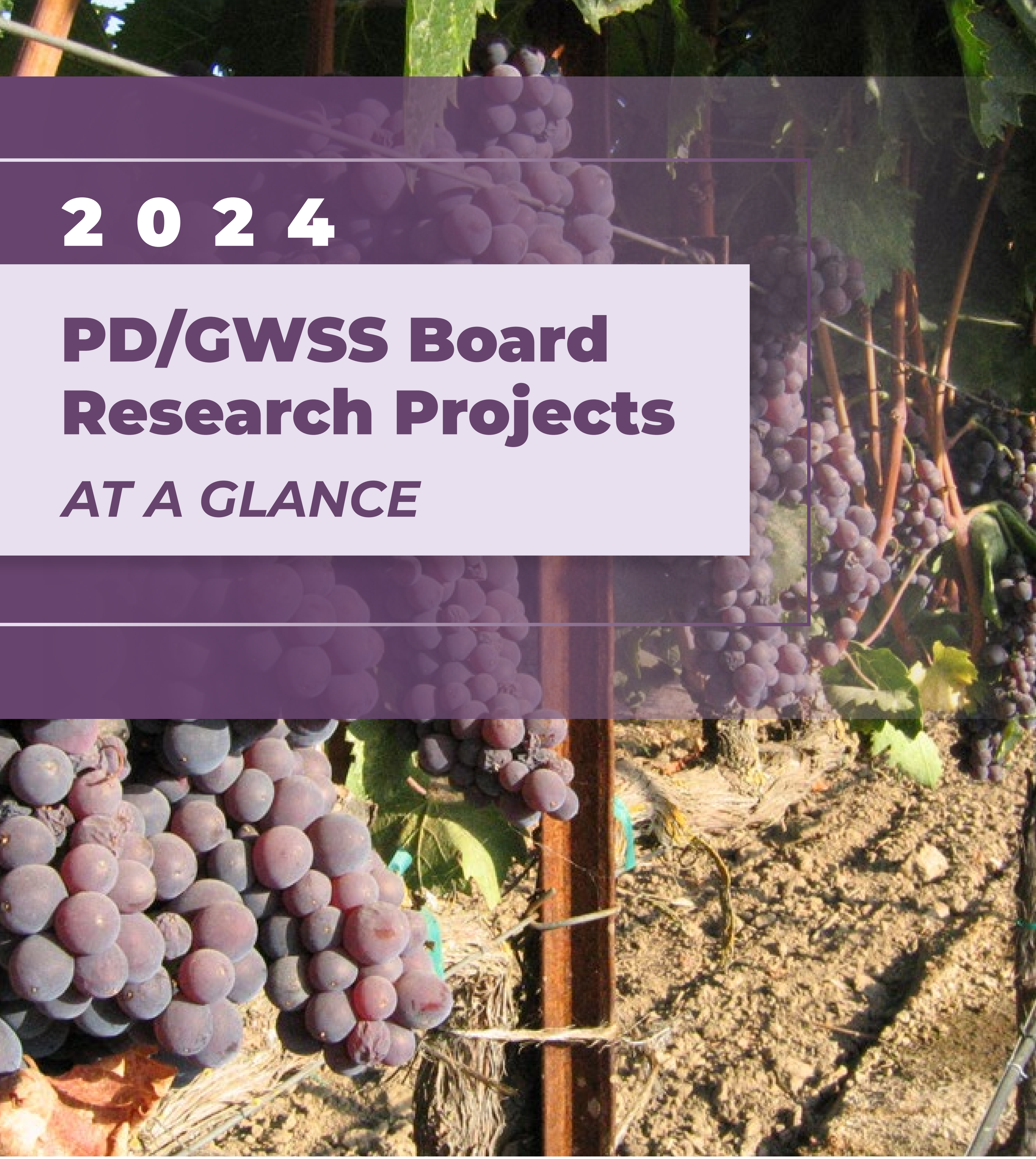

2024

PD/GWSS Board Research Projects

AT A GLANCE

cdfa CALIFORNIA DEPARTMENT OF FOOD & AGRICULTURE

CALIFORNIA PD/GWSS BOARD
Partnership for Winegrape Pest Solutions

Introduction

In the face of increasing risks of invasive pests and diseases from around the nation and the world, California's winegrape growers turn to the innovative grower-government partnership created over two decades ago to protect their vineyard investment. The Pierce's Disease and Glassy-Winged Sharpshooter (PD/GWSS) Board invests in research and outreach to protect vineyards, prevent the spread of pests and diseases and deliver practical and sustainable solutions.

The consistent, reliable funding made possible by the winegrape grower assessment supports leading researchers in finding solutions to PD and other serious pests and winegrape diseases. Learn more at cdfa.ca.gov/pdcp/PD_GWSS_Board.html.

Research progress reports are compiled annually by the Pierce's Disease Control Program and are available online at cdfa.ca.gov/pdcp/Research.html. The following is an overview of the 2024 research progress reports.

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All Research Projects

Research on Pierce’s Disease and the Glassy-Winged Sharpshooter

Modeling the Bacterial Transmission Process by Integrating New Behavioral Insights into Computational Fluid Dynamics Simulations

Project Leaders and Cooperators*: Rodrigo P.P. Almeida and Elizabeth G. Clark, University of California, Berkeley; Andrew J. McElrone, University of California, Davis; Daniele Cornara, Università degli Studi di Bari; Craig R. Brodersen, Yale University; Brandt M. Gibson, University of Toronto, Mississauga; and Saad Bhamla, Georgia Institute of Technology*

Understanding how *Xylella fastidiosa* (*Xf*) is transmitted from sharpshooters to host plants is critical to preventing the spread of Pierce's disease. The team discovered a new approach for modeling insect feeding and transmission behavior linked to a specific electrical output from insects measured during feeding. This project will use a framework to model the insect inoculation of *Xf* into plants and highlight physical aspects of the plant and insect that promote transmission. Ultimately, this will allow the team to identify what behaviors of the insect or plant characteristics create opportunities for bacterial transmission.

