2023 PD/GWSS Board Research Projects

AT A GLANCE

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 world, California's winegrape growers can 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 prevent the spread of pests and diseases and deliver practical and sustainable solutions.

The consistent, reliable funding made possible by the winegrape grower assessment allows leading scientists to work toward finding solutions to PD and other serious winegrape pests and 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. Summaries of the 2023 research progress reports follow.

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ABBREVIATIONS

Clustered Regularly Interspaced Short Palindromic Repeats = CRISPR

Glassy-Winged Sharpshooter = GWSS

Grapevine Fanleaf Virus = GFLV

Grapevine Leafroll-Associated Viruses = GLRaV

Grapevine Leafroll Disease = GLD

Grapevine Red Blotch Disease = GRBD

Grapevine Red Blotch Virus = GRBV

Pierce's Disease = PD

Xylella fastidiosa = *Xf*



SECTION 1:
Xylella fastidiosa and Pierce's Disease


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

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

Despite years of study, we do not understand how mature grapevines respond to *Xylella fastidiosa (Xf)* infections under field conditions. There are two reasons for this: first, infecting mature vines with these pathogens presents risks to commercial agriculture that are difficult to mitigate, and second, the time it takes to establish an experimental vineyard for this purpose is prohibitive in areas where wine grapes are commercially grown. Because of this, we have a very limited understanding of disease progression in commercially relevant field conditions. This is a remarkable knowledge gap, both from an industry and academic perspective.

In this project, we use a 10-year-old vineyard with 13 wine grape cultivars grown under commercially relevant conditions at a UC research station to study Pierce's disease (PD) progression. We infected grapevines with *Xf* in May 2021 and report on the results of those infections across two years. We show that varieties respond differently to infection, that there is variability in plant winter recovery, and that strains appear to differentially survive the winter in plants. This is particularly important because winter curing is, still, the only mechanism that completely and permanently cures grapevines from *Xf* infections, yet we do not know the mechanism behind this plant response. We hope that our observations will lead to future research explaining this important phenomenon.

View the full report at https://piercesdisease.cdfa.ca.gov/reports.

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 Andrew Walker, University of California, Davis and Summaira Riaz, United States Department of Agriculture – Agricultural Research Service*

The overall goal of this work is to develop tools that help breeders develop Pierce's disease (PD)-resistant grapes that produce fruit with desirable flavor and aroma. We are studying a region in the genome

of a wild grape species called *Vitis arizonica*, which has shown resistance to PD. This region is called PdR1, and our analysis determined that it contains genes that are known to play a role in the plant's defense system. We 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, our goal is also to understand if the resistance in these species is driven by different genes. This information will be useful in creating grapes with a combination of multiple resistance functions to achieve durable field resistance to PD.

View the full report at https://piercesdisease.cdfa.ca.gov/reports.

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

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

The breakdown of the chemical containment of the glassy-winged sharpshooter due to the development of resistance to chemical pesticides poses a clear and present danger with the potential spread of Pierce's disease (PD) to high-value wine-growing regions of California. The focus of this project is to deploy a genetic solution to this problem by using bioengineered rootstocks that protect grafted varietal vines against PD.

The goal of this project is to evaluate grafted vines under field conditions in order to identify elite bioengineered rootstocks that systemically protect the grafted scion variety from developing and/or succumbing to PD. The 450 vines correspond to different bioengineered commercial grapevine rootstocks that express one of seven different systemic immunity strategies to combat PD development. The proposed extensive testing, including pathogen challenge, evaluation of plant health, disease development, and examination of systemic immunity mechanisms, will lead to the identification of superior bioengineered rootstock lines that could protect any grafted scion variety against PD and would be ready to enter the commercial pipeline at the end of this study.

View the full report at https://piercesdisease.cdfa.ca.gov/reports.

