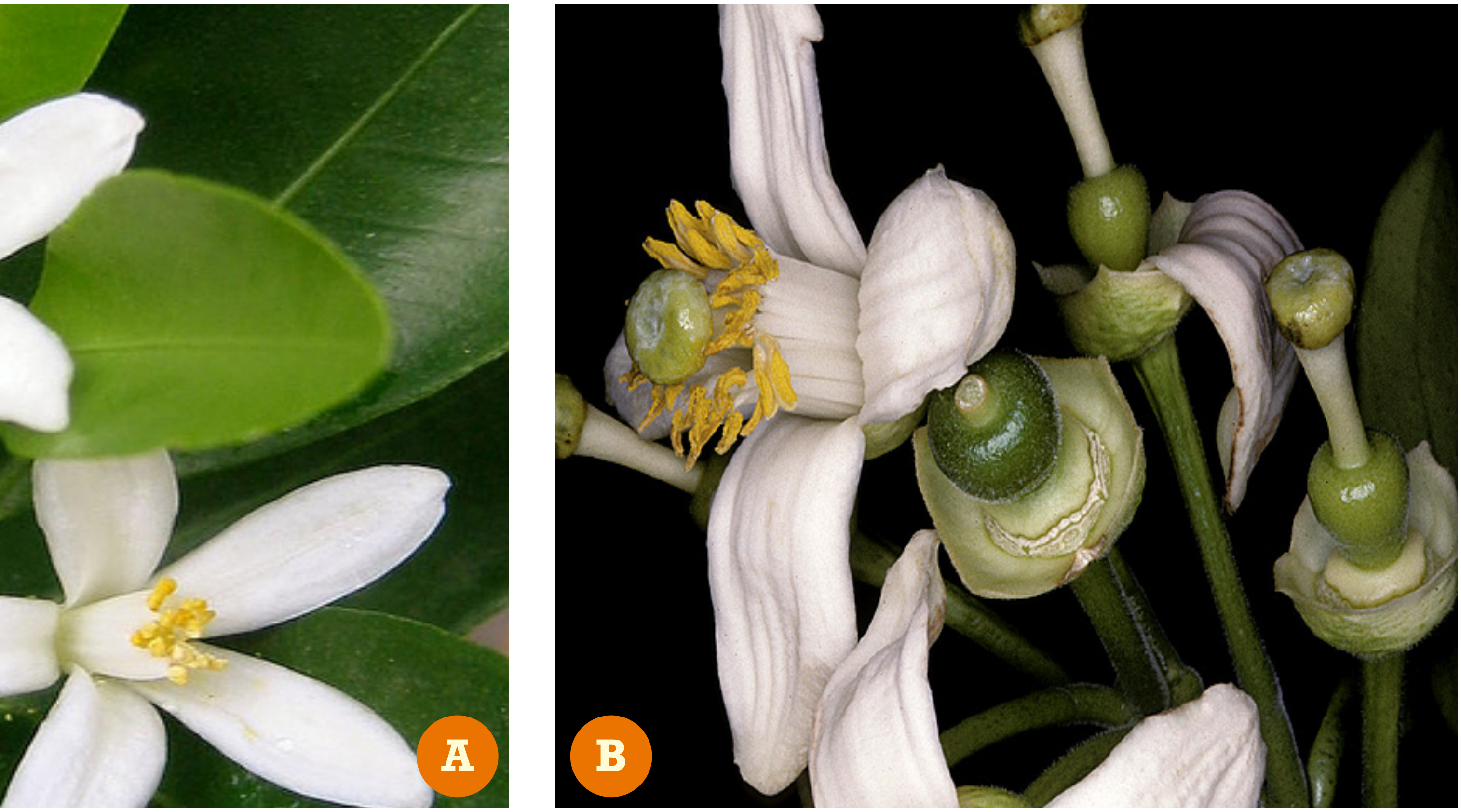
OTHER CULTIVARS OF MANDARIN

The potential efficacy of this foliar fertilization strategy when used on other cultivars of *C. reticulata* is suggested by the results obtained with a winter pre-bloom foliar application of urea (57.6 lbs./acre, 46% N) to Nour and Cadoux mandarin trees in Morocco. For both cultivars, the January application produced better results than the November or December applications and resulted in a significant increase in total yield, total number of fruit per tree and the yield (and number) of commercially valuable export-size fruit (fruit transverse diameter >2.04 inches). The net increase in total yield compared to the untreated control trees was 105 lbs./tree for Nour and 98 lbs./tree for Cadoux, with net increase in export size fruit of 43 lbs. and 68 lbs. per tree, respectively. At planting densities of 242 to 538 trees per acre, the winter pre-bloom foliar application of urea would have resulted in a net increase in export-size fruit ranging from 10,406 lbs. to 23,134 lbs. per acre for Nour and 16,456 lbs. to 36,584 lbs. per acre for Cadoux.

