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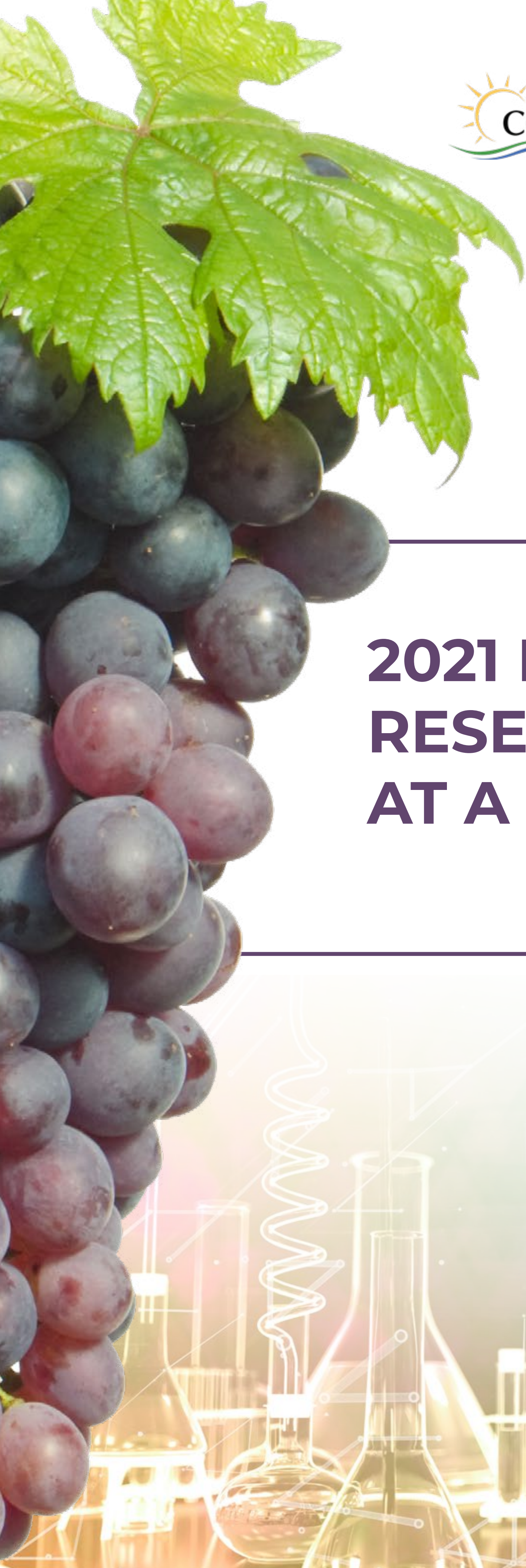
Partnership for Winegrape Pest Solutions



2021 PIERCE'S DISEASE RESEARCH PROJECTS AT A GLANCE

December 2021

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 2021 research progress reports follow.

