Citrus Center Highlights
FY 2016-2017

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Ray Prewett

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It has been a very busy year for the TAMUK Citrus Center, we have seen advancement on many fronts; including an increase in the number of graduate students, several new grant funded research projects, faculty and student awards, and many successful events. To share with citrus growers and industry leaders the events and achievements that took place during the year, I would like to welcome you to our first issue of ‘Citrus Center Highlights’. This issue is dedicated to you, our growers, and what you have done to support us throughout the past 70 years.

In this first edition of our magazine, you will get an insight into our research progress and current projects to better facilitate future partnerships and research collaborations while also highlighting the events and achievements that took place during the year. None of these accomplishments could be possible without the collaborations and support from our growers. We look forward to many more productive years serving you and the citrus industry.

Thank you,

Catherine Simpson

MESSAGE FROM THE DIRECTOR

During the past 12 months, the faculty, staff and students at the Texas A&M-Kingsville Citrus Center have been focusing their energy on performing research on a wide range of issues affecting our growers – some are immediate, such as Mexican fruit fly, Phytophthora and canker, while others are medium to long term, such as HLB, irrigation strategies and breeding. In addition to these activities, we tested thousands of samples in the diagnostic laboratory, provided a quarter of a million disease-free buds to nurseries, gave advice to growers on pest and disease control, and trained students and scouts. With all the hard work, commitment and service that our Center provides to the citrus industry of Texas, we hope that the citrus crop yields reflect that effort. In this issue, you will see a small glimpse of these endeavors and a few highlights of the achievements that have been received this year by both faculty and students.

We could not do our work without the support from the growers, who open the doors of their citrus orchards and let us use their groves as research labs. We also appreciate the support given to us by our university and industry leaders, who have been key to getting the voices of the citrus industry heard at the state and national level. Furthermore, our many collaborators across the USA and beyond, as well as the funding agencies have contributed significantly to our research and continued success. It is our mission to work together with the citrus industry to overcome challenges and ensure the future of Texas citrus. Thank you for your continued support.
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A Message from Texas Citrus Mutual

2017 has been a very busy year. The 85th Texas Legislature was in session, and once again budgets were being scrutinized. The industry fought along-side the Texas A&M Kingsville University folks at saving and protecting our industries most valued research asset, the Weslaco Citrus Center. Each session the budget line items come under intense review, and each session we must fight to protect what’s important. We escaped the budget axe this session, and have already begun to fight that axe for the 86th session that will begin in January 2019.

A study recently commissioned by Texas Citrus Mutual shows that without the Citrus Center and the work its team of scientists are doing, the losses to our industry could exceed $45 million total dollars. So we will work to fight to preserve this most valuable of assets to our industry, and we thank the team of scientists for their contributions.

Texas Citrus Mutual also worked the federal scene very hard for industry this year. TCM works very closely with the Florida and California Citrus Mutuals, to present an allied front in fighting for the U.S. citrus industries.

This year Texas received over $3 million in funding for various research, survey and education outreach needs through the Citrus Health Response Program (CHRP), over $7 million in MAC-HLB funding since 2015, and over $1 million in farm bill funding in 2017. These dollars are hard fought each year through the appropriations process, but are invaluable funding needs. These funds are used to support the Citrus Center, T.D.A. and the Texas Citrus Pest and Disease Management Corporations industry wide efforts at identification, solutions and cures.

A major accomplishment for TCM and industry this year was the successful appropriations of $47 million in the Omnibus budget package in Washington. For many years, TCM has worked hard to secure funding for construction of a sorely needed sterile fruit fly rearing facility. This year with the help and support of Congressmen Cuellar, Vela and Gonzalez, we achieved that goal. The USDA-APHIS facility is scheduled to break ground at Moorefield in 2019.

TCM through Representative Mando Martinez and Senator Chuy Hinojosa, introduced SB 1459 and saw its passage this legislative session. Recognizing a trend in minimally managed or abandoned groves valley-wide, the industry developed a plan by which landowners would be incentivized to remove these groves, but still be eligible to maintain a 5 year Ag use tax exemption. This is a win/win for industry and affected landowners, as it helps industry remove a growing source of untreated inoculum for pest and disease presence.

Texas Citrus Mutual will continue to work hard at preserving our industry with the help of the wonderful scientists at the Citrus Center in 2018 and beyond.

Dale Murden, President of Texas Citrus Mutual
Texas Citrus Producers Board has been busy bringing quality research and budwood to the Texas citrus production area since 1994. In the past 3 years TCPB has help fund research within the local community to provide novel ideas and solutions to many of the pests and diseases that could potentially harm the citrus industry.

With the help of our local scientist and the Board of Directors, TCPB has funded research for rootstock and scion evaluations for citrus greening resistance, effective phytophthora control, efficacy trials for new retrievable eradication devices to control Mexican fruit fly within residential settings and provided funding for clean certified budwood.

Not only has TCPB help fund these research topics they have also provided funding for sustainability of technical support for the Texas A&M Kingsville Citrus Center. This contribution is a major asset to the Citrus Center due to budgetary cuts and possible removal of the line item from the state budget. Without the Citrus Center and the work its team of scientists are doing, the losses to our industry could exceed $45 million total dollars.

Texas Citrus Producers Board would like to invite you to participate in round table discussions to help us understand what threats to the industry you see are major concerns and better understand the needs of our producers. Be on the lookout for upcoming events.
2016-2017 EVENTS

Lower Rio Grande Valley Irrigation Education and Outreach Program
May 9, 2017

2016-2017 EVENTS

Upcoming Events:
Dec 5—Blended preventative controls qualified individual training
Dec 6-7—Foreign supplier verification
Feb. 9 — Subtropical Ag & Environments Conference, Rio Farms
Feb. 23 — 3rd Annual Winter Texan Festival, Citrus Center
April 5-7—VIVA Fresh Produce Expo, San Antonio
May - Annual TCM Meeting, Mercedes Livestock Showgrounds
In February 2017, the Citrus Center and TCM hosted a CEU and ‘Meet the Scientists’ event. Representatives from Syngenta spoke about pest control products and sponsored lunch for attendees. Afterwards, scientists from the Citrus Center gave talks about their research findings and current projects.

