San Joaquin Valley Agricultural Sciences Center
9611 S. Riverbend Avenue, Parlier, California 93648-9757
(559) 596-2700 http://ars.usda.gov/pwa/sjvasc

SJVASC Update

October 2018

Research Highlight

Progress Toward a New Type of Grapevine Resistance to *Xylella fastidiosa*

Contacts: Elaine Backus, Elaine.Backus@ars.usda.gov, 559-596-2925

Fig. 1. Placing a GWSS on a plant for EPG recording.

The mission of the Crop Diseases, Pests and Genetics Research Unit is to identify new tools to reduce losses caused by pathogens and insect pests of grapevine, especially *Xylella fastidiosa* (*Xf*), the lethal, xylem-inhabiting Pierce's disease (PD) bacterium, and its vectors, glassy-winged sharp-shooter (GWSS) and blue green sharpshooter (BGSS). One of those tools, novel mechanisms of grapevine resistance to *Xf*, is the focus of research by **Dr. Elaine Backus**. Most *Xf* resistance research is aimed at developing grapevines resistant to bacterial infection, that is, multiplication and spread of *Xf* bacteria once they are in the plant. In contrast, Dr. Backus is searching for resistance that prevents bacteria from being inoculated (injected) into the plant when a sharpshooter carrying bacteria feeds on a healthy grapevine. Her goal is to develop a screening method that detects when the specialized feeding behaviors responsible for inoculating bacteria are performed less on certain grapevines. This screening method can be used by entomologists working with grape breeders to identify new genetic traits in wild grapes, which will cause the vector to change its behavior and reduce the likelihood of *Xf* inoculation.

Achieving her goal has required Dr. Backus to pursue four sequential objectives over the last 16 years. First, she co-invented and patented a new (AC-DC) version of an existing electronic technology, called electropenetrography (or EPG) to record in real-time the intricate details of sharpshooter feeding while their straw-like, piercing-sucking mouthparts (stylets) are inserted otherwise invisibly inside opaque plant tissues (**Figs. 1, 2**). A very thin, solid-gold wire is glued to a sharpshooter's back with electrically conductive glue, making the insect an electrode. The insect is placed on a plant that has been electrified by another electrode inserted into the plant's soil. When the insect probes its stylets into the plant, electrical signals pass into the monitor. The outputted voltage varies over time depending upon feeding behaviors of the insect, creating a waveform. Originally designed for tiny insects like aphids, Dr. Backus developed flexible settings on her monitor, so that all types of insects could be recorded using EPG. Each group of insects has its own, unique feeding waveforms. She's even adapting it to work with mosquitoes and ticks!

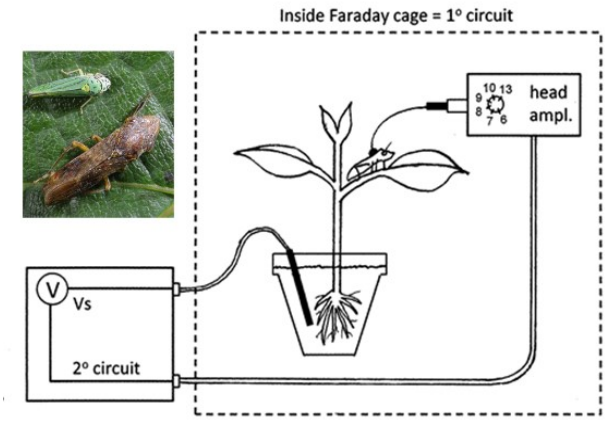


Fig. 2. (upper left) BGSS and GWSS side-by-side. (lower right) EPG monitor.