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

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

This is an ongoing project that manages the federal permit that enables the multi-investigator field testing of transgenic grapevines in California. The APHIS BRS federal permit for 5.4 acres that are currently being used to test transgenic grapevines was first approved on April 1, 2018, and the current permit was approved on April 1, 2022, with an end date of April 1, 2025. This new permit accommodates the field testing of transgenic rootstock genotypes 101-14 and 1103 grafted to the sensitive scion variety Chardonnay for validating their efficacy in protecting the scion from developing PD. Five *Vitis vinifera* varieties, Cabernet Sauvignon, Merlot, Pinot Noir, Sauvignon Blanc, and Zinfandel are also being tested as untransformed controls, either as scions grafted to the permitted rootstocks or as self-rooted plants.

View the full report at https://piercesdisease.cdfa.ca.gov/reports.

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; Swadeshmukul Santra and Jorge Pereira, University of Central Florida*

Disease management tools against Pierce's disease (PD) in grapes are very limited. The only chemical control options available against PD target the insect vectors and not the pathogen. There are no antibacterial compounds effective in planta against the pathogen *Xylella fastidiosa (Xf)*, because of the difficulty of reaching the vascular system by spray applications. The availability of an antibacterial chemical treatment easily applied in the field by soil drench or foliar spray would be a useful tool for growers to manage PD.

The team has tested a novel nano-sized formulation ("Zinkicide®, ZnK") against a different vascular (phloem) bacterial pathogen in citrus and has carried out preliminary tests of the same formulation against *Xf* in the greenhouse with promising results in blueberry and tobacco. The team is modifying the chemical composition of the nano-formulation to improve performance against *Xf* at lower doses and to test it in grapes.

Preliminary results indicate that both Zinkicide formulations TMN110 and TMN111 are causing phytotoxicity issues in grapevines. The novel chemistries used in nanoformulations tested currently showed high antibacterial activity in vitro and promising activity in tobacco plants, but still cause phytotoxicity in planta. They are currently developing variations of these products to avoid the negative effects in planta.

View the full report at https://piercesdisease.cdfa.ca.gov/reports.

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

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

Gene editing is promising biotechnology for genetic improvement. However, in vegetatively propagated crops like grapevine, the generation of genetically modified organisms makes it challenging to embrace the technology entirely. The recent development of performing gene-editing on naked plant cells (protoplasts) is a valuable option. However, it still requires streamlined methodologies to screen edited plant material and regenerate individual plants from protoplasts, which is still problematic in grapevine.

We aim to develop an innovative stepwise approach to generate a transgene-free gene-edited grapevine. The approach consists of two phases. In Phase 1, we will perform conventional genetic engineering to generate stable transformants via *Agrobacterium tumefaciens* transformation. However, the inserted genetic cassette containing the editing ingredients targeting specific gene(s) will be customized to be cut out, or “excised,” later. In Phase 2, we will ease the entry of a RiboNucleoProtein by using a peptide tag (Cell-Penetrating Peptide) targeting the inserted transgenic cassette for “excision” into intact regenerable edited plant materials from Phase 1.

View the full report at https://piercesdisease.cdfa.ca.gov/reports.

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 Anika Kinkhabwala, A&P Inphatec*

This project focuses on field testing of natural biological products for control of Pierce’s disease (PD). Biological products are microbes that live in association with plants. Our research group isolated one biocontrol from onion roots and two biocontrol species from grapevine wood and a mixture of bacteriophages. Bacteriophages are viruses that selectively infect and kill bacteria but do not infect plant or animal cells. Research showed that these organisms were able to reduce PD symptoms when inoculated to grapevines in greenhouse bioassays and field trials. This research aimed to generate the data needed for commercialization of new biopesticides that can be used by grape growers to manage PD. According to the first year’s field trial results, all the biocontrol treatments reduced disease symptoms.

View the full report at https://piercesdisease.cdfa.ca.gov/reports.

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*

A series of experiments to evaluate grapevines expressing potential Pierce’s disease (PD) suppressive transgenes under field conditions began in 2010. Mechanical inoculation of *Xylella fastidiosa (Xf)* established PD in susceptible control vines within 24 months and revealed five different DNA constructs gave protection against classic PD symptoms compared with non-transgenic control vines. This experiment was terminated in 2017 and a second field experiment began to test potential cross-graft protection of a PD-susceptible Chardonnay scion grafted to two adapted rootstocks (1103 and 101-14) that expressed paired (dual) combinations of the five transgenes. The successfully transformed rootstocks were bud grafted to Chardonnay and planted in USDA-APHIS-controlled area in 2018 and 2019.