Citrus Health Response Program (CHRP)
Critical Entry Point (CEP) Inspector Training
June 20-21, 2017

Citrus Center and AgriLife Extension scientists provided a training class for citrus pest and disease inspectors and scouts from the Texas Department of Agriculture and the industry in June.
Winter Texan Citrus Center Festival

A FUN & INFORMATIVE EVENT IN TOWN

Photos by Dr. Andrew Chow
In February 2017, the 2nd Annual Citrus Center Winter Texan Festival was held at the Citrus Center. This event is a fun opportunity to educate homeowners and Winter Texans about citrus health, care, pest and disease control. Faculty, staff, and students gave advice to Winter Texans from all over the U.S. and Canada to help them better maintain and care for their citrus during the winter and throughout the year. Informational booths on citrus nutrition and irrigation, new varieties, pest control, diseases, budwood, citrus varieties, and the greening control program were amongst the activities present, along with juice and fruit tasting and prize giveaways. The response was overwhelmingly positive with over 200 attendees. We look forward to seeing more attendees in February 2018.
The Everhard family has been involved in the citrus industry of Texas for three generations. His grandfather, Clay Sr., established the business which Clay later joined in 1960 after graduating from UT-Austin with a BBA. He also served in the Texas Air National Guard. The Everhard family maintained orchards in Brownsville, Mission and Carrizo Springs, a nursery in Mission and a packing shed in Carrizo Springs. In later years, he concentrated on the nursery operations, supplying both trees for orchards and garden centers. He has served on most citrus boards and committees, including Texas Citrus Mutual, including two terms as President, the Citrus Center Advisory Committee (1988-2006), and the Texas Valley Citrus Committee. He has been recognized for his contributions, serving as the Citrus Fiesta King in 1994, receiving the TCM Special Award and the Pott’s Award from the Rio Grande Valley Horticultural Society (2003). Currently he is still connected to the citrus industry as a real estate broker.

The Martin family’s introduction to Valley citrus can be traced back to the 1960’s, when James Ware bought a 20-acre citrus grove in Mission. In 1970, his son-in-law, Ken Martin took over as manager, and four years later he incorporated Rio Queen Citrus. The operation grew over the years to cover 5,000 acres of citrus, as well as vegetable production. Mike Martin, who graduated from Texas A&M in 1986, joined the family business and succeeded his father as president in 2003, and Rio Queen became one of the two major citrus producers in Texas. In addition to their own fruit, Rio Queen packed fruit for many other growers. In 2012, Paramount Citrus (now Wonderful Citrus) acquired Rio Queen’s citrus operations.

Mike recently established the Heino Brasch Memorial Scholarship for graduate students studying at the Citrus Center.

John Williams was born in Harlingen. His parents, Dan and Marcie Williams moved to the Valley from Virginia in 1935, and in addition to owning a grocery store, they farmed vegetables, and did some citrus grove care. John assisted with grove care operations, and after graduating from Texas A&M and working several other jobs, he was hired by the Lake Delta Citrus Association, overseeing harvesting in Cameron County. In 1975 he purchased the Bayview Orchard Service from Noel Ryall. The company takes care of 400 acres of citrus in Bayview, Rio Hondo and Los Fresnos areas. He and his wife Sue, founded Bayview Citrus Gardens, a citrus gift box shipper, and he also became a real estate agent. John has served on various boards, including Texas Citrus Mutual, Texas Valley Citrus Committee, Texas Producers Board, Southwest Soil & Water Conservation District #319 and Bayview Irrigation District #11. He was also key in the Diaprepes root weevil trials performed in conjunction with the Citrus Center.
Ray Prewett received the Texas Citrus Legend award from President Tallant during the 1st annual Citrus Center Grower Appreciation Day on October 26, 2016. Mr. Prewett has served the citrus industry as President of Texas Citrus Mutual for 31 years. During this time he was active in legislative initiatives and still serves as a strong advocate for the LRGV citrus industry.

Texas Citrus Legend, Jimmie Steidinger, has been a lifelong farmer and leader in agriculture. His dedication for farming, research, and extension has shown through his exemplary crops year after year. He has been an active member of the citrus industry and a proud supporter of the TAMUK Citrus Center, establishing the Barbara & Jimmie E. Steidinger Citrus Scholarship for agriculture students.

Dr. Tallant, President of Texas A&M University-Kingsville, presented Donald Thompson with the Texas Citrus Legend award during the 1st annual Citrus Center Growers Appreciation Day in 2016. Mr. Thompson has been a pillar of the citrus community since 1957 and a leader in innovative grapefruit production methods. He has served the citrus industry as Chairman for TCM, TVCC, and the TCE for many years.
Three members from the Texas A&M University—Kingsville Department of Agriculture, Agribusiness and Environmental Sciences received distinguished alumni awards from the Javelina Alumni Association. Dr. Veronica Ancona, Dr. John da Graça, and Dr. Greta Schuster attended the award banquet on October 14, 2016 to receive their awards and be honored by fellow Javelina alumni.

To see more visit: https://youtu.be/ubzWQUXG8lg
To see more about the TEEA:
https://youtu.be/mYXSYfZ6uZk
Faculty Awards

Dr. Madhura Babu Kunta
2017 Junior Researcher Award for the College of Agriculture

Student Awards

Julian Gonzales III
2017 Graduate Student of the Year Award for the College of Agriculture
2nd Place 4th Annual TAMUS AGEP Conference
2nd Place Texas A&M University System Pathways Student Research Symposium

Perla Duberney
3rd Place Poster, 28th Annual Texas Plant Protection Conference

Gabriela Sanchez
1st Place Poster, Subtropical Agriculture and Environments Conference
Recent Graduates

2016

Pallavi Vedasharan
Correlation of viability of Candidatus Liberibacter asiaticus with symptom development and age of leaf tissues
Advisor: Dr. Eliezer Louzada

Ana Olivares
Isolation and identification of root endophytic bacteria associated with citrus foot rot in south Texas
Advisor: Dr. Veronica Ancona

Mariana Uribe Bueno
IPM Reynosa
Bioinformatic and molecular analyses of the Tryptophan independent pathway for IAA biosynthesis in T. koningiopsis and T. asperellum.
Committee Member: Dr. Veronica Ancona

2017

Perla Duberney
Evaluation and control of the soil borne pathogens Phytophthora nicotianae and Fusarium solani of citrus in south Texas
Advisor: Dr. Veronica Ancona