CONTENTS

ABBREVIATIONS

Clustered Regularly Interspaced Short Palindromic Repeats = CRISPR

Glassy-Winged Sharpshooter = GWSS

Grapevine Fanleaf Virus = GFLV

Grapevine Leafroll-Associated Viruses = GLRaV

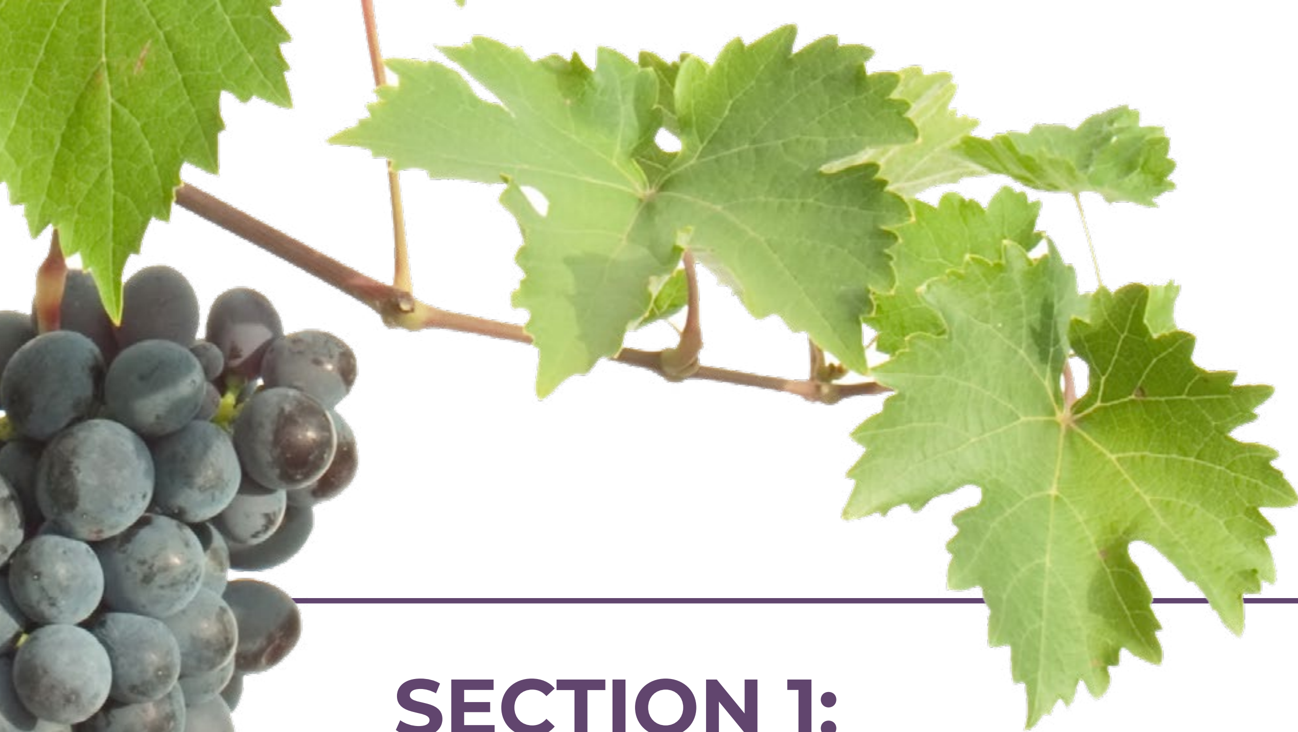
Grapevine Leafroll Disease = GLD

Grapevine Red Blotch Disease = GRBD

Grapevine Red Blotch Virus = GRBV

Pierce's Disease = PD

Xylella fastidiosa = *Xf*



SECTION 1:

Xylella fastidiosa, Pierce's Disease, and Glassy-Winged Sharpshooter

Addressing Knowledge Gaps in Pierce's Disease Epidemiology: Underappreciated Vectors, Genotypes, and Patterns of Spread

Project Leaders and Cooperators: Rodrigo P.P. Almeida and Alexander Purcell, University of California, Berkeley; Monica Cooper, University of California, Cooperative Extension, Napa County; Matthew Daugherty, University of California, Riverside; and Rhonda Smith and Lucia Varela, University of California, Cooperative Extension

Novel spatial Pierce's disease (PD) patterns have been observed, particularly at locations remote from riparian zones. We confirmed that spittlebugs commonly found in vineyards are vectors of *Xylella fastidiosa* (*Xf)* capable of acquiring the pathogen in the field. However, the research also showed that the phenology of these insects does not overlap with known windows of opportunity for PD spread in spring and early summer. The role of spittlebugs on PD spread is likely to be limited for most ecological scenarios; it is possible that shifts due to climate change may change the relative importance of these insects in the future.

It is also possible that PD ecology has been misunderstood, in that vectors may acquire *Xf* from grapevines in vineyards in the fall rather than hosts in the riparian zone in the winter. If that is the case, disease management recommendations would have to be modified. Research on this possibility is ongoing.

PD in California is caused by related but different populations of *Xf* and research is underway to determine if these populations are biologically distinct. In addition, infections of commercial grapevines in the Napa Valley indicate that disease symptoms progress differently from expected based on greenhouse research; this research is being continued with a larger number of varieties given the importance of understanding disease symptom development.

View the full report at piercesdisease.cdfa.ca.gov/.

Comparative Field Infection and Disease Development of the two Most Important Grapevine Vector-Borne Pathogens, *Xylella fastidiosa* and Grapevine Leafroll-Associated Virus 3

Project Leaders and Cooperators: Rodrigo P.P. Almeida, University of California, Berkeley; Monica Cooper, University of California, Cooperative Extension, Napa County; Matthew Daugherty, University of California, Riverside; and Maher Al Rwahnih, University of California, Davis

We still lack robust information on how *Xylella fastidiosa (Xf)* colonizes grapevines and how Pierce's disease (PD) symptoms develop under realistic and relevant field conditions. This information is critical to understanding the disease and developing and delivering sciencebased management guidelines. In addition to clarifying symptom progression, this project offers an opportunity to test hypotheses about *Xf* overwintering and climate adaptation.