Progression of Pierce's Disease Symptoms and *Xylella fastidiosa* Colonization of Grapevines Under Field Conditions

Project Leader and Cooperators*: Rodrigo P.P. Almeida, University of California, Berkeley; Monica Cooper, University of California, Cooperative Extension, Napa County; and Matthew Daugherty, University of California, Riverside*

This project fills a critical gap in understanding how mature grapevines respond to *Xylella fastidiosa* (*Xf*) infections under field conditions. This project takes advantage of a rare opportunity to study Pierce's disease (PD) in a 10-year-old vineyard at a UC research station, tracking disease progression across 13 wine grape cultivars. The team discovered new symptoms in some cultivars, including shriveled berries within the first year, and confirmed winter recovery varies based on grape cultivar and bacteria strains. The team also investigated how winter xylem sap affects *Xf* survival in potted vines and in the lab. Preliminary results suggest that sap composition during dormancy may affect bacterial persistence, and previously infected vines may be more resistant to reinfection, highlighting the complexity of PD progression in different environmental conditions.

Next, the team will refine predictive models to assess how temperature influences disease progression, continue bacterial culturing to pinpoint when *Xf* cells die during winter and further analyze the role of bacterial survival in reinfection risk. These findings could lead to significant breakthroughs in disease management, helping growers make informed decisions about cultivar selection and vineyard practices.

Costs of Pierce's Disease in the California Grape and Wine Industry and Benefits from the PD Control Program and Research and Development

Project Leaders*: Julian Alston and Devin Serfas, University of California, Davis*

This study found that if the current Pierce's disease (PD) and glassy-winged sharpshooter (GWSS) prevention, control and research efforts ended, California winegrape growers could see their annual losses from the fatal grapevine disease more than double from $48 million to $104 million. If GWSS spread unchecked, PD outbreaks could cost winegrape growers an additional $56 million a year in lost production and vine replacement. The study also highlighted the importance of grower-funded and long-term research investments, such as those led by the PD/GWSS Board since 2001.

The unique funding partnership the winegrape industry forged between growers, the state and federal government fuels the essential research and control operations that have significantly blunted the most severe impacts of PD and GWSS. Despite these efforts, PD still costs an estimated $110 million annually in California, including $45 million for control, prevention and research, $48 million in lost winegrape production and vine replacement and $17 million in lost table and raisin grape production and vine replacement. Without these critical programs, the industry's losses in production and vine replacement would be even higher, putting greater financial strain on growers and threatening the long-term stability of California's vineyards.

With industry and government support keeping PD and GWSS in check, grower-funded research continues to drive advancements in pest and disease management while supporting field trials for promising solutions. According to the study's authors, the economic burden of PD and GWSS could decrease as this research yields practical, vineyard-ready innovations.

Blocking the Acquisition and Transmission Cycle of *Xylella fastidiosa* by Glassy-Winged Sharpshooter Using Genetic Control

Project Leaders*: Peter W. Atkinson, Richard A. Redak, Linda L. Walling, Jason E. Stajich, University of California, Riverside and Rodrigo P. P. Almeida, University of California, Berkeley*

This project aims to use gene editing to create lines of the glassy-winged sharpshooter that cannot spread *Xylella fastidiosa* (*Xf*). The team identified target genes expressed in feeding and reproductive organs. The expression of proteins designed to block *Xf* from binding to the insect feeding organs should limit the spread of Pierce's disease. With the ability to edit and insert genes into the GWSS genome using CRISPR technologies, they will generate and test genetic control strategies in GWSS for the first time.

Future research activities include refining genetic tools and optimizing gene integration strategies to enhance efficiency and ensure stable inheritance. The team also aims to identify a germline-specific promoter for GWSS population control. Finally, the team plans to engage stakeholders, scientists, regulators and the public in discussing genetic pest control strategies.

Development of Tools for Precise Breeding of Grapevine Cultivars Resistant to Pierce's Disease

Project Leaders and Cooperators*: Dario Cantu, Mirella Zaccheo, Melanie Massonnet and M. Andrew Walker, University of California, Davis and Summaira Riaz, United States Department of Agriculture – Agricultural Research Service*

This work aims to develop tools that help breeders develop Pierce's disease (PD)-resistant grapes that produce fruit with desirable flavor and aroma. The team is studying a section of the genome (called PdR1) of a wild grape species called *Vitis arizonica*, which has shown resistance to PD. An analysis determined that PdR1 contains genes that play a role in the plant's defense system. These genes have been used to breed the first PD-resistant winegrapes. The team will confirm the function of these genes using genetic engineering approaches by either adding them to a susceptible grape variety or removing them from a resistant variety. Since PdR1 was found in different *V. arizonica* species, the team's goal is also to understand if different genes drive the resistance in these species. This information will help create grapes with a combination of multiple resistance functions to achieve durable field resistance to PD.

Enhancing Rootstock-Mediated Systemic Immunity Against Pierce's Disease in a Grafted Commercial Wine Grape Variety

Project Leaders and Cooperators*: Abhaya M. Dandekar, Paulo A. Zaini, Cecilia Aguero and Renata de A.B. Assis, University of California, Davis*

This project focuses on developing bioengineered rootstocks to protect grafted varietal vines against Pierce's disease (PD). The team evaluated grafted vines under field conditions to identify elite bioengineered rootstocks that protect the grafted scion variety from developing and/or succumbing to PD. The 450 vines correspond to different bioengineered commercial grapevine rootstocks expressing one of seven systemic immunity strategies to combat PD development. The team vines were inoculated with *Xylella fastidiosa* annually between 2021 and 2024, and annual evaluations of plant health and productivity were conducted from 2022 to 2024.

Management of the Federal Permits for Multi-Investigator Field-Testing of Transgenic Grapevine Rootstocks in California

***Project Leaders and Cooperators**: Abhaya M. Dandekar, Ana M. Ibáñez and Cecilia Aguero, University of California, Davis*

This project manages an Animal Plant Health Inspection Service-Biotechnology Regulatory Service (APHIS-BRS) Federal permit enabling the multi-investigator field testing of transgenic grapevines (see previous project and the project led by David Gilchrist further below). This project involves managing the documentation and submission process for the field permit that enables field testing, maintaining regulatory oversight and compliance with reporting requirements and regulatory compliance inspections at the field location. These activities include documenting the genotype of each grapevine line released for field testing and information for each transgene component incorporated in the genome of each grapevine line. The project also includes submitting mandatory reports and monitoring the field site while working closely with participating investigators and their crews.