Plants have been trained and maintained to commercial standards, inoculated with *Xf* in 2021 and 2022, and are being evaluated for bacterial infection, bacterial dynamics, and PD symptoms in the infected PD-susceptible scions on transgenic rootstocks compared with untransformed control rootstocks.

View the full report at https://piercesdisease.cdfa.ca.gov/reports.

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 project supports field activities related to the evaluation of resistance to Pierce's disease (PD) in transgenic grape rootstocks by expressing dual combinations of five unique transgenes that have shown positive protection against PD under field conditions. The new planting, managed to commercial standards, consists of untransformed PD-susceptible Chardonnay scions grafted to transgenic rootstocks, (Paulsen 1103 and MGT 101-14) expressing the paired constructs. The field experiment, conducted in a USDA-APHIS-regulated Solano County site, includes mechanical inoculation of *Xylella fastidiosa (Xf)* that was used successfully in past field experiments. PD symptoms and bacterial movement will be measured. A total of 721 transgenic and controls were planted in 2019. All plants displayed normal growth and morphology and were inoculated with *Xf* in July 2021 and two-bud pruned in March 2022 carefully leaving the inoculated branches tagged.

View the full report at https://piercesdisease.cdfa.ca.gov/reports.

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

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

The team has developed a stable antimicrobial peptide (SAMP) from the Australian finger lime that can directly kill pathogens, induce host defense response, and suppress insect vectors. Thus, it is a good candidate for developing into both a prophylactic and curative treatment for grapevines in areas under Pierce's disease (PD) pressure. The team confirmed that SAMP can efficiently and rapidly kill cultured *Xylella fastidiosa*, and most

importantly, that SAMP can inhibit PD in grapevines as a prophylactic treatment. Now the team is testing the most effective solutions and application strategies of SAMP in controlling PD. Greenhouse studies are being performed to assess the efficacy of SAMP as a prophylactic and post-inoculated treatment by using SAMP with different delivery methods, including foliar spray and injection. The priming effect of SAMP in grapevine will be examined and used as a clue for prophylactic treatment.

View the full report at https://piercesdisease.cdfa.ca.gov/reports.

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*

The team has demonstrated that early stimulation of certain sectors of the grapevine immune system leads to significantly less bacterial colonization and less disease in grapevines inoculated with *Xylella fastidiosa*. They studied this at the genetic level and determined which genes control this disease suppression in grapevine. The team is now determining if modulating the expression of these native grapevine genes can generate *Vitis vinifera* vines that are resistant to Pierce's disease.

View the full report at https://piercesdisease.cdfa.ca.gov/reports.

Protoplast-Mediated Gene Editing for Disease Resistance

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

CRISPR-Cas9 is a gene editing technology that allows one to make precise changes in a plant's genetic information. Protoplasts are plant cells which have had their cell walls removed, making it easier to utilize CRISPR-Cas9 for gene editing. The team previously established a protocol to generate protoplasts from grape tissue and stimulate these protoplasts to reform whole plants. They have also demonstrated they can edit the genetic blueprint of grapes by treating the protoplasts with DNA or Ribonucleoproteins (RNPs) prior to reforming whole plants from the protoplasts. Improving grape plants using RNPs is of particular interest since RNPs are proteins and RNA complexes and not DNA, meaning the resulting edited plants would not be considered genetically modified by current regulations. Now they are testing this technology to demonstrate that it can be used to create disease-resistant grapes.

Plants have susceptibility genes that pathogens use to infect a plant. By knocking out these genes in the plant, the pathogen's ability to infect the plant is compromised. A group of these susceptibility genes are called MLOs. These genes are required for the pathogen powdery mildew to infect grape plants. By targeting this group of genes and knocking them out in the grape plant, we hope to produce plants resistant to powdery mildew. This proof-of-concept study will lay 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.

View the full report at https://piercesdisease.cdfa.ca.gov/reports.

