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Researchers provide their latest progress report in a multi-year in-depth study of desert lemon varieties. In this issue, we take a look at the results of experimental and commercial fruit pack-out, exterior and interior quality, nutrient issues and fruit storage life. See "When Life Gives You Lemons, It’s Time to Make a Profit!" on page 48.
THE MISSION OF THE CITRUS RESEARCH BOARD:
ENSURE A SUSTAINABLE CALIFORNIA CITRUS INDUSTRY FOR
THE BENEFIT OF GROWERS BY PRIORITIZING, INVESTING IN AND
PROMOTING SOUND SCIENCE.

CITRUS RESEARCH BOARD MEMBER LIST
BY DISTRICT 2014-2015  (TERMS EXPIRE JULY 31)

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<tr>
<th>District 1 – Northern California</th>
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<tr>
<td><strong>Member</strong></td>
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<td>Etienne Rabe</td>
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<td>John Konda</td>
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<td>Toby Maitland-Lewis</td>
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<td>Jack Williams</td>
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<td>Joe Barcinas</td>
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<td>Alan Washburn</td>
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<td>Mark McBroom</td>
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June 25
CRB Board Meeting, Marriott Hotel, Ventura, California. For more information, contact the CRB at (559) 738-0246.

June 26
UCCE/CRB Grower Education Seminar, Santa Paula Community Center, Santa Paula, California. For more information, visit www.citrusresearch.org.

June 30
UCCE/CRB Grower Education Seminar, UCR Palm Desert Center, Palm Desert, California. For more information, visit www.citrusresearch.org.

July 1
UCCE/CRB Grower Education Seminar, Exeter Veterans Memorial Building, Exeter, California. For more information, visit www.citrusresearch.org.

July 6
CRB Board member nominations – District 2, Ontario, California. For more information, contact CDFA at (916) 900-5018.

July 7
CRB Board member nominations – District 1, Tulare, California. For more information, contact CDFA at (916) 900-5018.

July 8
CPDPP Board Meeting, Visalia/Exeter, California. For more information, contact CDFA at (916) 403-6652.
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If you are a California citrus grower interested in serving on the Citrus Research Board (CRB), now is your chance. Dates for the CRB nomination meetings have been set. Six seats will be expiring on July 31, 2015, and all six, plus one mid-term vacancy, require that public nomination meetings be conducted by the California Department of Food and Agriculture (CDFA).

District 1, Northern California, which has five seats expiring plus one mid-term vacancy, will hold its nomination meeting on Tuesday, July 7, at the Tulare County Agricultural Building. With the elimination of one position in District 2, Southern California – Coastal, only one seat is available, and that nominating meeting will be held Monday, July 6, at the Red Lion Hotel in Ontario, California. District 3, California Desert, will not have an open seat until 2016 if all terms are fully served by the incumbents. The public member also will be appointed by the Board this year.

The detailed list of seats expiring this year can be found on page 6, where the current board roster appears by name, district and term expiration. An official notice of the meeting will be sent by the CDFA to all known citrus producer addresses in the state.

For more information on voting at nomination meetings or serving as a board member, see Citrograph, Spring 2015, page 14; “CRB Board Structure Updated.”

Chad Collin is director of board and grower communications at the Citrus Research Board.
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The Board welcomes your comments and observations of the Citrus Research Program. The above table lists the audited financial statement for the 2013 and 2014 fiscal years, respectively. The restated 2012 fiscal year is included, as well. A complete copy of the audit is available for viewing at the CRB office at 217 N. Encina, Visalia, California. You are welcome to visit us at any time to discuss any elements of the program and see what we are doing. This is your program, and we look forward to hearing from you.
THAT'S HOW MOVENTO® INSECTICIDE MAKES ORANGES FEEL.

Movento® insecticide delivers powerful two-way systemic action that moves throughout the tree to protect the parts pests seek most, from new shoot growth to roots. This results in long-lasting, reliable protection against above- and below-ground pests, including Asian citrus psyllid, red scale and nematodes. With Movento as part of your ongoing pest management program, you’ll have stronger, healthier trees that produce a higher quality crop year over year.

For more information, contact your retailer or Bayer representative or visit www.Movento.us.
Huanglongbing (HLB, also known as citrus greening) is an economically serious disease that affects and threatens production in many citrus-growing regions around the world, including Florida and Texas in the U.S. A single HLB-affected tree was detected in California in 2012 and was eradicated. Despite continual survey and analyses of plant material, no HLB-affected citrus trees have been detected in California since that time.

Three forms of the disease are recognized. The Asian form of HLB occurs in several countries in Asia, Africa and Oceania, as well as South (Brazil), North (Florida, Texas) and Central America. The African form of HLB occurs in South Africa, while the American form occurs in Brazil.
Although the causal agent(s) have not been conclusively determined, HLB is associated with several ‘Candidatus Liberibacter’ species, namely ‘Ca. L. asiaticus’ (CLas; Asian HLB form), ‘Ca. L. africanus’ (CLaf; African HLB form) and ‘Ca. L. americanus’ (CLam; American HLB form). Only CLas is known to occur in the United States. CLas and CLam are transmitted by the Asian citrus psyllid Diaphorina citri (ACP), and CLaf is transmitted by the African psyllid Trioza erytreae. However, under controlled conditions, both insect species can transmit all three HLB-associated Liberibacter species. CLaf is heat-sensitive at temperatures of 32°C and above, while CLas and CLam are not heat-sensitive.

Currently, there is no cure or sustainably effective control for HLB. Accordingly, a key factor for managing HLB is early disease detection and diagnosis or detection of HLB-associated Liberibacter in psyllid vectors and plant tissue to prevent establishment of the disease.

The Citrus Research Board (CRB) had the foresight, starting in 2006, to fund research projects that sought to develop methods to detect Liberibacter earlier than the currently accepted method of quantitative polymerase chain reaction (qPCR). Now that these methods have come to fruition, the CRB formed an Early HLB Detection Task Force in the Fall of 2014. Good progress has been made by researchers in detecting trees infected with HLB-associated CLas. The overall purpose of the Task Force is to determine the path(s) forward for researchers to move ahead quickly and effectively to develop the most likely technologies for improved early HLB and CLas detection.

Regulatory-based detection of CLas currently is based on analyses of plant tissue and ACP samples using the polymerase chain reaction (PCR), either conventional PCR (cPCR), or real-time or quantitative PCR (qPCR), for CLas-specific DNA sequences.

Recent research on early detection technologies (EDTs) focused on detecting host plant responses to CLas infection and on detecting CLas itself. HLB detection based on host plant responses that appear to be uniquely produced upon CLas infection includes small RNAs, volatile organic compounds (VOCs) and metabolites. A potentially more sensitive and reliable PCR assay, digital droplet PCR (ddPCR), with potentially improved sensitivity and reliability for detecting CLas at low titer (i.e., amount of bacteria within a plant) is being developed. In addition, serological methods have been developed to detect proteins secreted by CLas upon infection and structural proteins of CLas cells.

However, critical evaluation of the comparative reliability (e.g., sensitivity, specificity, high through-put capability) of these HLB EDTs under controlled environmental conditions has been difficult. Results had been often times conflicting, protocols had not been standardized, and other biotic and abiotic conditions may have been complicating factors, as well.

Accordingly, the Task Force recommended that a comparative evaluation of the existing HLB and CLas EDTs be conducted in a field setting. Known CLas infected and uninfected trees were available for this comparative evaluation in Texas. The primary output of this study is determination of which available HLB/CLas EDT has a field level reliability similar to or better than qPCR, including determination of rates of false negatives and false positives. The study was designed by M. Szczerba (Roll Global/Paramount Citrus). The Organizing Team included M. Szczerba, K. Olsen, (Paramount Citrus), J. da Graça and M. Setamou, (Citrus Research Center, Texas A&M University, Kingsville), K. Godfrey (University of California, Davis), G. Vidalakis and J. Morse (University of California, Riverside) and C. LeVesque (Citrus Research Board, Riverside, California). LeVesque also served as Project Coordinator. The researchers who conducted the various EDT assays were C. Davis (University of California, Davis), C. Slupsky (University of California, Davis), W. Ma (University of California, Riverside), H. Jin (University of California, Riverside), C. LeVesque, M. Keremane (USDA-ARS, Riverside, California), J. Hartung (USDA-ARS, Beltsville, Maryland) and R. Fink (Applied Nanotech, Inc., Austin, Texas).

The comparative HLB/CLas EDT study in Texas included testing or assaying plant material and visual assessment of

Tagging a tree in the field for sample collection.
trees (sweet orange, grapefruit) confirmed by PCR, to be HLB-affected. All of the EDTs were used to assay identical sub-samples.

Samples were collected in December 2014 and analyzed in January and February. Results were compiled and analyzed by the Task Force.

Recommendations for the HLB EDT study in Texas included:

1. Explore ways of improving qPCR and ddPCR technology including protocol, threshold values and primers to raise the confidence level of reliability for early HLB/CLs detection. In addition, a process called “planned deviation” to assess the current qPCR threshold for detection of CLas-specific DNA sequences will be developed by State, Federal and university scientists, including C. Hollingsworth, (USDA-APHIS, Raleigh, North Carolina), M. Keremane, (USDA-ARS, Riverside, California), C. Blumquist and L. Kumagai, (CDFA, Sacramento, California), G. Vidalakis (University of California-Riverside); G. McCollum (USDA-ARS, Ft. Pierce, Florida), M. Cilia (USDA-ARS, Geneva, New York) and C. Levesque (CRB, Riverside, California). The purpose of the “planned deviation” is to change the regulatory action point.

2. Provide support for the collection of regulatory-qualifying (if possible) ACP and plant tissue samples in the Hacienda Heights area in southern California and test for CLas by qPCR and ddPCR. qPCR is to be performed by CDFA, and ddPCR is to be performed by M. Keremane (USDA-ARS, Riverside), G. McCollum (USDA-ARS, Ft. Pierce, Florida), M. Cilia (USDA-ARS, Geneva, New York) and C. Levesque (CRB, Riverside, California).

3. Request that CDFA provide the Task Force historical data regarding analyses of the Hacienda Heights ACP and plant tissue samples (actual Ct values of PCR analyses).

4. Request that USDA-APHIS and CDFA share with the Task Force the analyses of the CDFA data regarding the testing for CLas as performed by D. Bartels (USDA-APHIS). These analyses were provided.

5. Re-sample San Joaquin Valley (Fresno, Kern and Tulare Counties) and possibly San Diego County sites where samples analyzed using the metabolite technology rendered inconclusive results (C. Slupsky, University of California-Davis). Test plant material using qPCR.
and ddPCR performed by USDA-ARS, Riverside (M. Keremane), USDA-ARS in Florida (G. McCollum), USDA-ARS in New York (M. Cilia), and CRB (C. LeVesque).

In conclusion, the HLB EDT Task Force will continue to monitor research progress and evaluate the results to help determine the overall reliability of detecting HLB/CLas in clinically asymptomatic field trees as part of the overall HLB management in California. Results from the researchers have been received. Much of the work looks promising; however, more work is needed to validate the results.

Ed Civerolo, Ph.D., is interim president of the Citrus Research Board; Bob Atkins is the CPDPP Statewide Grower Liaison Coordinator; and MaryLou Polek, Ph.D., is vice president of science and technology for the Citrus Research Board.
GROWER SEMINAR SERIES COMING SOON

As chairman of Citrus Research Board’s Communications Committee, I am again honored to be a part of producing this issue of *Citrograph*. Over the past five years, the CRB has worked very hard to produce a magazine specifically for citrus growers and other interested parties.

ANNUAL SEMINAR SERIES AIDS GROWERS

Along with *Citrograph*, live seminars disseminate the progress made in CRB research projects. The upcoming grower seminar series is scheduled for this coming June and July in Santa Paula, Palm Desert and Exeter. Topics to be covered will be timely and informative, thus, hopefully, encouraging your attendance. Planned subjects include an update on the Asian citrus psyllid (ACP) biological control project, which will discuss the production and release of *Tamarixia radiata* and *Diaphorencyrtus aligarhensis* and the progress collaborative programs are making with respect to raising millions of these predatory wasps that specifically attack ACPs. Paired with that session is an update on the progress on the California version of Psyllid Management Areas (PMAs) and area-wide control measures designed to curtail the spread of ACP within the state’s citrus growing regions.

Additionally, an update on the recent International Research Conference on HLB held last February in Florida (see page 28) will be provided, including details on on-going research toward finding a solution to the HLB problem. There appears to be some exciting progress being made in this arena.

Also, discussions with respect to the continuing drought and how growers can manage their groves is planned. Salinity management, tree canopy discussions, fruit quality, the groundwater nitrate programs and the appropriate response to pest management will be provided at all venues.

A session about the Department of Pesticide Regulations update regarding the new Chlorpyrifos (Lorsban) usage restrictions also will be presented, as this has been a valuable tool for citrus growers. Finally, a discussion on food safety and the GAP certification programs will be presented by an unbiased organization, which hopefully will clarify the requirements marketers are requesting and requiring of growers.
We hope that these topics will peak your interest and encourage your participation at the upcoming regional meetings. Please mark your calendars and plan to attend in order to get the latest information on these subjects at the most convenient location for you.

CITROGRAPH KEEPS INDUSTRY UPDATED

Available on a more frequent basis than the seminar series, Citrograph continues to be the source growers can depend on to become informed about industry-funded research and the subsequent results being realized. It’s not all doom and gloom. Many of the on-going projects are producing valuable information and results that we, as growers, can implement immediately to stay ahead of the pest, disease and cultural issues we currently face.

At the Citrus Research Board, we continue to strive to produce a magazine that growers will enjoy, learn from and hopefully keep for future reference. Please feel free to contact the Board with any suggestions, comments or otherwise with regard to your own thoughts for topics to include in future issues.

Personally, as a small Central Valley grower, being a member of the Citrus Research Board for the past five years has been a wonderful educational experience for me. I encourage any and all of you to become more involved with the industry. It really is amazing to see what the many scientists are working so diligently on to solve the problems we growers are facing today.

The future of the industry is in all of our hands. With dedication and cooperation, we can at least slow the threat of HLB into California citrus-producing areas by implementing the solutions we are all working so hard to develop.

Dan Dreyer is the chair of CRB’s Communications Committee, a Board member and a grower based in Exeter, California.

JOIN US AT THE CITRUS GROWERS EDUCATION SEMINAR SERIES

Presented by Citrus Research Board and UC Cooperative Extension

Central Coast
8:30 a.m. – Noon Friday, June 26
Santa Paula Community Center
530 W. Main St.
Santa Paula, CA 93060

Southern California – Desert
8:30 a.m. – Noon Tuesday, June 30
UCR Palm Desert Center
75080 Frank Sinatra Dr.
Palm Desert, CA 92211

Central California
8:30 a.m. – Noon Wednesday, July 1
Exeter Veterans Memorial Building
324 N. Kaweah Ave.
Exeter, CA 93221

Agenda and details at www.citrusresearch.org

No registration fee, but RSVPs are appreciated!
Please call the Citrus Research Board at (559) 738-0246 or email: Info@citrusresearch.org at your earliest convenience.
Florida Citrus Mutual recently hosted a group of growers from California who wanted to learn about growing citrus when HLB is endemic in your state. Florida growers Larry Black and Vic Story were relentlessly optimistic during the tour of their groves.