In all experiments, the winter pre-bloom foliar application of urea tended to increase juice total soluble solids and the ratio of total soluble solids to acid each year. No negative effects on any fruit quality parameter measured (individual fruit size and weight, peel quality, peel thickness, total juice and percent juice per fruit, total soluble solids [ºBrix], percent acidity, total soluble solids to acid ratio) resulted from foliar application of urea. Regreening was not observed.

THE INFLUENCE OF ALTERNATE BEARING ON THE WINTER PRE-BLOOM FOLIAR APPLICATION OF UREA

For navel orange trees and the Nules Clementine mandarin trees located in Grapevine, the winter pre-bloom foliar application of



Navel orange flowers at (A) anthesis and (B) petal fall.

urea had a significant effect on yield and fruit size, respectively, for each year of the experiment, despite the fact that the orchards were alternate bearing. In contrast, for the Nules Clementine mandarin orchard in Fresno, the positive effect of the January winter pre-bloom foliar urea application was due to a significant increase in the pounds and number of larger fruit per tree in the lower-yielding, off-crop year and a numerical but non-significant increase in the yield of larger-size fruit in the higher-yielding, on-crop year (> 1,000 fruit per tree).

In the experiments presented above, the winter pre-bloom foliar application of urea had a significant effect on fruit size only when the crop load of the untreated control trees was less than 1,000 fruit per tree. This result is consistent with research demonstrating that gibberellic acid (GA3) was only effective in the low-yield years (less than 1,000 fruit per tree) of two Clementine mandarin orchards. In these cases, increased fruit size was achieved even when foliar-applied urea or GA3 increased fruit number above 1,000 fruit per tree.

The effect of crop load on the response of Clementine mandarin to foliar fertilizer and GA3 treatments was likely due to the differences in the number of flowers produced at bloom in the lower and higher yield years. The intense bloom resulted in a large number of fruit being set that likely saturated the carrying capacity of the trees and made it difficult to enhance fruit set further with foliar-applied urea or GA3. The capacity of the winter pre-bloom foliar application of urea to increase yield of commercially valuable-size fruit when yield was less than 1,000 fruit per tree was likely due to supplying nitrogen early in the season, when soil conditions could compromise uptake of nitrogen from the soil, to support fruit growth at the cell-division stage of fruit development. In addition, it is important to point out that the winter pre-bloom foliar application of urea did not initiate or exacerbate alternate bearing in any orchard.

POTENTIAL INCREASES IN THE YIELD OF LARGE-SIZE FRUIT WITH A SUMMER MAXIMUM PEEL THICKNESS

A single summer application of urea (54 lb/acre, 46% N, ≤ 0.25% biuret) to the foliage of navel orange trees in early July significantly increased the total number of fruit per tree with a numeric, but not significant, increase in total lbs. per tree, and increased yield of commercially valuable-size fruit of packing carton sizes 88 (fruit transverse diameter 2.7-2.9 inches), 72 (fruit diameter 3.0-3.1 inches) and 56 (fruit diameter 3.2-3.5 inches) as both pounds and number of fruit per tree compared to control trees. The treatment resulted in a net increase of 76 lbs. of fruit in the combined pool of fruit 2.7 to 3.5 inches in diameter per tree. At a planting density of 96 trees per acre, the summer application would have resulted in a net increase of commercially valuable large-size fruit (packing carton sizes 88-56) of 7,296 lbs./acre in a single year, of which 2,947 lbs./acre was fruit of packing carton size 56.

GROWER INCOME

The increases in yield of commercially valuable-size fruit obtained with the foliar urea fertilization strategies described herein should increase grower revenue. Low-biuret urea is relatively inexpensive. Depending on application costs, properly-timed foliar urea applications have the potential to increase grower net income in a given year and especially over time.

Additional information is provided in the chart below. It is designed to aid in deciding whether to use foliar-applied urea as a production management tool in a given year. Please keep in mind that this information is the best interpretation of our results and those of others to date and that they are subject to modification as additional results are obtained. Application time is important. Results for a specific cultivar in a specific production area might be improved by determining the best application time for the cultivars growing in that area. However, keep in mind that the time of irreversible commitment to flowering or maximum peel thickness, the two phenological stages targeted for urea application, can occur earlier or later than is typical due to the greater variations in climate that are occurring as a result of global climate change. Thus, despite all our best efforts, there will be some years in which the urea sprays are not properly timed, and the only benefit derived from the foliar application of urea is increased tree nitrogen status.

