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What we have learned about Huanglongbing from research collaboration in a historically endemic region in Guangdong, China

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Citrus HLB (Huanglongbing, also called yellow shoot disease or citrus greening disease) is threatening citrus production worldwide. In the U.S., HLB was first diagnosed in Florida in 2005 and spread rapidly thereafter. In California, the first HLB-affected citrus tree was found in Hacienda Heights of Los Angeles County in 2012, and more HLB-affected trees have been detected since then. Public concern about HLB is high, demanding effective control. One challenge in controlling HLB is our limited knowledge about the biology of the disease . Since 2006, Dr. Jianchi Chen, in the Crop Diseases, Pests and Genetics Research Unit, has been collaborating with scientists in Guangdong, China, where HLB has been endemic for over a hundred years, on a research project to learn about HLB and develop management strategies.

Guangdong is one of the major citrus-producing provinces in China. Before the 1990's, citrus production was mainly in the coastal Chaoshan and Pearl River Delta areas. An HLB epidemic occurred in the 1930-40's, but subsided in the late 1940's due to the decrease of citrus acreage during World War II. In the 1950s, citrus production rebounded, and so did HLB, triggering a debate about HLB etiology being physiological (for example, a nutrient deficiency) or pathological (caused by a disease agent). A milestone was set by Professor Kung-Hsiang Lin at the South China Agricultural College in 1956 that HLB was an infectious disease with an unknown viral etiology and transmitted by an unknown insect(s). Based on the "infectious" theory, three key measures for HLB control were proposed: use of clean nursery stocks with quarantine, removal of diseased trees, and insect vector control. The three key measures worked effectively under the collective agricultural production system used in China at the time that assured area-wide uniform pest control operations. In the late 1970's, agricultural production in China returned to individual family-run farms, HLB reemerged, and citrus production in Guangdong dropped substantially in the 1980's. Starting in the mid-1990's, major citrus production moved to the inland hillock mountainous north and west regions with a highly marketable local mandarin cultivar, Shatangju. Citrus quickly reached about 346,000 acres by 2010, but, unfortunately, HLB control did not follow the acreage expansion. HLB-affected nursery stocks spread widely. As a result, HLB began to reemerge, and

Terraces of Shatangju trees in the hillock and mountainous west region of Guangdong (A and B). A Huanglongbing (HLB)-affected tree in the lowland shown from a distance (C) and a close-up view showing yellow shoots (D). (E) An orchard with a high incidence of HLB.

citrus production in Guangdong is now facing another downturn.

The challenge of HLB etiology research was noted in the 1930s in Guangdong by Prof. C.P. Chen who described HLB as citrus tip-chlorosis. Dr. Jose Bove's group in France identified "*Candidatus* Liberibacter asiaticus" (CLas), an unculturable α-proteobacterium, as the most probable pathogen in 1994. Two years later, the association of CLas with HLB in Guangdong was confirmed by Prof. W. Tang and his graduate student X. Deng in South China Agricultural University. Vigorous research on HLB bacterial etiology continues today. However, documentation on "physiological" etiology of HLB was and is rare. There are occasional legendary stories that HLB-symptomatic trees were cured through improved nutritional management by experienced citrus growers. These "successes" are always questioned as to whether "true" HLB-affected trees were encountered, again raising the argument of what causes HLB. Impacts of nutrition management on HLB development, particularly at an early stage of infection, are very difficult to evaluate due to the woody and perennial nature of citrus and the lack of efficient research tools. There is, however, general agreement that good nutritional management along with various levels of pest management on HLB-affected trees can prolong fruit production. This information is welcomed by growers, particularly when the price of citrus fruits is high, and provides justification for further HLB research. As molecular and genomic technologies become more readily available, we are embracing a new round of HLB research to reveal more details of HLB etiology that will benefit current HLB management in the U.S. and around the world. Dr. Jianchi Chen (SJVASC) visits Guangdong each year to help guide research on HLB and bring back new results for application in the U.S., and he hosts visiting scientists and students from China at SJVASC to help both countries learn more about the disease to improve management.