Black even went so far as to say that his company has planted more trees in the past three years than they ever have.

And that’s just the medicine our beleaguered industry needs; more trees in the ground. To continue to support the citrus infrastructure in Florida – think packinghouses and processing plants – some economists believe growers need to plant more than 20 million trees over the next five years.

I know what you are thinking; asking growers to plant trees in this environment is like politely asking a psyllid to pass up new flush. Not going to happen.

But in fact, growers like Black ARE planting trees spurred on by good fruit prices and a host of government and private sector incentive programs. Many growers are investing in the industry by using the latest production techniques such as high-density plantings, and they are confident they will see a return on investment.

The USDA’s Tree Assistance Program (TAP) is offering significant cost sharing for tree removal and other associated activities to help mitigate the risk of planting. I can’t thank U.S. Department of Agriculture Secretary Tom Vilsack and Florida Farm Service Agency Executive Director Rick Dantzler enough for their hard work in making the TAP program happen. Already, almost 900 growers have been approved for close to $25 million.

Plus, on the private side, Minute Maid (Coca-Cola) and Florida’s Natural, two of our three biggest brands, are providing significant incentive programs. Florida’s Natural made $10 million available to support the planting of one million new orange trees through the 2016-17 citrus season. In order to provide a swifter return, the program encourages denser tree planting – from 270 to 350 trees per acre, up from about 180 per acre. The program will reimburse growers $10 for each new tree planted.

Coca-Cola is offering a floor price of $2 per pound solid on any new plantings that are part of their incentive program. The goal is to get 25,000 acres of trees planted. I’ve personally visited two huge 2,500-acre groves taking part in the Coke program, and it is a beautiful sight.
In addition to these incentive programs, Florida Citrus Mutual is pushing a proposal to tweak the IRS code regarding citrus tree planting that would provide another carrot to growers. The measure will allow growers to immediately expense new plantings.

Under present law, citrus growers generally are required to capitalize the costs of developing a grove prior to the close of the fourth year following the planting. After this pre-productive period, the grove may be depreciated over a ten-year period.

Instead, we suggest growers should be able to elect to immediately expense the costs of acquiring, planting, cultivating, maintaining and developing a citrus grove and the associated drainage, irrigation and infrastructure costs for a temporary period of ten years. The proposal would be effective for ten years.

Black has called this proposal a “game changer” that would spur an unprecedented round of plantings. No doubt, Florida Citrus Mutual, with the help of our colleagues in California, Arizona and Texas, will be pushing the tax incentive on the Hill in the upcoming months.

The incentive programs dovetail nicely with some of the success we are having on the research front. Some of the research could help the new trees should they become infected with HLB. Florida citrus growers have spent $90 million over the past nine years to fund research. This shows the level of commitment to the future of this industry. Plus, due to the great work of the Florida, California and Texas Congressional delegations, the Farm Bill authorized $125 million over the next five years in citrus research funding.

Consequently, we have the best and brightest researchers working on the puzzle, and they are making headway. Antimicrobials are looking like a viable therapy for diseased trees. Thermal therapy heat treatment is showing positive results at knocking down the bacteria, as well. Scientists also are finding rootstocks that are tolerant to HLB.

Farmers want to farm, and I’ve outlined some of the tools that should help them do what they do best. I’m not quite calling the future bright, but with several incentive programs designed to get trees in the ground and cutting edge research to keep them alive and bearing fruit, light is at least peeking through the clouds in Florida.

*Michael W. Sparks is the executive vice president and CEO of Florida Citrus Mutual, which is based in Lakeland, Florida.*
As most folks in the California citrus industry are now aware, the Asian citrus psyllid (ACP) is the most serious threat our orchards have ever faced. The ACP spreads a deadly plant disease known as huanglongbing (HLB or citrus greening disease), which kills trees and currently has no cure. Psyllids, which can spread the disease from tree to tree as they feed, have spread throughout southern California and gradually are taking hold in the Central Valley.

One of the best ways to limit the spread of HLB is to keep the ACP populations as low as possible so that they are unable to pick up the disease and move it. To accomplish this objective and to preserve California’s long, proud citrus heritage will take industry-wide vigilance and cooperation.

Currently, growers in many regions of California are using insecticides in 800-meter areas in an effort to locally control the pest as it is detected. When psyllids recur in a local area, and eggs and nymphs also are discovered, ACP is considered established; and treatments over a wider area become necessary. This is where the area-wide management program comes into play.
Area-wide management is a process in which neighboring growers coordinate treatments within a designated, short timeframe to achieve maximum protection against the ACP. This effort allows growers to respond swiftly as a group in the event that the psyllids infest an area and protects groves better since the treatment extends to acreage where the Asian citrus psyllids likely exist, but have not yet been found on trap cards or through visual inspection. The concept is based on the successful management of other pests in California, and it is gratifying to note that some growers in the southern part of the state already are working together to implement area-wide treatments.

**WHY AREA-WIDE IS HELPFUL**

Individual orchard treatments are not as effective as area-wide ones. Most pesticide residues break down in three to four weeks or less. If these treatments occur in a patchwork fashion, ACP re-invade from neighboring areas when the residues break down.

However, if treatments occur over a large area during a short timeframe, they have the biggest impact on psyllids, which then have nowhere to run and nowhere to hide.

**HOW IT WORKS**

First, a Psyllid Management Area (PMA) is established. These are small, manageable zones that share a number of factors such as temperature, a grower network and landscape or topography. Area growers work together to identify a captain who then leads communication throughout the zone. When psyllid activity dictates treatment is necessary, the captain contacts the growers in the PMA and schedules a treatment within a two-week timeframe. ACP-effective insecticides are suggested from the menu provided in the UC IPM Guidelines. The selection of treatments will vary by season, other pest pressures that currently are present, chemistry rotation and other factors.

**WHO ENDORSES THE PROGRAM**

Although area-wide management is not a mandated system, many industry leaders such as the University of California, Citrus Research Board, California Citrus Mutual and the Citrus Pest and Disease Prevention Program (CPDPP) agree with the approach. It boils down to neighbors helping neighbors. We all believe that area-wide treatments will have the most impact on lowering populations of the Asian citrus psyllid and ultimately give the California citrus industry a better chance of defending against ACP and ultimately the deadly HLB disease.

The CPDPP has employed grower liaisons in key regions of the state to keep growers and industry members up-to-date on ACP and HLB. These individuals keep growers and pest control advisors informed of psyllid detections and can help determine if and when an area-wide treatment approach is best.

For questions regarding the program, contact your local grower liaison. If you don’t know who that is, please access the following link: http://citrusinsider.org/grower_liaisons/

Get to know your neighbors; collaborating with them will help save California citrus from ACP and HLB. We’re all in this together.

**Additional Information**

https://youtu.be/fzZgNvCi8MY

Judy Zaninovich is a licensed pest control advisor and the grower liaison for Kern County.
The 2015 Citrus Showcase chalked up another successful year drawing hundreds of growers and industry members to the Visalia Convention Center on March 5. The Showcase, hosted by California Citrus Mutual (CCM), is the single largest educational forum for the California citrus industry. All of the educational workshops, luncheon and 100+ exhibitor tradeshow marked it as the biggest Showcase ever.

For the second consecutive year, CCM and the Citrus Research Board (CRB) teamed up to provide workshops on the biggest issues facing the California citrus industry. The following provides a synopsis of the key points in the presentations.

**CCM WORKSHOPS**

**WATER: A “PARTIAL” DISCUSSION**

On the heels of the Bureau of Reclamation’s announcement that Central Valley Water Project users will receive a second year of zero percent allocation, industry members filed into the water workshop to hear from three movers and shakers in the water policy arena.

First, Gary Bardini, the deputy director for Department of Water Resources’ (DWR) Integrated Water Management spoke
generally about California’s drought outlook and explained that snowpack and water storage numbers are worse than they were in 2014. DWR and the other state and federal agencies have been working collectively to find new ways to soften the impacts of the drought. Bardini spoke of the process for which water projects funded through Proposition 1 (Water Bond) will be evaluated by DWR. The only exception is the $2.7 billion for storage projects that will be overseen by the California Water Commission.

State Water Resource Control Board Member Dee Dee D’Adamo presented information on the new groundwater legislation implementation process. Much of the requirements are tied to deadlines that are yet to be announced, but it is important for local water interests to begin communicating about the make-up of their Groundwater Sustainability Agency (GSA) and the components of their Groundwater Sustainability Plan (GSP). The goal is to have the groundwater basins in “balance.” This will be particularly difficult given a lack of a surface water and subsequent reliance upon groundwater. D’Adamo urged local groups to work together in their efforts to comply with the new regulations, because if they are unable to do so, the State Water Resource Control Board will step in and create a management program.

George Soares, partner at Kahn, Soares & Conway, wrapped up the session with his summary of water efforts in Sacramento. The legislative and regulatory environment in our Capitol is challenging, and Soares discussed some of the dynamics at play. He summarized the water bond efforts and then the groundwater legislative initiative. He mentioned that the groundwater legislation includes the word “sustainability” 411 times and that particular word has yet to be defined in a court of law. That is just the tip of the iceberg of problems that will develop as a result of this new legislation. There is no debate as to the need to protect our water basins, but the particular legislative path used last year was a poor route. Soares’ straight talk about the challenges and impacts of the new regulations resonated deeply with the 100+ growers in attendance.

WHAT IS DPR THINKING?
In the afternoon workshop, “What is DPR Thinking,” Department of Pesticide Regulation (DPR) Director Brian Leahy presented an excellent overview of his department and how it is adapting to California’s changing landscape. Leahy summarized the many reasons pesticides are important and the importance of science in regulating pesticides. DPR’s primary goal is to ensure pesticide safety. He stressed the progress that has been made in fostering reduced-risk pest management, integrated pest management, and the licensing and training of professional pesticide applicators. Following Leahy’s presentation, CCM Vice Chairman Andrew Brown moderated a “question and answer” session where attendees expressed concerns about recent actions, such as reclassifying chlorpyrifos as a restricted use material.

CRB WORKSHOPS

LATEST AND GREATEST IN THE FIGHT AGAINST ACP AND HLB
CRB Board members reported “grower to grower” on their take-home messages from the recent International Research Conference on Huanglongbing (HLB) in Florida, also known as IRCHLB. The February conference brought scientists together from around the world to share research progress to find solutions for this devastating disease. To date, approximately $250 million has spent on HLB research. No magic bullets yet, but there is reason to be optimistic.

Jack Williams presented on HLB management that utilizes therapies – antibiotics, bactericides and thermotherapy strategies. This strategy subscribes to the belief that HLB infection and/or impact on tree productivity can be “managed”
by either killing bacteria or enhancing tree health and the host’s immune response. Benefits exist for each method, but there is no permanent solution, with mega-nutrient regimens ultimately becoming uneconomical within three to five years of showing symptoms. Thermotherapy and antimicrobials can be effective, but regulatory hurdles and actual cost-effectiveness in the field are unknown.

Canine detection of HLB was covered by Jack Williams. To date, dogs have demonstrated 99.97 percent accuracy in detecting infection by “sniffing out” Volatile Organic Compounds (VOCs), even in pre-symptomatic trees. However, research is still in the early phase and aimed at analyzing accuracy of detecting “newly” infected trees (very low titers/bacteria levels). Ten dogs are in training with three field-ready and seven nearly field-ready at the time of the March report.

Ed Civerolo was optimistic as progress has been made in developing chemotherapeutic treatments for managing HLB. This includes new and novel materials, new formulations, novel combinations and delivery strategies. These treatments need more extensive evaluation in commercial grove settings to validate effectiveness, but are additional tools for use as part of integrated HLB management strategy.

John Konda spearheaded the host plant resistance/tolerance to HLB topic. In Florida, he had participated in a lively discussion led by Ed Stover on the current status and evaluation of field blocks for resistance/tolerance. CRB is moving toward bringing in varieties from Florida for evaluation in California. Another take-away was that pre-existing Phytophthora infection increases severity of HLB disease expression.

Wrapping up the session was Jim Gorden, summarizing the Florida psyllid testing project, which tracked the rapid spread of HLB in Florida due to the delayed symptoms in infected trees (incubation period). The take-home messages for California are:

1. Keep psyllid populations as low as possible, for as long as possible.
2. Avoid moving psyllids long distances. Moving unprocessed fruit and unprotected plants means risking moving psyllids with undetected HLB.
3. Since latency and incubation periods are long, we should NOT rest assured when told that no more HLB positive plants have been detected.

For more info on key messages from the IRCHLB, see page 28.
FEATURED CRB-FUNDED PROJECTS

CRB’s second session featured two funded projects that directly impact the California grower. The food safety issue was addressed by Trevor Suslow (UC Davis) as he seeks to strengthen industry Best Practices. He discussed the survival of food borne pathogens (such as *Salmonella*) during both pre-harvest and post-harvest times. His research will continue to answer such questions as: Do these pathogens survive pressure washes and other methods of sanitation in the packing house? Can food-borne pathogens be detected in the final pack of citrus fruit waiting to be placed on grocery store shelves?

Last, Jim Adaskaveg (UC Riverside) spoke on *Phytophthora syringae* (brown rot) management in relation to the Chinese export market that was closed to California citrus in April 2013 and Tulare County in February 2015. He highlighted cultural practices such as resistant rootstocks, planting on berms for good drainage, avoiding over-irrigating, tree skirting, fumigation and fungicides. Complete Good Agricultural Practices (GAPs) for the management of *Phytophthora* can be found at the California Citrus Quality Council’s web site, www.calcitrusquality.org.

EXHIBITORS

The Citrus Showcase has grown during its 20-plus-year history, and 2015 had more exhibitors than ever. Growers and industry members connected with businesses that cater directly to the California citrus industry. Nurseries, chemical companies, irrigation and equipment suppliers, plus many more were in attendance.

LUNCH AND KEYNOTE SPEAKERS

Lunch always attracts hundreds of growers with a good meal and a powerful keynote speaker. This year followed that tradition, but broke the solitary speaker model with a three-man panel discussing the first-hand details of the September 11, 2012, attack in Benghazi, Libya. The three American security officers featured in the book *13 Hours* offered their harrowing, true account of repelling the terrorist attack on the US State Department Special Mission Compound. It was a riveting story and dialogue.

Also announced at lunch, California Citrus Mutual and Bayer CropScience are partnering on a social media campaign called #CitrusMatters to raise awareness among homeowners about ACP and HLB. For every time the hashtag #CitrusMatters
is shared on social media, Bayer CropScience will donate $1 – up to $25,000 to CCM in support of biological control programs for the Asian citrus psyllid.

