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SJVASC Update

April 2018

Research Highlight

Blueberry Research at the San Joaquin Valley Agricultural Sciences Center

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Historically, the Central Valley of California is not known for production of blueberries because of mild winter weather in the region. However, establishment of low-chill southern highbush blueberry cultivars in California's warm climate has significantly increased the acreage of blueberry production in central California in the past 15 years. California is now a major southern highbush blueberry production region in the U.S. with 5,700 harvested acres in 2015. The vast majority of blueberries grown in California are destined for the fresh market, with a 2015 production value of $117 million, ranked #2 in the value of utilized production in the nation.

Highbush blueberry fruit are susceptible to postharvest fruit rots caused by various fungal pathogens, limiting the storage ability of the fruit and affecting postharvest fruit quality. As the production continues to grow in California, the blueberry industry has a need to retain postharvest fruit quality and prolong the storage life of blueberries to extend the marketing period and increase exports. Certain insect pests also can pose trade barriers for export of blueberries to other countries. To meet industry's needs, we initiated research to address the following issues that the blueberry industry is facing: 1) control of postharvest fruit rots, 2) maintenance of postharvest fruit quality of blueberries, and 3) mitigation of pest-related barriers for movement or export of blueberry fruit. Below are some highlights of recently completed and ongoing research.

The occurrence and prevalence of fruit rot diseases in stored blueberry fruit are influenced by the climate and postharvest handling practices. The first step in developing effective measures to control postharvest fruit rots and retain fruit quality is to determine which diseases are most prevalent. Thus, we conducted a survey in 2013 and 2014 to determine major postharvest diseases that affect blueberries grown in the Central Valley of California. Our survey indicated that gray mold caused by the fungus *Botrytis cinerea* and Alternaria rot caused by the fungi *Alternaria* spp. are the two most important postharvest diseases affecting blueberries grown in the region and that control measures should target these pathogens.

Gray mold and Alternaria rot cause problems on blueberries after harvest or in the marketplace, but infections of the fruit by these pathogens start in the field. To control these pathogens, growers apply fungicides in the field during the fruit growing season. However, *B. cinerea* is a high risk for the development of resistance to certain groups of fungicides. Fungicide resistance in the pathogen often causes the failure of disease control. We collected *B. cinerea* isolates from major blueberry production areas and determined resistance of those isolates to boscalid, cyprodinil, fenhexamid, fludioxonil, and pyraclostrobin, representing five different fungicide classes for control of *Botrytis* diseases. We found that 26 and 30% of the isolates from blueberry were resistant to two and three classes of fungicides, respectively, but no fludioxonil resistance was detected. Our findings are useful in guiding and implementing fungicide resistance management spray programs for control of gray mold in blueberry.

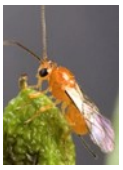
Various postharvest practices have been or are being evaluated for control of postharvest fruit rots on blueberries.

Postharvest treatments with continuous ozone at low concentrations significantly reduced postharvest fruit rots and limited spread of gray mold among the fruit in clamshells during cold storage. We also evaluated the effectiveness of sulfur dioxide (SO_2)-emitting packaging materials (pads, liners, etc.) alone or in combination with modified atmosphere packaging bags (MAP) for control of gray mold and other diseases and for maintenance of fruit quality. In one study, Dual SO_2-emitting pad (SO_2 was initially released up to 10 ppm and then declined and stabilized at a low concentration), Slow SO_2-emitting pad (SO_2 was released at a low and constant concentration), or MAP bag alone significantly reduced fruit rots during cold storage compared to the untreated control. Dual SO_2 pad in combination with MAP or Slow SO_2 pad in combination with MAP provided even better control, but Dual SO_2 pad can cause bleaching on blueberry fruit due to SO_2 injuries. Our results suggested that the combination of Slow SO_2-emitting pad and MAP bag is a promising method for control of fruit rots while maintaining blueberry fruit quality during storage. In another study, SO_2-emitting liners with modified atmosphere capability were effective in reducing fruit rots and maintaining fruit quality. Currently our research efforts in collaboration with manufacturers are to design use patterns and labels for registration of sulfur dioxide fumigation as well as sulfur dioxide-emitting sodium metabilsulfite products (liners, pads, bags) for blueberries.