New Scientist

Dr. Lindsey Burbank has joined the Crop Diseases, Pests and Genetics Research Unit as a Research Plant Pathologist. In her new research assignment, Dr. Burbank will address gene function in *Xylella fastidiosa*, the fastidious bacterial pathogen causing Pierce's disease of grapevines. Dr. Burbank earned her Ph.D. degree in Plant Pathology from the University of California-Riverside in 2014 under the direction of Dr. Caroline Roper. Her dissertation research included characterization of global genetic regulatory mechanisms in the bacterial pathogen *Pantoea stewartii*, including transcription factor regulation of siderophore-mediated iron acquisition, oxidative stress response, and other virulence processes. Dr. Burbank's postdoctoral work focused on molecular genetics of *X. fastidiosa* in relation to progression and persistence of Pierce's disease. Specifically, she investigated *X. fastidiosa* genes involved in response to cold-induced stress. This research led to identification of a cold-shock protein homolog important for pathogen survival and virulence. Additionally, Dr. Burbank worked on functional characterization of *X. fastidiosa* toxin-antitoxin systems that modulate virulence, plasmid stability, and growth of the pathogen. This research also led to development of plasmid shuttle vectors suitable for gene expression and complementation in *X. fastidiosa* that are stable *in planta* in the absence of antibiotic selection. In her new assignment, Dr. Burbank will continue to study molecular mechanisms responsible for *X. fastidiosa* virulence and response to environmental stresses.

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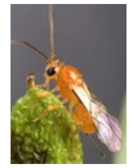
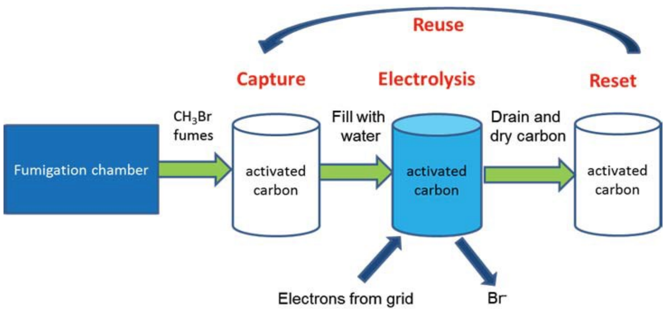
Research Updates



Destruction of methyl bromide sorbed to activated carbon by thiosulfate and electrolysis

Submitted to: Environmental Science and Technology

Authors: Y. Yang, L. Yuanqing, S. Walse, W. Mitch

Methyl bromide (MB, CH_3Br) is a postharvest fumigant that is highly effective for control of insect and microorganism pests. MB is also an atmospheric source of reactive bromine gases, which deplete stratospheric ozone. Anthropogenic utilization of MB is regulated by international agreement under the Montreal Protocol. In instances where postharvest chamber fumigations are permitted, contribution(s) to ozone depletion can be minimized, or eliminated, by removing MB from the ventilation effluent via activated carbon sorbent. As part of a larger research project to optimize activated carbons for this use, we conducted experiments to determine how sorbed MB was destroyed by aqueous solutions of thiosulfate as well as by electrolysis. Aqueous solutions of thiosulfate effectively destroyed MB, yet a relatively costly amount of thiosulfate was required and disposal of methyl thiosulfate generated as a reaction byproduct would be required. Electrolysis, a potentially cheaper approach, resulted in >80% debromination of MB in ~30 h. Finding cost-effective techniques for eliminating MB emissions into the atmosphere may help ensure that the continued use of MB has minimal environmental impact.