Heidi Arteaga
Assessment of citrus production management in commercial groves and residential areas with special reference to Huanglongbing mitigation efforts with biological control in Texas
Advisor: Dr. Mamoudou Setamou

Ana Olivares
Isolation and identification of root endophytic bacteria associated with citrus foot rot in south Texas
Advisor: Dr. Veronica Ancona

Heidi Arteaga
Assessment of citrus production management in commercial groves and residential areas with special reference to Huanglongbing mitigation efforts with biological control in Texas
Advisor: Dr. Mamoudou Setamou
The Physiology and Sustainability Lab is dedicated to conducting whole systems research on citrus, abiotic and biotic stress, post-harvest issues, and providing producers and the scientific community with solutions to physiological problems while promoting sustainable production. This year has been very productive for my lab. We currently have 6 active projects and 3 additional projects were recently funded. I currently have 4 Master’s students, 2 Ph.D. students, and I am committee member for another Master’s student. We are working on a wide variety of projects in citrus physiology, environmental stress, water conservation, and subtropical crop production. The following is a brief summary of a few highlighted projects.

Management Strategies and Citrus Production
One of our main projects is studying how management strategies using raised beds and groundcovers affect citrus growth and yield. In the past 3 years we have found that yield, growth rates, soil moisture, root development, and soil temperatures have increased when groundcovers and raised beds are used in young citrus production. One of the most promising findings has been the significant increase in root production in raised beds with groundcovers when compared to the traditional flat bed, no groundcover method. In one of our Texas Water Development Board funded projects we have found that we can increase water savings up to 6.4% during young tree establishment by using groundcovers. While the trees are still in the establishment phase, they have shown increased growth and have larger canopies. Collaborator: TWDB (grant #1513581823)

Citrus Sunburn Prevention
Fruit sunburn has increased in recent years due to higher temperatures and more frequent sprays to prevent pests and diseases. To mitigate the damage to fruit we have begun testing products that help reduce fruit sunburn as well as studying the underlying causes which will help us understand how to manage sunburn more effectively. We have found that sunburn is more prevalent on the west side of trees even though higher fruit temperatures were recorded on the south and east sides of the canopy. While there is a correlation between fruit temperatures and sunburn incidence, we are still investigating other factors that are involved. Our results have also shown that Reflections™, a calcium based product, can reduce the incidence of sunburn while also reducing fruit temperatures. Collaborator: Miller Chemical and Fertilizer, LLC.
**Developing Arduino Based Environmental Monitors**

Arduino based microcontrollers are an inexpensive way to develop equipment suited to various needs in the field. After extensive research, we found that there are no available environmental monitors that measure fruit temperatures and environmental conditions continuously in the field. After taking a short course in Arduino programming, we decided to develop our own equipment to monitor environmental conditions in citrus trees to better understand the microclimate that affects fruit development, sunburn, stress, and ultimately fruit quality and yield.

**Drones to Monitor Citrus Diseases and Yield**

Unmanned aerial vehicles (UAVs or drones) have many applications that can be applied to agriculture. Often we can only see what our groves look like at the ground level and even then it is only a relatively small portion of the grove at a time. Drones can be used as a tool to monitor groves, over time, from a birds eye view. The data they collect can then be used to track disease progression, yield, tree health or water status, and many other factors. In one of our current projects we are using drones to monitor HLB and Phytophthora progression in a citrus grove over time. We are collecting field level data and comparing it with the drone data to verify accuracy and determine if drones can be a more efficient means of monitoring and managing citrus throughout the year.

Collaborators: TAMU Corpus Christi, USDA-NIFA HSI

**Subtropical Fruit Trials**

Citrus is one of the predominant crops grown in the LRGV, however, many growers are seeking to diversify their acreage with alternative crops. To evaluate suitable crops and how to manage them for production in the LRGV, we are evaluating several different species and cultivars to determine which is most suitable for our conditions. We will be evaluating several plants such as peaches, avocados and papaya for their performance and ability to produce in our challenging environment. We hope to provide information on best management practices and production advice to diversify the valley fruit industry.

**Collaborative Research**

In addition to this research, I work with several colleagues on collaborative projects. I am currently working with Dr. Ancona examining the effects of *Trichoderma* on citrus growth and physiology. I am also conducting a small project on boron toxicity in citrus with Dr. Louzada, evaluating the effects of calcium on psyllid populations with Dr. Setamou, and working with AgriLife extension on irrigation education and drone technology in citrus. Funding: TCPB and NIFA.

**Future Projects**

I recently received a grant from the TWDB to conduct research on innovative new systems for improving irrigation for small scale citrus producers. Dr. Ancona and I will be conducting a research project through the MAC program to evaluate thermotherapy in conjunction with antibiotic compounds in south Texas. I will also be conducting a product trial on biostimulants to improve citrus health and yield during stress conditions. Furthermore, a product trial to compare zinc and calcium products and their relative absorption in citrus is in the works right now.
I joined the TAMUK Citrus Center as Faculty member on September 2014 and since then, I have been working closely with citrus growers to study and develop solutions to the plant disease issues affecting the citrus industry of Texas. My lab is working on several projects including the evaluation of control treatments against Phytophthora and the evaluation of therapies to mitigate Citrus Huanglongbing (HLB) disease, including the use of antimicrobials and heat therapy. Additionally, my lab is studying the microbial communities associated with citrus roots to identify bacteria that antagonize soil borne diseases of citrus. Below you will find brief descriptions of the ongoing research in the Pathology lab.

**Evaluation of control methods against Phytophthora infections**

Production of grapefruit trees is negatively affected by Phytophthora foot and root rot diseases. Overall fruit yield (quantity and size) per tree is significantly reduced with increased disease severity. With the high incidence and severity in Texas citrus groves, it is imperative to evaluate the efficacy of the current commercially available chemistries, and study alternative strategies to manage infection. With the support from the Texas Citrus Producers Board (TCPB), chemical companies (Syngenta, Bayer, OroAgri) and collaboration with citrus growers, we have evaluated the effectiveness of products against Phytophthora propagules in the soil. Results show that the commercial products available to growers are effective at lowering the populations of the pathogen in the soil (Fig 1).

![Fig 1. Phytophthora propagule counts one month post application.](image1)

Alternative methods of management include *Trichoderma* spp., a common fungus found within the microbial communities from cultivated soils, which possess antifungal activity against many phytopathogens. My lab has isolated several *Trichoderma* species from citrus orchards across Texas and found one isolate has strong antifungal activity against Phytophthora (Fig 2).