Freshly-picked blueberries can taste amazing, but those purchased in the store can often be somewhat disappointing in comparison. To address the loss of fruit quality, we have initiated research into determining why this happens with the goal of improving the eating quality of blueberries in the marketplace. Preliminary results indicate that changes in flavor components progressively occur during storage that may act to alter the flavor of the fruit. Part of the research effort regarding blueberries also has been the development of procedures to enable the assessment of the blueberry sensory quality by a semi-expert taste panel. Upcoming research will utilize a taste panel to evaluate the characteristics of blueberries that have been stored for varying durations and determine the timing and degree of flavor loss that occurs over time. The results from this work will then be compared to the profiles of flavor components within the fruit to determine potential reasons for the changes in flavor and possible means for slowing or preventing the changes.

To address insect-related trade barriers, we continue to coordinate postharvest research activities through the California Blueberry Commission, the U.S. Highbush Blueberry Council, and various service companies. Projects include an effort, directed through the USDA Animal and Plant Health Inspection Service, to gain market access for California, Oregon, and Washington State fresh blueberries to Australia, a key reciprocal trading partner. Even though an efficacious methyl bromide postharvest fumigation for spotted wing drosophila (SWD) was developed and presented to Australian officials in 2015, no export program is currently active. Australia has indicated a desire to only grant access for the entire U.S., not just the Western region that is free of blueberry maggot. Toward this end, research is being conducted in the Eastern U.S., primarily in Michigan, to demonstrate the efficacy of the "SWD methyl bromide schedule" toward the blueberry maggot. Methyl bromide alternatives for the control of SWD have also been developed, such as a postharvest fumigation with phosphine. Importantly, we have teamed with industry, IR4, and the U.S. Environmental Protection Agency to expand the phosphine label, with a final ruling expected within a year that allows treatment of all types of fresh fruits and vegetables.


Research Updates




Restoring product quality in powdery mildew-resistant raisins through backcrossing

Submitted to: Journal of Horticultural Science and Biotechnology

Authors: C. Ledbetter, S. Lee

All cultivars of raisin grapes are susceptible to powdery mildew, and control of the disease represents a major production expense for raisin growers. There are wild species of grape that naturally resist infection of the powdery mildew fungus, but these grapes have very poor fruit quality characteristics. A breeding effort was initiated to combine the powdery mildew resistance from wild grape species with high fruit quality raisins that had already been developed in the breeding program. At each cycle of breeding, seedlings were exposed to powdery mildew to identify and eliminate seedlings that were susceptible to the disease. Resistant seedlings were then fruited, and vines possessing the desired combination of fruit quality traits and production characteristics were used in the next cycle of breeding. After two cycles of breeding, raisin selections were identified with fruit quality traits similar to those of commercial raisin cultivars. Elite powdery mildew-resistant raisin selections are currently being trialed under different pruning regimes to identify selections with acceptable yield. Utilization of powdery mildew-resistant raisins in California will significantly reduce fungicide applications in this important commodity.

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Development of a duplex ddPCR assay for detection of '*Candidatus* Liberibacter asiaticus'

Submitted to: PLoS One

Authors: V. Selvaraj, Y. Maheshwari, S. Hajeri, J. Chen, T. McCollum, R. Yokomi

Huanglongbing (HLB) (aka citrus greening) is a devastating citrus disease associated with '*Candidatus* Liberibacter asiaticus' (CLas), an unculturable bacterium transmitted by the Asian citrus psyllid (ACP). Quarantines in California require that HLB-affected trees be removed immediately upon detection to eliminate pathogen reservoirs and to limit further spread by ACP. The current regulatory protocol for CLas detection is quantitative real time PCR (qPCR) targeting 16S rRNA (16S). A critical need exists to improve early detection of CLas to assist in timely HLB eradication. A duplex assay was developed that targeted the 16S and ribonucleotide reductase (RNR) genes in a droplet digital polymerase chain reaction (ddPCR) format. This assay resulted in simultaneous absolute quantification of the two targeted genes of CLas in the same reaction mixture. The duplex format improved diagnostic performance and precision by elimination of non-specific amplification in the sample. CLas detection by ddPCR resulted in direct pathogen quantification without need for standards. In contrast, qPCR only provides an indirect relative measure of the pathogen titer and requires adding target standard dilutions to interpolate pathogen titer. Low titer detection of CLas-infected citrus leaf and insect tissues was shown to be significantly greater with the duplex assay than the singleplex assay. The duplex ddPCR for simultaneous detection of the 16S and RNR targets in the same sample showed great advantages which are critical for regulatory samples being tested for CLas infection.