When to use a winter pre-bloom foliar application of low-biuret urea

- **When your production goal for the year is to increase yield**
- **When your current harvest is a heavy on-crop and you anticipate a low return bloom and an off-crop; spray urea in the winter preceding the anticipated off-bloom**
- **When you hang your fruit late, which reduces return bloom, a winter pre-bloom urea application will provide nitrogen to support the mature fruit and flower development**
- **If we have a mild winter with insufficient chilling to induce a good bloom**
- **If winter soils are cold and/or wet; if this condition occurs in February and March, late foliar urea applications can be beneficial for increasing yield and fruit size**
- **When you wish to increase total soluble solids in your fruit or pounds solids per acre**

When to use summer maximum peel thickness applications of low-biuret urea

- **When your production goal for the year is to increase fruit size**
- **When you are carrying a heavy on-crop and need to increase fruit size**

Other considerations

- **When cost is an overriding consideration, low-biuret urea is an inexpensive fertilizer, and only one application is required to increase yield of commercially valuable-size fruit or increase fruit size; use low-biuret urea after considering the above**
- **When your production goal is to increase fruit size, do not use a winter pre-bloom urea spray as the increased yield will make it more difficult to increase fruit size; use urea as a summer application only**
- **If maturing fruit have rough, thick peels, do not use urea as a winter pre-bloom spray when the mature fruit are still on the tree; similarly, if young developing fruit exhibit this peel disorder, do not use urea as a summer maximum peel thickness spray**
- **When spring soils are cold and wet and trees are yellow, use urea as a winter pre-bloom spray after considering the above; the trees could be nitrogen deficient until the soils warm up sufficiently for nitrogen and other nutrients to be available to the roots and for the roots to be able to take them up; nutrient deficiencies, even transient or incipient deficiencies, need to be corrected quickly to prevent negative effects on citrus tree physiology, flowering, yield, fruit size or quality**
- **If you have high leaf nitrogen concentrations (>3.3% N), do not apply urea: (a) it will likely have no effect on yield; and (b) it could reduce fruit size, especially if leaf potassium is not sufficient or not in balance with leaf nitrogen**

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GO GREEN TO SAVE GREEN

Staff Report

More and more California growers are now turning to electrostatic sprayer technology, which is enabling them to save money via reduced time, water, labor, fuel and material.

Additionally, the newer spray technology is much more environmentally friendly. With standard equipment, drift is a serious problem that can affect non-targeted organisms and may occasionally be detected on plants and animals miles away from the application. New sprayers provide better coverage with less drift, according to Mark Ryckman, sales manager and owner of Progressive Ag Inc in Modesto, California.

HISTORY OF COMMERCIAL USE

Over the past half-century, there had not been much evolution in spraying agricultural pesticides. Significant income has been lost by growers using standard spray equipment that yields drip, drift and splash problems. As a result, according to the web site of GenZ Technology, a spray tech company, California crop growers annually use more than 100 million tons of chemicals on the top 10 orchard and vineyard crops. More than 70 percent of these pesticides miss their target and are lost into the environment. Money evaporates.

Electrostatic spray technology actually has been used in other applications since the 1930s. For many years, the automotive industry has employed it as a more efficient way to paint vehicles. These spray systems eventually were modified for agricultural use in the 1980s, beginning with cotton. However, adaptation of the technology for use on citrus is much more recent.

"We have been serving the grape, blueberry, raspberry, strawberry and row crop industry for more than a decade," said Willie Hartman of On Target Spray Systems in Mt. Angel, Oregon. "Two years ago, we modified our designs for the orchard industry and currently offer vertical tower and V-trellis sprayers for the grove market."

HOW IT WORKS

Growers are using the technology to apply insecticides, fungicides, oils, foliar nutrients and miticides.

According to Hartman, electrostatic sprayers utilize the natural law of attraction. "Highly-charged drops are electrically attracted to the grounded plants," he explained. "Since like charges repel and opposite charges attract, these droplets repel from one another creating a fine spray mist less prone to run-off and coalescence. The opposite charges of the plants attract the particles to them, drawing the spray deep inside the plant's canopy. Once the plant is fully and evenly covered in spray, the surface charges even out, resulting in complete, uniform coverage of crops, inside and out."

Ryckman of Progressive Ag added more detail. "High velocity air, moving at speeds over 200 mph, passes by unique air-shear nozzles where chemical-laden liquid is pulled into the air stream," Ryckman said. "Small, fine droplets, approximately 50 micron in size, are created and take on a charge as they pass by electrostatic electrodes. As the charged cloud comes near the plant, it has a magnet-like effect. The result is more chemical-laden droplets deposited on the plant surface, especially in the under-leaf and hard-to-reach areas."

While conventional sprayers have unwanted run-off and drift, the new electrostatic sprayers provide complete wrap-around coverage in less time.

Additionally, spray manifolds can be mounted and adjusted to parallel canopies, although Hartman believes that spraying from the top down "may be the future."