Once an instrument was invented that could record sharpshooter feeding, Dr. Backus moved on to her second objective, to define the biological meanings of the sharpshooter waveforms. This work required several years of recording both GWSS and BGSS waveforms and correlating them with specific stylet behaviors (like salivation, various types of stylet movements, and fluid uptake) or positions of the stylets (especially on the journey towards and inside of xylem

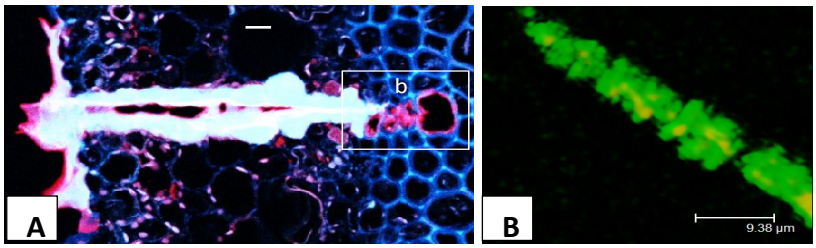


Fig. 3A. The solidified salivary sheath of a GWSS feeding on grape; the sheath is secreted by and surrounds the stylets as they penetrate plant tissues. Pink=antibody-labelled salivary enzyme called β-endoglucanase, which breaks down cell walls and *Xf* bacterial cement; blue=plant cell walls and solidified gelling saliva; white=combined gelling saliva and endoglucanase. Note that the endoglucanase is injected into the large, round xylem cell at the end of the sheath. **B.** GFP-expressing *Xf* cells in a smear of watery saliva ejected onto the cover of an artificial diet by a GWSS carrying the bacteria in its mouth.

cells). Various techniques were used, such as: 1) video-recording stylets moving, sucking up fluids, and secreting saliva inside transparent artificial diets; 2) microscopically examining fed-upon grape tissues to identify cells penetrated; and 3) dissecting thousands of GWSS salivary glands to raise antibodies to saliva, then microscopically tracing where saliva is injected into various cells in the plant (**Fig. 3A**), and using green fluorescent protein (GFP)-expressing bacteria to trace when and how *Xf* cells are expelled from the stylets when feeding on artificial diets (**Fig. 3B**). Over 20 different waveforms were defined, but the most important were the family of **pathway** waveforms (behaviors performed while the sharpshooter is searching for a xylem cell), **ingestion** (sucking up and swallowing xylem sap, the insect's preferred food), and an interruption waveform between groups of swallows, named the **X wave**. The biological meaning of the X wave proved very important, because it represents a sequence of behaviors: xylem sap uptake, swishing sap plus saliva around in the insect's mouth cavity (probably to taste plant compounds in the xylem), and then dribbling and spitting it back into the xylem cell (**Fig. 4**). This correlation research led Dr. Backus to hypothesize that the X wave represents the *Xf* inoculation behavior by sharpshooters.

The third objective of Dr. Backus's work is to thoroughly test (and hopefully prove) the X wave hypothesis. She just completed four years of EPG recordings to associate the X wave with bacterial inoculation into grape plants. Briefly, she fed clean BGSS on artificial diets containing *Xf*, then allowed them to make a single stylet probe into a tiny grape plant while being EPG-recorded. The insects were allowed either to make X waves in xylem or were prevented from doing so. All probed leaves were collected and tested by polymerase chain reaction (PCR) to detect any *Xf* injected into the leaves. The remaining stem and leaf of each plant was held for 5-6 months to grow and develop PD symptoms, and then more leaves were collected and tested by PCR. The first year's PCR tests have now been analyzed, and 95% of plants with confirmed *Xf* were exposed to probes containing X waves. If the next three years of tests show similar results, the X wave hypothesis will be proven.

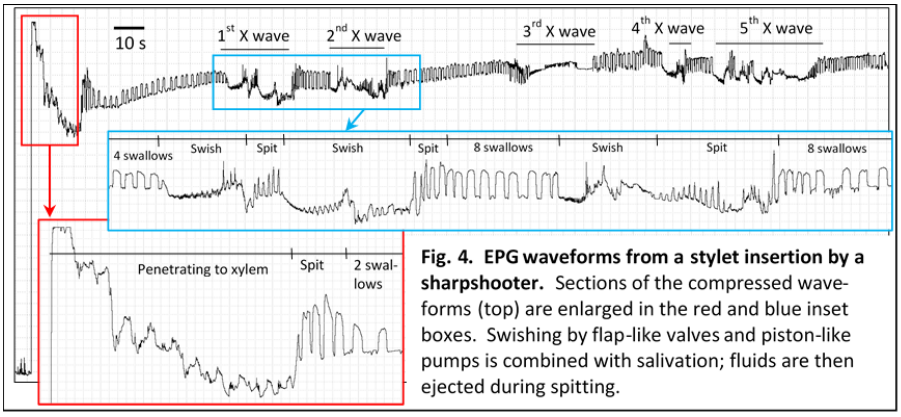
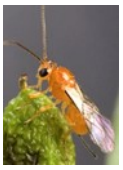