This campaign will utilize an educational web site, www.citrusmatters.bayercropscience.us, and the hashtag #CitrusMatters in social media to educate California homeowners with backyard citrus trees about the important role they play in helping protect California citrus from ACP and HLB.

The industry is encouraged to join in on social media by sharing why #CitrusMatters with family, friends and neighbors who have citrus trees in their yards.

Alyssa Houtby is the director of public affairs for California Citrus Mutual. Chad Collin is with the Citrus Research Board, where he serves as director of board and grower communications, and also as associate editor of Citrograph.
Our products are formulated for citrus and work together as a program trusted by growers and researchers for higher yields, quality fruit and effective disease control. K-PHITE 7LP provides superior disease protection and plant health while stimulating plant vigor. RENEW is the most effective way to provide the plant with the essential combination of phosphorous and potassium during the pre-bloom and early fruit sizing period. K-Cellerate is designed to maximize the potassium within the plant system required for cell enlargement, cell wall strength, fruit expansion and reduced post-harvest decay. With a unique patented continuous-reaction process and molecule that achieves unmatched purity, the PFS program is unequalled in plant response.
During February 9-13, 2015, the 4th International Research Conference on Huanglongbing (IRCHLB) was held at the Caribe Royale Conference Center in Orlando, Florida. This conference has been held about every two years, beginning in 2008, and was once again hosted by Florida Citrus Mutual. Plans for IRCHLB V, which will be held at the same location in 2017, are already underway.

The IRCHLB IV was attended by 505 researchers, government officials, industry representatives and growers from 25 nations, including several members of the Citrus Research Board and the Ventura County Farm Bureau. Three keynote speakers were featured: Josy Bové, Ph.D., (France), speaking on, “Half a century on HLB: learning about the disease and trying to control it;” Chris Gilligan, Ph.D., (England), “An epidemiological perspective for the integrated management of HLB;” and Neil McRoberts, Ph.D., (California), “Embedding epidemiology and technology in their
socio-economic context to assist with strategic planning of HLB management.” Attendees listened to 106 15-minute scientific presentations, viewed 116 posters, and engaged in 20 discussion sessions, including topics such as host resistance and tolerance, therapeutics, culturing Liberibacters, area-wide management and early detection technologies.

Although more than $250 million has been spent on ACP/HLB research to date, there are still no cures or magic bullets for the disease. However, participants left the conference with a sense of optimism that good progress had been reported. What follows is a brief synopsis of some of the most interesting and/or promising areas.

**THERMOTHERAPY**

The use of heat to cure citrus trees of HLB-associated bacteria was described in a previous *Citrograph* article (Xia, et. al. 2012. July-August, pp 44-49). Agricultural engineers have been busy designing equipment that is easy to use and can efficiently and effectively treat hundreds of acres of trees in a short length of time.

Three different methods were described including solarization, steam heat and hot water trunk wraps. Solarization involves placing an enclosure of thick plastic around the tree for about two weeks, allowing the sun to heat up the enclosure naturally. While it is the least expensive method, there is no control over the temperature and availability of sunlight. Also, this method causes the most damage to the tree. Steam heat involves more equipment, but the heat therapy process takes only one to two minutes per tree. A great deal of experimentation has been conducted to determine the duration and temperature limitations per tree canopy volume. In addition, scientists are evaluating whether pulsing the steam heat will minimize the adverse effects of heat therapy. Specialized equipment has been designed that can easily move through a grove of mature trees at a rate of 100 trees per day (Figure 1). A third...
The process wraps irrigation tubing around the trunk of a tree and recycles hot water through the tubing. The intent is to kill the bacteria as it moves up and down the trunk within the phloem and may address, to a limited extent, bacteria residing in the roots of a tree (Figure 2).

The limitations of thermotherapy must be considered. If too much heat is administered, the tree can die. Often, excessive leaf drop occurs followed by prolific flushing. This situation requires immediate pesticide treatment to control psyllid populations. Yield reduction has occurred in some heat-treated groves. Depending on the method and the geographical location of the citrus grove (e.g., July in the Coachella Valley), the use of thermotherapy may be limited to certain times of the year.

The major advantage to thermotherapy is that no approval or regulatory action is necessary to implement any of the above methods. The methods are easy to use and can be relatively inexpensive.

**CANINE DETECTORS**

USDA HLB Multi-agency Coordination (MAC) Group funds have been used to train dogs to “sniff” out trees affected by huanglongbing. Conference participants were treated to a real-time demonstration and were able to meet Mira and Bello and their handler (Figure 3). It was reported that the ability of canines to detect plant diseases (including citrus canker and HLB) may be as high as 99.97 percent. Within the year, HLB-trained canines will visit California.
ANTIMICROBIALS

Florida has funded a great deal of research in the area of antimicrobials. More than 100 compounds have been evaluated including antibiotics, biocides, peptides, fungal compounds and other assorted compounds. Nine have proven highly effective against HLB.

Whereas a few antibiotics have proved effective (penicillin G, oxytetracycline and streptomycin [Kasugamycin]), it is difficult to see their implementation in the California citrus industry. First, the delivery system is problematic. Trunk injections are the most effective method, but these severely damage the tree. The antibiotic activity is short-lived and the break-down products often are harmful. The compound itself or the by-products can accumulate on or in fruit. It will require many years to acquire the data necessary for regulatory approval, and it is questionable whether or not treated fruit would be accepted by trade partners and consumers.

A new therapeutic called “Zinkicide”, which is a formulation of zinc oxides, is being evaluated in Florida field trials. Preliminary results indicate it is long-lived and produces little phytotoxicity. It is also effective against citrus canker.

Several antimicrobial peptides (AMP) have been developed. So far, the only delivery method is through genetic modification of the host plant. AMPs have been placed into both scion and rootstock varieties, where they target structural components in the bacteria that prohibit proper membrane formation. The effects are probably long-lived as the plant will continue to produce the AMP.

A combination of therapies has proven more effective than one method used alone. When antimicrobials were applied almost immediately after a thermotherapy treatment, there was a synergistic effect, and the longevity of the tree was increased while the amount of pre-mature fruit drop decreased.

HOST PLANT RESISTANCE AND TOLERANCE

Citrus varieties, hybrids and citrus relatives naturally occurring and those developed by conventional breeding have been evaluated for their potential HLB resistance and/or tolerance. Several varieties look promising, and the CRB is in the process of bringing some of these varieties into California for evaluation under California conditions.

PHYTOPHTHORA INTERACTIONS

One of the most alarming presentations described the synergistic effect of Phytophthora with HLB. Plants that already are infected with species of Phytophthora succumb to HLB sooner and develop more severe disease symptoms than those plants not infected with the root-rotting fungus. Furthermore, HLB infection significantly reduces the effectiveness of chemical treatments for Phytophthora. Despite this, Florida growers must continue to treat for both pathogens in order to get a few more years of productive life from their trees.

ACP BIOLOGY

It is known that when psyllid nymphs acquire HLB-associated bacteria, the bacterium is retained for the life of the psyllid and the adults are more likely to transmit the bacteria to other plants. Susan Halbert suggested that when an adult female psyllid lays her eggs on new flush, she will also feed on the host plant and transmit the bacteria in the process. This results in a localized infection. As the eggs hatch and the nymphs begin to feed, it is highly likely that a large number of these nymphs will acquire the bacteria from these localized infections. Considering that a female will lay hundreds of eggs at a time, it is unbearable to think about how many infested psyllids then disperse from that original tree. Dr. Halbert used this concept to explain how HLB spread so rapidly throughout Florida. Psyllids collected in retail nurseries tested positive nine months prior to plants testing positive for HLB-associated bacteria.

LIVING WITH HLB

Scientists from Brazil and Florida presented data in support of the “Three-Pronged Control” strategy. This includes only planting known, disease-free trees, continuous chemical control of the psyllid and continuous removal of infected trees. They identified two major obstacles to the success of this program; grower-neighbors who do not follow this program and proximity to residential areas with backyard citrus trees. Area-wide management of the psyllid is extremely dependent on the timely cooperation of the growers who are part of a management district.
The reports regarding citrus production in Florida were depressing. Production and mitigation costs have significantly increased, while yield and fruit quality have significantly decreased. Florida growers are experiencing a two percent increase in pre-mature fruit drop this year and are packing an average of 19 more fruit per box resulting in a reduction of four million boxes. One of their big questions is – can replanting keep up with the attrition rate?

Nutritional programs do not prevent citrus trees from infection, but they can help the plant to fight it off. Stressed trees succumb to HLB sooner than healthy trees. Therefore, maintaining healthy trees PRIOR to infection will prolong the productivity of a grove. Knowing soil type and maintaining the root health of trees are vital to staying in business.

**CLUSTER ANALYSIS**

*Citrograph* readers will hear more about the “Cluster Analysis” performed by David Bartels. Using data collected in Texas and California, he analyzed the relationship between the locations where psyllids were trapped and the results of laboratory tests of the insects for HLB-associated bacteria. The psyllid test values initially were above the threshold for being Liberibacter positive. However, these ACP test values declined for one or two years before plants were diagnosed as HLB-positive in Texas. He reported that a similar trend seems to be occurring in southern California.

**FINAL WORDS**

HLB IS COMING! Plant disease epidemiologists predict that additional trees in California will test positive for HLB-associated bacteria by the end of this year. As growers, you must increase your vigilance in searching for Asian citrus psyllids in your grove, especially breeding populations. When ACP are detected, contact CDFA and/or your local treatment coordinator immediately. If you think your trees are showing suspect symptoms, contact CDFA. A trained inspector will visit your grove.

Additional Reading


MaryLou Polek, Ph.D., is vice president of science and technology for the Citrus Research Board in Visalia, California.
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novasource.com
An on-line course highlighting how pesticide resistance develops among pests is now available on the University of California Agriculture and Natural Resources Statewide Integrated Pest Management (UC Statewide IPM Program) web site, www.ipm.ucanr.edu. Created primarily for pest control advisors and other licensed pesticide applicators, this course describes the mechanisms of resistance in pathogens, insects and weeds and discusses ways to manage resistance within the different disciplines.

Divided into three narrated presentations followed by a final test for each section, this course has been approved for two continuing education units in the “Other” category from the Department of Pesticide Regulation.

The course is based on a series of workshops held in Davis, Fresno and at the Kearney Agricultural Research and Extension Center during the spring of 2014 presented by Doug Gubler, Ph.D., (Department of Plant Pathology, UC Davis); Larry Godfrey, Ph.D., (Department of Entomology and Nematology, UC Davis); Beth Grafton-Cardwell, Ph.D., (Lindcove Research and Extension Center and UC Riverside); and Kassim Al-Khatib, Ph.D., (UC Statewide IPM Program).

It also is important to note that there are six citrus-specific pest courses available on www.ipm.ucanr.edu.

Cheryl Reynolds is senior editor and interactive learning developer for the University of California Agriculture and Natural Resources Statewide Integrated Pest Management Program in Davis, California.
MANAGING CITRUS BROWN ROT FOR EXPORT TO CHINA
From a Chore to a Necessity

J. E. Adaskaveg, H. Förster, W. Hao and D. Cary

PROJECT SUMMARY
Some doors just will not open easily; others close easier still! As the California citrus industry tries to navigate global markets, some doors have to be reopened with science to satisfy trading partners so that unwanted microbes or insects do not hitch a ride with our fruit. The most recent problem with California’s trade of oranges has been in China with a common disease called brown rot. In late 2012, brown rot caused by a species of Phytophthora that is a quarantine pathogen in China was detected and subsequently was reported in early 2013. Needless to say, the market was closed. Despite numerous attempts to resolve the crisis during the summer and fall of 2013, it was not until the summer of 2014 that a two-year agreement was reached to re-open trade with China. In this research project, we are improving our understanding of diseases caused by Phytophthora species and are developing new approaches to manage Phytophthora brown rot and root rot to help growers meet the demands of exporting fruit to international markets.
Brown rot of citrus fruit is caused by species in the genus *Phytophthora* that are members of the kingdom Stramenopila. These organisms are fungal-like, but differ in numerous characteristics from true fungi and have evolved adaptations to life in aquatic environments. In sub-tropical climates like in California, brown rot-causing species include *P. citrophthora*, *P. syringae*, *P. parasitica* (= *P. nicotianae*) and *P. hibernalis*. These species also cause other diseases of citrus such as *Phytophthora* root rot, foot rot and gummosis. They are present in most citrus-growing areas of California, and fruit damage mostly occurs in the winter season. Brown rot may be present alone or in combination with *Phytophthora* root rot, foot rot or gummosis.

*P. parasitica* and *P. citrophthora* have also been reported from citrus in China, but not *P. syringae*, and so it is considered a quarantine pathogen. *P. syringae* was first described from lilac (*Syringa vulgaris*) in 1881. It has a wide host range of more than 31 species in 19 genera of flowering plants, mainly in temperate climates around the world including Europe, North America, parts of South America, Asia (e.g., Korea) and New Zealand. Major diseases caused by *P. syringae* include collar and fruit rot of apple, leaf spot and dieback of lilac, pruning wound canker of almond, and root rot and fruit brown rot of citrus. On citrus species, *P. syringae* has been reported in Argentina, Brazil, Greece, Italy, Morocco, Peru, Portugal and the United States. Detailed studies on the occurrence of *Phytophthora* species are not available from China, and additional species may be on quarantine lists with other trade partners. Therefore, a general approach to the biology and epidemiology of *Phytophthora* diseases on citrus is most appropriate for developing brown rot management programs.

**SYMPTOMS AND IMPACT ON FRUIT INTENDED FOR THE FRESH MARKET**

Infections by *Phytophthora* spp. cause an olive-brown discoloration of the rind, and the fruit has a distinctive pungent, aromatic odor. Fruit remain firm and leathery, but they can be invaded by secondary decay organisms that cause the fruit to deteriorate rapidly. Fruit infected on the tree usually fall to the ground. At very high humidity, fruit become covered by a delicate white growth of the fungus.

Losses due to brown rot can be very high, but are sporadic and are associated with periods of heavy rainfall. Although losses can occur in the orchard, the most serious aspect of the disease is that fruit infected before harvest may not show symptoms at the packinghouse. Mixing infected fruit with non-infected fruit on the pack-line allows the pathogen to spread quickly throughout large batches of fruit while in storage and during transit. In addition, brown rot-infected fruit are readily colonized by wound pathogens, such as *Penicillium* and *Geotrichum* spp.

**DISEASE CYCLE AND EPIDEMIOLOGY**

*Phytophthora* species that cause brown rot can survive in the soil as persistent chlamydospores (*P. parasitica* only), oospores (*P. parasitica*, *P. syringae* and *P. hibernalis*); or as mycelium (*P. citrophthora*) in decaying roots or fallen, diseased fruit. Free water is required for dissemination and infection of the host. All species produce sporangia (Figure 1A) that develop within 18 hours from chlamydospores, oospores (Figure 1B) or mycelium at high soil moisture content. Therefore, *Phytophthora* diseases are exacerbated by wet soil conditions. Swimming zoospores that are formed in the sporangia are released and may be splashed up onto low-hanging fruit in the tree. For *P. syringae*, a continuous wetness period of three to four hours at temperatures between 57° and 73°F (14° and 23°C) is necessary for fruit infection. Under fluctuating temperatures, total wetness of 24 to 30 hours is needed for infections to occur. New generations of sporangia will again release zoospores if wet conditions persist and serious brown rot epidemics may occur.