SAVORING THE SAVINGS

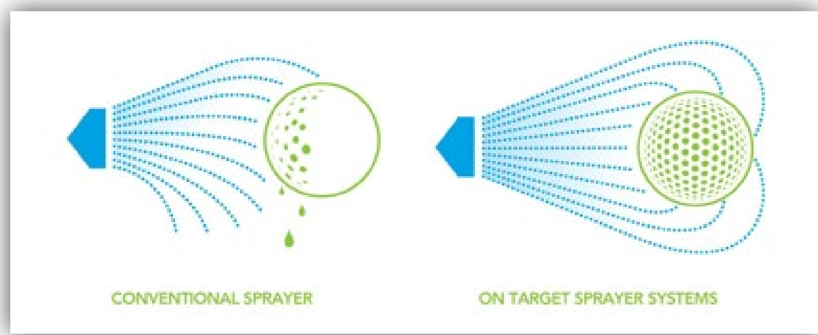
The new sprayers are perfectly suited for sustainable agriculture because of their environmental, social and economic benefits.

"A reduced need for the number of applications and chemicals results not only in environmental benefits, but also decreased fossil fuels and cost savings,"
Hartman said.

Progressive Ag's LectroBlast® Electrostatic Sprayers travel faster than most conventional sprayers, because they use high-velocity air to get chemical-laden droplets to the plant surfaces. Conventional sprayers use low-velocity air and high-pressure spray. Due to the high-velocity air and charged spray cloud, LectroBlast® sprayers produce superior canopy penetrations while traveling at higher speeds.

"A reduced need for the number of applications and chemicals results not only in environmental benefits, but also decreased fossil fuels and cost savings."

"Additionally, our On Target sprayers are much quieter than conventional sprayers and reduce runoff and drift, making them socially equitable."



Conventional sprayers often result in drift and uneven coverage. The newer electrostatic sprayer systems achieve full coverage with dramatically-reduced drift.

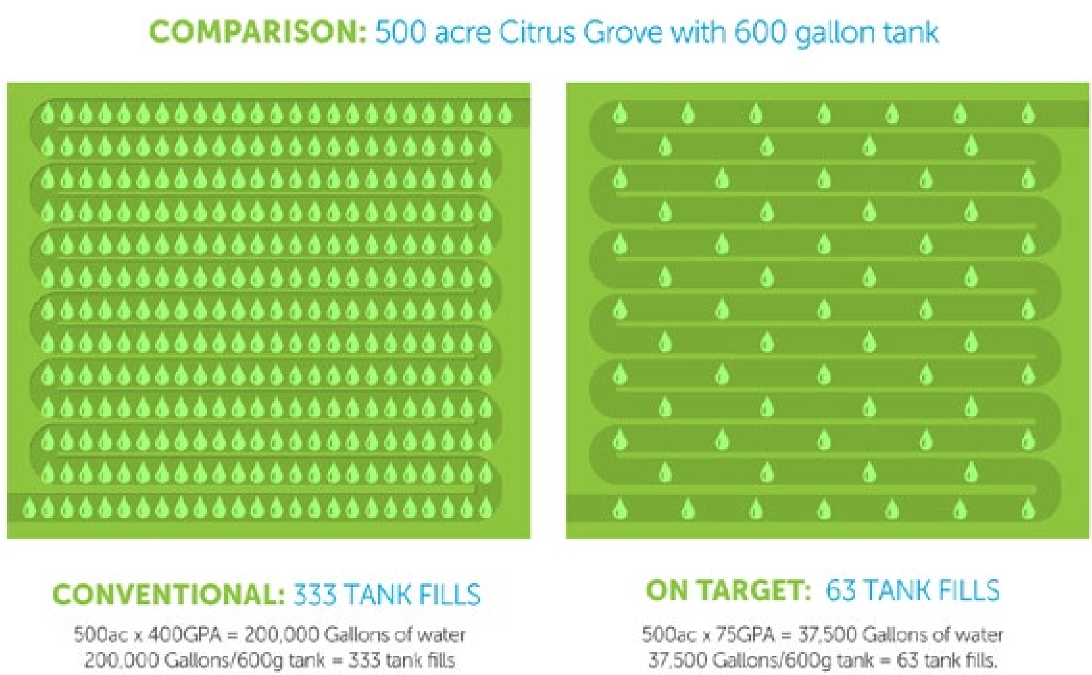
He noted that, on all crops, his customers are achieving complete wrap-around coverage, using 70-80 percent less water, an especially important savings during this time of severe drought. Also, they are spending more time spraying and less time filling the tank, thus resulting in labor savings. "Our sprayers require 50 percent less horsepower than conventional equipment, which adds up to substantial fuel savings," Hartman explained.

Ryckman agreed that better coverage is only part of the story.