Systemic Formulations of Antibacterial Nanoparticles for Pierce's Disease Management

***Project Leaders and Cooperators**: Leonardo De La Fuente and Deepak Shantharaj, Auburn University; Lindsey Burbank, United States Department of Agriculture – Agricultural Research Service; and Swadeshmukul Santra and Jorge Pereira, University of Central Florida*

The only chemical control options against Pierce's disease (PD) target the insect vectors, not the pathogen, *Xylella fastidiosa* (*Xf*). Antibacterial compounds have not been effective against *Xf* because spray applications have difficulty reaching the vascular system, but the availability of an antibacterial chemical treatment that is easily applied in the field by soil drench or foliar spray would be a useful tool for growers to manage PD.

The team tested a novel nano-sized formulation ("Zinkicide®, ZnK") against a different vascular bacterial pathogen in citrus, and saw promising results after testing the same formulation in blueberry and tobacco. The team modified the chemical composition of the nano-formulation to improve performance against *Xf* at lower doses and to test it in grapes. The products showed high antibacterial activity in the lab and some combinations showed reduced phytotoxicity in plants. They are currently developing variations of these products to avoid the negative effects on living plants. These formulations will need to be tested in the field to assess if the observed phytotoxicity is caused by excessive accumulation of active compounds in pots.

Development of a Protoplasts-Based Platform to Knock-in Agriculture Relevant Genes into Grapevines

***Project Leaders and Cooperators**: Juan Debernardi, Dario Cantu, David Tricoli, Danielle Inchaurregui, Noe Cochetel, Mirella Zaccheo and Melanie Massonnet, University of California, Davis*

Grape production faces significant challenges from plant diseases and environmental factors, highlighting the need for disease-resistant cultivars. However, traditional breeding methods are limited as they disrupt the fidelity of clonal germplasms in grapevines. This project aims to create a proof of concept for a CRISPR-based platform that enables the insertion of large DNA fragments from grape wild relatives, conferring resistance to Pierce's disease (PD), into *Vitis vinifera* varieties. This method could rapidly incorporate additional genes that confer resistance to other diseases and environmental stresses, benefiting the entire grape community.

Researchers have refined the knock-in process by testing various gene delivery formats and evaluating DNA repair mechanisms. Experiments demonstrated that double-stranded DNA (dsDNA) donor templates, generated through PCR, were more effective than plasmid DNA for targeted insertions. Successful knock-in events were confirmed via PCR and sequencing, validating the approach. Moving forward, the team will regenerate whole plants from edited protoplasts, evaluate gene expression and resistance to PD and continue to refine the knock-in methodology for broader application in grapevine breeding.

Advancing Biopesticides for Management of Pierce's Disease

***Project Leaders and Cooperators**: Akif Eskalen and Andrew Richards, University of California, Davis; Steven Lindow, University of California, Berkeley; Philippe Rolshausen, University of California, Riverside; and Jean Rodriguez, A&P Inphatec*

This project explores advancing biopesticide technologies to manage Pierce's disease (PD). The team is conducting a comparative evaluation of combinations of three biological control agents and one bacteriophage under field conditions, optimizing the strategies to improve PD management options for growers and assessing potential for commercialization. Previous research demonstrated that these biological agents successfully reduced PD symptoms in greenhouse bioassays and early field trials. In the first year of field trials, most treatments effectively reduced PD symptoms. In the second year, all also showed a significant reduction in leaf scorch. The 2024 field trial tested 11 treatments by injecting biocontrol agents or bacteriophages into Cabernet Franc vines before and after infection with *Xylella fastidiosa*. Results showed earlier PD symptoms, including leaf scorch, matchsticks, berry shriveling and uneven lignification, likely caused by extreme summer heat. In contrast to previous years, treatments did not result in a statistically significant reduction in PD leaf scorch.

Field Evaluation of Cross-Graft Protection Effective Against Pierce's Disease by Dual DNA Constructs Expressed in Transgenic Grape Rootstocks

***Project Leader and Cooperators**: David Gilchrist, Abhaya Dandekar, James Lincoln and Bryan Pellissier, University of California, Davis*

This continuing project supports field activities that evaluate resistance to Pierce's disease (PD) in transgenic grape rootstocks by expressing combinations of transgenes that have shown positive protection against PD under field conditions (see other projects led by Abhaya Dandekar above and David Gilchrist below). The field experiments include inoculating plants with *Xylella fastidiosa* (*Xf*) and measuring PD symptoms and bacterial movement. Activities supported under this project include vine management, pest management, field supplies, irrigation and labor. Transgenic and control vines were planted at the highly regulated field site in 2019. All plants displayed normal growth and morphology, then plants were inoculated with *Xf* in starting in 2021 and 2-bud pruned each spring, carefully leaving the inoculated branches. The project's field phase reached its planned conclusion in 2024, with plant removal and debris disposal per APHIS permit guidelines scheduled for 2025.

Transgenic Rootstock-Mediated Protection of Grapevine Scion Against Pierce's Disease by Dual DNA Constructs

***Project Leader and Cooperators**: David Gilchrist, James Lincoln and Bryan Pellissier, University of California, Davis*

This project evaluates the potential of genetically modified rootstocks to confer resistance to Pierce's disease (PD) in non-transgenic grape scions through cross-graft protection. Initial field trials (2010-2015) demonstrated that five transgenes provided consistent protection against PD when introduced into whole plants. Based on these results, a second-phase experiment was launched to assess whether dual-transgene combinations in adapted rootstocks could suppress PD in grafted, non-transgenic Chardonnay scions. Over 700 transgenic rootstock plants were established in a USDA-APHIS-controlled field, and mechanical inoculation with *Xylella fastidiosa* (*Xf*) was performed to monitor bacterial movement, infection severity and potential cross-graft protection.