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Prevalence and incidence of postharvest diseases of blueberries in California

Submitted to: Acta Horticulturae

Authors: C. Xiao, S. Saito

Recent establishment of low-chill southern highbush blueberry cultivars in California's warm climate has significantly increased the acreage of blueberry production in the Central Valley of California, which is now a major southern highbush blueberry production region in the United States. The vast majority of blueberries grown in California are destined for the fresh market. As the production continues to grow, the blueberry industry has a need to prolong the storage life of blueberry in order to extend the marketing period. However, the postharvest life of blueberry fruit is limited by fruit rots caused by fungal pathogens. In this study, we conducted a 2-year survey to determine the major postharvest diseases affecting blueberries in the region. We collected blueberry fruit at harvest from 42 and 62 grower lots in 2013 and 2014, respectively; stored the fruit at 0-2°C for 5 weeks; and then evaluated for the presence of fruit rots. We found that incidence of decay ranged from 23 to 74% in 2013 and from 3 to 74% in 2014. One or more fungi were isolated from each decayed fruit. On average, *Botrytis* spp. (mainly *B. cinerea*), *Cladosporium* spp., *Alternaria* spp. (mainly *A. alternata* and *A. arborescens*), *Aureobasidium pullulans*, *Rhizopus stolonifer*, and *Penicillium* spp. were isolated from 21-46%, 6-13%, 25-38%, 12-29%, 2-26%, and 2-8% of the decayed fruit sampled from conventional grower lots, respectively; and from 20-73%, 5-18%, 11-46%, 7-19%, 5-25%, and 3-4% of the decayed fruit sampled from organic grower lots, respectively. Our results indicated that *B. cinerea*, *Alternaria* spp., *Aureobasidium pullulans*, and *Cladosporium* spp. were the major pathogens as they were consistently isolated from decayed fruit from most grower lots during the 2-year survey and that control measures should target these pathogens.

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Evaluation of sulfur dioxide-generating pads and modified atmosphere packaging for control of postharvest diseases in blueberries

Submitted to: Acta Horticulturae

Authors: S. Saito, C. Xiao

Postharvest fruit rots are a limiting factor of storage and shelf life of blueberries. Gray mold caused by the fungus *Botrytis cinerea* is one of the most important postharvest diseases in blueberries grown in California. In this study, we evaluated the effectiveness of sulfur dioxide (SO_2)-generating pads (designated as Dual or Slow release pads) alone or in combination with modified atmosphere packaging (MAP) for control of gray mold and other diseases. Freshly harvested fruit were placed in clamshells in cardboard boxes, the treatments were applied, and the fruit were then stored at 1°C for 5 weeks. No gray mold was observed on the inoculated fruit treated with the Dual pad, thus there was no spread of gray mold. Although gray mold developed on the inoculated fruit treated with the Slow pad or MAP bag alone, gray mold spread was significantly reduced compared to the control. Incidence of decayed fruit was significantly reduced with either the Dual or Slow pad, especially in combination with the MAP bag, compared to the control. However, more than 20% of blueberries treated with the Dual pad exhibited bleaching due to SO_2 injuries, and fruit became significantly softer than those treated with the Slow pad or the controls. Our results suggest that the combination of the Slow pad and MAP bag is a promising method for control of fruit decay while maintaining blueberry fruit quality during storage.

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Impact of changing wax type during storage on mandarin flavor and quality attributes

Submitted to: Acta Horticulturae

Authors: D. Obenland, M. Arpaia

Packers of mandarins sometimes apply a storage wax designed to limit water loss during the initial part of storage and then replace it with a higher shine pack wax prior to shipment of the fruit. Mandarins are prone to developing off-flavors in storage following waxing and are damaged more easily during handling so it was important to determine if multiple wax applications would harm or improve fruit quality. 'Tango' mandarins were harvested twice during the first season and subjected to various combinations of storage wax and pack wax, with storage times up to seven weeks. Similar experiments were conducted in the second season of the study using 'Tango' and 'W. Murcott' mandarins with a single harvest. Evaluations of visual and internal fruit quality, including flavor, were conducted at the end of each storage period. In the first season, application of multiple waxes caused many of the fruit to have unsightly brown marks on the peel, likely due to additional mechanical impacts from the fruit running down the pack line twice. In addition, the flavor quality was sometimes judged to be worse than fruit with pack wax alone. These problems were not evident in the second season. Multiple wax applications have the potential to damage both mandarin appearance and flavor, and care must be taken in the implementation of these treatments to maintain mandarin quality.