We showed that *Xf* populations infecting grapevines in California may be adapted to regional climates. Historically, the northern limit of PD in California has been Napa and Sonoma, attributed to *Xf* intolerance to cold winter temperatures. However, in 2020 our team isolated *Xf* from multiple sites near in Mendocino County. We hypothesize that these strains may have adapted to successfully overwinter in cooler areas of Northern California and will compare overwintering and symptom progression of pathogen strains that belong to distinct phylogenetic groups. Using the 14 cultivars at this site, we will test the hypothesis that cold curing differs by cultivar more comprehensively.

View the full report at piercesdisease.cdfa.ca.gov/.

Modeling of *Xylella fastidiosa* Transmission and Grapevine Susceptibility Using Fluid Dynamics Simulations

Project Leaders and Cooperators: Rodrigo P.P. Almeida, Elizabeth G. Clark, and Daniele Cornara, University of California, Berkeley; Andrew J. McElrone, United States Department of Agriculture, Agricultural Research Service; Leonardo De La Fuente, Auburn University; Craig R. Brodersen, Yale University; and Dula Y. Parkinson and Harold S. Barnard, Lawrence Berkeley National Laboratory

Xylella fastidiosa (Xf) is transmitted from plant to plant by insects feeding predominantly on xylem. However, little is known about how bacteria are transferred from the infected insect to the plant during the probing and feeding process. This is due to the fact that much remains to be understood regarding how insects feed on xylem sap. Here, we are integrating 3D digital models with tools to simulate fluid and particle transmission to infer how bacterium transmission occurs.

We are approaching this through performing several different experiments integrating synchrotron-based micro-CT and digital modeling. Preliminary results suggest that this approach will be an effective technique to illuminate critical structural aspects of xylem sap-feeding insects and plants susceptible to Pierce's disease. This may reveal how and why some insects are more effective at transmitting *Xf* than others.

View the full report at bit.ly/3gz7p7m.

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

Project Leaders 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

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 very limited understanding of disease progression in commercially relevant field conditions.

In this project we use a 10-year-old vineyard with 14 wine grape cultivars grown under commercially relevant conditions at a University of California research station to study the progression of Pierce's disease in inoculated vines. We infected grapevines with *Xf* in May 2021. We are still processing samples but the preliminary data show that the *Xf* field infections were successful. We have also observed early disease symptoms in some varieties in the inoculated canes; interestingly, disease symptoms varied substantially based on variety. These early results also suggest that varieties differ in their responses to *Xf* infection.

View the full report at bit.ly/33ajdd2.

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

Project Leaders and Cooperators: Peter W. Atkinson, Richard A. Redak, Linda L. Walling, and Jason E. Stajich, University of California, Riverside and Rodrigo P. P. 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. We have solved this issue for the glassy-winged sharpshooter (GWSS). We will use a CRISPR-based technology in a proof-of-concept strategy to generate strains of GWSS that break the transmission cycle of Pierce's disease.

Our ability to develop genome-edited GWSS strains is an indication of the impact that CRISPR-based technology has on modern genetics and novel genetic control mechanisms for insect pests, such as GWSS. We have shown that it is possible to insert a gene into the GWSS genome using two distinct CRISPR-based technologies. With our technical breakthrough of high-frequency editing of GWSS, we are setting the foundations to generate and test, for the first time, genetic control strategies in this important pest insect species of California agriculture.

View the full report at bit.ly/34HQUTM.

Management of the Federal Permit for Field Testing Transgenic Grapevine Rootstocks in California

Project Leader and Cooperators: Abhaya M. Dandekar, Ana M. Ibáñez, and Aaron Jacobson, 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. This permit accommodates transgenic rootstock genotypes 101-14 and 1103P grafted to the sensitive scion variety Chardonnay for validating their efficacy in protecting the scion from developing Pierce's disease. The permit was amended to also allow the inclusion of five *Vitis vinifera* varieties that can be planted as untransformed controls, either as self-rooted plants or as scions grafted to the permitted rootstocks. Timely reporting and inspections are conducted on an ongoing basis to maintain compliance with federal permit conditions.