![Fig 2. Antagonism of *Trichoderma asperellum* (T) against *Phytophthora* (P) after A—D) 2, 3, 4, 5, 6 and 7 days.](image2)
**Evaluation of therapies to mitigate Citrus HLB**

Huanglongbing (HLB) is a devastating citrus disease that is putting the citrus industries of the world at risk. In Texas, HLB was found in 2012, and control methods have been focused on suppression of the insect vector. Although the rate of decline of HLB infected trees in Texas is slower than in Florida, HLB symptoms are starting be more prominent (Fig 3) becoming an imminent threat to the citrus industry of the state.

![Fig 3. HLB symptoms. A) Blotchy yellowing of the leaves B) inverted fruit color.](image)

This year, in collaboration with ARS-USDA at Fort Pierce FL (USDA-NIFA grant#2017-70016-26142), my lab is performing field evaluations of selected antimicrobial compounds to determine their effectiveness at suppressing HLB bacterium under Texas conditions. Along those lines, we will start a project during the spring 2018 to evaluate heat therapy treatments in conjunction with commercially available antimicrobials to test their usefulness in mitigating HLB symptoms and recovering citrus tree health and productivity in Texas (NIFA-APHIS-PPQ grant # AP17PPQS&T00C166).

Another collaborative project is with the University of California-Riverside, UC Davis, University of Florida, (USDA-NIFA grant #2016-70016-24833) were my lab is involved in the development of an antibody-based HLB detection method, investigate the pathogenicity mechanisms of HLB bacterium and generate HLB-resistant citrus varieties.

To engage citrus growers and obtain feedback on their views on the deployment of edited citrus, my lab performed a small survey. Most of the interviewed growers agreed that resistance to HLB by edited citrus is a viable strategy to combat the disease, but were concerned about consumer acceptance and the productivity of the newly developed varieties.

**Microbial ecology within citrus roots**

With the aim of finding antagonistic bacteria to suppress *Phytophthora* infection, the pathology lab is studying the microbial communities associated with citrus roots. From our preliminary analysis of infected trees, we found increased abundance of bacterial species associated with citrus roots when compared to non-infected trees (Fig 5). Identification of those bacteria has revealed several candidates for their activity against fungal pathogens and we will continue our research on them.

![Fig 4. Dr. Ancona discussing with citrus grower Mr. Hoffman about strategies to combat HLB in Texas.](image)

![Fig 5. Citrus bacterial isolations are significantly higher in roots of Phytophthora infected trees versus healthy trees.](image)

**Acknowledgements**

None of this work would have been possible without the support form the citrus industry, grower collaborators, TCPB, funding agencies, and the hard for of current and past students.
The main goals of the state of the art USDA-certified diagnostic laboratory are 1) Detection of exotic citrus pathogens to prevent the spread of pathogens and emerging diseases to maintain sustainable production, 2) Support Texas citrus budwood program by testing for pathogens to enable disease-free budwood distribution, and 3) Develop and validate improved pathogen detection techniques.

**Validation of early detection method and development of HLB diagnostic method using root samples.** Kunta & Park et al.

Funding sources: USDA APHIS HLB MAC

We have developed a method for pre-symptomatic detection of HLB disease using root samples. The project has been conducted on different citrus varieties at different ages grown under different geographic locations and at different seasons for 2 years both in Texas and Florida. The results from this research will be a great benefit for citrus growers and researchers to deploy better strategies for HLB control.

**Improving Early Detection of HLB via ACP Nymph/Citrus Flush Sampling.** MCollum & Kunta et al.

Funding source: Citrus Research Board

In the absence of HLB symptoms, citrus flush with ACP nymphs is the tissue most likely to be infected with CLas and may provide the greatest diagnostic reliability. Our results demonstrate that qPCR can reliably detect CLas (HLB pathogen) infections well before HLB symptoms develop if proper tissue is sampled. If no HLB symptoms, ACP nymphs or adults are present, shoot tips (most recently fully expanded leaves) are the best tissue for diagnostics.

**Development of a sensitive real-time PCR detection method for citrus tatter leaf virus (CTLV)**

A sensitive assay to detect CTLV was developed and can be adopted for a routine high throughput CTLV screening for the production of virus-free budwood and plant eradication programs.

**Development of field tools for capture of testable psyllids and sensitive detection of huanglongbing.** Ramadugu, Kunta & Setamou et al.

Funding sources: USDA NIFA

Field deployable Bio Ranger and visual LAMP technology to detect HLB.

**First Report of Colletotrichum queenslandicum on Persian lime causing leaf anthracnose in the USA**

Persian lime, Ponderosa lemon, Palestine sweet lime, avocado, and papaya were susceptible. Rio Red grapefruit, Mexican lime, Marrs sweet orange, Australian finger lime, variegated pink Eureka lemon, Pomona seedless lemon, seedless Lisbon lemon, Etrog citron, and coffee did not develop lesions in pathogenicity assays.
Screening for Phytophthora nicotianae resistant rootstocks. Kunta & Louzada

Funding sources: TCPB

Sour Orange, C22, Sarawak pummelo x RioRed grapefruit, and Sarawak pummelo x Bower mandarin; were exposed to high amounts of *P. nicotianae* cultures to screen for resistance. Evaluation of the rootstocks eighteen months post-inoculation resulted in one surviving sour orange plant out of a total of 3,564 seeds and seedlings.

Quantification of live Candidatus Liberibacter asiaticus (HLB pathogen) in citrus. Kunta & Louzada

Several parameters were evaluated to develop a PMA-qPCR method to determine live CLas concentrations in citrus leaf tissue. This research is important to evaluate the efficacy of any HLB disease control strategies and to understand the disease epidemiology. Using this method, live CLas populations in leaves at different maturity and symptoms, and under different seasons were estimated in Texas grapefruit trees.

A Draft Whole Genome Sequence of “Candidatus Liberibacter asiaticus” strain TX2351 from Asian Citrus Psyllids in Texas, USA. Kunta & da Graça et al.

The TX2351 genome has the size of 1,252,043 bp, 36.5% G+C content, 1,184 predicted open reading frames, and 51 RNA genes.