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*

Several strategies are being deployed to reduce incidence of Pierce's disease (PD), but these rely mostly on controlling the vectoring insect or developing resistant grapevine varieties. Due to its importance, research to develop additional management options is warranted. One such option could be the use of beneficial fungi, such as *Trichoderma* spp., that could enhance the ability of grapevines to protect themselves from *Xylella fastidiosa* infections or reduce the development of PD symptoms. *Trichoderma* spp. have been observed to enhance plant protection against several diseases, as well as enhance plant growth and development, but have not been extensively tested as products to prevent PD.

This project utilizes a unique collection of *Trichoderma* spp. isolates collected from California that have already been observed to provide biological control activity against grapevine fungal canker pathogens, and therefore could protect vines from multiple threats. Genomic sequencing of promising *Trichoderma* isolates also will occur to discover genes associated with improving plant health.

View the full report at https://piercesdisease.cdfa.ca.gov/reports.

SECTION 2:

Pierce's Disease Vectors

Modeling Sharpshooter Feeding Behavior with a Novel 3D Approach to Insect Behavioral Visualization

***Project Leaders**: Rodrigo Almeida and Elizabeth Clark, University of California, Berkeley; Andrew McElrone, United States Department of Agriculture – Agricultural Research Service; and Leonardo De La Fuente, Auburn University*

Xylella fastidiosa (*Xf*) is transmitted from plant to plant by insects feeding on xylem sap. However, little is known about how bacteria are transferred from the infected insect to the plant during feeding. The team used 3D imaging to illuminate the anatomy of three important insect vectors of *Xf* and used these 3D reconstructions to generate 3D computational fluid dynamics simulations to understand how xylem sap-feeding occurs. This work provides a basis for understanding the physics of fluid flow from the xylem vessel and the insect feeding complex. This approach can be applied to explore comparative elements of their functional anatomy, such as how and why some insects are more effective at transmitting *Xf* than others.

View the full report at https://piercesdisease.cdfa.ca.gov/reports.

CRISPR-Mediated Genome Modification of *Homalodisca vitripennis* for the Genetic Control of Pierce's Disease

***Project Leaders and Cooperator**: Peter Atkinson, Richard Redak, Linda Walling, and Jason Stajich, University of California, Riverside and Rodrigo Almeida, University of California, Berkeley*

Genetic-based control strategies for pest insects have been severely constrained by the ability to alter an insect's genome at precise locations. This hurdle has been overcome by the development of CRISPR-based technology when there is a physical means for delivering this technology into an insect's genome.

In order to develop and establish genetic control strategies for the glassy-winged sharpshooter (GWSS), the team first applied CRISPR/Cas9 technologies into the pest to establish a reliable, robust genetic platform. With their technical breakthrough of high-frequency editing of GWSS, the team is setting the foundations to generate and test, for the first time, GWSS genetic control strategies.

The team developed a rapid somatic assay screen for testing promoter activity. They performed precise dissections of multiples tissues from which RNA-seq libraries will be constructed and sequenced in order to identify promoters strongly expressed in the foregut, cibrarium and pre-cibrariuim of GWSS, the tissues to which *Xylella fastidiosa* (*Xf)* binds. Next the team will construct and test anti-*Xf* acquisition genes for their ability to block *Xf* acquisition and transmission.

View the full report at https://piercesdisease.cdfa.ca.gov/reports.

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, mate recognition and localization are mediated exclusively via substrate-borne vibrational signals transmitted through the plant. Previous studies show the potential of using vibrational disruption for pest management, but existing knowledge on blue-green sharpshooter (BGSS) vibrational communication is insufficient to implement a management program for this pest.

This project will provide a detailed description of BGSS communication signals that are relevant for the management of Pierce's disease (PD). In the context of BGSS reproduction, descriptions will aid the identification of signals used in mate recognition, finding, choice, and/or acceptance. These will likely include signals produced by duetting individuals and/or signals produced during male-male competitive interactions. Outside the context of reproduction, competitive or cooperative interactions may arise to facilitate access to feeding sites. These interactions may be mediated by signals used to repel or attract conspecifics to feeding sites. Finally, the project will provide practical recommendations on the exploitation of BGSS vibrational communication as a novel method to suppress populations.