Fig. 4. EPG waveforms from a stylet insertion by a sharpshooter. Sections of the compressed waveforms (top) are enlarged in the red and blue inset boxes. Swishing by flap-like valves and piston-like pumps is combined with salivation; fluids are then ejected during spitting.

Assuming that X waves represent *Xf* inoculation, Dr. Backus's fourth objective is to collaborate with grape breeders at ARS Parlier and the University of California-Davis to test wild and domestic grape varieties for decreased performance of X waves by sharpshooters. She has already EPG-recorded sharpshooters feeding on: 1) Chardonnay domestic grape, 2) the wild grape *Vitis arizonica*, and 3) three Chardonnay x *V. arizonica* hybrids; the wild and hybrid grapes are resistant to *Xf* multiplication and spread inside plants. Preliminary results support her hypothesis that fewer X waves are performed on the *Xf*-resistant grapes. If future studies continue to support these findings, then Dr. Backus will use multivariate statistical analysis to develop an index, similar to a grade point average in school, to boil all the EPG data down into a single number that can be easily compared among EPG recordings of different grape varieties. Brief EPG tests, combined with this resistance index, will be the screening method that can be used to identify grapes capable of resisting a vector's ability to inoculate *Xf*. Using this screening method, grape breeders and entomologists can introduce an all-new resistance mechanism that, when combined with other resistance mechanisms, will make a durable, effective grapevine resistance that lasts longer in vineyards and eliminates the need for insecticides.


Research Updates




Subsurface drip irrigation reduced nitrous oxide emissions in a pomegranate orchard

Submitted to: Environmental Science and Pollution Research

Authors: S. Gao, A. Hendratna, C. Zejiang, Y. Duan, T. Pflaum, R. Tirado-Corbala, C. Phene

Nitrous oxide (N_2O) is a potent greenhouse gas, and agriculture is a major source for its production through fertilization. Effective field management practices should be developed as mitigation strategies. This research determined if high-frequency subsurface drip irrigation and fertilization can reduce N_2O emissions in comparison with traditional surface drip irrigation. After two years of data collection in a pomegranate orchard, the subsurface drip resulted in substantially and consistently lower N_2O emissions than surface drip irrigation. Results also showed that higher N_2O emissions resulted from higher nitrogen (N) fertilizer application rate. Thus, N use efficiency can be improved by applying N-based fertilizers on demand as needed.

Contact: Suduan Gao, Suduan.Gao@ars.usda.gov, (559) 596-2870

Long-term productivity of early season peach trees under different irrigation methods and postharvest deficit irrigation

Submitted to: Water Resources Research

Author: D. Wang

Deficit irrigation has long been recognized as a means of water savings for crop production, but its long-term impact on crop productivity is unknown. This study investigated the effect of deficit irrigation and different methods of irrigation on peach production over a 10-year period. Up to 40% water savings was achieved with deficit irrigation for 8-9 years, while fruit yield and quality were not significantly impacted. In general, no differences were found on fruit yield or quality between different methods of irrigation. The results highlight the unrealized potential of deficit irrigation as an on-farm management strategy in greatly increasing water use efficiency.