Pathogenicity of Zygosaccharomyces bailii and other yeast species to Mexican fruit fly. Kunta &

Yeasts were isolated from samples of egg suspensions, single larva or single pupa, diet, and indoor-air collected from the Mexican fruit fly Mass Rearing Facility (MFFR) in Edinburg, TX, CPHST Mex Fly Methods Development Laboratory (MD) in Mission, TX or from egg suspensions from Petapa-Guatemala. The highly pathogenic yeast to MFF was determined. The results of this research is highly important for rearing MFF for the sterile insect technique used to eradication of MFF.

Occurrence of a citrus canker strain with limited host specificity in south Texas. Da Graça & Kunta et al.

A W strain of citrus canker bacterium was confirmed on Mexican lime trees in Rancho Viejo, TX. Based on the flushing cycles and branch diameters, the age of canker lesions was estimated to be 5-6 years old. Mexican limes and alemow were the only citrus plants that developed definitive canker lesions in pathogenicity assays.

Future projects

1. Determination of prophages in Texas CLas isolates. This information is valuable for developing new HLB management strategies as the prophages are involved in bacterial virulence.
2. Transcriptome studies in orange jasmine to understand host plant responses to CLas infection.
3. Determine causal agent for concave gum disease and study gene expression in host plant infected with concave gum.
4. Training citrus growers in using newly developed HLB detection technologies.
My research focuses on the development of sustainable and integrated management strategies for economically significant mite and insect pests affecting citrus in Texas. The development of any effective control strategy relies on an understanding of the pest bio-ecology.

Thus guided by the principle of pursuit of developing sustainable multi-target control, my research interests include both fundamental and applied questions aimed at providing practical solutions to the many pest problems facing our growers. I conduct fundamental studies for a better understanding of factors regulation pest infestations and their population fluctuations. At that aim, my research focuses on bio-ecological studies of mite and insect pests, and on factors governing their host plant selection.

My applied research include: a) the development of attract and kill approaches for pest management using semiochemical and pheromone based attraction and environmentally-friendly killing stations, b) the development of grove management strategies for pest exclusion, c) the quantification of pest densities and development of practical and effective sampling plants based on pest distribution, d) enrichment of pest biological control by either augmentative releases of natural enemies or incorporation of flowering plants in growers for natural enemy recruitments, e) testing and optimization of pesticide efficacy, f) pesticide resistance management, g) evaluation of plant protectant to prevent pathogen acquisition and/or transmission, h) development of season long organic pest control through the combined use of biopesticides, pest exclusion methods and biological control, and i) area-wide pest surveys for early detection of invasive species and identification of spread of endemic pests.

Testing of pesticide efficacy

In collaboration with several chemical companies including BASF, Bayer CropScience, Dow AgroSciences, Dupont, Gowan, Nichino, NuFarms, Syngenta and Valent, we have tested several insecticides, miticides and antimicrobials for the control of various insect, mite and plant pathogens. Notably, we have established the high efficacy of Nealta (a.i. cyflumetofen), Portal (a.i. Fenpyroximate) for the control of spider mites in general and specifically for citrus red mite.

Nealta used in combination of Movento (a.i. spirotetramat), Agrimek (a.i. abamectin), Envidor (a.i. spirodiclofen), Vendex (a.i. fenbutatin oxide), Micromite (a.i. diflubenzuron) or Nexter (a.i. pyridaben) provided good knock down and residual control of all mites including citrus rust mites in groves. Minecto Pro is a new binary insecticide/miticide with active ingredients abamectin and cyantraniliprole that has provides broad spectrum control of many citrus pests. In combination with Nealta, it also resulted in stellar results.

Testing of efficacy of wind break for psyllid control

Fig. 1. Type of artificial windbreaks to reduce psyllid colonization in groves and wind scarring of citrus fruit
The Asian citrus psyllid exhibits strong border effects in its grove colonization and niche occupation. Higher psyllid densities are generally observed on trees along grove borders. We developed the border spray strategy that can be implemented between major flush cycles to complement whole grove sprays at the onset of flush cycles. However, deployment of artificial windbreaks in the form of 10 to 12 feet high insect-resistant screen along grove borders (Figure 1) resulted in dramatic reduction of psyllid populations (60-70%) relative to grove with no windbreak. This non-chemical approach can be implemented in young plantings and mature groves with immediate benefits in psyllid control and possibly a reduction in wind scarring that is a major problem in South Texas Citrus. These artificial windbreaks can be established along the live ones, the time these latter windbreaks grow. In addition to preventing psyllid colonization, these windbreaks can be tailored to also lure psyllids onto insecticide-impregnated mesh (yellow area on right Figure 1) as killing surface. The insecticide used in these mesh is beta-cyfluthrin, a potent pyrethroid that is protected with UV protectant to prolong its effectiveness for up to 60 days, which can be replaced to ensure efficacy.

**Implementation of a novel planting design to boost tree growth and hasten production**

Through a grant funded by the USDA-HLB-MAC, the Texas Pest and Disease Management Corporation in collaboration with Texas A&M University-Kingsville Citrus Center and Texas AgriLife Extension is evaluating in grower participatory trials, the effectiveness of a new citrus planting design for rapid tree growth and many other agronomic benefits. This system consists of raised planting bed covered with black plastic mulch. The system stemmed from a Diaprepes control program funded by the Texas Department of Agriculture in 2009. The objective of that successful program was to deploy the black plastic mesh under the tree canopy to break the life cycle of Diaprepes and other root weevils in citrus groves. Extensive damage resulting from the feeding damage of root weevil larvae is a major problem in Texas citrus. Such root weevil infestations affect the health of citrus trees and promote Phytophthora infection. However, subsequent to the deployment of black plastic mesh, not only Diaprepes was eradicated from groves within two years, all root weevils were effectively controlled, and additional agronomic benefits were recorded in the grove including but not limited to moisture retention due to lower evaporation, weed control due to the plastic blocking sunlight and acting as a barrier for weed growth, soil preservation as a result of reduced erosion. Altogether these benefits led to healthier tree and better production.

This observation has led to the development of the currently funded USDA-HLB-MAC project to field test the new planting design. In collaboration with seven growers, groves with this new planting design (Figure 2) are established throughout of South Texas encompassing a total of 100 acres. Tree growth parameters obtained from these young trees clearly demonstrated better tree growth and reduced infestations of several insect pests including root weevil with the new planting design. Evaluation is ongoing and yield data will be collected to evaluate the production benefits of this novel strategy.
Development of new red grapefruit cultivar
A new redder grapefruit is in process of being patented. It is redder than Rio Red and children will like it because of the sweet and mild flavor.