View the full report at https://piercesdisease.cdfa.ca.gov/reports.

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, City University of New York; Monica Cooper, University of California Cooperative Extension, Napa County; and Cindy Kron and Lucia Varela, University of California Cooperative Extension, Sonoma County*

In most Napa and Sonoma vineyards Pierce's disease (PD) is transmitted by sharpshooters. However, some vineyards with PD have no sharpshooters and the most likely vector is a spittlebug of the genus *Aphrophora*. These spittlebugs, which closely resemble western pine spittlebugs, have been shown to be able to transmit PD. The vineyard and pine spittlebugs look identical, but sometimes species that look identical can be genetically very different.

The purpose of this work is to seek consistent DNA differences that allow differentiation of the vineyard spittlebug from the western pine spittlebug. The results from this study will allow, 1) defining the vineyard spittlebug as a new species, 2) understanding the population spread of spittlebugs across the vineyards in the Sonoma and Napa region and 3) paving the way for a simple DNA test that would permit agricultural investigators to identify these bugs for PD control studies.

View the full report at https://piercesdisease.cdfa.ca.gov/reports.



SECTION 3:
Other Pests & Diseases of Winegrapes

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. The goal of this project is 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.

The greenhouse will house approximately 2,000 vines, or 250-750 selections depending on the number of vines per selection, and industry-ranked selections are being prioritized. Propagation efforts are progressing as planned. Between 2022 and 2023 propagations, the team has reached its year one goal of completing propagation of 200 high-priority selections. These selections are now entered in the testing pipeline, with testing initiated in summer 2023. Tissue culture of priority selections is also progressing as planned, and they plan to bring at least 50 more selections into tissue culture in year two.

View the full report at https://piercesdisease.cdfa.ca.gov/reports.

Autonomous Field-Scouting of Virus-Infections in White Varieties and Pre-Symptomatic Vines

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

The only management response to viruses is the early detection and eradication of infected plants, but scouting individual plants is time-consuming and not possible on a large scale and in intensively mechanized systems. Despite the similarity of the symptoms of numerous grapevine problems, an expert eye can recognize infection in red varieties. It is much harder to assess infection in white varieties. In order to reduce the source of spread in vineyards, growers urgently need a tool to identify virus infections in white varieties and earlier in the season for red varieties.

Hyperspectral imaging coupled with machine learning can be used to assess infection by grapevine leafroll-associated viruses and grapevine red blotch virus, the two most problematic viruses in grapevine, in

vineyard conditions more efficiently, rapidly, and affordably than traditional diagnostic methods. Although previous work has focused on red varieties, this method has also shown promising results in assessing infection before symptoms are visible to the human eye. The team will apply hyperspectral imaging obtained from autonomous aerial and ground vehicles to the identification of infected grapevines in white varieties and in pre-symptomatic red varieties. This is a very novel attempt that will provide growers with an unprecedented resource for fighting these diseases.

View the full report at https://piercesdisease.cdfa.ca.gov/reports.

Genomics Resources for Identification, Tracking, Surveillance, and Pest Management of Vine 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. It is necessary to explore alternative control strategies based on a detailed understanding of pest biology. DNA sequence information for vine mealybug will enable the development of new pest control technologies. A high-quality vine mealybug reference genome was created from single insect DNA extraction. Work is ongoing to annotate the reference genome using RNAseq data from male and female vine mealybugs. Although little genetic diversity was observed initially based on microsatellite markers, new genomic references will be used to screen for additional marker candidates.

This project will expand DNA sequence information for vine mealybug representative of pest populations across California. This information will be used to track pest populations, evaluate the prevalence of insecticide resistance, and develop new pest control technologies based on novel genetic targets.

View the full report at https://piercesdisease.cdfa.ca.gov/reports.