Contact: Dong Wang, Dong.Wang@ars.usda.gov, (559) 596-2852

Fruit nutritional quality under deficit irrigation: The case of table grapes in California

Submitted to: Journal of the Science of Food and Agriculture

Authors: T. Centofonti, G. Banuelos, J. Ayars

Sustainable agricultural practices are needed to face current threats to agricultural production in areas where water scarcity, recurrent droughts, and decreases in soil quality are endangering productivity and food security. To survive increasing drought conditions, deficit irrigation (DI) practices (reducing irrigation water applied below full crop evapotranspiration losses) are being considered for use throughout a crop's growing season or at specific phenological stages of a crop. Does DI have any effect on nutritional quality of a given crop? In this 4-year study, we investigated the effects of DI irrigation on nutritional characteristics in berries of two different types of seedless grapes grown at two different locations in California. Different DI treatments were developed as a reduced percentage of water applied compared to the grower's irrigation practice of fully irrigating to replenish all evapotranspiration losses. Each year, berries were randomly picked from various clusters within the vines for each DI treatment at each site and were analyzed for mineral elements, berry weight, diameter and firmness, carbon and nitrogen content, pH, soluble solids, and total phenolic compounds. Results showed that DI treatments did not significantly increase or decrease nutraceutical compounds in grape berries, nor were there any significant decreases in size of either berry type. This study showed that DI practices can be safely

used in drought-susceptible areas like Central California for at least four years without affecting the quality of grape berry in 'Crimson seedless' and 'Sugraone' table grapes.

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

Harvest date influences off-flavor development in mandarins following postharvest wax application

Submitted to: Postharvest Biology and Technology

Authors: D. Obenland, M. Arpaia

Mandarins are prone to off-flavor formation following the postharvest application of wax, and it had been observed that fruit harvested later in the season sometimes have more off-flavor than fruit picked earlier. To more fully evaluate the seasonality effect on off-flavor and to determine potential causes for it, 'Owari' and 'China S-9', two Satsuma varieties, were harvested at three to four dates within the harvest season, waxed, and placed into storage. The testing was conducted over three seasons with 'W. Murcott', a non-Satsuma variety, being included in the third season. At the end of the storage period in the first two seasons, the fruit were evaluated for flavor using a semi-expert sensory panel. In all three seasons, analyses were conducted for various parameters related to fruit internal gas composition. Consistently, in seasons 1 and 2 where flavor was evaluated, both Satsuma varieties did not develop off-flavor or a loss in overall flavor quality in the initial harvest. In subsequent harvests, however, off-flavor became increasingly noticeable and was correlated with the decline in overall flavor quality that occurred. Advancing harvest date in all three seasons was associated with a decrease in oxygen inside of the fruit and an increase in internal carbon dioxide, except in the case of 'W. Murcott' where there was little or no change in internal oxygen and an increase in carbon dioxide. Enhanced production of compounds related to off-flavor within the fruit occurred in fruit from the later harvests in the form of higher ethanol in the Satsuma varieties. Measurements of the ability for gases to pass through the peel performed in season 3 showed no meaningful relationship with the internal gas concentrations measured during the season. Respiration rate, however, strongly increased during the season in the Satsuma varieties and decreased in 'W. Murcott', both changes being associated with the measured internal gas composition inside the fruit. It is concluded that seasonality exists for the propensity for off-flavor to develop in mandarins, but that it may not occur in every instance. The potential mechanism modulating the change in the three varieties examined in this study appeared to be changes in the rate of respiration, which, when respiration is sufficiently high, cause oxygen levels to decline to a level where fermentation occurs and off-flavor is observed. Knowledge that seasonality in off-flavor development exists offers a potential tool to utilize in the prevention of its occurrence in that attempts can be made to harvest those varieties that are susceptible to off-flavor early rather than late in the season.