Development of a seedless Golden nugget grapefruit.
We are developing a seedless golden grapefruit that is the most amazing fruit you will ever taste. It is as sweet as ice cream.

Development of a seedless Meyer lemon.
We already developed and will be patenting a new seedless Valley lemon (Meyer lemon).

Development of Phytophthora resistant Sour Orange rootstock.
Phytophthora has been killing thousands of trees in the Rio Grande Valley. To combat this disease, my lab has developed the first version of a Phytophthora resistant rootstock. The picture below is a root system of a sour orange seedling growing in a heavily contaminated soil.

Production of transgenic plants with broad spectrum disease resistance
To introduce a gene inside of a plant genome the most used method is called Agrobacterium tumefaciens–mediated transformation. Agrobacterium is a bacterium that causes crown gall disease in plants by introducing a piece of its own DNA in the plant genome. To use this bacterium, we remove all the pathogenic genes and put in place the gene we want to introduce in the plant (see photo below). We use what normally happens in nature to our benefit.

Using this methodology we produced citrus plants with resistance to Phytophthora, Citrus canker, and are testing for HLB.

Development of a method to eliminate undesirable genes from transgenic plants
In any genetic transformation we have to use some genes to allow us to identify which plant contains the gene we want. One of the most used methods is to introduce genes that give resistance to an antibiotic and another gene that give us a visual cue in addition to our gene and two other genes. When we culture the plants in a medium containing the antibiotic the plants that doesn’t have our gene will die. For visual cue we use a gene that will make the transgenic plant glow under an special light (mCherry gene).
Below is a transgenic plant that contains the desired genes under normal light and under special light.

**Development of a method to eliminate undesirable genes from transgenic plants**

Our lab decided to establish a methodology to eliminate any kind of gene that is not the gene of interest in the transgenic plant so that no antibiotic resistant gene or visual cue gene is present in the final transgenic plants. In cooperation with the USDA-ARS in Albany, CA, we designed a strategy where we used the *mCherry* gene cited above as a way to show the method works and we are able to remove any DNA we want from a transgenic plant. We created a new version of this gene where the sequence of DNA that drives the gene is interrupted by marker genes so that only if we remove the markers, the *mCherry* gene would work and make the plant glow. The photo below shows transgenic shoot primordia, after removal of the markers and proving that the method works.

**Establishment of genetic transformation in citrus using a Gene Gun.**

To speed up the process of evaluation of transgenic plants and bring the benefit of the improved plants to growers and consumers, we were funded by the California Citrus Research Board to establish a genetic transformation system that does not use *Agrobacterium* transformation. We decided to use a system that shoots the gene, coated in gold particles, into the plant cells. We purchased a Gene Gun driven by helium gas that, like a hand gun, shoots the gene to introduce it into the plant. We started the optimization of the Gene Gun procedure and were able to introduce the gene in the cell with high efficiency. The photo below shows the Gene Gun works.

*These projects have been funded by:*
1. Texas Citrus Research Board.
2. California Citrus Research Board.
3. National Institute of Food and Agriculture– Hispanic Service Institutions Grants Program.

**Collaborative Research**

All research projects described here has been in collaboration with the USDA-ARS-Crop Improvement and Genetic Research and the USDA Certified Citrus Diagnostic Laboratory at the TAMUK– Citrus Center.

**Future Projects**

A main endeavor to be initiated in 2018 is research to produce seedless mandarin like citrus fruits that are adapted to the Rio Grande Valley climatic and soil conditions. The citrus market worldwide is going towards seedless easy to peel mandarin like fruits, so our lab is putting effort in this area of research.
Ray Prewett has served the Texas citrus industry for many years, in this article he gives his perspectives on the changes and challenges we face.

You have been involved with the Texas citrus industry for over 30 years, how have you seen it evolve during that time?

Texas Citrus Mutual was the driving force in developing the tree insurance program (right before I started working for the industry). This program played in the survival of the industry when hit by the freezes in 1983 and 1989. With regard to the changes in the industry, one of the biggest has been the consolidation of grove ownership and packinghouses.

Today growers are pushing their groves to get the highest yield and pack-outs but that has not always been the case. When I started working in the industry in 1983 we had a large number of marginally productive groves. Some growers were originally attracted to the citrus business primarily because of favorable tax laws, but the law changed in 1986. For the industry as a whole, the quality of fruit and care of the groves is much better today than it was when I started in the industry.

In what ways do you think the citrus industry has changed for the better?

Fruit quality and pack-outs are better today but some smaller growers still find it challenging to utilize the latest technologies.

What do you see as the biggest challenge to the Texas citrus industry at this moment?

The ultimate impact of citrus greening on the Texas citrus is still a major unknown. Fortunately, so far, the disease has not had the same type impact on Texas citrus production as it has in Florida and other places around the world. The Mexican Fruit Fly has been another major challenge. Homeowners find it difficult to control citrus pests including greening and fruit flies. In most areas of the Valley dooryard citrus is adjacent to or is very close to commercial groves and many of the pest problems growers face are originating in the dooryard properties. The Valley shares its water resource with Mexico. Cities are growing rapidly and requiring more and more of that water resource. Valley agriculture needs to do more to maximize the efficiency of our irrigation system.
In your view, what has been the role and contribution of the TAMUK Citrus Center to the Texas citrus industry?

The development of the Rio Red grapefruit by the Citrus Center has been a major game changer for the industry and those benefits are still being realized. The center also played a major role the development of the area wide control program for the Asian Citrus Psyllid, the vector for citrus greening. Since that program started the population levels of the psyllid have been quite low and this is a major reason why the disease has not spread even more rapidly. The newly developed orchard planting design with raised beds and fabric is still being tested and perfected by growers but hopefully in the next few years this new technology will be validated and make a major contribution to the industry as a whole.

How do you think the TAMUK Citrus Center has contributed to the national citrus industry?

While the size Texas industry and the budget of the Citrus Center are small compared to other areas, the center is recognized nationally and internationally for many of its programs including but not limited to its world class variety development and pest management programs.

How can we best work together to improve citrus production in Texas?