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 Cooper, Jennifer Rohrs, Sarah McDonald, Hannah Fendell-Hummel, University of California Cooperative Extension, Napa County; Tom Shapland, Tule Technologies; Rodrigo Almeida, University of California, Berkeley; Keith Perry, Cornell University; Oakville Neighborhood Grower Group; and Rutherford Neighborhood Grower Group*

This project is advancing the use of emerging technologies to identify diseased vines and support grower-coordinated efforts to reduce the economic and environmental impacts of grapevine leafroll disease (GLD) and grapevine red blotch disease (GRBD). Successful roguing to mitigate leafroll and red blotch requires accurate identification of diseased vines, but this can be challenging when symptoms are confusing, asynchronous, or absent. This project is increasing the accuracy of visual assessments and improving vine removal efforts using artificial intelligence (AI) and an “in-house” (LAMP-GRBV) assay. The team’s AI application, Virus Vision, performed with 88% accuracy in eight vineyards with known infections of grapevine red blotch virus and 87% accuracy in five vineyards with known infections of grapevine leafroll-associated virus-3 (GLRaV-3). The LAMP-GRBV assay rapidly confirmed visual assessments of GRBD symptoms, with 99% agreement between visual ratings and diagnostic results.

The team is addressing fundamental questions of GRBD ecology through participatory research at 17 UCCE and 13 grower field sites. This is the first large-scale, multi-site, participatory GRBD research study to be conducted in California. Regional monitoring of the disease and vector, combined with network-based learning, is addressing uncertainties in vector biology, disease epidemiology, and management.

View the full report at https://piercesdisease.cdfa.ca.gov/reports.

Improving Extension Outcomes: Identifying Drivers and Barriers to Adoption of Management Practices Using Leafroll and Red Blotch Disease as Model Systems

***Project Leaders and Cooperators**: Monica Cooper and Malcolm Hobbs, University of California Cooperative Extension, Napa County; Larry Bettiga, University of California Cooperative Extension, Salinas County; Stephanie Bolton, Lodi Winegrape Commission; and Michelle Moyer, Washington State University*

Management guidelines for grapevine leafroll disease encourage the planting of virus-screened material, removal of diseased vines, and management of vector populations. Since grapevine red blotch disease was more recently identified, specific guidelines are under development, but will likely include similar practices. The uptake and implementation of these practices varies among growers and across regions.
To understand why, the team conducted a survey and interviews with growers in California and Washington and identified nine key economic, knowledge, and social-behavioral factors that affect adoption.

The cost of practices—in time, labor, and outlay—are important considerations. Therefore, individual or collective practices that reduce the economic burden of adoption can improve regional disease management outcomes. The most influential technical factor is the availability and acceptance of evidence-based management practices. Prioritizing research and outreach programs that develop and disseminate an evidence-based understanding of disease ecology and management can reduce the detrimental effects of viral diseases. Lastly, programs that improve regional camaraderie and collaboration, as well as supportive learning environments within individual organizations are social factors that can increase the adoption of management practices.

View the full report at https://piercesdisease.cdfa.ca.gov/reports.

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*

Grapevine red blotch virus (GRBV) possesses a single-stranded DNA genome of around 3,200 nucleotides encoding only seven proteins and depends heavily on the host cellular machinery. Due to their limited coding capacity, viral proteins must interact with "host factors" (susceptibility genes) with pro-viral activity for essential steps of the infection process, such as virus replication, movement, or virion formation. Many host factors have been extensively studied as potential targets for viral control because mutations impart resistance against viral pathogens. In addition, some of these host factors are essential for virus infection but not for plant growth and development. These non-essential host factor genes represent opportunities to engineer resistance to viruses through transgene-free gene editing methods.

CRISPR/Cas9 editing of host factor genes successfully produced virus-resistant plants against several RNA viruses. The application of CRISPR editing technology has not been attempted for GRBV resistance in grapevine because of the lack of knowledge on grapevine genes that act as proviral host factors during viral infection, which is critical to developing GRBV control measures. The team is addressing this significant knowledge gap by profiling the host factors interacting with GRBV replication proteins, which are the most critical viral proteins during the initial infection phase. They will also characterize the host factors by generating CRISPR knockouts and examining the viral replication in the mutant plants. This knowledge of host factor genes, without which GRBV cannot replicate in grapevine, will lead to developing non-transgenic grapevine lines with durable genetic resistance against GRBV.