Results so far indicate that transgenic rootstocks show differences in bacterial presence compared to untransformed controls, with lower *Xf* populations detected in scions grafted onto modified rootstocks. Initial findings suggest that transgene expression may influence bacterial dynamics, though further analysis is needed. Key observations include bud failure in inoculated plants, movement of *Xf* into rootstocks and variations in bacterial load based on rootstock genotype.

Using a Stable, Plant-Derived Antimicrobial Peptide to Control Pierce's Disease

Project Leaders*: Hailing Jin, Caroline Roper and Luis de Luna, University of California, Riverside*

The team has developed a stable antimicrobial peptide (SAMP) from the Australian finger lime that can directly kill pathogens and induce host defense response. Originally developed as a control solution for citrus greening or Huanglongbing, it is also a candidate for developing preventive and curative grapevine treatments under Pierce's disease (PD) pressure. Experiments demonstrated that SAMP treatments positively affect the health of plants inoculated with *Xylella fastidiosa*, significantly lowering the severity of PD symptoms in the late stages of the disease. SAMP-treated plants display healthier phenotypes overall than mock-treated plants and have lower bacterial contents and levels of xylem occlusion. The treatment also improved the long-term survival of the plants, with a 17% to 37% survival rate compared to 5% for untreated plants, suggesting the SAMP treatment is a reliable method to combat PD in both newly inoculated and sick plants.

Substrate-Borne Vibrational Signals in Intraspecific Communication of the Blue-Green Sharpshooter

Project Leader: Rodrigo Krugner, United States Department of Agriculture – Agricultural Research Service

In leafhoppers, males and females find and communicate with each other by sending vibrations through the plant. This project studied how blue-green sharpshooter (BGSS), an important native vector of Pierce's disease, communicates using vibrations. The foundational knowledge of BGSS behaviors learned during this project provides insights into developing targeted mating disruption techniques, potentially offering a more effective and environmentally friendly approach to BGSS population management in vineyards.

They identified the signals BGSS use to find and choose mates and those used in competition or cooperation, such as securing feeding spots, and explored the effectiveness of disrupting the insect's communication. Results showed that broadcasting a female signal immediately following a male's signal reduced the probability of mating, compared to the silent control treatment. A second approach investigated how signals could attract males to the source of the signals. Results showed that males perceive and reply to artificial playback of female signals and attempt to locate the source of the signals (electromagnetic shaker attached to plants), suggesting that further research is warranted on the development of "trap-and-kill" (also known as "trap out") method.

Using the Native Grapevine Immune System to Generate Pierce's Disease Resistant Grapevines

***Project Leader and Cooperator**: Caroline Roper, University of California, Riverside and David Tricoli, University of California, Davis*

This project aims to develop grapevines resistant to Pierce's disease (PD) by enhancing immune response to *Xylella fastidiosa* (*Xf*). Researchers use transgenic and CRISPR/Cas9 gene-editing approaches to introduce immune-related resistance factors or eliminate genes linked to disease susceptibility. So far, researchers genetically modified Thompson Seedless grapevine by introducing five native *Vitis vinifera* target genes using *Agrobacterium*-based transformation. They introduced the same genes into the thale cress plant for preliminary trials to accelerate testing. At the same time, they designed guide RNAs to knock out five genes associated with tylose formation, an excessive defense response that worsens PD symptoms. They are still refining CRISPR gene-editing methods for grapevines.

Researchers will propagate transgenic and gene-edited vines and conduct virulence bioassays to assess their resistance to PD. Using greenhouse bioassays, they will evaluate disease severity, bacterial colonization, and tylose formation in these modified plants. The goal is to create grapevines that enhance immune responses against *Xf* or prevent detrimental tylose overproduction, offering a dual strategy for PD resistance. Findings from this study could provide valuable insights for breeding or engineering more resilient grape varieties.

Interactions Between the Spotted Lanternfly and Pierce's Disease of Grapevines

***Project Leaders and Cooperators**: Cristina Rosa, Michela Centinari and Julie Urban, The Pennsylvania State University; Caroline Roper, University of California, Riverside and Carmen Gispert, UC Cooperative Extension, University of California, Riverside Palm Desert Campus*

Little is known about the spotted lanternfly's (SLF) ability to transmit pathogens to plants, especially grapevines. This project investigates whether the insect spreads *Xylella fastidiosa* (*Xf*) between grapevines and if it can spread other pathogens, such as grapevine red blotch virus.

In 2024, the team demonstrated that SLF that fed on Pierce's disease-infected grapevines tested positive for *Xf* DNA, suggesting they can pick up *Xf*. SLF were allowed to feed on infected grapevines for up to three days, then transferred to healthy vines. The highest number of insects positive for *Xf* was after three days of feeding on infected vines, and some insects still had *Xf* in their guts up to two days after being moved to healthy plants. The pest's ability to transmit *Xf* to healthy vines has not yet been confirmed as the experiment was conducted late in the season and the plants remained asymptomatic. Additionally, a behavioral study investigating SLF feeding preferences found no significant difference in preference between healthy and infected vines, though insect mortality was high due to the season and weather. Future research activities include confirming whether SLF successfully transmitted *Xf* to healthy vines, repeating the feeding experiment under summer conditions for more conclusive results and investigating grapevine red blotch virus transmission.

Taxonomic Status, Population Structure and Identification Methods for the Vineyard Spittlebug *Aphrophora* Sp., a Suspect *Xylella fastidiosa* Vector

Project Leaders and Cooperators*: Vinton Thompson, American Museum of Natural History; Manpreet Kohli, Baruch College and Graduate Center, City University of New York and American Museum of Natural History; Cindy Kron, UC Cooperative Extension, Santa Rosa; Lucia G. Varela, University of California Agriculture and Natural Resources, Davis and Monica Cooper, UC Cooperative Extension, Napa*

This project aims to identify and understand the genetic structure of the vineyard spittlebug *Aphrophora* sp., a potential vector of Pierce's disease (PD) in Napa and Sonoma vineyards, to contribute to PD control efforts and targeted management strategies. The primary focus is differentiating the vineyard spittlebug from the Douglas-fir spittlebug (*A. permutata*) and developing a DNA marker for identification. The team has completed a successful sample collection, DNA extraction and DNA sequencing of 48 samples. Findings confirmed the existence of a separate genetic cluster from *A. permutata* in the vineyards. Additional sequencing of the samples is necessary, and data analysis is underway.