*P. parasitica* is most active in the warmer seasons, whereas *P. syringae* and *P. hibernalis* are mainly present during cooler environments, namely in the fall and winter. *P. citrophthora* can cause disease throughout the year. These seasonal occurrences
of the four species correspond with the optimum growth conditions of the organisms.

**MANAGEMENT**

Management of *Phytophthora* brown rot requires an integrated approach of controlling all phases of the disease including root rot and foliar and trunk diseases. General pre-plant strategies include crop rotation, soil fumigation and using clean plant material to establish the orchard, and also selection of *Phytophthora*-tolerant rootstocks such as the hybrids of Carrizo or C-35. Planting on berms is often done to improve soil drainage of water away from the root system once irrigation is completed. Irrigation systems should be set up using drip or micro-sprinklers to allow for adequate watering with minimal durations.

The focus of our research is to evaluate post-planting seasonal practices specifically for brown rot control, including traditional practices, procedures under the USDA APHIS-AQSIO (US-China) two-year agreement and future strategies.

**A) Traditional Management Practices for Brown Rot.** Brown rot of citrus can be effectively managed with field applications of Bordeaux mixture (copper sulfate and hydrated lime) prior to rainy periods during the harvest season. Many commercial fixed or neutral coppers (e.g., copper hydroxide, copper oxide) are also very effective. Copper sprays should contain 0.6 to 0.8 lbs. (272 – 363 grams) of metallic copper per 100 gallons (378.5 liters) of water and should be applied at high-volume (typically 400 gallons or 1,514 liters per acre). In order to prevent copper phytotoxicity, add 0.3 to one pound (136 – 454 grams) of hydrated lime per one pound (454 grams) of metallic copper fungicide to neutral coppers. Depending on the amount of rainfall, more than one application of copper may be necessary.

Pre-harvest treatments of the canopy or soil with systemic phosphonate fungicides can also provide effective control of brown rot before harvest when applied up to several weeks prior to conditions suitable for infection. A number of products are available, but because phosphonates are currently not registered in China, this group of chemicals containing phosphite cannot be used for orchard blocks or lots planned for export to China or other markets where the minimum residue levels (MRLs) have not been established. Another pre-harvest fungicide for soil treatment is phenylamide mefenoxam. This compound is usually applied twice a year at the time of root flushes (e.g., for citrus in California, this is in late spring and late summer/early fall).

**B) Procedures under the US-China Agreement - Summer 2014.** The two-year agreement for export of oranges to China is based on pre-harvest management practices. Because the species of *Phytophthora* occurring on citrus in California are soil-borne and have limited aerial dissemination, fruit lower to the ground are more vulnerable to infection from splashing water. A common practice to avoid lower fruit infection is “tree skirting,” where the lower branches of the tree are removed.

The University of California Integrated Pest Management Guidelines for Citrus Brown Rot recommends tree skirting at 24 inches (61 cm). To ensure that lower fruit are not exported to China, the agreement also states that fruit sent to China should be harvested above 20 inches (50 cm), whereas lower fruit should be sent to other markets.

The agreement also provides guidelines to manage pathogen populations in the soil and root rot by using rootstocks tolerant to *Phytophthora* root rot and fungicides (i.e., mefenoxam as described above). Orchard blocks with tolerant rootstocks should be prioritized for export to China because pathogen populations in the soil are generally lower than in orchards with susceptible rootstocks. Lower soil populations result in a lower potential for brown rot. Foliar and fruit applications with copper also should be done, and new treatments should be developed (see “Future Strategies” on next page).

The first application is mandatory and should be done at the beginning of the navel orange season after the first rain. This is usually between October 15 and November 30 for most of the southern Central Valley of California where oranges are produced. Additional applications should be based on environmental conditions that favor the disease (e.g., high rainfall and cool temperatures during late fall and early winter). Persistence of copper can be empirically predicted. In Florida, a Citrus Copper Application Scheduler was developed (available at [http://agroclimate.org/tools/Citrus-Copper-Application-Scheduler/](http://agroclimate.org/tools/Citrus-Copper-Application-Scheduler/)). In this model, date of application, amount of copper used, volume of application per acre and the amount of rainfall after the application determine the amount of residual copper present over time (i.e., weeks after application). As in any risk model, threshold levels for determining re-application are indicated. Our goal is to adapt this model for California conditions.
Numerical risk model for forecasting brown rot caused by P. syringae

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<th>(°C)</th>
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<th>19-25</th>
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Figure 2. Preliminary numerical risk model for forecasting brown rot of oranges caused by Phytophthora syringae based on cardinal temperatures, temperature range for growth and wetness periods for infection. Numerical risk values are assigned for each rain event as follows: 0 = no infection; 1 = low risk; 2 = moderate risk; and 3 = high risk for infection. To use the model, risk values are accumulated over time and a threshold value is used to determine overall risk.

Another component of the US-China agreement is the monitoring of orchards for symptoms of brown rot in two- to four-week intervals throughout the navel harvest season. Shorter intervals are recommended during periods with more favorable environments for disease. Also, orchards should be surveyed one week before harvest to ensure low disease levels of fruit planned for export to China. Risk assessments for brown rot on harvested fruit have been established in our studies during the last two years. Thus, risk of brown rot is low, moderate or high when less than 10, 10 to 20 or more than 20 brown-rotted fruit per acre are present, respectively. Fruit from high-risk orchards should not be shipped to China. Caution should be followed with moderate disease risk assessments, whereas low-risk orchards should be considered as high priority for fruit export.

A forecasting model for P. syringae infection periods is being developed based on environmental conditions conducive for the pathogen. Figure 2 illustrates a preliminary risk model. Temperatures between 41° and 77°F (5° and 25°C) and more than 25 hours of wetness represent high risk for a brown rot infection period. Wetness periods of 10 to 18 hours for the same temperature range are less conducive and represent a moderate risk; whereas less than 10 hours of wetness result in the lowest risk. Temperatures of less than 41°F (5°C) or higher than 77°F (25°C) are not favorable for growth at all indicated wetness periods in the table. Numerical risk values are assigned for each event as follows: 0 = no infection; 1 = low risk; 2 = moderate risk; and 3 = high risk for infection. To use the model, risk values are accumulated over time and a threshold value (tentatively set at 10) is used to determine overall risk.

C) Future strategies. For the development of season-long disease management strategies, new soil, foliar and post-harvest fungicide treatments are being evaluated and developed in our research program. In the United States, phosphonate fungicides are exempt from tolerance, and they can be used in the orchard and for post-harvest treatment in the packinghouse. In part, the reason for the two-year US-China agreement is that China will need to set MRLs for the phosphonate chemicals, and this will take about two years according to Chinese regulators. Currently, therefore, we cannot use phosphonate fungicides in California for exporting fruit to China. Once MRLs are established in China, we plan to develop guidelines for pre-and post-harvest use for these products.

Pre- and post-harvest uses of phosphonates will most likely follow existing labels in the United States because we asked China to use these rates to set their MRL levels. For post-harvest use, we plan to use the phosphonate fungicide potassium phosphite by itself or in combination with heat treatments for integrated citrus post-harvest brown rot management. We also worked with IR-4 to establish international MRLs in a residue program with our Pacific Rim trading partners. Historically, the IR-4 program only developed pesticide tolerances for the United States. Thus, for the first time with any pesticide, IR-4 is working on this challenging international residue project.

In our research, we are developing heated and non-heated post-harvest treatments with 2,000 to 4,000 mg/L potassium phosphite that can be mixed with our standard post-harvest fungicides. Used at ambient temperature, the low rate of potassium phosphite was very effective when applied within 20 hours after inoculation (using high inoculum levels and constant optimum environments for fungal development prior to treatment) and reduced the incidence of decay by >96 percent as compared to the control. Under fluctuating temperatures and lower inoculum levels that are more typical in California orchards, the fungicide will be effective even after longer periods of incubation following an infection period. If the fungicide treatment was heated to 131°F (55°C), then less than one percent of the fruit developed brown rot. When this post-harvest treatment is integrated with pre-harvest management strategies in a “systems approach,” we determine that we will be able to obtain greater than 99.9 percent control.
New pre-harvest fungicides belonging to several chemical classes also are being developed so that rotational programs of different modes of action can be integrated into pre- and post-harvest management strategies. For this, we are screening several exciting materials with very high activity against species of *Phytophthora*. Several compounds are shown in Table 1 and compared to mefenoxam (Ridomil Gold) as a standard fungicide for their *in vitro* activity. Oxathiapiprolin is one of the most active compounds that we ever evaluated against *Phytophthora* species with EC50 values of less than 0.001 mg/L (< 1 ppb – Note: The lower the value, the more active the compound).

Research is ongoing with these fungicides as pre-harvest treatments to manage root rot and brown rot diseases. For this, we are conducting field trials with fluopicolide and oxathiapiprolin as soil treatments to reduce the incidence and severity of root rot, and we are testing mandipropamid and oxathiapiprolin as pre-harvest foliar treatments for brown rot control. Based on our efforts, fluopicolide and mandipropamid are in the IR-4 federal specialty crop registration program as pre-harvest soil and foliar treatments of citrus, respectively. Oxathiapiprolin is being registered directly by the registrant for both soil and foliar applications on citrus.

Table 1. *In vitro* toxicity of selected new fungicides as compared to mefenoxam (Ridomil Gold) against mycelial growth of several *Phytophthora* species causing brown rot of citrus in California. Values are effective concentrations (mg/L) to inhibit growth by 50 percent (EC50). Fungicide Resistance Action Committee (FRAC) Groups of fungicides represent different modes of action. Oxathiapiprolin is a new mode of action.

<table>
<thead>
<tr>
<th>Fungicide</th>
<th>FRAC</th>
<th><em>P. parasitica</em></th>
<th><em>P. syringae</em></th>
<th><em>P. citrophthora</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mefenoxam</td>
<td>4</td>
<td>0.080 – 0.280</td>
<td>0.004 – 0.030</td>
<td>0.024 - 0.058</td>
</tr>
<tr>
<td>Fluopicolide</td>
<td>43</td>
<td>0.040 – 0.080</td>
<td>0.020 – 0.050</td>
<td>0.033 - 0.049</td>
</tr>
<tr>
<td>Mandipropamid</td>
<td>40</td>
<td>0.003 – 0.008</td>
<td>0.002 – 0.006</td>
<td>0.004 - 0.005</td>
</tr>
<tr>
<td>Oxathiapiprolin</td>
<td>New</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

J. E. Adaskaveg, Ph.D., is a professor of plant pathology, H. Förster, Ph.D., is a project scientist, W. Hao, Ph.D., is a postdoctoral scholar and D. Cary is a staff research associate in the Department in the Plant Pathology and Microbiology, University of California, Riverside.

CRB Project No. 5400-148

Literature


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DEVELOPMENT OF DISEASE-RESISTANT CITRUS ACCEPTABLE TO CONSUMERS

Eliezer Louzada and James Thomson

SUMMARY

The second year of this CRB-funded project is focused on the development of citrus cultivars that exhibit resistance to multiple diseases such as HLB, Phytophthora disease complex and citrus canker. We are using precise genetic engineering to introduce into disease-susceptible commercial citrus varieties, potential citrus genes to create resistance. Even though these genes are already in citrus, they normally do not respond quickly enough or produce enough protein to provide the required resistance level. By copying these genes and reintroducing them back into citrus, we can make the genes work more quickly, produce more protein and, therefore, provide resistance to several diseases simultaneously. While this project is producing genetically modified plants (GMOs), the use of only citrus genes may help with consumer acceptance.

We also are creating transgenic citrus cultivars that will be devoid of resistance markers (i.e. antibiotics or herbicides), specifically to improve public acceptance and to help with federal deregulation. Figure 1 shows an example of this concept where a gene from citrus (called CSM-1 gene) was re-introduced into Ruby Red grapefruit, and the transgenic plant became resistant to root rot (it still needs to be tested for HLB resistance and other diseases). We are using this and other citrus genes in an attempt to create citrus varieties that exhibit broad-spectrum disease resistance. Moreover, we are testing a new method of introducing the genes into citrus that will facilitate commercializing the final product.
INTRODUCTION

Citrus production has been plagued by many diseases, including those caused by the *Citrus tristeza virus*, *Phytophthora* spp., *Xanthomonas citri* subsp. *citri* (citrus canker) and more; however, the industry could manage these diseases and survive. With the introduction in the United States of the disease huanglongbing (HLB), also known as citrus greening, this scenario has changed completely.

Today, the industry faces one of its greatest challenges. The Florida citrus industry has been devastated by HLB, and the prognosis for other citrus-producing states is no different if a permanent solution is not found. Unlike other diseases, surviving with HLB has proven difficult and not commercially viable. It is a consensus opinion that only resistant varieties will permanently solve the problem, but as yet, no strong resistance has been found in citrus. The use of genetic engineering to introduce disease resistance genes into commercially important citrus varieties is the best approach, because it is a precise method to introduce a trait without changing the genomic makeup of the plant and, therefore, trait quality remains unaffected. This is unlike a breeding scheme where the traits of interest from the parent line are generally lost or mixed in undesirable ways.

There are currently many ongoing projects with these objectives, but the majority use genes that are not from citrus; and in addition to the gene of interest, they also contain marker genes (i.e. antibiotics/herbicides) to ensure the gene has been inserted. Considering that the public opposition to genetically modified organisms (GMOs) is still strong, a desirable approach is to use genes that are from citrus and to remove antibiotic resistance genes during production. Furthermore, most methodology used to make genetic transformation is patented by private organizations, which can make commercialization of a final product expensive due to the licensing fees required to use the technology.

OBJECTIVES

There are three main objectives of this project:

1. Develop new lines of transgenic plants from commercially important citrus scion and rootstock varieties that are resistant to HLB using genes derived from citrus.
2. Make these plants such that the selection markers (i.e. antibiotics/herbicides) are removed from the genome prior to commercialization.
3. Optimize a technique (electroporation) that will allow production of transgenic plants without requiring licensing fees from a private company, thereby lowering the cost of commercialization.