View the full report at https://piercesdisease.cdfa.ca.gov/reports.

Mechanisms of Grapevine Fanleaf Virus Symptom Development for Novel Resistance Strategies

Project Leader*: Marc Fuchs, Cornell University*

Grapevine fanleaf virus (GFLV) is managed in diseased vineyards by using rootstocks that are resistant to *Xiphinema index*, the ectoparasitic nematode of GFLV. This approach is somewhat unsatisfactory because, although the debilitating effects of the virus on vine growth and production

are substantially delayed, the vines eventually get infected. Therefore, there is a need to explore innovative disease management strategies.

The team is investigating how GFLV interacts with its plant hosts for the development of disease symptoms and modifications of the root system by using symptomatic and asymptomatic GFLV strains, as well as the model herbaceous host *N. benthamiana*. Candidate host genes involved in GFLV-induced leaf symptoms were identified, and specific changes in the root system architecture, such as fewer root tips or thicker roots, were documented. Dysregulated root genes upon GFLV infection are involved in important biochemical pathways, including protein synthesis and hormone and immune responses. Altering the expression of key plant host genes and proteins that are indispensable for the virus to cause disease symptoms and complete its infectious cycle may help imagine new disease management options for GFLV.

View the full report at https://piercesdisease.cdfa.ca.gov/reports.

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

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

The objective of this project is to develop grapevines resistant to grapevine leafroll-associated virus 3 (GLRaV3) and to the grape mealybug and the vine mealybug, the two most important vectors of GLRaV3 in California, by using RNA interference (RNAi). RNAi is a potent mechanism for regulating gene expression 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.

For GLRaV3, RNAi targets essential for the virus to complete its infection cycle were selected and used in grapevine transformation experiments for the recovery of stable transformants. Some transgenic plants of rootstock 110R and 101-14, and of *Vitis vinifera* cultivars Cabernet franc and Pinot noir were obtained and characterized. For mealybugs, two RNAi gut gene targets critical for successful insect feeding on grapevine phloem sap were selected and a third target was chosen for mealybugs to enhance the efficacy of the two gut RNAi targets. Target genes were identified for the grape and the vine mealybugs and characterized. Results showed a significantly increased mortality of grape mealybug nymphs feeding on an artificial diet supplemented with dsRNA constructs against grape mealybug genes. Similar work with the vine mealybug led to the identification and characterization of a new RNAi target for optimal RNAi efficacy. This new target has been used in combination with the

three RNA constructs from the grape mealybug. Subsequently, bioassays revealed a significantly increased mortality of vine mealybugs on excised grapevine leaves treated with these dsRNA constructs. Transformation efforts are pursued by using stacked dsRNA constructs against the mealybugs and GLRaV3, and by directing their expression to the phloem using a tissue-specific plant promoter. The production of grapevine plants stably transformed with dsRNA constructs against GLRaV3 and the two mealybug pests is progressing.

View the full report at https://piercesdisease.cdfa.ca.gov/reports.

Epidemiological Characteristics of Grapevine Red Blotch Disease

Project Leaders and Cooperators*: Marc Fuchs, Cornell University and Monica Cooper, University of California Cooperative Extension, Napa County*

Grapevine red blotch virus (GRBV) is transmitted by vegetative propagation, grafting, and by the three-cornered alfalfa treehopper (TCAH). Little information is available on how GRBV interacts with the TCAH for transmission in the vineyard. A low transmission rate of GRBV by the TCAH was documented in the vineyard, but infected vines did not exhibit disease symptoms even 14 months post-exposure to viruliferous TCAHs. The TCAH seems to retain GRBV for at least 30 days after virus acquisition on infected plants and feeding on healthy plants.