Contact: Dave Obenland,

David.Obenland@ars.usda.gov, (559) 596-2801

Development and validation of a Loop-Mediated Isothermal Amplification technique (LAMP) for the detection of *Spiroplasma citri,* the causal agent of citrus stubborn disease

Submitted to: European Journal of Plant Pathology

Authors: M. Drais, Y. Maheshwari, V. Selvaraj, L. Varvaro, R. Yokomi, K. Djelouah

Citrus stubborn disease (CSD) is a bacterial disease caused by *Spiroplasma citri*. CSD-affected trees are stunted and low yielding with reduced fruit quality. However, *S. citri* spread by leafhopper vectors is slow such that good fertilization and horticultural care may offset CSD yield losses. A rapid and simple test to accurately detect *S. citri*-infected trees is needed to initiate mitigation actions and to distinguish CSD from Huanglongbing (HLB), a devastating citrus disease. To this end, a Loop-Mediated Isothermal Amplification technique (LAMP) was developed to detect *S. citri*, targeting the spiralin gene. LAMP is a simple, cost-effective technique for detection of specific DNA sequences without thermal cycling and can be carried out using a small portable instrument. The protocol for *S. citri* LAMP detection was conducted using the BioRanger device and was optimized to use crude plant extract samples to facilitate on-site field testing. The LAMP assay detected DNA from *S. citri* to a level of 100 fg/µl with no inhibition by crude plant extract. The LAMP assay was validated with field samples

with detection efficiency and percentage yes/no calls comparable to that obtained by real-time PCR conducted with DNA extracted and purified from the same sample. The LAMP procedure allows growers, pest control, or diagnostic services to rapidly test for *S. citri* in the field.

Contact: Ray Yokomi, Ray.Yokomi@ars.usda.gov, (559) 596-2990

Fungicide-resistant phenotypes in *Botrytis cinerea* populations and their impact on control of gray mold on stored table grapes in California

Submitted to: European Journal of Plant Pathology

Authors: S. Saito, T. Michailides, C. Xiao

Gray mold caused by the fungus *Botrytis cinerea* limits the storage and shelf life of table grapes grown in the Central Valley of California. Control of gray mold in table grapes has long been dependent on postharvest fumigation with sulfur dioxide. Preharvest use of fungicide sprays may provide an alternative to the control of postharvest gray mold. However, fungicide resistance in *B. cinerea* can result in the failure of disease control. Determining the frequency and phenotypes of fungicide resistance in the current *B. cinerea* populations is important to the development of effective chemical control programs. In this study, isolates of *B. cinerea* were collected from table grape vineyards and tested for resistance to selected fungicides on fungicide-amended media, and baseline sensitivity to the newer fungicide fluopyram was also tested. Seven fungicide-resistant phenotypes were detected; 85.0, 23.1, 13.7, and 94.8% of the isolates were resistant to boscalid, cyprodinil, fenhexamid, and pyraclostrobin, respectively. All isolates were sensitive to fludioxonil. Only 5.2% of the isolates were sensitive to all fungicides tested, whereas 8.9, 56.1, 23.6 and 6.1 were resistant to one, two, three, and four modes-of-action fungicides, respectively. All tested isolates were sensitive to fluopyram. Most fungicides failed to control gray mold on detached table grapes inoculated with respective fungicide-resistant phenotypes. Our results suggest that alternation of sprays using different classes of fungicides will be needed to control postharvest gray mold, and that fludioxonil and fluopyram could be effective fungicides integrated into a preharvest fungicide spray program for control of gray mold in table grapes in the Central Valley of California.

Contact: Chang-Lin Xiao, Chang-Lin.Xiao@ars.usda.gov, (559) 596-2722

Evaluating insecticide coverage in almond and pistachio for control of navel orangeworm (*Amyelois transitella*) (Lepidoptera: Pyralidae)