Protoplast-Mediated Gene Editing for Disease Resistance

Project Leader and Cooperator*: David Tricoli, Juan Debernardi and Mirella Zaccheo, University of California, Davis*

This proof-of-concept research demonstrated the efficient editing of disease susceptibility genes in grapevine protoplasts and successful regeneration of whole plants with targeted gene edits using plasmid DNA or Ribonucleoproteins (RNPs). Improving grape plants using RNPs is of particular interest, since RNPs are proteins and not DNA, and resulting edited plants would not be considered genetically modified. The team's technique allows them to target multiple genes in a single experiment allowing them to produce a collection of plants within six months in which they have turned off various combinations of genes. Plant pathogens use plant susceptibility genes to allow them to infect a plant. By knocking out these genes in the plant, the pathogen's ability to infect the plant is compromised. One of these susceptibility genes is called MLO, which is required for the pathogen powdery mildew to infect grape plants and was used as a model system that could be applied to other disease-associated genes in the future. By knocking MLO genes out in the grape plant, they created genome edited Thompson Seedless, Colombard, Malbec and Merlot that should be resistant to powdery mildew. This research lays the groundwork for using protoplast-mediated gene editing to knock out other susceptibility genes in grapes to create plants that are resistant to various diseases.

Identification of Novel Californian *Trichoderma* Isolates for Biological Control of Pierce's Disease

***Project Leader**: Christopher Wallis, United States Department of Agriculture – Agricultural Research Service*

This project explores the use of beneficial fungi *Trichoderma* spp. to enhance the ability of grapevines to protect themselves from *Xylella fastidiosa* (*Xf*) infections and reduce Pierce's disease (PD) symptoms. The team has conducted greenhouse and field experiments for the past three years to screen a unique collection of Central Valley Trichoderma isolates for their ability to limit *Xf* infections and fungal pathogen development. Greenhouse studies showed that prior inoculation by two *Trichoderma* isolates significantly reduced titers of *Xf* in grapevines. PD symptoms were low across all treatments and controls, but data suggested a trend of two isolates being associated with reduced PD symptoms. Final measurements of *Xf* titers from the latest greenhouse trials will be completed in 2025. Field trials assessed the ability of *Trichoderma* strains to colonize grapevine pruning wounds and suppress fungal pathogens, with results from 2023 and 2024 confirming that multiple strains effectively colonized pruned spurs and outcompeted fungal pathogens, highlighting their potential as long-term protectants for vineyards.

In addition to evaluating disease suppression, sequencing efforts have characterized various *Trichoderma* isolates, identifying well-known and previously uncharacterized species with potential biocontrol properties. Notably, SL01-1 was found to contain genes associated with antibiotic production, and further analysis is underway to isolate bioactive compounds that may inhibit *Xf* and other grapevine pathogens. Genome annotations for all isolates and species are ongoing, with complete draft genomes expected to be assembled in 2025. Future work will isolate individual antibiotic compounds using high-performance liquid chromatography-fraction collection and testing their efficacy against *Xf* and other pathogens.

Research on Other Winegrape Pests and Diseases

Development and Validation of Hiplex Assays for Improved Detection of GLRaVs and GRBV in Grapes

***Project Leaders and Cooperators**: Maher Al Rwahnih, Raied Abou Kubaa, Kristian Stevens and Juliana Osse De Souza, Foundation Plant Services, University of California, Davis*

This project aims to develop a more efficient and accurate diagnostic method, Hiplex PCR, for detecting grapevine leafroll-associated virus 3 and grapevine red blotch virus. By improving virus detection through a cost-effective, high-throughput approach, the project seeks to enhance quarantine and certification programs, ensuring sustainable disease management.

So far, the team has designed 55 primer pairs to improve virus detection, incorporating genetic diversity to enhance accuracy. A comprehensive reference database of viral genomes was built, ensuring robust assay design. Additionally, a quality control system was integrated using internal positive controls. The test protocol and pipeline have been established, and targeted sample selection, nucleic acid extraction and sequencing have been completed. Preliminary sequencing results indicate successful amplification of viral targets with ongoing quality control analysis to finalize detection thresholds. Next steps include refining the detection pipeline and comparing Hiplex PCR with qPCR and high-throughput sequencing.

Propagating the Premier US Grape Collection for Protection in a Foundation Greenhouse

***Project Leader**: Maher Al Rwahnih, University of California, Davis, Foundation Plant Services*

Foundation Plant Services (FPS) is the primary source for certified, virus-tested and true-to-variety grapevine plant material distributed to nurseries under the California Department of Agriculture's Grapevine Registration and Certification (R&C) Program, which provides most grapevines planted in the United States. Due to the occurrence of insect-vectored grapevine red blotch virus (GRBV) at FPS's Russell Ranch Vineyard, efforts are underway to protect grapevines in greenhouse culture. This project aims to propagate, test and, if necessary, treat priority grapevine selections that can be maintained in the FPS greenhouse as a source of high-quality, healthy grapevine stock.

In 2024, the team moved vines representing 1,186 selections into the greenhouse. Five hundred priority selections were established in pots housed in the greenhouse with DNA ID samples collected and processed, with data now under review. Programmers also developed a database to help manage collection and propagation. While no new microshoot-tip culture therapy was initiated in this period, the team acclimated six selections to soil, with four selections still growing on media in the tissue culture lab. Finally, the team completed virus testing of 500 priority propagates with DNA ID results pending.