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Deficit irrigation strategies and their impact on yield and nutritional quality of pomegranates

Submitted to: Fruits

Authors: T. Centofanti, G. Banuelos, C. Wallis, J. Ayars

Deficit irrigation (DI) is often conducted as a response to water shortage, inconsistent water supply, or recurrent droughts. The cultivation of drought-tolerant crops and the application of DI strategies are necessary agronomic measures for sustainable agriculture in arid regions of the world and in areas that are experiencing recurrent water shortages (i.e., Central California). Pomegranate is a drought-tolerant fruit tree and is extensively cultivated in arid and semi-arid regions of the world. In this study, pomegranate trees grown in Central California were treated with four different DI treatments [35, 50, 75, and 100% of evapotranspiration (ETlys)]. Tree physiological responses were evaluated relative to fruit yield and nutritional quality including pH, soluble solids, total phenolic compounds, anthocyanin and non-anthocyanin compounds, and mineral elements. Results of this 2-year study showed that DI strategies, as low as 35% of ETlys, did not affect yield, fruit color, pH, concentration of soluble solids, total phenolic compounds, anthocyanin and non- anthocyanin compounds, or mineral elements. The application of deficit irrigation may be a strategy to reduce water usage and increase agricultural sustainability on arid and drought-stricken regions. Longer-term studies are needed to better predict physiological responses to water deficit in crops and trees relative to nutritional quality and productivity.

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Emission, pest control, and crop response in an almond orchard fumigated with reduced rates of Telone® C-35 and low permeability film

Submitted to: Journal of Crop Production

Authors: S. Gao, D. Doll, R. Qin, S. Rana-Dangi, J. Gerik, D. Wang, B. Hanson

Many specialty orchard crops rely on pre-plant soil fumigation to control soil-borne pests, and adequate pest control is required for successful fumigation with alternative fumigants to methyl bromide. This research investigated the application of a totally impermeable film for reducing fumigant emissions, reducing fumigant rates, and improving tree performance after fumigation and replanting. Results showed that the impermeable film was effective in reducing emissions, and the 66% fumigant rate provided similar pest control and yield as the full rate. Thus, the 66% rate can be used without compromising yield. This research also identified that delivering fumigants to deeper soil depths for improved fumigation efficiency can be a challenge.

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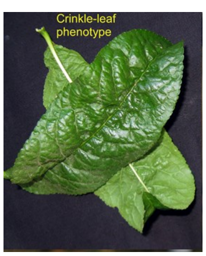

Structure-function characterization of the crinkle-leaf peach wood phenotype: a future model system for wood properties research?

Submitted to: JOM: The member Journal of The Minerals, Metals & Materials Society

Authors: A. Wiedenhoeft, R. Arevalo, C. Ledbetter, J. Jakes

In a plant breeding program, unexpected mutations are sometimes discovered within a family of plants. Most of these chance mutations are not useful, but occasionally, one is identified that can be put to good use. A leaf mutation called 'crinkle-leaf' was discovered in peach that co-segregates with weak, brittle wood, providing a unique research opportunity. Wood samples of wild-type and crinkle-leaf trees were examined in an attempt to identify specific properties that differed between the two types. While wild-type trees had wood with longer vessels and fibers, wider rays, and slightly higher specific gravity, these differences did not fully account for the gross differences in wood strength apparent to workers pruning the trees. This co-segregation provides a unique opportunity to utilize the crinkle-leaf mutation as an observable vegetative marker for altered wood properties. As a model system, crinkle-leaf trees and their wild-type counterparts can be used to examine macroscale wood properties relative to the interactions of various wood component properties, thus providing valuable information on structure-function relationships and assisting in the development of a global predictive model for wood properties based on wood cellular anatomy.