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A zinc, copper and citric acid biocomplex shows promise for control of *Xylella fastidiosa* subsp. *pauca* in olive trees in Apulia region (southern Italy)

Submitted to: Phytopathologia Mediterranea

Authors: M. Scortichini, J. Chen, M. De Caroli, G. Dalessandro, N. Pucci, A. L'Aurora, M. Petriccione, L. Zampella, F. Mastrobuoni, D. Migoni, L. Del Coco, C.R. Girelli, F. Pia-

cente, N. Cristella, P. Marangi, F. Laddomada, . Di Cesare, G. Cesari, F.P. Fanizzi, S. Loreti

The bacterium *Xylella fastidiosa* subsp. *pauca* is associated with olive quick decline syndrome in southern Italy. To search for a method to control the disease, a compound containing zinc and copper complexed with citric-acid hydracids (Dentamet®) was evaluated for bactericidal activity and movement inside plants. A 3-year field trial of integrated management including regular pruning and soil harrowing with spring and summer spray treatments with Dentamet® was carried out. The results showed reduction of symptom severity, suggesting these treatments may be beneficial.

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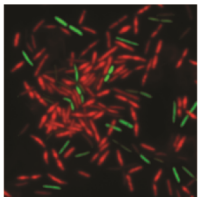

Distinct contributions to biofilm development and pathogenesis by toxin-antitoxin system mqsR-mqsA of *Xylella fastidiosa*

Submitted to: Applied and Environmental Microbiology

Authors: M. Anderson, L. Burbank, D. Stenger

Toxin-antitoxin (TA) system proteins function in cellular adaptation for prokaryotes living in changing, and sometimes harsh, environmental conditions. While TA system proteins from human-associated microbes have received considerable attention, TA systems encoded by plant pathogenic bacteria are less well studied. Toxin MqsR is an endoribonuclease capable of degrading RNA *in vitro*, and binding by antitoxin MqsA impedes this activity. Here, data are presented on mqsRA of *Xylella fastidiosa*, an economically important phytopathogen. Results indicated that mqsR mediates cell survival and biofilm formation, two vital functions for the lifestyle of *X. fastidiosa* in nature. Alternately, mqsA inhibited biofilm formation. Finally, a role for mqsRA in pathogenesis of grapevine was demonstrated, indicating this TA system is necessary for the progression of disease symptoms *in planta*. In sum, the research suggest TA system proteins MqsRA are active mediators of cellular functions essential to the survival of *X. fastidiosa*.

Contact: Drake Stenger,

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EPG waveform library for *Graphocephala atropunctata* (Hemiptera: Cicadellidae): Effect of input resistor and voltage levels on waveform appearance and probing behaviors

Submitted to: Journal of Insect Physiology

Authors: F. Cervantes, E. Backus, T. Perring

Blue-green sharpshooter (BGSS) is a native pest of grapevines in California. This insect transmits the bacterium that causes Pierce's disease of grapevines, severely affecting production. Development of blue-green sharpshooter- and Pierce's disease-resistant varieties of grape depends in part upon better understanding of the means by which the insect feeds on the plant and the feeding mechanisms involved in transmission of the plant pathogen. Electropenetrography (EPG), one of the most useful techniques to study insect feeding behavior, has been used with BGSS in the past. However, the technology used in previous studies had limitations and was not standardized, making it difficult to compare results from different studies. Using the most current electropentrograph available (the AC-DC instrument), an updated waveform library was created that standardizes the methodology. Results strongly support that the instrument settings and type of adhesive can influence appearance of waveforms and quality of EPG information for BGSS. These findings will improve the use of EPG for BGSS to better support studies of host plant resistance, which will aid ultimately in development of BGSS- and Pierce's disease-resistant grape varieties.