Ecology of Grapevine Red Blotch Disease

***Project Leader and Cooperators**: Rodrigo P.P. Almeida, University of California, Berkeley; Monica Cooper, University of California, Cooperative Extension, Napa County and Matthew Daugherty, University of California, Riverside*

The project aims to develop and refine disease management practices by understanding how grapevine red blotch virus (GRBV) spreads in vineyards and surrounding landscapes. Building on prior work led by Monica Cooper, researchers have collected approximately 600 GRBV samples from Napa Valley and identified two different clades of GRBV, with Clade I showing a significant geographic-genetic correlation, suggesting localized spread. At the same time, Clade II appears more randomly distributed, possibly due to past planting of infected vines. Interestingly, all sampled infected grapevines only contained single genetic variants, with no evidence of co-occurrence of multiple variants in the same plant. One possible explanation for the different patterns between the clades could be if Clade I were more effectively transmitted by insect vectors, such as the threecornered alfalfa hopper (TCAH). Additionally, vector infectivity data indicated that TCAH will most likely transmit GRBV between June and August, providing insight into optimal timing for disease intervention.

Next, the team will study how TCAH spreads GRBV, focusing on its movement across large areas and how the environment, such as nearby plants and farming practices, affects insect populations and disease spread. Future research will focus on how the disease moves over time and space and whether changes in insect infection rates from year-to-year impact how quickly the disease spreads.

Early and Autonomous Field-Detection of Virus Infections in White and Black Grape Vines

***Project Leaders**: Luca Brillante, California State University, Fresno and Marc Fuchs, Cornell AgriTech*

Grapevine red blotch virus and grapevine leafroll disease can cause significant damage to vineyards, but early detection for effective management and mitigation remains challenging. This project aims to give growers early, accurate and easy-to-understand maps of infected vines, giving them a powerful tool to manage disease outbreaks more effectively.

The team is using advanced imaging technology and artificial intelligence methods to identify infected plants more accurately. By analyzing the light reflected from grapevines in different parts of the spectrum, they found that infected vines show spectral differences before symptoms appear, even for white grape varieties, whose symptoms are not visible to the human eyes. The team created high-resolution maps of infected areas in commercial vineyards. Testing their accuracy against lab-based PCR tests and growers' field assessments showed the method is reliable and practical. Further research will refine machine learning models and expand datasets to improve disease prediction accuracy, as well as investigate varietal differences in metabolic responses to infection.

Genomics Based Technology for Identification, Tracking, Insecticide Resistance Surveillance, and Pest Management of Vine Mealybug and Grape Mealybug in Vineyards

***Project Leaders**: Lindsey Burbank, Rachel Naegele and Mark Sisterson, United States Department of Agriculture – Agricultural Research Service and Dario Cantu, University of California, Davis*

Continued use of chemical control for insect pests such as mealybugs is likely to lead to the development of insecticide resistance. Detailed understanding of the pests' biology is critical to exploring alternative control strategies. The team created a high-quality vine mealybug reference genome from a single insect DNA extraction. Genome annotation was also completed. This reference genome sequence is publicly available for future research through the web portal (https://grapegenomics.com/). The team also compared populations of vine mealybug collected from several San Joaquin Valley and Central Coast locations. Little genetic diversity was observed initially based on microsatellite markers. Still, the vine mealybug sequences produced by this project will be available for later comparison to other insect samples from different regions or countries. Challenges with rearing grape mealybug delayed genome sequencing for this second species.

Improved Decision-Making for Grapevine Leafroll and Red Blotch Diseases Using Rapid Identification Tools and a Regional Approach to Monitoring and Management

***Project Leaders and Cooperators**: Monica L. Cooper and Jennifer K. Rohrs, UC Cooperative Extension, Napa; Tom Shapland, Tule Technologies; Rodrigo Almeida, University of California, Berkeley; Oakville Neighborhood Grower Group (Chris D'Alo); Nord Vineyard Services; Rutherford Neighborhood Grower Group (Justin Leigon); and Piña Vineyard Management*

This project aims to improve the detection and management of grapevine red blotch virus (GRBV) and leafroll disease using artificial intelligence (AI) and the LAMP-GRBV assay. These advancements will help the grape industry make data-driven decisions, reducing viral disease's economic and environmental impact in vineyards.

Researchers developed an AI-based tool for detecting diseased vines through image analysis, with a beta version tested by growers in 2023. In 2024, collected images were placed in an open repository, and a manuscript on the tool's accuracy and user feedback was accepted for publication. The team also advanced the use of LAMP-GRBV to complement visual assessments, refining sampling guidelines and validating trunk cambium sampling to detect pre-symptomatic infections. They applied adaptive cluster sampling to map disease spread, improving zonal roguing strategies for vineyard management.

Field studies in 2024 continued monitoring GRBV incidence and threecornered alfalfa hopper populations at vineyard sites. The team assessed visual symptoms, analyzed spatial spread patterns and tracked insect feeding damage. Results indicated that disease spread remains highly localized, supporting the need for zonal roguing rather than vine-by-vine removal. Additionally, real-time data on vector activity and disease progression were shared with growers.

Next steps include refining AI models for disease detection, further evaluating the effectiveness of zonal roguing and publishing findings on disease ecology and management strategies. The team will also expand educational outreach and continue collaborations with growers to enhance disease monitoring and mitigation efforts.

Developing an Efficient DNA-Free, Non-Transgenic Genome Editing Methodology in Grapevine

Project Leaders*: Laurent Deluc and Satyanarayana Gouthu, Oregon State University*

Genome editing is an advanced plant breeding innovation that enables precise and rapid modifications to plant genomes, enhancing yield and disease resistance traits. The objective of this project is to develop methods to use this technology in grapevine while complying with government regulations. In grapevines, introducing loss-of-function mutations in 'Mildew resistance Locus O' (*mlo*) genes provides broad-spectrum and durable resistance to powdery mildew. Development of a transgene-free mlo-edited microvine using a two-phase genome editing approach (insertion and excision) would pave the way for using this technology for other grapevine diseases.

The team has achieved the two major research milestones of the project. First, mlo genes were effectively edited, resulting in stable, transformed grapevines with partial to near-complete resistance to powdery mildew. Second, cell-penetrating peptides (CPPs) were evaluated for their ability to facilitate the delivery of ribonucleoproteins (RNPs) into intact plant cells. The final phase, which involves excising the inserted T-DNA while preserving the beneficial mutations, is currently in progress. The locations of the insertions have been identified, and Cas9/RNP will soon be delivered to the edited grapevine cells. The outcomes of this final step will be presented in a comprehensive report in 2025.