"With today's electrostatic spray technology, you're looking at a number of advantages," he said, mentioning the following:

- **improved canopy penetration**
- **increased under-leaf coverage**
- **reduced spray drift and waste of costly chemicals**
- **ability to cover more ground in less time**
- **fewer fill-ups**
- **easy calibration**
- **low maintenance**
- **dilute capability**
- **reduced fuel costs**
- **less worker exposure to hazardous materials**

On Target's Hartman recommends that citrus growers consider switching to the technology. "It is precise, efficient and has a quick return on investment."



An On-Target spray system efficiently and effectively coats an orchard.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TART, TANGY AND TASTY

ADD LEMONS TO YOUR MENU YEAR-ROUND

It's time to brighten your winter with some spectacular lemon dishes, courtesy of Sunkist. Fresh, flavorful California and Arizona lemons comprise approximately 95 percent of the U.S. crop and are available throughout the year. Major varieties are the Lisbon and the Eureka. We're also providing a recipe that utilizes Meyer lemons, which are thin-skinned and slightly less acidic than their Lisbon and Eureka cousins. Meyers actually are a cross between lemons and possibly oranges or mandarins.

Sunkist, which is owned by thousands of citrus growers across California and Arizona, is a leading international supplier of fresh fruit. Founded in 1893, it is the oldest operating citrus cooperative in America.

GIN WITH HOUSE-MADE BITTER SUNKIST® LEMON AND SODA

1 serving

From Kathy Casey (Liquid Kitchen – Seattle, Washington)

Ingredients:

House-Made Bitter Lemon

- 4 large Sunkist® Lemons
- 1 1/2 cups sugar
- 2 cups water

Cocktail

- 1 1/2 ounces gin
- 1 ounce House-Made Bitter Lemon
- 2 ounces chilled sparkling water
- Garnish: Sunkist lemon slice and fresh thyme

Directions:

House-Made Bitter Lemon

- Wash lemons and cut the ends off. Slice each lemon in half lengthwise and each half into 4 slices.
- Combine lemons, sugar and water in a blender cup.
- Process in a high speed blender by pulsing until almost smooth, mixture should still have some texture.
- Place mixture in a medium non-corrosive pan and bring to a boil over medium high heat. Let boil for 1 minute, then remove from heat and let steep for 1 hour.
- Strain through a fine strainer. Store refrigerated for up to 2 weeks.

Cocktail

- Measure the gin and Bitter Lemon into a mixing glass.
- Fill glass three-quarters with ice. Shake vigorously.
- Add the sparkling water and swirl in mixing tin.
- Strain into a tall Collins glass filled with fresh ice.
- Garnish with lemon slice and fresh thyme.

HOT SUNKIST® MEYER LEMON SOUFFLÉ

6 Servings

Ingredients:

3 Tbsp butter
3 Tbsp all-purpose flour
1 cup milk
½ Sunkist® Meyer lemon, zest only
1 Sunkist® Meyer lemon, juiced
4 egg yolks
6 egg whites
½ tsp cream of tartar
½ cup sugar
Warm lemon sauce (see recipe below) – optional

Directions:

Preheat oven to 350 degrees. In a saucepan, melt the butter. Lower the heat, and stir in the flour. Cook for a few minutes stirring constantly. Gradually blend in the milk. Turn the heat to medium, and cook, constantly stirring until thick. Add lemon zest and juice. Remove from heat and cool slightly.

In a bowl, use an electric mixer to beat the egg yolks until thick and light in color. Gradually stir a small amount of warm lemon mixture into beaten egg yolks until incorporated. Add egg yolk mixture back into remaining lemon mixture in the pan, blending well.

With clean beaters, beat the egg whites with the cream of tartar until foamy. Gradually add the sugar, beating until soft peaks form. Gently stir about one-fourth of the egg whites into the lemon-yolk mixture. Then, fold in the remaining egg whites. Pour into a buttered and sugared 1-1/2-quart soufflé dish. Bake at 350° F for 30 - 35 minutes, or until set. Serve with warm lemon sauce.

WARM SUNKIST MEYER LEMON SAUCE RECIPE

Ingredients:

1/3 cup sugar
2 Tbsp cornstarch
1/8 tsp salt
Dash of nutmeg (optional)
3/4 cup water
1/2 Sunkist® Meyer lemon, zest only
1 Sunkist® Meyer lemon, juiced
1 Tbsp butter

Directions:

In a small saucepan, combine the sugar, cornstarch, salt, and nutmeg. Gradually blend in the water, lemon zest and juice. Add the butter. Cook over medium heat stirring until thick.

SUNKIST® LEMON SEAFOOD PAELLA

8 Servings

Seafood paellas should be made with a variety of fish and shellfish, each adding its own flavor and texture. Because the seafood is used in smaller sized portions, end cuts and scrap pieces of fresh seafood are ideal.