Contact: Elaine Backus, Elaine.Backus@ars.usda.gov, (559) 596-2925

Effects of nymphal diet and adult feeding on allocation of resources to glassy-winged sharpshooter egg production

Submitted to: Environmental Entomology

Authors: M. Sisterson, D. Stenger

The glassy-winged sharpshooter (GWSS) is an invasive insect capable of transmitting the bacterial pathogen *Xylellela fastidiosa* to grapevine. Introduction of the GWSS to California resulted in epidemics of Pierce's disease of grapevine in two grape growing regions of California. In response, an area-wide suppression program was initiated

that relies on application of insecticides. In this study, effects of juvenile diet on timing and magnitude of GWSS egg production were evaluated. While juvenile diet affected adult size and development time, juvenile diet did not affect time to first oviposition or quantity of mature eggs available at time of first oviposition. Results suggest that the nutritional resources required for egg production were gained by females during the first week after adult emergence via adult feeding. Identifying periods where resource acquisition is critical for GWSS egg production will aid in predicting GWSS population dynamics and may suggest novel targets for control.

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Two poplar hybrid clones differ in phenolic antioxidant levels and polyphenol oxidase activity in response to high salt and B irrigation

Submitted to: Phytochemistry

Authors: K. Nguyen, C. Cuellar, P. Mavi, D. Leduc, G. Banuelos, M. Sommerhalter

In some regions of the westside of the San Joaquin Valley of Central California, irrigation with saline- and boron (B)-laden water has led to the need to identify alternative crops that can tolerate irrigation with such poor-quality waters. In the last decade, as part of a general phytoremediation strategy, a multi-year screening program was initiated to test hybrid poplars for their ability to tolerate high salt/B irrigation water. Hybrid poplars are fast growing trees with economic relevance for the veneer, lumber, and paper industry. Ideally, a poplar plantation could be used to recycle salt- and B-laden waters, e.g., drainage water, while the harvested tree products could provide an economical resource. In the multi-year study, two poplar clones were identified that can grow well under high salt and boron growing conditions. It was unclear as to which mechanisms and plant responses were involved in their boron and salt tolerance. With these two clones, we determined that tolerance antioxidant levels responded respectively differently to cope with stressful growing conditions like high salinity and boron as part of their coping mechanism. This type of evidence suggests there are likely other physiological responses besides increased antioxidant activities that are also participating in the salt and boron tolerance exhibited by these two poplar clones. To acquire a better understanding for identifying plant species for their salt and boron tolerance, more research is needed to evaluate antioxidant and other activities in other plant species that potentially exhibit salt and boron tolerance.

Contact: Gary Banuelos, Gary.Banuelos@ars.usda.gov, (559) 596-2880

Spatial variations in soil selenium and residential dietary selenium intake in a selenium-rich county, Shitai, Anhui, China

Submitted to: Nutrients

Authors: Z. Long, L. Yuan, Y. Hou, G. Banuelos, Y. Liu, L. Pan, X. Liu, X. Yin

Selenium (Se) is an essential trace element for human beings. Since Keshan disease in northeast China was shown to be linked to Se deficiency, Se has received increasing worldwide attention. Researchers have studied the distribution of Se in elderly persons in China and determined that Se-deficient counties in China in which people experience Keshan diseases tended to have the fewest elderly populations compared to Se-sufficient counties. In China, Dashan village in Shitai County (Anhui) is known as a longevity village. In addition, the rates of cancer are very low and the percentage of elders (>80 years) is high (about 12% of the total population). To explore the potential relationship between longevity and naturally occurring Se in Shitai County, the Se content in Shitai's food chain (including soils and foods) was determined and the daily Se intake and hair Se content were calculated for the residents in Shitai and in other nearby villages. Based on this study, soil Se levels could be classified as Se rich in Dashan. Thus, the Se concentrations of most foods produced in Dashan were also high. The daily Se intake of Dashan residents reached 298.4 μg/d/adult, which was 6-10 times higher than that at other village study sites, and 5 times higher than the current recommended Se intake values in China (60 μg/d/adult). Although a significant positive correlation was found between soil Se content and longevity indexes by some researchers, there are still few data available comparing longevity and Se nutrition under natural field conditions. Thus, the present study demonstrated that the study site, Shitai county, had significant variations in Se levels of soils, foods, and resident dietary intakes, and the region should be used as a natural laboratory to continue to investigate the relationship between Se and human health.