Thus, transgenic plants will be produced using genes exclusively from citrus, and will be devoid of the gene for antibiotic resistance. Furthermore, to make these transgenic plants available to receive additional genes if needed in the future, in exactly the same place where the disease resistance gene was introduced, a technique called recombinase mediated cassette exchange (RMCE) will be used. RMCE is a technique that uses enzymes that can cut and paste DNA without the gain or loss of genetic material; very precise and fast. Think of them as scissors and tape for DNA. (Read more about this technique in an article by James Thomson in *Citrograph*, January/February 2013, pp. 38-39.)
During the production of the initial disease resistant plants, small inert DNA sequences will be added to the piece of DNA inserted into the plant. These small sequences are now the landing pad for the recombinase enzymes. One of these enzymes will be used to remove the antibiotic resistance gene from the plant genome during initial plant production. Later, more DNA can be added to these transgenic citrus at the exact spot where the DNA landing pad has been inserted. The process is designed to be self-removing, meaning that only the DNA of interest (disease resistance) is left in the plant genome. All other genes (i.e. antibiotic and recombinase genes) are removed at the end of the RMCE process.

To ramp up the production of transgenic plants, the electroporation technique will be optimized for citrus. This technique does not have patents that require licensing in order to use; therefore, it will be much easier and more rapid to implement this technique at commercial production levels.

Electroporation is a technique where electricity is passed through the plant tissue. The passage of the electric current produces small holes in the plant’s cells allowing DNA (i.e. disease-resistance gene) to enter and join with the genome. This technique has been used in the past to transform citrus protoplasts and to transform embryos of corn and other monocotyledons (grass) species. In citrus, the procedure needs to be optimized because it is difficult to obtain citrus protoplasts and subsequently, to get them to produce shoots (totipotent). *Agrobacterium tumefaciens*-mediated genetic transformation is a commonly used procedure, but it is patented; it will be the back-up system to transform citrus in case the electroporation technique is problematic.

**PRELIMINARY RESULTS**

The first year of this project was dedicated to setting up procedures, testing the regeneration of plants from various citrus tissues, preparing the DNA materials to be introduced into the plants, and finding the best way to perform the electroporation of citrus tissues.

The citrus tissue chosen for testing were seedling stem sections (explants) ranging from 1.0 to 3.0 mm (*Figure 2*), to be able to fit into the electroporation cuvette (*Figure 3*), which has a gap of only 4 mm. Using the small size, we were able to fit 15 explants per cuvette.
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Seedling stem sections (explants) were cultured in the dark for 14 days and then transferred to light to test their regeneration capacity. Figure 4 shows the progression of plant regeneration from a 2 mm thick cv. Carrizo stem section. Explants in the range of 2-3 mm produce more plants per explant than the 1 mm length. The efficient regeneration observed for these explants will be important for the subsequent electroporation procedure.

The electroporation procedure for explants consists of the application of an electric pulse to the explant to open holes in the cell. Once these holes are formed, the disease resistance gene can get inside the cell and integrate into the plant genome. The electric pulse needs to be calibrated so that the cell is not damaged, but remains strong enough to allow a good amount of DNA to go inside. Electric pulses ranging from 250 to 500 V/cm resulted in good regeneration of transgenic plants. During this optimization procedure, a marker gene called the GUS gene, which produces a blue color in the transgenic tissues (Figure 5), was used to observe results.

The back-up Agrobacterium-mediated genetic transformation system was used to introduce the CSM-1 disease resistance gene into the rootstock Carrizo citrange to hasten the evaluation of HLB resistance, while optimizing the electroporation technique. Finally, transgenic cv. Carrizo with the CSM-1 disease resistance gene have been obtained, and molecular characterization is underway. We achieved success in obtaining Carrizo seedlings that contain the CSM-1 disease resistance gene, and molecular characterization is in progress. It is expected that disease resistance testing will begin by the end of 2015.

Eliezer Louzada, Ph.D., is a professor of breeding and molecular biology at Texas A&M University-Kingsville Citrus Center. James Thomson, Ph.D., is a research geneticist with the USDA Agricultural Research Service-Crop Improvement and Genetics Research in Albany, California.

Glossary

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protoplast</td>
<td>Plant cell devoid of cell wall</td>
</tr>
<tr>
<td>Totipotent</td>
<td>Ability of the tissue to regenerate shoots</td>
</tr>
</tbody>
</table>
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Update on Lemon Selection Evaluation for the California Desert

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

As the nation’s leader in lemon production, California lemons continue to be the highest-value citrus crop per acre for the state. In 2013-2014, the 46,000 bearing acres of lemons in California had a value of $596,124,000, in contrast to the $670,548,000 value for the 133,000 bearing acres of Navel oranges. Although lemons are grown predominantly in the coastal and desert regions of the state with smaller numbers of acres in the San Joaquin Valley due to frost sensitivity, this range of climatic regions in the state provides the market with year-round production. Among these regions, lemons grown in the desert occupy an important early-season market niche and are an important source of fruit for packinghouses located there and in other areas of the state.

The choice of lemon cultivar plays an important role in production issues such as fruit quality, yield and ability for fruit to occupy important market niches. In the 2005-2006 season, we initiated a replicated
evaluation trial entitled “Evaluation of Lemon Selections for the Desert” (CRB Project 5200-127) with funding from the Citrus Research Board (CRB) to provide the industry with information on the tree growth, yield, pack-out, fruit quality characteristics and other important traits for selected lemon varieties in the California desert.

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.” The first summary of our initial results from planting in 2006 through the 2011-2012 season was published in the Winter 2014 issue of Citrograph. This initial article provided results comparing the 12 lemon selections (Table 1) for freeze tolerance, tree growth based on canopy volume, fruit yield and earliness.

This trial update will provide results of experimental and commercial fruit pack-out, exterior and interior quality, nutrient issues and fruit storage life. All data were analyzed statistically using univariate and multivariate analysis of variance. Means separations were analyzed using Duncan’s Multiple Range Test at α=0.05.

BACKGROUND

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 (Table 1). Trees of the twelfth selection, Limonero Fino Largo (95), were planted in April 2007 (Table 1). 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 22 feet by 17.5 feet, and the experimental design is randomized complete block.

LEAF NUTRIENT CONCENTRATIONS

Leaves were collected for nutrient analysis once annually in August or September from the 2011-2012 season through the 2014-15 season. Leaf nitrogen (N), phosphorus (P), potassium (K), sulfur (S), calcium (Ca), magnesium (Mg), zinc (Zn), iron (Fe), manganese (Mn) and copper (Cu) concentrations were determined each season, and leaf boron (B) only for the 2014-15 season. We found no statistically significant differences in leaf nutrient concentrations related to lemon selection.

For N, leaf concentration values were 2.2 percent in 2011-12, but dropped in subsequent years, reaching 1.8 percent in 2014-15. No deficiency symptoms were noted. All these N levels were well below the optimum range of 2.4 to 2.6 percent. While leaves collected in August to September would be expected to have a lower N concentration, due to heavy demand from the developing leaves and fruit, these values are low. We are attempting to increase leaf N through more frequent applications of foliar N and application of liquid fertilizer with a higher N levels.

Leaf P was well within the optimal range of 0.12 to 0.16 percent for 2011-12, 2012-13 and 2014-15. For 2013-14, leaf P was high at 0.2 percent. Leaf K was generally higher than the optimal range of 0.7 to 1.09 percent. In 2011-12, leaf K was 1.63 percent, increasing to 2.4 percent in 2013-14, and dropping to 2.2 percent in 2014-15. Leaf S, Ca and Mg levels were within the optimum ranges of 0.14 to 0.19 percent, 3.0 to 5.5 percent and 0.26 to 0.60 percent respectively.

Leaves were generally low in Zn and Mn. Annual values of 13 to 19 parts per million (ppm) were slightly below the optimum Zn concentration of 25 to 100 ppm, and annual values of 16 to 21 ppm were slightly below the optimum Mn concentration of 25 to 200 ppm. For 2011-12, 2012-13 and 2013-14, leaf Fe levels were 216, 250 and 186 ppm, respectively. These levels are high in comparison with the recommended optimum range of 60 to 120 ppm. However, in 2014-15, leaf Fe concentrations dropped to 102 ppm. Leaf Cu levels for the four years were within the optimal range of five to 16 ppm. Leaf B levels in 2014-15 were 86 ppm, within the optimum range of 31 to 100 ppm.

LEMON FRUIT QUALITY DATA

**Fruit shape:** We collected fruit shape data using the portable fruit sizer. Fruit shape is the length to width ratio of each fruit, photographed as it passes through the sizer. A spherical fruit would have a value of 1.0. Fruit shape for all the selections was within a range of 0.73 to 0.79. Variegated Pink Eureka consistently had the most spherical fruit, followed by Interdonato and Messina (Table 2). Limonero Fino 49 had the most non-spherical fruit (Table 2).

**Fruit color:** The sizer camera also collects peel color data from each fruit. The data presented here are the ratio of the red to green color in the peel. A redder (or yellower) peel has a greater value than a rind that is greener. Not surprisingly, peels of Variegated Pink Eureka fruit had the most color (Table 2). Most of the other selections had similar values, but the Yen Ben had the most yellow peel, while the peel of Limonero Fino 49 fruit had the most green (Table 2).

**Juice content:** We selected 20 pieces of whole fruit per tree for additional quality analysis. Fruits were weighed and juiced, and we then calculated the percent juice by dividing the fruit weight by the juice weight. Percent juice for all selections was in a relatively narrow range from about 47-51 percent (Table 2). Variegated Pink Eureka had the greatest percentage juice and was the only selection with more than 51 percent juice, but Seedless lemon, Limonero Fino 49, Allen Eureka and Limoneira 8A Lisbon fruit had more than 50 percent juice (Table 2). Yen Ben fruit only approached 47 percent juice (Table 2).

**Juice pH:** Juice pH data was taken, but there were no statistically significant differences among the selections (Table 2).
### Table 1. Description of 12 lemon selections planted in Thermal, California.

<table>
<thead>
<tr>
<th>Selection</th>
<th>Variety Introduction (VI) Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allen Eureka</td>
<td>227</td>
<td>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.</td>
</tr>
<tr>
<td>Corona Foothills</td>
<td>VI Number Pending</td>
<td>Femminello Santa Teresa originated in Italy as a selection of Femminello Comune. This selection is resistant to the Mal Secco disease found in Italy and purportedly has a greater percentage of its fruit in the late summer as compared to other Femminello selections. There is a registered bud source of this variety at Lyn Citrus Seed, but the CCPP bud source is pending.</td>
</tr>
<tr>
<td>Interdonato</td>
<td>667</td>
<td>Originated in Italy, and considered to be a lemon x citron hybrid. This variety is resistant to the Mal Secco disease and considered to be the earliest of the Italian varieties. This variety was not previously tested in California or Arizona.</td>
</tr>
<tr>
<td>Limoneira 8A</td>
<td>380</td>
<td>The other commercial standard for this trial and the most commonly planted Lisbon selection in California and Arizona. Trees Limoneira 8A are considered to be vigorous and produce early season fruit.</td>
</tr>
<tr>
<td>Limonero Fino 49</td>
<td>480</td>
<td>The earliest yielding lemon variety and chief winter lemon of Spain. Limonero Fino 49 trees are considered to be 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.</td>
</tr>
<tr>
<td>Limonero Fino 95</td>
<td>674</td>
<td>According to the Spaniards, similar to the Limonero Fino 49, but harvest is about two weeks earlier and productivity is lower. Imported into California in 2003. Not previously tested in Arizona or California.</td>
</tr>
<tr>
<td>Messina</td>
<td>661</td>
<td>Purportedly resistant to the Mal Secco disease, Messina is extremely early bearing but yields poorly.</td>
</tr>
<tr>
<td>Seedless</td>
<td>492</td>
<td>Introduced to California as a cutting from Lassocks Nursery, South Australia, 1939, but did not become a registered variety until 1985. This lemon was formerly known as Seedless Lisbon, but is now believed to be not a Lisbon-type lemon at all. It has since adopted the name “Seedless Lemon.”</td>
</tr>
<tr>
<td>Variegated Pink Eureka</td>
<td>486</td>
<td>Selection 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 has yields distributed from late winter to early summer and is less vigorous than a conventional Eureka.</td>
</tr>
<tr>
<td>Walker Lisbon</td>
<td>415</td>
<td>A selection of Lisbon located at the Lindcove Research and Extension Center that appeared to be precocious and early yielding. Walker is mentioned as a Lisbon selection of lesser importance in the Citrus Industry Volume I (Hodgson 1967), but has performed well in Arizona trials.</td>
</tr>
<tr>
<td>Yen Ben</td>
<td>586</td>
<td>A Lisbon lemon sport selection that originated in Queensland, Australia, in the 1930s. This variety is known to be precocious and a heavy yielder, but produces small fruit.</td>
</tr>
</tbody>
</table>

All cultivars, either introduced or developed through breeding program, go through a rigorous “Variety Introduction–VI” disease testing and therapy program under quarantine at the Citrus Clonal Protection Program (CCPP). Varieties that successfully complete the VI process receive a unique VI identification number that permanently accompanies the budwood that is made available to growers, nurseries, researchers, and others as Protected Foundation Block Budwood from the CCPP.
Juice soluble solids, acids and soluble solid to acid ratio: Soluble solid data were collected from the juice samples using a digital refractometer, while juice acid data was collected using a benchtop titrator. Soluble solids were in a narrow range ranging from 7.15 to 7.75 percent (Table 2). Because of the quantity of the data, there were statistically significant differences between the selections. Walker Lisbon, Corona Foothills, Limonero Fino 49, Seedless lemon and Interdonato had the greatest amounts of soluble solids, while Femminello Santa Teresa, Messina and Limonero Fino 95 had the least (Table 2). For acids, Corona Foothills, Limonero Fino 49 and Walker Lisbon had the greatest, followed by Seedless lemon, Femminello Santa Teresa and Limoneira 8A. Limonero Fino 95, Yen Ben and Variegated Pink Eureka had the least acidity (Table 2). The Total Soluble Solids:Total Acid (TSS:TA) ratios were also in a narrow band, ranging from 1.30 to 1.41 (Table 2). This is much lower than normal for oranges, mandarins and grapefruit. Interdonato and Yen Ben were the two selections with TSS:TA ratios of 1.40 or higher, while Femminello Santa Teresa, Limoneira 8A Lisbon Allen Eureka and Limonero Fino 49 were the only selections with ratios of 1.32 or less (Table 2). Ratios of all the other selections ranged from 1.33 to 1.37.

Seeds per fruit: We selected 10 fruit of the original sample of 20 per tree for seed count. Seedless lemon was virtually true to its name, having about one seed per fruit. Just over two seeds were found in a typical Yen Ben and Messina fruit, while we found about five seeds in a typical Variegated Pink Eureka and Limonero Fino 95 fruit (Table 2). All the other selections had from about 11 to 18 seeds per fruit (Table 2).

Peel thickness: We used calipers to take two peel thickness measurements on each of the 10 fruit from which we counted seeds. Interdonato, Variegated Pink Eureka and Yen Ben had noticeably thin peels, just over two millimeters thick (Table 2). At the other extreme was Messina, which had a peel of about 4.5 millimeters thick (Table 2). Corona Foothills Femminello Santa Teresa and Limonero Fino 95 had peels approaching or just surpassing 4.0 millimeters (Table 2). The thickness of fruit peel of all the rest of the selections ranged from 3.5 to about 3.8 millimeters (Table 2).