The team also studied the behavior of the TCAH in a vineyard ecosystem by analyzing its gut content to make inferences on its dietary preferences and identify which plant hosts it feeds on before visiting a vineyard. Plants species of the families Asteraceae (prickly lettuce, dandelion, thistle, spotted knapweed, etc.), Fabaceae (burr clover, sweet clover, vetch, sweet pea, alfalfa, bird's foot trefoil, etc.), and Vitaceae (free-living vines and *Vitis vinifera*) dominated the TCAH gut. This research indicates a wide range of feeding hosts of TCAH in vineyard ecosystems, stressing the need to remove the virus inoculum in infected vineyards as an optimal disease management strategy.

View the full report at https://piercesdisease.cdfa.ca.gov/reports.


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

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

The team is using grapevine virus-based RNA interference (RNAi) approaches to target grapevine leafroll-associated viruses. They have successfully achieved the ability to perform all the assays directly in grapevines instead of needing to rely on model plants; deliver expression plasmids and viral vectors directly into greenhouse-grown grapevine plants without relying on regeneration from somatic embryos and/or in vitro micropropagation; and developed two grapevine virus infectious clones that give asymptomatic infections in greenhouse-grown grapevines.

Now they are testing the optimal insertion site of specific grapevine leafroll-associated viruses (GLRaV) targeting sequences in the viral infectious clones. The team successfully used the viral vectors to target grapevine virus A (GVA) in *N. benthamiana* and grapevine plants. The silencing effects in grapevine plants using those viral vectors are being tested and monitored in the grapevine varieties: Salt Creek (rootstock) and Colombard (scion). They will continue to test the capacity of the viral vectors to suppress the target viral infection/replication and will use the viral vectors to deliver and enhance the RNAi efficacy in grapevine rootstocks and scions against GLRaVs.

View the full report at https://piercesdisease.cdfa.ca.gov/reports.

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*

Grapevine red blotch virus (GRBV) causes a delay in ripening events in grapes and affects key metabolic pathways, leading to significant decreases in sugar accumulation, color development, and aroma compound accumulation, all important factors for wine quality.

However, there is little known about the impact of GRBV on cell wall composition and structure. It is known that during ripening the grape


cell wall changes in composition and integrity, which impacts phenolic extractability during winemaking. In addition, it has been shown that pathogens such as fungi, bacteria, and viruses alter cell wall modifications. Consequently, 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 and this could result in binding reactions to phenolic compounds such as tannin. The team is studying the impact of GRBV on pathogenesis-related proteins, cell wall composition, and cell wall enzymatic processes to aid in understanding plant-virus interactions and potential mitigation strategies to alleviate the impact of GRBV on grape composition and wine quality.

View the full report at https://piercesdisease.cdfa.ca.gov/reports.

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*

Research in the Oberholster group indicates that grapevine red blotch virus (GRBV) causes a delay in ripening events in grapes, leading to significant decreases in sugar accumulation, anthocyanin concentrations, and aroma compound accumulation. GRBV also affects key metabolic pathways that are responsible for the production of compounds that are important to the color, flavor, and mouthfeel of a final wine. 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 has any impact on this relationship. Using an omics approach and multivariate statistics, the link between virus titer, ripening phase, years of infection, and GRBV disease outcomes will be investigated over multiple seasons. 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.

View the full report at https://piercesdisease.cdfa.ca.gov/reports.


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

Project Leaders and Cooperator: *Frank Zalom and Mysore Sudarshana, University of California, Davis and Cindy Kron, University of California Cooperative Extension, Sonoma County*

The findings from this project are expected to confirm preliminary results that suggest that the treehopper *Tortistilus albidosparsus* is a vector of grapevine red blotch virus (GRBV). The team conducted systematic field sampling of GRBV-infected vineyard blocks where *T. albidosparsus* has been found in abundance and vegetation surrounding and within those blocks from bud break through summer to determine the seasonal cycle of this treehopper and when virus transmission is most likely to occur. Variables relevant to GRBV acquisition and transmission for both *T. albidopsparus* and *S. festinus* (three-cornered alfalfa hopper) will be studied in greater detail using an artificial transmission system and salivary gland dissections, in addition to traditional transmission to grapevines. These data will contribute to the management of GRBV by improving the timing of controls and treatments based on treehopper biology and knowledge of when transmission is most likely to occur.

View the full report at https://piercesdisease.cdfa.ca.gov/reports.