Ingredients:

Sauce

1-32 oz can clam juice
1 cup dry white wine
1 tsp saffron threads

Paella

3 Tbsp olive oil
Salt and pepper
1lb. of firm, fresh pieces of whitefish such as tilapia, bass, halibut and swordfish, cut into 16-21 pieces
1lb. or 16-21 mussels, cleaned and de-bearded
1lb. or 16-21 medium sized clams, rinsed
1 ½ cups minced onion
3 Sunkist® lemons, zest and juice
3 cloves garlic, minced
½ tsp crushed red pepper
3 cups uncooked short-grain rice such as Valencian, Arborio, or Calrose
1 cup frozen green peas
1-8oz jar of sliced, roasted red peppers
½ cup chopped fresh parsley

To prepare broth, combine clam juice, white wine and saffron threads in a saucepan. Bring to a simmer (do not boil). Keep warm over low heat.

Directions:

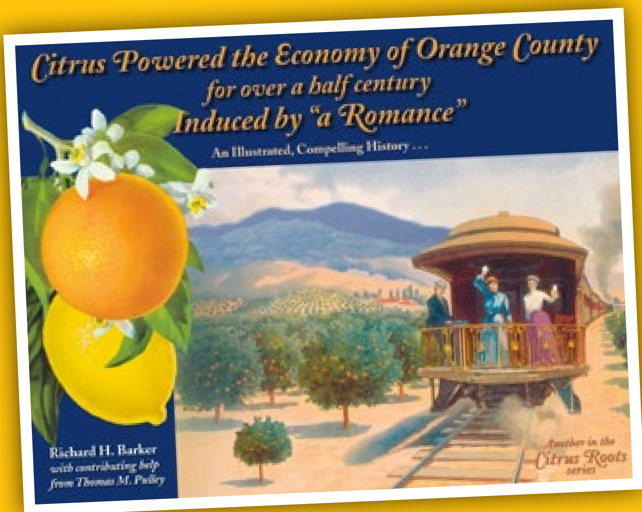
To prepare paella, heat 3 tablespoons olive oil in a large paella pan or large skillet over medium-high heat. Season fish with salt and pepper; add to pan, and sauté 1 minute. Remove fish from pan, reserve and keep warm. Add onion to pan, and sauté 5 minutes. Add the lemon zest, garlic and crushed red pepper, and cook 5 minutes. Add rice, and cook 1 minute longer, stirring constantly. Stir in broth, bring to a low boil and cook 10 minutes, stirring frequently.

Stir in the peas and red pepper slices. Add mussels and clams to pan, nestling them into rice mixture. Cook 5 minutes or until shells open; discard any unopened shells. Stir in the reserved fish and chopped parsley, and cook 5 minutes. Remove from the heat, stir in lemon juice and check for seasoning. Cover with a towel, and let stand 10 minutes before serving.

CITRUS ROOTS

Preserving Citrus Heritage Foundation

OUR MISSION IS TO ELEVATE THE AWARENESS OF CALIFORNIA'S CITRUS HERITAGE THROUGH PUBLICATIONS, EDUCATION AND ARTISTIC WORK.