Identification of Grapevine Host Factors with Pro-Viral Activity to Target for Resistance Against Red Blotch Virus Through CRISPR Gene Editing

Project Leaders*: Laurent Deluc and Satyanarayana Gouthu, Oregon State University*

This research fills a critical knowledge gap in grapevine red blotch virus (GRBV)-host dynamics and lays the foundation for CRISPR-based gene editing approaches. If successful, this work will enable the development of non-transgenic, GRBV-resistant grapevines. While CRISPR/Cas9-based gene editing has successfully created resistance to other plant viruses, it has not yet been applied to GRBV due to a lack of knowledge about which grapevine genes support infection. This research aims to identify these key host factors using yeast-two-hybrid (Y2H) screening and in-plant validation.

The team has made significant progress in identifying potential host targets for gene editing. GRBV replication proteins were cloned into yeast vectors, creating a comprehensive prey library for Y2H screening. Initial results revealed strong interactions between GRBV proteins and several grapevine genes. Further validation confirmed these interactions, providing new insights into how GRBV hijacks host functions for replication and spread. Additionally, the team optimized a protoplast generation

protocol for BiFC assays to study these interactions in living cells. The next steps include validating the most promising host interactors, refining gene annotations and screening the GRBV C3 protein for additional interactions.

Foundations to Develop New Grape Cultivars Resistant to Grapevine Fanleaf Decline

***Project Leader and Cooperator**: Luis Diaz-Garcia, University of California, Davis and Marc Fuchs, Cornell University*

Current management strategies for grapevine fanleaf virus (GFLV) are ineffective under high nematode pressure, making developing resistant grapevines a priority. This project focuses on leveraging rgflv1, a resistance factor found in Riesling clone 49 that provides complete resistance to GFLV. Researchers are conducting field and greenhouse trials to confirm its effectiveness, identify its presence in additional Riesling clones and genetically map its location to develop molecular markers for future breeding efforts.

In 2024, the team began vineyard surveys to detect *rgflv1* in Riesling clones. Initial sampling at two commercial vineyards tested negative for GFLV, leading to the interplanting of new vines in areas with high nematode pressure and infected vines. Vines will be sampled and tested every two months throughout 2025 to monitor infection and resistance. Additionally, controlled nematode inoculation studies are underway in greenhouse-grown potted plants. So far, all samples have tested negative for GFLV, and ongoing monitoring will include bi-monthly testing of select plants. The team also cultivates plants from two mapping populations to pinpoint the *rgflv1* resistance gene. Future genotyping and phenotyping for GFLV resistance will depend on securing additional funding.

Resistance to Grapevine Leafroll-Associated Virus 3 and its Major Mealybug Vectors

***Project Leaders**: Marc Fuchs and Greg Loeb, Cornell University*

This project aims to develop grapevines resistant to grapevine leafroll-associated virus 3 (GLRaV3), grape mealybug and vine mealybug using RNA interference (RNAi). RNAi is a potent gene expression regulation that targets and degrades specific RNA molecules. The team's strategy is to use RNAi against the virus and the two insect vectors by combining multiple double-stranded RNA (dsRNA) constructs.

Validation experiments for the grape and vine mealybugs using excised grapevine leaf discs revealed the need for not only the dsRNA constructs to be mealybug species-specific to be efficacious. The team isolated and cloned $AQP1_{Plf}$ and $NUC1_{Plf}$ constructs and documented their efficacy at increasing the mortality of vine mealybug crawlers in bioassays. Additional transgenic events were also developed and characterized in both rootstock and winegrape varieties. Some of these events reduced the infection rate of GLRaV3 following whip budding in the greenhouse and increased the mortality rate of the grape mealybug. The team shared research progress on leafroll disease management with the grape and wine industries through discussions and the publication of an extension article.

Investigating Fanleaf Symptom Development and Nematode Transmission to Imagine Novel Management Strategies

***Project Leader**: Marc Fuchs, Cornell University*

Current management methods for grapevine fanleaf virus (GFLV) only slow its effects rather than prevent infection. To develop better control strategies, this project explores how GFLV interacts with its plant hosts and triggers disease symptoms above and below ground. By comparing symptomatic and asymptomatic GFLV strains, researchers analyzed changes in proteins and gene activity in the leaves and roots of benth (*Nicotiana benthamiana*), a model plant that supports systemic GFLV infection.

The team identified key genes and proteins involved in symptom development, particularly in plants infected with virus strains containing a lysine at position 802 of the viral protein 1EPol. This protein was found in the cytoplasm and nucleus of infected cells, suggesting a significant role in the disease process. Researchers also discovered that GFLV strains causing significant root structure changes were less efficiently transmitted by *Xiphinema index*, the nematode responsible for spreading the virus, compared to strains with little or no effect on root growth. Ongoing experiments with infected grapevines will determine whether the same leaf and root changes observed in *N. benthamiana* also occur in grapevines, providing further insights into GFLV infection and potential control measures.

Transmission of Grapevine Red Blotch Virus and Behavior of its Insect Vector

***Project Leader**: Marc Fuchs, Cornell University*

Grapevine red blotch virus (GRBV), the cause of red blotch disease, poses a significant threat to vineyard productivity and sustainable viticulture. This project seeks to deepen our understanding of how GRBV is transmitted by the threecornered alfalfa hopper (TCAH) to inform more effective disease management strategies.

Through a series of controlled experiments, researchers examined how TCAH spreads GRBV. They discovered that, in addition to flying, TCAH primarily moves by walking and jumping, leading to virus transmission occurring near the initial infection source. Male TCAHs were identified as the primary drivers of virus spread, transmitting GRBV at much higher rates than females. The study also revealed that free-living grapevines (*Vitis californica* and its hybrids) serve as feeding and reproductive hosts for TCAH, unlike cultivated wine grapes (*Vitis vinifera*). Additionally, researchers determined that when GRBV infects the rootstock, it takes at least two years for symptoms to appear in scion leaves.

To further investigate the long-term effects of GRBV transmission, researchers will continue testing experimental vines for the virus while closely monitoring symptom development.