Table 2. Interior fruit quality parameters and peel characteristics of twelve lemon selections harvested annually from 2009-10 to 2014-15 at Thermal, CA.

<table>
<thead>
<tr>
<th>Selection</th>
<th>Fruit Shape</th>
<th>Peel Color</th>
<th>Juice Content (%)</th>
<th>Juice pH</th>
<th>Total Soluble Solids (%)</th>
<th>Total Acids (%)</th>
<th>TSS:TA</th>
<th>Seeds per fruit</th>
<th>Peel Thickness (mm)</th>
<th>Peel Smoothness Rating</th>
<th>Oil Content (lbs./ton)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allen Eureka</td>
<td>0.75 bc²</td>
<td>1.09 b</td>
<td>50.29 ab</td>
<td>2.35</td>
<td>7.24 e</td>
<td>5.51 bc</td>
<td>1.32 de</td>
<td>10.82 e</td>
<td>3.62 d</td>
<td>3.32 bc</td>
<td>5.15</td>
</tr>
<tr>
<td>Corona Foothills</td>
<td>0.74 cd</td>
<td>1.08 bc</td>
<td>49.05 abc</td>
<td>2.41</td>
<td>7.71 ab</td>
<td>5.79 a</td>
<td>1.33 cde</td>
<td>14.24 bc</td>
<td>4.27 ab</td>
<td>3.24 bcd</td>
<td>14.13</td>
</tr>
<tr>
<td>Femminello Sta. Teresa</td>
<td>0.76 bc</td>
<td>1.08 bc</td>
<td>48.11 bc</td>
<td>2.37</td>
<td>7.43 cd</td>
<td>5.67 ab</td>
<td>1.30 e</td>
<td>15.01 b</td>
<td>4.12 bc</td>
<td>3.52 b</td>
<td>12.39</td>
</tr>
<tr>
<td>Interdonato</td>
<td>0.77 b</td>
<td>1.06 cd</td>
<td>49.05 abc</td>
<td>2.34</td>
<td>7.56 abc</td>
<td>5.36 cd</td>
<td>1.41 a</td>
<td>13.39 de</td>
<td>2.37 g</td>
<td>2.04 e</td>
<td>5.67</td>
</tr>
<tr>
<td>Limoneira 8A Lisbon</td>
<td>0.74 cd</td>
<td>1.09 b</td>
<td>50.14 ab</td>
<td>2.43</td>
<td>7.49 bc</td>
<td>5.67 ab</td>
<td>1.32 de</td>
<td>12.42 cde</td>
<td>3.52 de</td>
<td>3.36 bc</td>
<td>----</td>
</tr>
<tr>
<td>Limonero Fino 49</td>
<td>0.73 d</td>
<td>1.05 d</td>
<td>50.33 ab</td>
<td>2.41</td>
<td>7.65 ab</td>
<td>5.77 a</td>
<td>1.32 de</td>
<td>12.77 cd</td>
<td>3.80 cd</td>
<td>3.52 b</td>
<td>11.17</td>
</tr>
<tr>
<td>Limonero Fino 95</td>
<td>0.73 d</td>
<td>1.09 d</td>
<td>48.78 abc</td>
<td>2.41</td>
<td>7.18 e</td>
<td>5.21 d</td>
<td>1.37 abc</td>
<td>5.54 f</td>
<td>3.96 bc</td>
<td>3.52 b</td>
<td>11.59</td>
</tr>
<tr>
<td>Messina</td>
<td>0.77 b</td>
<td>1.09 b</td>
<td>49.14 abc</td>
<td>2.39</td>
<td>7.26 de</td>
<td>5.33 cd</td>
<td>1.36 bcd</td>
<td>2.51 g</td>
<td>4.58 a</td>
<td>3.08 cd</td>
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<tr>
<td>Seedless Lemon</td>
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<td>1.08 bc</td>
<td>50.39 ab</td>
<td>2.42</td>
<td>7.64 ab</td>
<td>5.68 ab</td>
<td>1.35 bcd</td>
<td>1.02 g</td>
<td>3.51 de</td>
<td>3.00 d</td>
<td>14.32</td>
</tr>
<tr>
<td>Variegated Pink Eureka</td>
<td>0.79 a</td>
<td>1.33 a</td>
<td>51.24 a</td>
<td>2.36</td>
<td>7.15 e</td>
<td>5.35 cd</td>
<td>1.34 cde</td>
<td>5.52 f</td>
<td>3.05 f</td>
<td>3.88 a</td>
<td>10.85</td>
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<tr>
<td>Walker Lisbon</td>
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<td>48.14 bc</td>
<td>2.50</td>
<td>7.75 a</td>
<td>5.75 a</td>
<td>1.34 cde</td>
<td>18.22 a</td>
<td>3.83 cd</td>
<td>3.36 bc</td>
<td>13.23</td>
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<tr>
<td>Yen Ben</td>
<td>0.76 bc</td>
<td>1.10 b</td>
<td>46.99 c</td>
<td>2.44</td>
<td>7.46 bc</td>
<td>5.29 d</td>
<td>1.40 ab</td>
<td>2.53 g</td>
<td>3.20 ef</td>
<td>2.04 e</td>
<td>10.19</td>
</tr>
</tbody>
</table>

² Values indicate the ratio of length to width. A perfect sphere would have a value of 1.0.
³ Means separation using Duncan’s Multiple Range test. Values within a column associated with the same letter are not significantly different at α=0.05.
⁴ Values indicate the ratio of red to green in the peel. A greater value indicates that the peel color is redder.
⁵ There was no significant effect of selection upon juice pH.
⁶ Values are a subjective rating from 1 to 10 of peel smoothness. A greater value indicates that the peel is less smooth.
⁷ Peel oil content data was collected only in the 2011-12 season. A single sample was taken per selection, thus no statistical test can be performed. Sample for ‘Limoneira 8A’ Lisbon was inadvertently lost.
During the 2011-12 season, we sent packed fruit samples of 11 of the 12 selections to a juice plant, operated by Ventura Coastal in Tipton, California. The sample for Limoneira 8A Lisbon was lost. Peel oil content ranged from 5.15 lbs. per ton for Allen Eureka to 14.32 lbs. per ton for Seedless lemon.

FRUIT STORAGE LIFE

Fourteen to 19 days after the first harvest of the 2011-12, 2012-13 and 2013-14 seasons, a 37.5 lb. carton of commercially packed fruit was collected for 11 of the 12 selections from the packinghouse (Richard Bagdasarian Inc, Mecca, California). For the Variegated Pink Eureka selection, one eight lb. carton of fruit was received annually in 2011-12 and 2012-13, but not in 2013-14. For the October 11, 2011 harvest, the fruit was received on October 28, 2011; for the October 17, 2012 harvest, the fruit was received on November 5, 2012; and for the October 22, 2013 harvest, the fruit was received on November 5, 2013. Fruit size in the cartons ranged from 75 to 200, and the fruit quality was #1 grade, except for the Variegated Pink Eureka fruit, which was #2 grade. All fruit was moved to the University of Arizona Yuma Agriculture Center. Variability in the receiving date, and the fruit size and grade were at the discretion of the managers of Richard Bagdasarian.

Upon arrival in Yuma, the total fruit from each variety was divided into three replicate groups, each containing an equal number of fruit. The number of fruit in a group ranged from 13 to 68, depending on how much was received from the packinghouse. All fruit was then stored in a walk-in cooler maintained at 45°F (7.2°C).

One week after the fruit were placed in the cooler, they were removed by replicate group; each fruit was inspected for evidence of disease, and the number that appeared to have disease was recorded. Fruit with disease were returned to their respective replications. For the 2011-12 and 2012-13 seasons, the incidence of diseased fruit was recorded three and five weeks after fruit was placed in the cooler. For the 2013-14 season, incidence of diseased fruit was recorded at three and six weeks after the fruit was placed in the cooler. The predominant diseases found were Penicillium mold (Penicillium italicum and/or P. digitatum) and sour rot (Galactomyces citri-aurantii). Sometimes, more than one of these diseases was found on a fruit. No other diseases were found.

Because of the variability in the numbers of fruit counted per selection, data is presented as the percent of diseased fruit per replication.

Penicillium mold: For 2011-12, incidence of Penicillium spp did not surpass three percent for any of the selections. For the first date of data collection, 25 days after harvest, disease incidence in Limoneira 8A Lisbon fruit was less than one percent, and none of the others had signs of the fungi (Figure 1). Two weeks later, at 39 days post-harvest, Limoneira 8A Lisbon and Allen Eureka fruit had less than one percent disease.
incidence, while the Variegated Pink Eureka had a disease incidence level approaching three percent (Figure 1). At 53 days after harvest, there was no increase in disease incidence (Figure 1). Fruit of the other selections had no incidence of the disease in 2011-12 (Figure 1).

For the 2012-13 season, the infestation levels were generally similar to that of the previous year. Twenty-six days after harvest, less than three percent of the Corona Foothills, Femminello Santa Teresa, Interdonato, Limoneira 8A Lisbon, Limonero Fino 49, Messina, Variegated Pink Eureka, Walker Lisbon and Yen Ben fruit were still less than three percent; but for Interdonato, almost five percent of the fruit was infested with *Penicillium spp* (Figure 1). Seedless lemon was not affected by *Penicillium spp* in 2012-13 (Figure 1).

In 2013-2014, there was no evidence of *Penicillium spp* for any of the lemon selections by November 12, 2013, which was 21 days post-harvest (Figure 1). Two weeks later, or 35 days after harvest, only the Limoneira 8A Lisbon showed any *Penicillium spp* at a low rate of about one percent (Figure 1). On December 18, 2013, 56 days post-harvest, disease levels of Limoneira 8A were stable at about one percent, and levels of the disease in Allen Eureka had increased to about 1.5 percent (Figure 1). However, *Penicillium spp* in Interdonato and Yen Ben fruit had increased to about seven percent (Figure 1). The other selections were not affected by these fungi in 2013-14 (Figure 1).

*Sour Rot:* In 2011-12, no fruit was affected by sour rot on November 4, 2011, 25 days after harvest (Figure 2). By 39 days post-harvest, Limonero Fino 49, Yen Ben, Limoneira 8A Lisbon and Allen Eureka fruit showed evidence of sour rot, but that incidence was less than two percent (Figure 2). However about 2.5 percent of the Variegated Pink Eureka fruit had the disease (Figure 2). Two weeks later, at 53 days post-harvest, 1.5 percent or less of the Limonero Fino 49, Limoneira 8A Lisbon, Interdonato, Messina and Allen Eureka were affected, while the incidence of diseased Yen Ben fruit had increased to about four percent, and the incidence of diseased Variegated Pink Eureka had increased to about five percent (Figure 2). Corona Foothills, Femminello Santa Teresa, Limonero Fino 95, Seedless Lemon and Walker Lisbon fruit were not affected by sour rot in 2011-12 (Figure 2).

At 26 days after the first harvest of the 2012-13 season, only Yen Ben, Interdonato Corona Foothills and Limonero Fino 95 fruit showed evidence of sour rot, all at levels of less than three percent (Figure 2). By December 3, sour rot appeared on those four selections, as well as on Seedless Lemon, Variegated Pink Eureka and Femminello Santa Teresa; but for all selections, the disease incidence was at about 2.5 percent.
or less (Figure 2). In the subsequent two weeks, the sour rot flourished on the Interdonato fruit, affecting 16 percent of the fruit by December 17, 2012 (Figure 2). Sour rot also increased on the Yen Ben fruit, doubling to almost six percent four days after harvest (Figure 2). For Allen Eureka, Corona Foothills, Femminello Santa Teresa, Limonero Fino 95, Messina, Seedless Lemon, Variegated Pink Eureka and Walker Lisbon fruit, disease incidence remained at 2.5 percent or less (Figure 2). Sour rot did not affect Limoneira 8A Lisbon or Limonero Fino 49 fruit in 2012-13.

For the 2013-14 season, sour rot was not found by November 12, 2013, 21 days after harvest. Two weeks later, it had appeared on Allen Eureka, Interdonato, Limonero Fino 95 and Seedless Lemon fruit, but at levels of less than 1.5 percent (Figure 2). By December 18, 56 days post-harvest, less than three percent of the Allen Eureka, Corona Foothills, Femminello Santa Teresa, Limoneira 8A Lisbon, Limonero Fino 95 and Seedless Lemon fruit had sour rot (Figure 2). Sour rot incidence was six percent in the Interdonato fruit and about 30 percent in the Yen Ben fruit by December 18 (Figure 2). It is worth noting that no Limonero Fino 49, Messina or Walker Lisbon fruit were affected by sour rot in 2013-14.

**YIELD DATA**

For the past seven seasons, from 2008-09 through 2014-15, harvest dates were based on market conditions and determined by officials at Richard Bagdasarian Inc. (RBI). Due to the large fruit load during the 2010-11 season, we decided that it would be appropriate to begin conducting two harvests per season. 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. Yield data from each group of four trees were collected by counting the numbers of whole and fractional picking sacks harvested from each group. Yield is reported based on pounds per tree for each of the seven harvest seasons.

During those seasons, Corona Foothills, Walker Lisbon, Limonero Fino 49, Femminello Santa Teresa, and the controls, Allen Eureka and Limoneira 8A Lisbon, had the highest yields; and Yen Ben, Interdonato, Seedless Lemon, Limonero Fino 95, Messina and Variegated Pink Eureka had the lowest yield (Figure 3). Although the cultivar with the highest yield varied over the years, for the past three seasons, Corona Foothills had the highest yield. Except for the most recent season, Variegated Pink Eureka had the lowest yield of the 12 cultivars (Figure 3). This was not surprising, since the variegated pattern of the leaves for this cultivar caused the light green and white portions of the leaf surface to have less chlorophyll and less capacity for photosynthesis. The highest yields during the seven seasons occurred in 2012-13. For the past two seasons, yields have decreased compared to those in the 2012-13 season for almost all cultivars except for Variegated Pink Eureka and Yen Ben (Figure 3). The gain or loss in yield between 2013-14 and the 2012-13 season ranged from +4 percent for Yen Ben to -58 percent for Variegated Pink Eureka.
percent for Limonero Fino 95 (Figure 3). In contrast, the gain or loss in yield between 2014-15 and the 2012-13 season ranged from +18 percent for Variegated Pink Eureka to -75 percent for Interdonato (Figure 3).

Some of the reductions in yield can be attributed to the exceptionally high yield in 2012-13; and it would not be surprising that the following season might be lower, due to lack of carbohydrates to sustain a second large crop. However, if this were the only cause, yields would be expected to have rebounded in 2014-15, but they did not. Some of the reduction may have been due to the warm fall in 2014, which led to greater than normal fruit drop. Yet the most likely main cause was shading due to over-growth, which can lead to reduced flower production. Recently, we began to correct the shading problem by conducting substantial pruning between and inside trees, which now allows additional light into the interior of trees.