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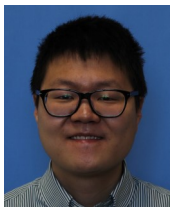
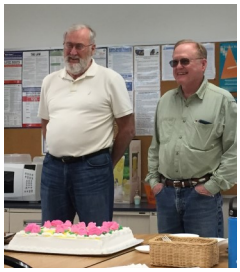
New Visiting Scientist



Dr. Ross Zhan will be working with Dr. Hong Lin in the Crop Diseases, Pests and Genetics Research Unit as a Postdoctoral Research Associate. Dr. Zhan received his Ph.D. in December 2017 from the Department of Botany & Plant Pathology at Purdue University where his research focused on the cloning and characterization of the maize lesion mimic mutant les23 and its suppressor slm1. Here, Ross will continue his research interests in understanding and characterizing interactions of host-pathogens underlying molecular mechanisms associated with grape Pierce's disease and citrus HLB.

Retirement of Dr. Jim Ayars



Dr. Jim Ayars retired on 3 April 2018 after 41 years of Federal service as an agricultural engineer working to improve use of water for agricultural purposes through the adoption of more efficient irrigation and drainage systems, particularly in semi-arid and arid areas. The use of drainage water for crop irrigation was an early hallmark of his research when he showed through a 5-year experiment that cotton and sugarbeet crops had a tolerance for salt; that research found direct application not just by American farmers in the San Joaquin Valley of California, but also in parts of Asia. He expanded his studies of crop salt tolerance and shallow groundwater and, in the process, developed a new crop coefficient that makes precision irrigation possible both on land with high water tables and land with shallow saline groundwater. In further studies, he characterized the relationship between irrigation management, drain flow, and drain water quality, and he demonstrated that salt load was independent of the flow and that reductions in loads of salt, boron, and selenium are directly proportional to reductions in deep percolation and drainage flow. After carrying out a 3-year study of cotton and tomato crops grown in the presence of shallow groundwater, he was able to develop a fully automated subsurface drip irrigation (SDI) system that could be controlled by an irrigation algorithm that included *in situ* crop water use. This SDI system is now in use on several thousand acres of the San Joaquin Valley. Another of his studies led irrigation specialists to rethink their traditional design of drainage systems for arid regions away from deep placement and wide spacing of plants toward shallower, lateral placement, which results in less salt and fewer dissolved minerals being transported away from the irrigated area and requiring removal. Through a 27-year study in California, he established a comprehensive set of best management practices for irrigation and drainage systems in arid areas. His recommendations, now used by the World Bank and the Natural Resources Conservation Service, incorporate plant stress indicators to schedule watering, a redesign of the ways that water is delivered to individual plants, and integrate controlled, subsurface drains. His knowledge of irrigation and drainage management resulted in him being called upon to provide expert advice to irrigation projects in Egypt, Israel, Jordan, Kazakhstan, Uzbekistan, and elsewhere, and to serve on numerous advisory, project, and program committees.

Passing of Dr. Edwin L. Soderstrom

Dr. Edwin L. Soderstrom passed away 26 February 2018 in Fresno, CA. Ed earned his M.S. (1959) and Ph.D. (1962) in Entomology from Kansas State University, where he studied the biology of stored-product insect pests. This prepared him for a career with ARS in Fresno investigating the biology and control of insect pests of dried fruits and nuts until his retirement in the late 1990's. The emphasis of his research was on use of alternatives to insecticides for insect control, such as high or low temperatures and modified atmospheres.

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