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Towards the elucidation of the cytoplasmic diversity of North American grape breeding programs

Submitted to: Molecular Breeding

Authors: J. Fresnedo-Ramirez, Q. Sun, C. Hwang, C. Ledbetter, D. Ramming, A. Fennell, A. Walker, J. Luby, M. Clark, J. Londo, L. Cadle Davidson, G. Zhong, B. Reisch

In plants, interactions among genetic material from the nucleus and the so-called "cytoplasmic genomes", which include DNA from organelles such as mitochondria and plastids, may impact breeding strategies and outcomes. Genetic analysis of cytoplasmic genomes for grape breeding has been limited. The understanding of how diverse grapevine is with respect to cytoplasmic genomes will help to address those limitations. Here, cytoplasmic diversity was analyzed among 6,073 grapevines, with 52 DNA markers revealing five distinct cytoplasmic groups. These groups included wine grapes, raisin/table grapes, and three groups of interspecific hybrids. Ten of the 52 DNA markers were predicted to affect gene function, which may have an impact on the traits of known cultivars or breeding selections. The results will aid further study of cytoplasmic genomes and breeding in grapevine.

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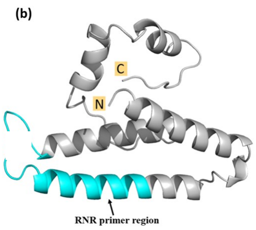

Unusual five copies and dual forms of nrdB in "*Candidatus* Liberibacter asiaticus": biological implications and PCR detection

Submitted to: Scientific Reports

Authors: Z. Zheng, M. Xu, M, Bao, F. Wu, J. Chen, X. Deng

Citrus Huanglongbing (HLB, yellow shoot disease, also known as citrus greening disease) is currently threatening citrus production worldwide. HLB is associated with an unculturable bacterium "*Candidatus* Liberibacter asiaticus" (CLas). Research in CLas biology is challenging because the bacterium cannot be cultivated in artificial media, a standard technique for bacteriological research. Effective HLB management relies on comprehensive knowledge of CLas. In this study, the whole genome sequence of CLas strain A4, originating from Guangdong, China, was analyzed. A unique gene (nrdB) was found to have an unusually high copy number of five in two forms among bacteria in general. This gene encodes a protein called ribonucleotide reductase (RNR) ß-subunit, which is essential for bacterial growth. Analyses on RNR gene revealed several new biological features of the bacterium. The high copy number feature was further used to develop a sensitive and stable PCR detection system for CLas. A total of 262 HLB samples collected from China and the U.S. were evaluated to test a primer set based on this gene. The RNR system tripled the detection sensitivity of the currently used primer set. It is expected that the RNR detection system will significantly enhance current efforts for early and accurate HLB diagnosis.

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New Postdoctoral Scientist

Dr. Melissa Anderson is a new Research Molecular Biologist working in the laboratory of Dr. Drake Stenger in the Crop Diseases, Pests and Genetics Research Unit. Her research will focus on understanding the role of *Xylella fastidiosa* toxin-antitoxin systems in biofilm formation and plant pathogenesis. Prior to coming to Parlier, Dr. Anderson conducted her postdoctoral studies at the University of California-San Diego in the California Center for Algae Biotechnology working on nuclear transcription regulation and genetic engineering techniques in the microalga *Chlamydomonas reinhardtii* and also completed the Micro-MBA certificate program from UCSD's Rady School of Management. Dr. Anderson completed her doctoral degree at the University of North Carolina-Chapel Hill in the School of Medicine's Department of Microbiology and Immunology in 2013 under the direction of Dr. Peggy Cotter. For her thesis research, she studied the effects of competition and cooperation on community development during biofilm formation mediated by Contact-Dependent Growth Inhibition (CDI) systems in species of *Burkholderia* including the Tier 1 Select Agent *Burkholderia pseudomallei* which required biosafety level 3 certification. Dr. Anderson has a bachelor of science degree in Cell and Developmental Biology from the University of California, Santa Barbara.

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