During this current season, trees were harvested on September 29, 2014, and again on November 24, 2014. For the first harvest, Corona Foothills, Walker Lisbon, Femminello Santa Teresa, Limonero Fino 49 and Allen Eureka had the greatest yields; while Yen Ben, Variegated Pink Eureka and Interdonato had the lowest fruit production. For the September harvest, yields for the five best performers ranged from 161 percent to 102 percent of that of Limoneira 8A Lisbon standard, while the three worst performers ranged from 35 percent to 23 percent of the standard. For the second harvest, production of Limoneira 8A was greatest, followed by Walker Lisbon, Yen Ben and Corona Foothills, while yields of Interdonato and Messina and Limonero Fino 95 were the least yields. The four selections with the greatest yields for the entire 2014-15 season were (in order) Corona Foothills, Walker Lisbon, Femminello Santa Teresa and Limoneira 8A. The five cultivars with the least production for this season were Interdonato, Variegated Pink Eureka, Limonero Fino 95, Yen Ben and Messina.

**PACK-OUT DATA**

For the past seven seasons, from 2008-09 through 2014-15, 30-35 lb. subsamples of fruit for each cultivar were collected for pack-out data. Pack-out data were collected with a portable single-line fruit sizer (Aweta America 1998). For the first set of harvests conducted September 1 through November 15 each year from 2008-09 through 2014-15, Messina had the largest sized fruit, peaking on sizes 75 and 95, while Corona Foothills, Interdonato, Limonero Fino 49 and Limonero Fino 95, Walker, Allen, Seedless and Femminello Santa Theresa fruit size peaked on 95 and 115 (Figure 4). Variegated Pink-Fleshed Eureka and Yen Ben had the smallest sized fruit, peaking on sizes 140 and 165 (Figure 4). When we examined the pack-out
results just for the most recent season, 2014-15, the pattern for this first harvest was very similar to the pattern for the average pack-out for the first harvests for the seven years from 2008-09 through 2014-15.

For the second set of harvests conducted November 16 through January 31 each year from 2008-09 through 2014-15, Messina again had the largest size fruit, peaking at size 75 (Figure 5). Allen, Corona Foothills, Femminello Santa Theresa, Interdonato, Limoneira 8A, Limonero Fino 49 and Limonero Fino 95, Seedless Lemon and Walker peaked on size 95 (Figure 5). As was the case for the first set of harvests, Variegated Pink Eureka and Yen Ben had the smallest fruit, peaking on sizes 140 and 165 (Figure 5). When we examined the pack-out results just for the most recent season, 2014-15, the pattern for this second harvest also was very similar to the pattern for the average pack-out for second harvests for the seven years from 2008-09 through 2014-15.

COMMERCIAL RETURNS

We received commercial return data from Richard Bagdasarian Inc. for the 2011-12, 2012-13 and 2013-14 harvest seasons. Commercial return data for the 2014-15 season are not yet available, but will be included with the final report of this project.

Commercial return data include details as to the numbers of standard cartons in four quality grades – Sunkist (#1), Choice (#2) and Standard (#3) – as well as the equivalent cartons that are not packed but sent to be processed into juice, oil and other citrus byproducts. Within each quality grade, the returns provide specifics about the numbers of cartons in each fruit size category (75, 95, 115, 140, 165, 200 and 235) and the price for which each carton was sold based on quality and size. Marketing, hauling, packing and other charges are deducted from the total price, and a net return is calculated. Numbers of field bins, standard cartons, field boxes (60 lb.) and a net return per field box are also provided. From these data, we calculated an estimated return per acre.

It is worth noting that Richard Bagdasarian Inc. has agreed to keep each of the 12 lemon selections harvested separate during the picking, hauling and packing process. Thus, we receive a page for each of the selections, containing the data for just 20 trees. It is not easy to keep track of no more than 12 bins or 200 cartons each of the 12 selections, when thousands of bins and millions of cartons move through the
packinghouse annually. We appreciate the efforts on the part of the management and employees of RBI.

2011-12 SEASON

For the 2011-12 season, Walker Lisbon, Limonero Fino 49 and Corona Foothills each had yields of more than 100 field boxes. Femminello Santa Teresa, Allen Eureka and Yen Ben had between 80-100 field boxes. Five other selections had between 60-80 field boxes, and the Variegated Pink Eureka had 16 field boxes. Variegated Pink Eureka had the greatest net return per field box for the season, in excess of $30 per box. This is certainly due to the unique exterior and interior color of the fruit that makes it desirable. Corona Foothills, Limonero Fino 49 Messina and Yen Ben all had returns greater than $10 per field box because of good sizes, early fruit or, in the case of Yen Ben, due to good-quality small fruit when small fruit is at a premium in the market. All of the remaining selections had returns of less than $10. Seedless lemon had the lowest return of about $5 per field box. Return per acre was the greatest for both Limonero Fino 49 and Corona Foothills – just over $5,000 per acre. Walker Lisbon and Yen Ben had returns exceeding $4,000 per acre, while the other selections had returns between $1,500 and $3,000 per acre.

2012-13 SEASON

In 2012-13, Corona Foothills, Limonero Fino 49 and Walker Lisbon each had yields of more than 150 field boxes. Allen Eureka, Femminello Santa Teresa, Limoneira 8A Lisbon and Limonero Fino 95 had between 100-150 field boxes. All the other selections had fewer than 100 field boxes, and Variegated Pink Eureka had the least – about 25 field boxes. As in 2011-12, Variegated Pink Eureka had the greatest return per field box, at approximately $14. All the other selections had similar returns from about $5-$7 per box. Net estimated returns were the greatest for Corona Foothills and Walker Lisbon, with more than $4,000 per acre, and returns for Limonero Fino 49 were just under $4,000. Returns for Limoneira 8A Lisbon, Femminello Santa Teresa and Allen Eureka surpassed $3,000, while the rest of the selections had net returns per acre ranging from $2,200 to as low as $1,500 per acre.

2013-14 SEASON

Results for the 2013-14 season were similar to those of the past two seasons. Corona Foothills had more than 140 field boxes, while both Limonero Fino 49 and Walker Lisbon each had more than 100. The remaining selections had between 40-80 field boxes, except for the Variegated Pink Eureka that had just 16. Returns for this season were much higher than those in the previous two seasons. Variegated Pink Eureka again had the greatest return per field box of almost $40, while Corona Foothills and Limonero Fino had returns of more than $20. Allen Eureka, Limoneira 8A and Walker Lisbon all had returns surpassing $15, while returns for the remaining selections ranged from $9-$14 per field box. Considering both yield and return, it is not surprising that Corona Foothills had an estimated return per acre of more than $13,000, while the per acre return for Limonero Fino 49 was about $10,000 and the per acre return for Walker Lisbon was about $7,200. For the other selections, returns ranged from about $2,000-$4,000 per acre.

SUMMARY OF THE ATTRIBUTES OF EACH SELECTION

Allen Eureka fruit had good size with oblong shape, average exterior quality, average juice content, average seed count, peel thickness and peel smoothness. Allen Eureka had middle-of-the-pack yield, returns and cumulative yield.

Corona Foothills fruit were oblong in shape with excellent exterior fruit quality, average juice content, high seed count, high peel thickness and average peel smoothness. Corona Foothills fruit had good size, were relatively early in maturity and had the best first harvest yield and next-to-best total yield and returns with excellent cumulative yield.

Femminello Santa Teresa had rounder fruit, average juice content, high seed count, average peel thickness with a peel that was not particularly smooth. Fruit maturity was not quite as early as some of the others. This selection had smaller first harvest fruit size and average exterior quality. It also had good first harvest and second harvest yield and returns, good total yield and cumulative yield.

Interdonato fruit were rounded in shape with pointed mammilla, average juice content and average seed count. The peel was extremely thin and nicely smooth. Interdonato fruit had average fruit size, with excellent exterior quality. This selection had middle-of-the-pack total yield and cumulative yield. Returns were not exceptional. This selection is quite susceptible to post-harvest diseases.

Limoneira 8A Lisbon had rounder fruit with average juice content, seed count and peel thickness. Additionally, the peel was not particularly smooth. Fruit size and exterior fruit quality was good. There was average return per field box.
and average estimated return per acre. This selection also had average first harvest yield, second harvest yield and total yield. Cumulative yield and fruit were not particularly early maturing.

**Limonero Fino 49** has oval-shaped fruit, average juice content, high seed count, average peel thickness and a not particularly smooth peel. This selection had excellent first and second harvest fruit size and good exterior quality. The trees of this selection are thorny. There were excellent first harvest yields and good second harvest yields and returns, excellent total yield and cumulative yield. Along with Corona Foothills and Walker Lisbon, Limonero Fino 49 was the earliest of the selections.

**Variegated Pink Eureka** fruit were the most spherically shaped fruit of all selections, with the greatest juice percentage and about eight seeds per fruit. This variegated selection had the most color in the peel, which was thin, but not smooth. Of all the selections evaluated, Variegated Pink Eureka had the smallest fruit size and average exterior fruit quality. Yields for the first and second harvests were poor, as was the case for cumulative yield. This selection has exceptional return per field box, but average estimated return per acre. It was not early maturing.

**Walker Lisbon** fruit were oblong in shape with average juice content, the highest seed count (19 per fruit), and average peel thickness and smoothness. Fruit size and exterior fruit quality were good. Walker Lisbon had good first harvest yield and good total yield. In 2014-15, Walker had second-highest yield after Corona Foothill. Walker had good returns and relatively early in maturity.

**Yen Ben** fruit were relatively round in shape with low juice content, low seed count (about 1.5 per fruit) and relatively thin, but smooth peel. This selection had small fruit size, but good exterior quality. Yen Ben had poor first harvest yields and unremarkable second harvest yields with poor total yield and returns and average cumulative yield. Yen Ben is not an early selection and is highly susceptible to post-harvest diseases.

### The Future

The plan is to maintain this replicated lemon trial for the California desert for at least ten years, so that yield and quality of production can be evaluated. This past winter, at the request of the desert citrus growers and with the support of Peggy Mauk, a grower advisory group was formed and met at CVARS. This group provided advice that led to a number of changes in farming practices so that this replicated trial will better reflect farming practices of desert lemon growers. Information obtained from this trial will help to further define performance of these selected cultivars grown based on common practices of lemon growers in the California desert.

As part of the Integrated “Citrus Breeding and Evaluation for California” core project and based on results of this desert lemon trial, last year, rootstocks were grown and trees were budded at Lindcove Research and Extension Center for a set
of second generation multi-location lemon trials. These trials were planted at four sites: CVARS, Santa Paula, Lindcove and UCR in the spring of 2015. These trials include the following cultivars: Corona Foothill, Walker Lisbon, Limonero Fino 49, Yen Ben, Interdonato, Limoneira 8A (control) and Limoneira 8A IR1. The rootstocks vary based on location. At Lindcove and Riverside, rootstocks are Carrizo, C35 and Rich 16-6 trifoliate; at Santa Paula, they are Carrizo, Citrus macrophylla and Swingle; and at CVARS, the rootstocks are Carrizo, Citrus macrophylla and Volk. The trials have row and column design with five or six replicates per location each with single-tree plots. In addition, at Lindcove, there is a demonstration block adjacent to the trial with two trees of each scion rootstock combination next to each other.

The grower advisory group for CVARS desert lemon trial also has provided advice for this new set of trials for the Riverside and CVARS locations. These multi-replicated scion trials will extend our knowledge about the performance of selections that demonstrated commercial potential in the desert at other locations in the state. This set of second generation multi-location trials also 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 pack-out and fruit for data collection on post-harvest and consumer acceptance in later years of a ten-year cycle.

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Tracy L. Kahn, Ph.D., is a principal museum scientist in the Department of Botany and Plant Sciences, and curator of the University of California, Riverside Citrus Variety Collection. Vince Samons is a principal superintendent of agricultural operations at the University of California, Riverside. Glenn C. Wright, Ph.D., is an associate professor and tree fruit specialist at the University of Arizona – Yuma Agricultural Center.
The U.S. citrus industry is faced with several pathogens that seriously threaten production. Huanglongbing (HLB) has been known in China and other Asian countries for more than 100 years, but was contained there for most of that time. In recent years, the disease has spread to Brazil and now to North America with outbreaks in the United States, Mexico and Central American countries. Current survey methods to detect the disease (based on visual inspection) do not do so sufficiently early, thereby allowing it to progress throughout an area before any action can be taken; especially if there is no strategy in place to control the insect vector.

Now the vector, the Asian citrus psyllid (ACP), *Diaphorina citri*, and the bacterial pathogen (*Candidatus Liberibacter* spp.) have been detected in California. The first ACP was trapped in San Diego County in September 2008, and the only tree testing positive for the pathogen was detected in April 2012. Early detection using cost-effective surveillance techniques is crucial to successfully fighting the disease.

One strategy for the early detection of HLB funded by the Citrus Research Board focuses on analyzing the host plant’s innate immune responses that are induced early during infection and manifest as changes in the plant’s odor signature. The odor signature of citrus trees and other living organisms is made up of a bouquet of volatile organic compounds (VOCs). Some examples of citrus VOCs are limonene, methyl salicylate and linalool. The odor signature for HLB infection is a combination of some of these compounds being expressed more strongly (up regulated) and others being suppressed (down regulated). It is similar to smelling fruit from a vendor to determine freshness or ripeness. Likewise, there is much current active research to find odor markers that may be used to diagnose or monitor, in a non-invasive manner, human disease states such as breath or body odor analysis for diabetes, asthma, lung cancer, early stages of pressure ulcer wounds and infections.
VOC biomarkers that are associated with such responses are present locally near the tree. The innate immune system found in plants, fungi, insects and primitive multi-cellular organisms constitutes an evolutionarily conserved defense strategy against diseases and pests. Dandekar et al. give an early report outlining a strategy (i.e. identification of early-stage disease biomarkers and development of platform technologies to enable rapid detection and diagnosis of HLB and other pathogens in specialty crops). A recent review by Jansen et al., on using VOC analysis of detection of diseased plants, argues that although a certain plant species may emit similar VOCs upon induction by different diseases and, furthermore, different plant species may emit the same VOCs after being challenged with a similar disease, the total VOC blend emitted may be specific for a certain plant-pathogen interaction. This supports the need for a tool capable of broad spectrum VOC analysis. One such tool is the mobile analytical platform being developed by Applied Nanotech in Austin, Texas, branded EZKnowz®.

Using the EZKnowz® platform, researchers at the University of California, Davis, have established an HLB-specific VOC signature in sweet orange. They claim that they can correctly classify a tree as HLB-positive about 90 percent of the time during the summer and winter, and about 80 percent of the time during the rest of the year. Furthermore, they have seen different VOC signature alterations that distinguish the HLB VOCs from other citrus diseases, such as tristeza (CTV) and citrus variegated chlorosis (CVC).