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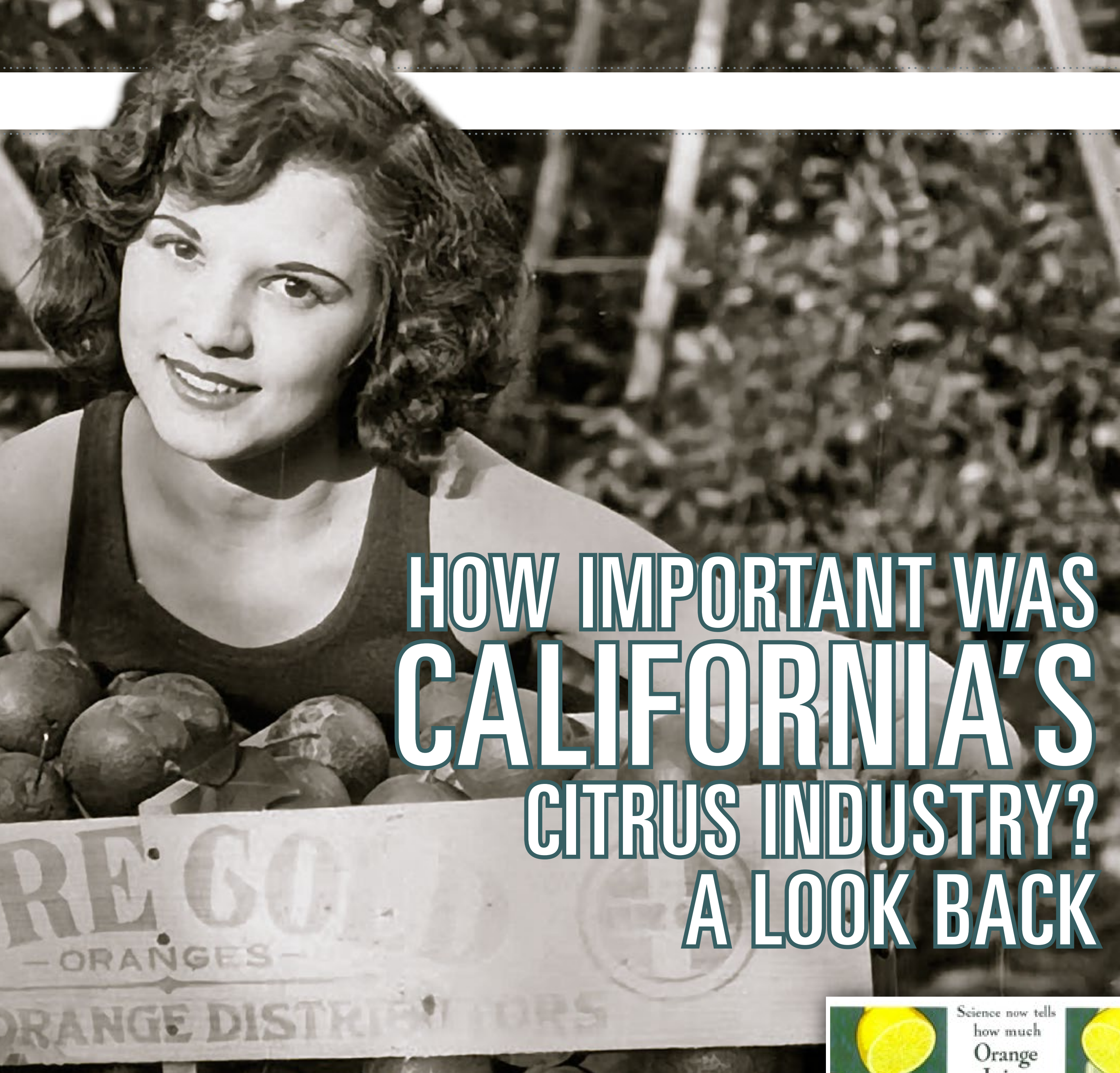
Applying annual citrus pest control provided additional labor.

Richard Barker

Just what did the California citrus industry contribute during its period of dominance that extended from the Roaring Twenties through the aftermath of World War II? How many families directly received their livelihood from citrus? Approximately how many were indirectly linked to the industry? This article, which is the first in a series, takes a look at the 1930s.

By 1930, the grower-owned cooperative serving the California citrus industry was considered one of the biggest, most successful businesses in the United States. This evaluation combines the California Fruit Growers Exchange, Mutual Orange Distributors and smaller organizations into one industry serving California and Arizona. There is no question that this amalgamation in aggregate was one of the largest business entities within the two states.

Today, very few people realize how widely returns were distributed, and what proportions went to other industries for labor, materials and services. In the 1930s, citrus was grown from the Mexican border to the very northern counties of Butte, Tehama and Sierra. The industry furnished the livelihood for an estimated 250,000+ people. Growers and their families comprised far less than half the total. An even greater number of peripheral



HOW IMPORTANT WAS CALIFORNIA'S CITRUS INDUSTRY? A LOOK BACK

The $150 million citrus crop was "pure gold" to Arizona and California.

individuals sought their livings as jobbers by selling fruit and vegetables. They numbered 4,000-5,000 and served half-a-million retail "mom and pop" grocery stores spread throughout every state and Canada. The sale of citrus was a meaningful source of income to wholesale/retail merchants' families.

Paul S. Armstrong, General Manager of California Fruit Growers Exchange, addressed the Fruit Growers and Farmers Convention in 1937. Excerpts of his speech help to provide an understanding of the size of the California/Arizona citrus market. As Armstrong featured, the 1936 citrus crop mainly originated from California and was sold in primary markets for more than **$150 million**, which would equate to more than **$2.51 billion** in 2014 dollars.* This determined the source of funds, which flowed through the U.S. economy as follows (2014 equivalents are listed in parentheses):

- **$40 million ($670.3 million)** covered rail freight and refrigeration. More than half of this sum went to cover wages of rail labor, which was spread over the three main routes that served California.

The health benefits of citrus were being widely marketed in 1931 by Sunkist.

During the Great Depression, the Fernstrom Paper Mills in Pomona, California, boasted 350 employees.

• **$5 million ($83.8 million)** was dispersed for marketing, primarily in California, Chicago and New York. Advertising played a big role in increasing the annual per capita consumption of oranges to 79 by 1936. (As an addendum, in 1939, the Exchange became the first western advertiser to win first prize in the national outdoor advertising art exhibit.)

• **$30 million ($502.7 million)** went to local wages for labor, which was divided roughly between $18 million for grove labor and $12 million for packinghouse labor.