The new orchard planting design project is a great example of how the growers and the industry are working together. There are fewer small growers than in the past but these growers need a wide range of assistance on things with their pest management programs and many other areas including how to maximize their fruit quality. Larger growers also need help from the Citrus Center particularly in working with scientists to develop and test new technologies. There is a need to improve the yields and quality of the oranges we produce in Texas. To remain competitive all growers are going to need to maximize the amount of fresh fruit they produce. These are but a few of the areas where cooperation between growers and the citrus center will continue to be needed.

How can the TAMUK Citrus Center facilitate better partnerships between growers, industry leaders, as well as state and federal agencies?

Over the years the Citrus Center has reached out to all growers with educational programs and there is still a need for these efforts. There is a need to find a better solution and method for the Citrus Center to provide more assistance to growers. A few years ago, the university changed their expectations for facility members to prioritize teaching and attracting outside dollars for research if they want to be promoted. Under this system, interaction between faculty and growers tends to take a backseat to the other priorities. Some faculty have still managed to spend a significant amount of time assisting growers but this is not easy.

What do you think the future holds for the Texas citrus industry?

The future looks bright for the Texas citrus industry because we still produce the best grapefruit in the world. Fresh grapefruit has great health benefits. Feeding the growing population in the world is a huge challenge. New technology in agriculture is exploding. The Texas citrus industry is relatively small and faces big challenges to remain competitive with other citrus producing areas of the world. The Citrus Center, in cooperation with other citrus research institutions, will be key to meeting this challenge.
Grower Perspectives: Building Partnerships

By Dale Murden and Catherine Simpson

For the first issue of ‘Citrus Center Highlights’ we felt that the perspectives from two presidents of Texas Citrus Mutual would be a great way to start. I interviewed Dale Murden and Ray Prewett to get their views on the Texas citrus industry: past, present, and future as well as how we can build strong partnerships for the future. Dale Murden is the current president of Texas Citrus Mutual and a citrus grower in the valley. Here is his perspective on the industry and building partnerships to enhance the future of Texas citrus.

You have been involved with the Texas citrus industry for a while now, how have you seen it evolve during that time?

I started my citrus career in 1980 with the first ever IPM Citrus Program with Extension Service. The 1983 freeze changed everything. We've gone from 20 plus packing houses to 4.

In what ways do you think the citrus industry has changed for the better?

The industry has always taken advantage of natural disasters to improve what wasn't working at that time. Whether it was marketing issue, planting density issues, varietal issues etc.

What do you see as the biggest challenge to the Texas citrus industry at this moment?

Pest and disease issues and pressure from Mexico are the immediate threat. Land development and water are always a threat.

In your view, what has been the role and contribution of the TAMUK Citrus Center to the Texas and national citrus industries?

Industry would not be where it is today without the scientific community and in our case in Texas its been a huge plus that we've had the Citrus Center here locally all these years. Too many contributions to name.

How can we best work together to improve citrus production in Texas?

By working together and with the other citrus producing states and universities.

How can the TAMUK Citrus Center facilitate better partnerships between growers, industry leaders, as well as state and federal agencies?

I think the Citrus Center is a bright star in the TAMUK system and having President Tallant along with state representatives in Austin whom are also huge fans has been a huge help to industry....we just need to keep our issues at the forefront of everyone's mind. If we don't do that, nobody will for us.

What do you think the future holds for the Texas citrus industry?

The future is bright. Planting is on the rise. Obviously we have many challenges to address, but US grown and Texas grown fruit can be here to stay if we set our minds to it.
In the summer of 2016, Christopher Barbola did agricultural research with Dr. Veronica Ancona at the Texas A&M Citrus Center in Weslaco as part of the South Texas College STEP2 grant program. After the summer research in the program ended, Dr. Ancona hired him to continue working in her lab. In the spring of 2017, Chris applied for a USDA research internship program and was accepted. For the internship, Chris worked at USDA’s Agriculture Research Center (ARC) in Beltsville, Maryland for the summer of 2017. The STEP2 grant supported his travel, housing, and meals while in Maryland. At the Beltsville research center, he got the opportunity to work in the post-harvest pathology lab, and gained valuable knowledge in the field of fungal biology (see pics below). After returning to the valley, he presented his research in August 2017 at UTRGV in a presentation to faculty and students from UTRGV and STC, and also related his experiences from his internship in order to encourage other students to apply. According to Chris, his summer internship at USDA ARC-Beltsville will remain his most memorable experience, one in which he made life-long friends and grew as an independent human being and gained confidence.

Chris has returned to work in the fall of 2017 with Dr. Ancona at the Citrus Center. In Dr. Ancona’s words, “Besides being a great student, his passion for learning and his openness to new projects makes him a great asset in my lab.” Chris is currently taking classes at STC to complete his Associates Degree in Biology. He then plans to continue his education at UTRGV, TAMU Kingsville, or College Station and finish his Bachelor’s degree with biology major. He sees himself working in the USDA or EPA as a scientist in the field of Agriculture or Environmental Science. Chris is a fine example of a student who has taken the opportunity from the STEP 2 grant to launch his path toward a career as a scientist.
The Citrus Budwood Story

Historically, all citrus nurseries until very recently were open field operations, and budwood was collected from selected orchard trees. In the 1990’s our industry was threatened by the possible introduction of the brown citrus aphid from Florida; this is a highly efficient vector of Citrus tristeza virus, and severe strains cause quick decline of citrus on sour orange rootstock, the primary one used in Texas. Because of this, the industry lobbied the Texas legislature and passed House Bill 2807 in 1997 to establish a mandatory certified budwood program. The Citrus Center was designated to manage the program on behalf of the state.

Field nurseries in Texas

To prepare for this, the Citrus Center and the industry selected the best budwood source trees of the most important varieties. Dr. Mani Skaria invited Dr. John da Graça to come to the Citrus Center as a visiting scientist in 1994, and they conducted shoot-tip grafting on the major varieties grown in Texas to eliminate any viruses and viroids.

In 1998, the first foundation and increase budwood trees were planted at the Citrus Center, and the first certified buds were cut in 1999.