Applied Nanotech has found similar evidence that infection of CTV and HLB and co-infection can be distinguished, in work funded under a USDA Small Business Innovation Research program. Figure 1 shows an EZKnowz® spectrum from an HLB-infected Valencia tree in a greenhouse study at the USDA-ARS Agricultural Research Center, in Beltsville, Maryland. On this spectrum are circles that represent different VOC analyte peaks. Some of these analyte peaks change in intensity relative to other peaks, depending on if the tree is healthy or infected with HLB or CTV. Presumably, this extends to other disease states, as well. Table 1 documents which peaks remained the same (=) or increased in intensity (+) or decreased in intensity (-) relative to healthy, non-infected trees.

EZKnowz® signatures of HLB-infected, CTV-infected, HLB and CTV co-infected and non-infected trees are visually distinguishable. Statistical analysis of the plots (partial least squares analysis), is able to separate the odor profiles among the four different health states. One complicating factor is that the trees were not all inoculated at the same time, which may be the situation in the grove, as well. In this study, the co-infected trees were inoculated about four months prior to VOC odor testing; the HLB- and CTV-infected trees had been inoculated for two years or more.

A critical issue is at what disease stage is HLB-induced odor print strong enough for detection by the EZKnowz®. There are currently two benchmarks: visual symptoms that appear about one year or more after infection and polymerase chain reaction (PCR) techniques that may detect the presence of the bacterium in citrus leaves within several months after infection. The UC Davis team sees a very high classification accuracy rate for symptomatic (PCR positive for infection and show visual symptoms) and asymptomatic trees (PCR positive for infection, but otherwise appear healthy) in studies conducted in Florida. These tests were performed on field samples, so the exact time of infection was unknown. Initial greenhouse studies have shown that the VOC signature for HLB is present before PCR tests are positive for the infection. A study to address this specific question is being initiated at the USDA Davis containment facility at UC Davis (CRB 1500-159 “Longitudinal Study of HLB-Induced Volatile Organic Compound (VOC) Release;” Citrograph: Winter 2014, pp. 28-34).

The EZKnowz® trace chemical analyzer uses a gas chromatograph (GC) combined with a differential ion mobility spectrometer (DMS) in a form that can be made small and robust, suitable for
portable applications. The units supplied to UC Davis were early-stage prototype machines that required a laptop for operation and significant post-test analysis using algorithm software to generate a conclusion.

Applied Nanotech is also working to provide an EZKnowz® product prototype that will be hand-portable, robust and fast. The equipment used for analysis in Hacienda Heights required about 12 minutes of sampling and analysis, and data needed to be sent to another location for analysis against a library of odor signatures. The goal of our current work is to cut that time down to one to two minutes. In addition to making the tool faster, we also are working to make it more user-friendly by eliminating radioactive isotopes typically used in ion mobility instruments. We are also developing a handheld sampling unit (Figure 2) that would collect VOC odors from a tree, improving workflow efficiency by separating the steps of sample collection from the steps of odor analysis.

Further, the Applied Nanotech–UC Davis team is working to achieve operation control and full data analysis at the point of testing, eliminating the need to send raw data to another location for analysis, thus providing near real-time response. The final end-product is expected to be a VOC detection system that can identify various citrus pathogens and is deployable in the field as a cost-effective, near real-time survey tool. The tool could be handheld (for backyard survey) or it could be mounted on a small vehicle for surveying a managed orchard. It is intended to be a stand-alone unit complete with a microprocessor and software algorithms sufficient to generate a conclusion and communicate its findings to a centralized system for generating reports and disseminating information. The conclusion could be red-yellow-green, i.e. infection detected, infection suspected or no infection detected.

Long-term applications of this technology will reach beyond agricultural needs; we are also exploring application of this technology for human health benefits. As an example, Applied Nanotech and UC Davis have teamed on an NIH-funded program for early detection of pressure ulcers, also known as bedsores, to direct more effective and timely treatment before the ulcers have fully emerged. Pressure ulcers are a serious problem in clinical care, causing infections and amputation. Pressure ulcers result in costs of $1 billion to the U.S. healthcare system and $3 - 5 billion worldwide. Success of this technology for the citrus industry will drive other applications to the benefit of all users.

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DEVELOPING NOVEL BLOOD AND CARA CARA-LIKE CITRUS VARIETIES

Kasturi Dasgupta, Roger Thilmony and James G. Thomson

SUMMARY

In 2012, the Citrus Research Board took a unique approach to developing novel citrus cultivars—a biotech approach. Blood and Cara Cara oranges are special cultivars distinguished by their color and distinctive flavor. They offer variety in flavor, taste and health benefits, and are often in high demand when in season.

Despite increasing consumer interest, production of these citrus varieties remains unreliable due largely to dependency on stringent growing conditions for full color and flavor formation. To offset this issue, our lab proposed to generate Blood orange and Cara Cara-like cultivars via genetic engineering by enhancing the expression of naturally occurring compounds in citrus and promoting their presence in the fruit using citrus genetic components. The compounds of interest are anthocyanin and/or lycopene. Anthocyanins are pigments naturally occurring in Blood oranges and many berry species such as raspberries, cherries and grape, while lycopene is found in Cara Cara navel oranges along with tomato, cherries and strawberries.

The required enzymes necessary for anthocyanin and lycopene production are characterized, and the citrus genes responsible for producing these enzymes are known. Activation of a single gene (MybA) has been shown to turn on the anthocyanin pathway in many species, including citrus. Lycopene production, on the other hand, requires at least three genes for biosynthesis in most plants. It has been shown that proper activation increases in the metabolite’s production. With this information, isolation and identification of the required genes was the first goal for the project.
PART 1: EXPRESSION OF THE **MYBA** GENE

Anthocyanins are natural pigments found typically in red, purple and blue fruit, as well as flowers. Among complex gene expression factors, the **MybA** gene appears to be the major influence for anthocyanin accumulation. We have cloned the **CsMybA** citrus sequence, as well as a novel **PfeMybA** gene from plum. We have further isolated the known **MybA** genes from **Arabidopsis** (**AtMybA**) and grape (**VvMybA**) to act as positive controls for our research.

Our results using a constitutive promoter (which turns on the gene everywhere) show that the various **MybA** genes display varying intensity of anthocyanin accumulation and expression pattern in the leaves and flowers (Figure 1). Non-transgenic tobacco plants (used as model plant due to speed of growth) did not show any anthocyanin accumulation, whereas expression of the citrus **CsMybA** gene resulted in dark purple coloration in the leaves, stem and strongly in flowers (Figure 1A, 1C). Similarly, **Arabidopsis** **AtMybA** and Plum **PfeMybA** expression showed accumulation of anthocyanin in leaves, flowers, stem and roots. Grape **MybA** expression resulted in deep red coloration in the flowers and excessively dark coloration of the stem and leaves (Figure 1B, 1C). Despite the differences in anthocyanin accumulation between species, our results show that the isolated **MybA** genes are functional and can be easily used to generate blood orange-like citrus cultivars.

METABOLIC ENGINEERING OF **LYCOPENE** PRODUCTION

Carotenoids, including lycopene and xanthophyll, are the main pigments responsible for the color of the peel and pulp of citrus. They greatly contribute to the fruit’s nutritional and antioxidant value. Citrus fruits are a rich and complex source of carotenoids. Most orange varieties predominantly accumulate xanthophylls, specifically violaxanthin in the pulp of mature

![Figure 1. Visual comparison of transgenic tobacco plants expressing various MybA genes. (A) Control (Wild type tobacco), transgenic tobacco plant lines expressing citrus CsMybA, plum PfeMybA, respectively. (B) Transgenic tobacco lines expressing grape VvMybA and Arabidopsis AtMybA used as controls. (C) Comparison of flowers from different transgenic tobacco lines expressing MybA from grape, citrus, Arabidopsis and plum, respectively.](image-url)
fruit, and are yellowish orange in color (Figure 2). A massive increase in lycopene production occurs naturally during the ripening process, which is eventually converted to the other carotenoids in the peel of orange and mandarin fruit. The change occurs during the transition from green fruit (chloroplast) to ripe fruit (chromoplast) and is largely due to the induction of several genes including phytoene synthase (PSY). Modification of the lycopene metabolite is the key point in the carotenoid production where the citrus fruit determines if it will be rosy/pink or yellowish orange. Two genes encoding lycopene modification are the cyclases and have been identified in citrus (ε-LCY and β-LCY) as those responsible for this transition.

In this work, we have expressed PSY to increase metabolic production and reduce the expression of lycopene cyclases ε-LCY and β-LCY to inhibit the shift in color away from rosy/pink. This increased lycopene production in fruit should lead to the generation of Cara Cara-like cultivars. The vectors containing the modified phytoene synthase (PSY) and cyclases ε-LCY/β-LCY transgenes to test the lycopene accumulation and biotech production of Cara Cara-like citrus cultivars are currently under investigation.

**PART 2: FRUIT-SPECIFIC PROMOTERS TO TARGET PIGMENT PATHWAYS**

With the existing citrus lycopene and anthocyanin genes in hand, the proposed research required the accumulation of the metabolites (anthocyanin or lycopene) in citrus fruit at a level that could be easily seen. This required precise gene expression of the metabolic pathways for improved fruit quality using citrus-derived DNA driven by fruit-specific promoters. A promoter is a region of DNA, which regulates a gene by controlling where, when and how much to turn a gene on or off.

Constuitive promoters may be suitable for proof of concept experiments, but have potential disadvantages for use in crop breeding. Fruit-specific promoters enable manipulation of metabolic pathways in the fruit alone, avoiding potential adverse effects. Our objective here is to restrict the expression of lycopene and anthocyanin to fruit instead of turning the whole plant purple/pink, which is energetically unfavorable for plant growth and development.

We have identified five citrus candidate fruit-specific promoters from citrus using bioinformatic tools and gene expression databases. All five promoters, selected from fruit-specific genes, have been isolated and cloned into our test system. Control promoters from tomato and back-up promoters from plum, tobacco and Arabidopsis also have been isolated. Due to the difficulty of transformation in citrus and the long juvenile period before fruiting, the activities of the above promoter-reporter gene constructs were examined in tomato fruit. These results will be used to confirm the promoter strength and fruit-specific expression pattern. Independent transgenic tomato lines were analyzed for GUS staining with proper controls. GUS is a sensitive reporter gene that allows promoter expression to be visualized and stains the tissue blue.

The promoter-reporter constructs were tested either by injection directly into tomato fruit tissues for transient assay or used to generate transgenic plants for stable assays. The GUS staining pattern in sliced tomato fruit, but not in the leaf, gives good evidence that the promoter will perform as desired. Wild type used as a control did not show any GUS staining, whereas 35S::GUS stable transgenic lines showed strong blue staining in fruit.
leaves and flowers (Figure 3A, 3B). Fruit specific promoters from tomato E8 and PG showed strong expression of GUS in transgenic fruits and very weak expression in leaves (Figure 3C and 3D) as expected. PG promoter, however, had stronger expression in the ripe fruit than the unripe fruit, while E8 was strong throughout the fruit, regardless of age. Figure 3E shows citrus candidate #3 with weak expression in leaf and strong expression in unripe, as well as ripe, fruit. Citrus candidate #5 showed some blue stains toward the base of the leaf and strong expression on the skin/rind and seeds of unripe tomato fruit. Moderate expression was seen in the ripe fruit of these plants (Figure 3F). Citrus candidate #6 showed weak expression throughout the leaf, but strong expression in the immature fruit and moderate expression in the inner tissues of mature fruit (Figure 3G). A plum candidate fruit-specific promoter also was tested in transgenic tomato lines (Figure 3H). The leaves showed an interesting pattern of GUS expression having blue stains in the mid-rib and the base of the leaf. Both unripe and ripe fruit however showed strong GUS expression throughout the fruit.

Citrus candidate #1 and candidate #4 currently only have been tested by transient Agrobacterium injection to assess the functionality of the promoters in tomato fruit. As shown in Figure 3I, GUS expression was not detected in wild-type tomato fruit injected with empty vector control, whereas strong expression was detected in fruit injected with citrus candidate promoter constructs. Citrus candidate #1 promoter generated GUS staining mostly in the seeds of the unripe fruit, but strong staining was detected in the inner tissues and seeds of the ripe fruit. Citrus candidate #4 showed strong GUS expression throughout young immature fruit. However, mature ripe fruit had expression mainly in the seeds. These results suggest that the cloned candidate citrus and plum fragments contain active promoter sequence elements regulating GUS expression pattern in tomato fruit. Using
these candidate citrus fruit-specific promoters, constructs have been generated to test the anthocyanin and lycopene expression in transgenic citrus fruit.

PART 3: INDUCTION OF EARLY FRUITING

Most citrus cultivars need 5–15 years to begin flowering, which delays regular fruit production for many years. Alternatively, early flowering has been achieved in transgenic trees, including citrus plants, by constitutively over-expressing flower meristem identity genes. The FLOWERING LOCUS T (FT) gene is a key regulator of the flowering transition. It already has been shown that the FT over-expression induces an extremely early fruiting phenotype and two fruiting cycles per year in sweet orange plants (Figure 4A). Therefore, we are introducing the FT gene into our constructs to reduce the flowering time period and more quickly assess citrus fruits accumulating lycopene or anthocyanin compounds. We generated transgenic tobacco lines to quickly test the functionality of AtFT in our constructs. As shown in Figure 4B, transgenic tobacco lines without FT expression, flower normally as wild-type (approximately three weeks after transfer from rooting media to soil), whereas lines expressing AtFT showed an early flowering phenotype (three to four days after transferring from rooting media to soil; preliminary data). Transgenic lines expressing AtFT, however, showed a ~50 percent dwarfing in biomass compared to WT or transgenic lines without FT overexpression. The flowers, seed pods and seed formation, however, were not affected in plants overexpressing the FT gene.

CONCLUSION

Metabolic engineering for enhanced production of naturally-occurring metabolites using plant-specific genes offers significant promise for improved citrus cultivar varieties, decreases production costs due to strict growing conditions, and increases the nutritional value of fruit. The results obtained from this work are promising for future citrus biotechnology research and the citrus community. The constructs generated are being used as molecular tools for producing citrus cultivars that will accumulate the healthy phytonutrients in their fruit. Blood orange and Cara Cara-like cultivars generated using native citrus genes and promoters will help to standardize regulatory procedures and hopefully increase consumer acceptance as the global impact of these nutritionally-enhanced staple fruit is realized.

Kasturi Dasgupta, Ph.D., is a postdoctoral scholar, Roger Thilmony, Ph.D., is a research molecular biologist at USDA-ARS-WRRC in Albany, California, and James G. Thomson, Ph.D., is a research geneticist at USDA-ARS-WRRC in Albany, California.

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Figure 4: (A) Induction of early flowering in transgenic sweet orange plant containing a sweet orange FLOWERING LOCUS T (AtFT) over-expression cassette. FT over-expressing line exhibiting an early flowering and fruiting phenotype compared with the WT control of the same age (Adapted from Pons et al., 2013). (B) Transgenic tobacco plants without overexpression of FT (left side) and transgenic plants overexpressing AtFT showing early flowering phenotype with reduced biomass (right side).
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