Submitted to: Pest Management Science

Authors: J. Siegel, M. Strmiska, F. Niederholzer, K. Giles, S. Walse

Insecticide application is an essential component of insect control in the California almond and pistachio industry. Excellent insect control is essential because tree nuts are valuable (estimated farm gate value exceeding $7 billion), and improving nut quality is an ongoing challenge that must be met to ensure continued demand. The acreage planted in tree nuts has increased dramatically over the past decade, but the purchase of spray rigs to manage these acres has lagged behind. Over the past 6 years, the temperature during the growing season has increased, and this change has been favorable to a major pest of tree nuts, the navel orangeworm. The research presented in this paper focuses on describing the insecticide coverage produced by ground and air application for control of this pest. Several consistent patterns were identified: ground applications fail first in the upper canopy of the tree, and air applications fail first in the lower canopy. In the best ground applications coverage was consistently high throughout the canopy; this was not observed for application by air. Uniform coverage helps ensure that all nuts are protected by insecticide, while uneven coverage results in gaps that can lead to increased damage. The reduced coverage in the lower canopy may not be important if most nuts are growing in the upper canopy, but, if the nuts are evenly distributed, gaps in coverage are unacceptable. We also investigated the effect of water volume and speed on ground application. Using a volume of 200 gallons per acre provided better coverage than a volume of 100 gallons per acre, and coverage in the upper canopy failed when the speed exceeded 2 miles per hour. However, there are tradeoffs associated with the high volume of water; application will take longer because more stops will be necessary to refill the spray tank. Application in the field requires balancing several factors including the time to complete a spray, therefore lower volumes may be used, but

applicators must be made aware that they are sacrificing coverage. Finally, insecticides may be applied at several rates, and we investigated the effect of both minimum and maximum rate on contact toxicity. Toxicity in our assay system was highest when the maximum label rate was used. Growers facing high insect pressure should get the best control using the maximum label rate of insecticide applied at 2 miles per hour.

Contact: Joel Siegel, Joel.Siegel@ars.usda.gov, (559) 596-2735

Optimizing efficiency of aerosol mating disruption for navel orangeworm (Lepidoptera: Pyralidae)

Submitted to: Journal of Economic Entomology

Authors: C. Burks, D. Thompson

Experiments were conducted to improve cost-effectiveness of aerosol mating disruption for navel orangeworm, an insect pest of nut crops in California. Mating disruption is achieved by emitting pheromones into orchards so that male orangeworms can't find females, disrupting mating and, hence, reproduction. Small plot trials in 2015 revealed that increased frequency of emissions increase impact on male location of a pheromone source, and that emission over only key portions of the night was as effective as the then-current practice of emitting for 12 hours. In 2017, a small-plot experiment showed that aerosol emitters dispensing more concentrated pheromone more frequently suppressed male capture in pheromone traps as efficiently as aerosol dispensers placed more densely and emitting the same total amount of active ingredient less frequently over a longer period of the night. A season-long efficacy test in 2016 found that aerosol using parameters based on these experiments provided a near-complete suppression of navel orangeworm males in pheromone traps and significantly less navel orangeworm damage in plots treated with this modified aerosol mating disruption regime. These findings demonstrate that the same total amount of pheromone (active ingredient) dispersed in a more concentrated form, more frequently, and from fewer point sources can provide significant protection from navel orangeworm with greater cost efficiency.

Contact: Chuck Burks, Charles.Burkes@ars.usda.gov, (559) 596-2757

Impact of temperature on *Lobesia botrana* (Lepidoptera: Tortricidae) development

Submitted to: Journal of Economic Entomology

Authors: C. Preto, D. Bellamy, S. Walse, F. Zalom

European grapevine moth has the potential to impact the export of grapes and grape products from the United States. Phytosanitary treatments, conducted prior to export, are typically required to control this insect pest. The efficacy of such treatments must be demonstrated using the most treatment-tolerant life stage. Therefore, researchers must track development across life stages as a function of age, quantifying the relative distribution of life stages over the time-course of treatment and its assessment. Here, we evaluate the accuracy of using the measurement of larval head capsule width to diagnose a particular instar. Moreover, we use the measurement to describe inconsistencies in the maturation of specimens within an age group spanning only 24 hours. We then expand the description to identify the effect of temperature on divergence in rates of maturation within each life stage, and across the entire timespan of development. Results indicate that head capsule width is an accurate proxy, and that the development of European grapevine moth, reared as in this study, is generally predictable. Anomalies in development are briefly discussed, further emphasizing how this work aids the evaluation of how efficiently a treatment controls European grapevine moth.

Contact: Spencer Walse, Spencer.Walse@ars.usda.gov, (559) 596-2750

Subscribe or unsubscribe to the SJVASC Update or read past Updates at:

http://ars.usda.gov/pwa/sjvasc/subscribe