View the full report at bit.ly/3spwhnh.

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; and Evan Johnson, University of Florida

The only chemical control options available against Pierce's disease (PD) target the insect vectors, not the pathogen *Xylella fastidiosa (Xf)*. There are no antibacterial compounds effective in planta against the *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 will be a useful tool to be adopted by growers to manage PD.

We have tested a novel nano-size formulation ("Zinkicide®") against a different vascular bacterial pathogen in citrus. That formulation showed effective reduction of Huanglongbing

(HLB) symptoms in citrus in a field trial ongoing for five years. We have carried out preliminary tests of the same formulation against *Xf* in the greenhouse with promising results in blueberry and tobacco. In our experiments, Zinkicide® significantly reduced symptoms and pathogen populations. With this preliminary information, we proposed to modify the chemical composition of the nano-formulation to improve performance against *Xf* at lower doses and to test it in grapes. New nano formulations are being developed and will be tested soon. We hope to conduct experiments with grapes infected with *Xf* and treated with different nano formulations next year.

View the full report at bit.ly/3LuNzs6 and watch the presentation at youtu.be/5GSbsamZz8Y.

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

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

We are testing for potential cross-graft protection of a Pierce's disease (PD) susceptible Chardonnay 04 scion against the development of PD symptoms by expression of dual combinations of five previously identified PD suppressive transgenes in two adapted rootstocks. We are assessing both potential cross-graft protection of the non-transformed scion and the effect of the transgenes to protect the rootstocks against downward bacterial movement into the perennial tissue and plant death compared to equivalent combinations of untransformed rootstock/scion control combinations.

The experiment protocol include planting in a regulated field area, training the plants to commercial standards, and mechanically inoculating the plants with pathogenic *Xylella fastidiosa (Xf)*. The data to be collected will evaluate both disease and yield components in the PD susceptible scions. The first inoculation with *Xf* was completed in July 2021 and subsequent laboratory testing of random canes confirmed the bacteria were established in inoculated canes.

View the full report at bit.ly/3oyv91d and watch the presentation at youtu.be/RUU_U3OqqxQ.

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 is for direct support of 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 includes mechanical inoculation of *Xylella fastidiosa (Xf)* that was used successfully in past field experiments. PD symptoms, bacterial movement, and fruit yield will be measured during the experiment.

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. This project funds the costs of planting, training the plants to commercial standards, and all field costs associated with tilling, pest management, irrigation and other requirements dictated by the federal permit.

View the full report at bit.ly/34jV8GN.

Optimizing Biological Control of Pierce's Disease with *Paraburkholderia phytofirmans*

Project Leaders and Cooperator: Steven Lindow and Clelia Baccari, University of California, Berkeley and Caroline Roper, University of California, Riverside

A naturally occurring *Paraburkholderia phytofirmans* strain has been identified that grows and moves extensively within mature grape plants and greatly reduces disease severity when applied to plants either before, or even several weeks after pathogen inoculation in both greenhouse and in large field studies. This strain appears to induce disease resistance in the plant, causing eradication of the pathogen.

A variety of studies of the temporal and spatial patterns of movement of the biological control agent within the plant and of the resistance reaction by the plant to the presence of this beneficial bacterium are being undertaken to addresses the question of when and how a limited number of applications of *P. phytofirmans* might best be applied in field settings to control Pierce's disease. Initial studies reveal that inoculation of plants with P. phytofirmans from three weeks before inoculation with the pathogen to up to six weeks after inoculation with the pathogen provide equally great reductions in disease severity.

These findings suggest that a single application each year may provide substantial reduction in the likelihood of infection by the pathogen. Spray application of *P. phytofirmans* appears superior to that of direct inoculation into the plant, also indicating that biological control of disease with *P. phytofirmans* can be achieved by relatively simple application methods.

Given that this well-studied biological control agent is a naturally occurring strain recognized as a beneficial organism, the regulatory requirements for its commercial adoption should be relatively modest.

View the full report at bit.ly/3B5rM5Y and watch the presentation at youtu.be/0DaW7XimRwk.