Dr. da Graça returned to Texas in 1999 to assume the Center Deputy Director’s position (later changed to Director), and because of his experience with the South African budwood program, he assumed responsibility for the Texas program. The manager at the time was Craig Kahlke, who moved to New York State in 2003 to work for the Cornell extension service. John Watson, then nursery manager at the Center, took over this position until 2011 when he was promoted to Farm Superintendent. Mark Van Ness was then hired to direct the program, and applying his organizational skills from his years in the corn industry to citrus has benefited the program significantly.
Certified disease-free citrus budwood
Initially, all the foundation and increase trees were in the field, but the discovery of citrus greening (HLB) in Florida in 2005 prompted the Citrus Center to construct a screenhouse for foundation trees in 2008. We then obtained a grant from the Economic Development Administration to construct the main screenhouse complex which was built in 2012, and houses both foundation and increase trees.

Field budwood increase trees with the first screenhouse in the background

The citrus growers funded building a screened structure over existing field increase trees to ensure budwood supply while the new screened trees were growing. In 2013, a new regulation came into effect requiring all citrus nurseries in the Valley to be under screen; this will be extended to all of Texas in 2018.

Mark Van Ness with potted scion trees

Since 2012, nearly 1 million buds have been supplied to citrus nurseries. Until 2015, grapefruit was the predominant type, but demand for sweet orange varieties has increased significantly in the past 2 years.

Dr. John da Graça and Mark Van Ness examining budwood

A back up collection is maintained at the Texas A&M AgriLife Center in Stephenville. Currently, there are 100 or so varieties in the protected germplasm collection that were obtained from the California and Florida programs. New Texas varieties, such as the Texas Red grapefruit are undergoing shoot tip grafting to remove pathogens.
Texas Citrus Salsa

Recipes and pictures courtesy of: Edinburg Citrus Association and TexasSweet

Makes 6 Servings

**Ingredients:**

1 Texas Red Grapefruit, peeled, sectioned, and chopped  
1 large Texas Orange, peeled, sectioned, and chopped  
1 medium tomato  
1 cup diced green, red, and yellow bell pepper (for color contrast)  
1 jalapeno pepper, seeded and minced  
3 tablespoons chopped red onion  
1 tablespoon chopped fresh cilantro  
1 1/2 teaspoons sugar  
1/4 teaspoon salt

**Directions:**

Mix grapefruit, orange, tomato, pepper, onion, and cilantro and seasonings. Drain juice before serving.  
Makes approximately 2 cups. Excellent on grilled fish, chicken, or just with chips!

**Nutrition:**

Per cup

Calories:130; Fat: 0.5 g; Cholesterol: 0 mg; Carbohydrates: 33 g; Protein: 3 g
**Texas Grapefruit Yogurt Cake**

**Ingredients:**

<table>
<thead>
<tr>
<th>Cake</th>
<th>Grapefruit Syrup</th>
<th>Grapefruit Glaze</th>
</tr>
</thead>
<tbody>
<tr>
<td>3/4 cup greek yogurt</td>
<td>1/4 cup Texas Grapefruit juice</td>
<td>1/3 cup powdered sugar</td>
</tr>
<tr>
<td>Zest of 1 1/2 Texas Grapefruits</td>
<td>1 tbsp sugar</td>
<td>3 tbsp. Texas Grapefruit juice</td>
</tr>
<tr>
<td>3 large eggs</td>
<td>2 tbsp brown sugar</td>
<td>1 drop almond extract</td>
</tr>
<tr>
<td>1 1/2 cups all purpose flour</td>
<td>1 tbsp. butter</td>
<td>*food coloring optional</td>
</tr>
<tr>
<td>2 tsp baking powder</td>
<td>1-2 drops vanilla</td>
<td></td>
</tr>
<tr>
<td>1/4 tsp salt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/2 tsp vanilla</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/3 cup canola oil</td>
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</tbody>
</table>

**Directions:**

Preheat the oven to 350F. Spray a 8 inch round cake pan with cooking spray and dust with flour. Whisk the four baking powder and salt together then set it aside. In a small bowl, mix the grapefruit zest and sugar thoroughly until it is evenly distributed. In a large bowl, mix the yogurt, sugar, and zest. Then add the eggs and whisk thoroughly. Add the vanilla and stir again. Add the flour mixture to the wet ingredients until the flour is just incorporated. Add the oil and mix well. Pour the batter into the cake pan and bake for 30-35 minutes until a toothpick comes out clean.

For the syrup: In a small saucepan add the grapefruit juice, sugars, butter, and vanilla. Stir over medium heat until the sugar and butter is dissolved.

For the glaze: Whisk the powdered sugar, grapefruit juice, and a drop of almond extract*. Poke holes in the cooled cake with a toothpick. Slowly pour the syrup over the cake. Then add the glaze over the top of the cake.
The Texas A&M University – Kingsville Citrus Center has served the Texas citrus industry since 1948 after a group of local citizens and citrus growers approached Texas A&I to establish a research and training facility specializing in citriculture in the Lower Rio Grande Valley. Since then the Citrus Center has grown to over 200 acres located in Weslaco and Monte Alto. In 2010, the new Citrus Center building was dedicated and equipped with modernized labs and classrooms. Currently, the Citrus Center is staffed with 42 people who operate the office, research labs, farm, and budwood program.

The mission of the TAMUK Citrus Center is to serve the citrus industry of Texas by conducting basic and applied research and deliver innovative solutions which enable the citrus industry to remain competitive in an increasingly global marketplace. The Citrus Center is also dedicated to fostering scientific excellence and capacity building by incorporating undergraduate and graduate student training into its research programs to develop highly skilled professionals. Since 1988, 92 Master’s students 11 Ph.D. students have graduated from the program and many have gone on to take positions working within the citrus industry. Currently there are 16 Master’s students and 4 Ph.D. students working towards their degrees under the direction of the 6 faculty members.
Chair: Earl Neuhaus, President of Neuhaus & Co.

Vice Chair: Dennis Holbrook, South Texas Organics

Secretary: Dale Murden, President of Texas Citrus Mutual

Jim Hoffman, Chairman of Edinburg Citrus Association
Jeff Arnold, General Manager of Edinburg Citrus Association
Paul Heller, Vice-President of Wonderful Citrus Texas Division
Mark Fryer, Owner of Orchard Service and Nursery Ltd.
Laura Coffman, Precision Orchard Services

Jimmie Steidinger, former citrus producer and Citrus Legend
Jud Flowers, Lone Star Citrus Growers
Rick Garcia, Tommy Thompson Farms
Tommy Garcia, Garcia Farms
Becky Bonham, B&B Enterprises
James Bettiga, Wonderful Citrus
Bruce Sutton, Wonderful Citrus
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