• **$18 million ($301.6 million)** was paid out for orchard supplies such as fertilizers, water, pest controls, orchard heating, etc.; and the multiplier effect spread this sum over the U. S.

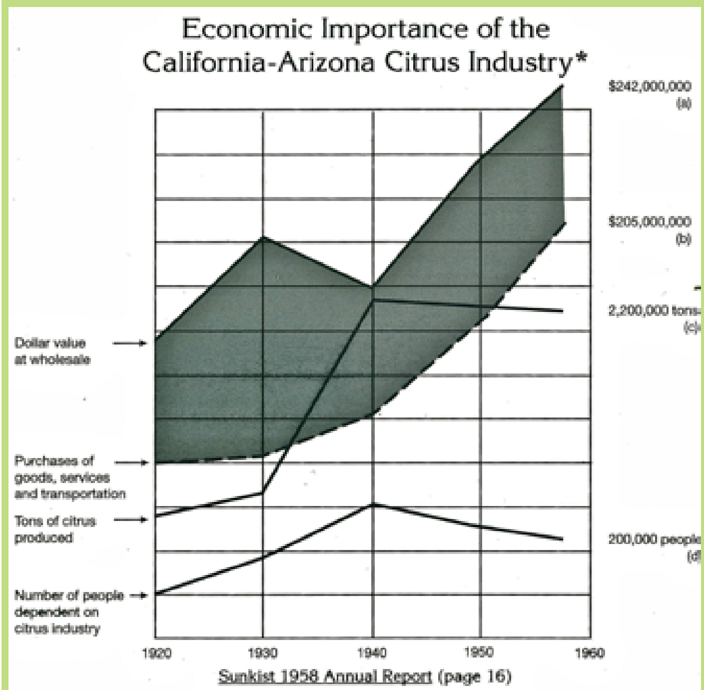
• **$10 million ($167.5 million)** was used for packinghouse materials, chiefly boxes, tissue wraps and processing materials. The treated tissue wrappers were made in Pomona by Fernstrom Paper Mills for Fruit Growers Supply Co. (FGSC); though the pulp supply came from sources within the U. S. (another example of inducing the multiplier effect); and the lumber for the boxes came from the mills of FGSC in Northern California. These dollars purchased goods and services in northern California and elsewhere.

• The remaining **$50 million ($837.9 million)**, or about one-third of the total, was paid to growers as financial fundamentals. A proportion would cover interest charges that had accrued through borrowed funds from financial institutions, or a return on capital. Further, it either covered depreciation of trees or funds for replacement, as well as a margin of profit.

As additional information (see "Economic Importance of the California-Arizona Citrus Industry" chart at right), one can look beyond the duration of Armstrong's speech, and verify that the California citrus industry was only minimally affected by losses from the Great Depression.

Consumer demand for citrus had held up miraculously well, even though the U. S. had gone through a widespread, long period of unemployment and great financial distress. The vigorous past emphasis on health had to be a strong stimulus for the appeal for citrus over other consumer goods. The slogans "Orange for Health - California for Wealth" and "Nature's Finest Beverage: Drink an Orange" could be heard echoing over and over. Billions of sales were booked during the duration of this chart by this industry, which was far more than any other agricultural commodity enterprise during this period. Citrus was a powering entity based on the force of the multiplier effect and the velocity of its monetary turnover. These catalysts drove the economies of California and Arizona to highs for a long period of time.

The California citrus industry was one of the state's largest industries, holding second or third place throughout the 1920s and '30s. An upcoming, concluding article will examine the industry at its apex regarding the bearing acreage of all citrus varieties, and its placement analogous to other industries in the state.



Legend to Graph Economic Importance of the California-Arizona Citrus Industry

* All figures are estimates based on industry sources: (a) wholesale value of citrus, which would equate to $1.9 billion in 2014 dollars, according to the U.S. Inflation Calculator; (b) includes all production, marketing and transportation costs and would equate to $1.6 billion in 2014; (c) includes oranges, lemons and grapefruit; (d) individuals whose income is derived directly or indirectly from citrus. (An increasing use of mechanical handling of fruit in packinghouses has occurred since 1940). In 1940 as a comparison, the entire population of the San Gabriel Valley and the Pomona area was 349,488, and the population of Southern California was 4,169,243.

Source: Sunkist 1958 Annual Report, p.16

** Source: U.S. Inflation Calculator*

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	Early Vegetative	Growth/ Flush	Flowering	Fruit set	Fruit fill	Post harvest
YaraMila® 15 - 15 - 15	20% of total N / 10% of total K_2O					30% of total N / 15% of total K_2O
YaraLiva® CN-9® / Tropicote®			20% of total N	30% of total N		
Ultrasol™ K Plus		20% of total K_2O		30% of total K_2O		25% of total K_2O
YaraVita®	Correction of nutrient deficiencies based on tissue analysis					

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