Virus-Based Delivery of Interfering RNAs Targeting Grapevine Leafroll-Associated Virus(es)

***Project Leader**: Yen-Wen Kuo, Bryce W. Falk, Maher Al Rwahnih and Kristine E. Godfrey, University of California, Davis*

This project offers a potential breakthrough in grapevine disease management, providing a scalable, innovative approach that could ultimately reduce the impact of grapevine leafroll disease (GLD). The team is developing a new strategy to combat GLD using grapevine geminivirus A (GGVA) viral vectors to deliver RNA interference (RNAi). They demonstrated that GGVA can effectively introduce gene-silencing sequences in grapevines without causing harmful symptoms. By successfully suppressing grapevine virus A (GVA) in test plants, they confirmed that GGVA is a promising tool for protecting grapevines from viral infections. They also refined a root infiltration technique, improving efficiency in treating multiple plants at once, which could enhance large-scale disease management efforts.

These findings lay the groundwork for future applications of GGVA-based RNAi to control GLD and its insect vectors, such as mealybugs. The team continues to optimize the viral vector system by testing different gene-silencing sequences for better efficacy.

Preparing for the Arrival of Spotted Lanternfly: Outreach and Grower Engagement About the Value of Coordinated Area-Wide Responses

***Project Leaders and Cooperators**: Neil McRoberts and Sandra Olkowski, University of California, Davis; Stephanie Bolton, Lodi Winegrape Commission; Kim Stemler, Monterey County Vintners and Growers Association; Jason Staling, Sonoma County Viticultural Technology Group; Eric Pooler, Napa County Farm Bureau; and Marc Fuchs, Cornell University*

A technical working group assessed the spotted lanternfly's (SLF) potential impact on California and the effectiveness of pest control strategies. The group's findings highlight the economic risks of SLF invasion and the importance of coordinated control efforts among growers to mitigate its impact. This project aims to provide extension support, bringing the key information from these findings to the industry on a broader scale, ultimately setting the stage for immediate and impactful outcomes in managing SLF after it arrives in the state.

Investigating the Impact of Grapevine Red Blotch Virus (GRBV) on Grape Skin Cell Wall Metabolism and Soluble Pathogenesis-Related Proteins in Relation to Phenolic Extractability

***Project Leader and Cooperators**: Anita Oberholster, Mysore Sudarshana, Larry Lerno and Cristina Medina Plaza, University of California, Davis*

The team is studying the impact of grapevine red blotch virus (GRBV) on pathogenesis-related proteins, cell wall composition and enzymatic processes to alleviate GRBV's impact on grape composition and wine quality.

GRBV delays grape ripening and affects key metabolic pathways, leading to significant decreases in sugar accumulation, color development and aroma compound accumulation, all crucial factors for wine quality. However, little is known about the impact of GRBV on cell wall composition and structure. During ripening, the grape cell wall changes in composition and integrity, which impacts phenolic extractability during winemaking. In addition, pathogens such as fungi, bacteria and viruses alter cell wall modifications. These changes in the grape cell wall can directly impact the extractability and final concentrations of phenolics in wines. Grapes infected with GRBV have significantly higher quantities of pectin (acidic heteropolysaccharide groups located in the cell wall) and soluble proteins, which could result in binding reactions to phenolic compounds such as tannin.

Investigating the Relationship Between Grapevine Red Blotch Virus (GRBV) Titer Levels, Years of Infection and Symptomology

***Project Leader and Cooperators**: Anita Oberholster, Mysore Sudarshana, Larry Lerno and Cristina Medina Plaza, University of California, Davis*

This project studies the link between grapevine red blotch virus (GRBV) levels, ripening phase, years of infection and GRBV disease outcomes over multiple seasons using an omics approach and multivariate statistics. Answers to these questions will increase understanding of plant-virus interactions and potential mitigation strategies to alleviate the impact of GRBV on the grape and wine industry.

GRBV delays grape ripening, significantly decreasing sugar accumulation, anthocyanin concentrations and aroma compound accumulation. GRBV also affects key metabolic pathways that produce compounds essential to the wine's final color, flavor and mouthfeel. GRBV alters pectin and soluble proteins in the grape skin cell walls, which could result in binding reactions to phenolic compounds such as tannin. Other research questions remain regarding the potential interaction between virus titer and observed symptomology and whether the duration of infection impacts this relationship.

Grape Germplasm Evaluation to Identify Potential Host Plant Resistance for Vine Mealybug

Project Leaders and Cooperators*: Summaira Riaz, Mark Sisterson, Claire Heinitz and Enrique Melenes, Agricultural Research Service, United States Department of Agriculture*

This work aims to provide the first comprehensive screening of a wide array of native North American grape germplasm species and accessions to identify genetic sources of vine mealybug resistance that can be used in future grape breeding efforts. Host plant resistance would provide a sustainable, long-term mechanism for VMB control for the industry, filling an essential need as insecticide use becomes restricted in the future. The team is screening 19 grape accessions for resistance to vine mealybug and establishing potential molecular markers associated with any sources of resistance found for further breeding and population studies.

Biology and Role of Treehoppers in Grapevine Red Blotch Disease with Emphasis on *Tortistilus albidosparsus*

Project Leaders and Cooperators*: Frank Zalom and Mysore R. Sudarshana, University of California, Davis; Cindy Kron, UC Cooperative Extension, Sonoma County and MacKenzie Patton, Central Sierra Cooperative Extension*

This study aims to confirm whether the treehopper *Tortistilus albidosparsus* plays a role in spreading grapevine red blotch virus in Northern California vineyards and to understand its behavior throughout the year. By understanding the insect and when it is most likely to transmit the virus, better cultural practices and treatments can be implemented to manage red blotch disease and reduce its spread in vineyards.

In 2024, researchers studied how the insect might spread the virus in lab and field settings. They also completed a second year of research on the movement of these treehoppers from winter and spring plants to grapevines. They confirmed that adult female treehoppers lay eggs in grapevines and the nymphs can develop into adults directly on the vines. Additionally, researchers tracked how long these treehoppers take to cause damage while feeding.