Geographic Distribution of Isolate Virulence in *Xylella fastidiosa* Collected from Grape in California and its Effect on Host Resistance

Project Leaders and Cooperators: Rachel P. Naegele, Lindsey P. Burbank, and Rodrigo Krugner, United States Department of Agriculture, Agricultural Research Service; Leonardo De La Fuente, Auburn University; and Rodrigo P.P. Almeida, University of California, Berkeley

Multiple isolates of the pathogen *Xylella fastidiosa (Xf)* from California were screened for differences in severity of disease they cause in grapevine both in the greenhouse and in field-grown plants. Tobacco plants were also used as a model host plant so that more *Xf* isolates could be screened in a shorter period of time. In both tobacco and grapevine, differences were observed in disease severity caused by different *Xf* isolates.

Virulence is not necessarily correlated with geographic region of isolation, as more and less-virulent isolates were obtained from the same region. Particularly in field-grown grapevines, variability in disease between plants inoculated with the same isolate can be as high as between isolates, suggesting that other plant and environmental factors are equally important in determining disease outcomes in the field. Three different sources of Pierce's disease resistance all showed consistently low disease across multiple isolates of *Xf.*

View the full report at bit.ly/33cncWA.

Generating Pierce's Disease Resistant Grapevines Using CRISPR/CAS9 and Traditional Transgenic Approaches

Project Leaders: Caroline Roper, University of California, Riverside and Dario Cantu, University of California, Davis

Through advanced molecular techniques, we have identified a piece of *Xylella fastidiosa (Xf)* on its cell surface that acts as a strong elicitor of the grapevine defense response. Using this piece, we have stimulated the grapevine's immune system and determined which grapevine genes are involved in protecting the vines from future encounters with *Xf.* This protection includes significantly less bacterial colonization and significantly less disease symptoms in the vines. The goal of the proposed work is to use the information about the grapevine immune response to *Xf* to generate Pierce's disease-resistant vines.

View the full report at bit.ly/3uPz8c4.

Development of a Gene Editing Technology for Grapevines Using Plant Protoplasts

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

We have previously established a protocol to generate protoplasts from grape tissue and stimulate these protoplasts to reform whole plants. In this project, we plan to use this methodology to edit the genetic code of grape plants by treating them with DNA or Ribonucleoproteins (RNPs). We first used DNA called mCherry to test if we could successfully introduce DNA into the "naked" plant cell. Using this test DNA, we optimized the parameters for getting DNA into the protoplasts. Next, we used these parameters to introduce DNA into the protoplasts to target a gene that when edited will result in dwarf plants.

We have identified our first successfully edited plants using this technique. When using DNA, a subset of the edited plants will be transgenic, since the CRISPR-Cas9 DNA can become incorporated into the cells genetic code. However, since RNPs are proteins and not DNA, no insertion of foreign DNA into the cells genetic code is possible and therefore the resulting edited plants will not be transgenic (non-GMO). We will be testing the use of RNPs to edit grape plants during the coming year. Successful editing and recovery of plants from grape protoplasts opens up a wide array of options for improving grape varieties for disease resistance, agronomic traits or quality improvement.

View the full report at bit.ly/3rKaNCN and watch the presentation at youtu.be/Gujc3QHAD2Q.

Breeding Pierce’s Disease Resistant Winegrapes

Project Leader and Cooperator: Andrew Walker and Alan Tenscher, University of California, Davis

We have made rapid progress breeding Pierce's disease (PD) resistant winegrapes through aggressive vine training, marker-assisted selection, and our rapid greenhouse screen procedures. These practices allowed us to produce four backcross generations with elite *Vitis vinifera* winegrape cultivars in our first 10 years. We have screened through thousands of seedlings that were 97% *V. vinifera* with the PdR1b resistance gene from *V. arizonica* b43-17 and selected only the most promising. Routinely, we first selected for fruit and vine quality and then moved the best to greenhouse testing, where only those with the highest resistance to *Xylella fastidiosa (Xf)*, after multiple greenhouse tests, were advanced to multi-vine wine testing at Davis and in PD hot spots around California. The best of these have been planted in vineyards at 50 to 1,000 vine trials with enough fruit for commercial scale winemaking.

We have sent 20 advanced scion selections to Foundation Plant Services (FPS) over the past five winters to begin the certification and release process. Five of them are available from certified grape nurseries and their patents granted. Five PD resistant rootstocks based on PdR1b were also sent to FPS for certification. Over 500 seedlings from our PdR1 x PdR2 stacked line have been evaluated with the four most promising sent of FPS for certification and multi-vine trials established for small lot winemaking. Stacking of PD and powdery mildew resistance is well underway.

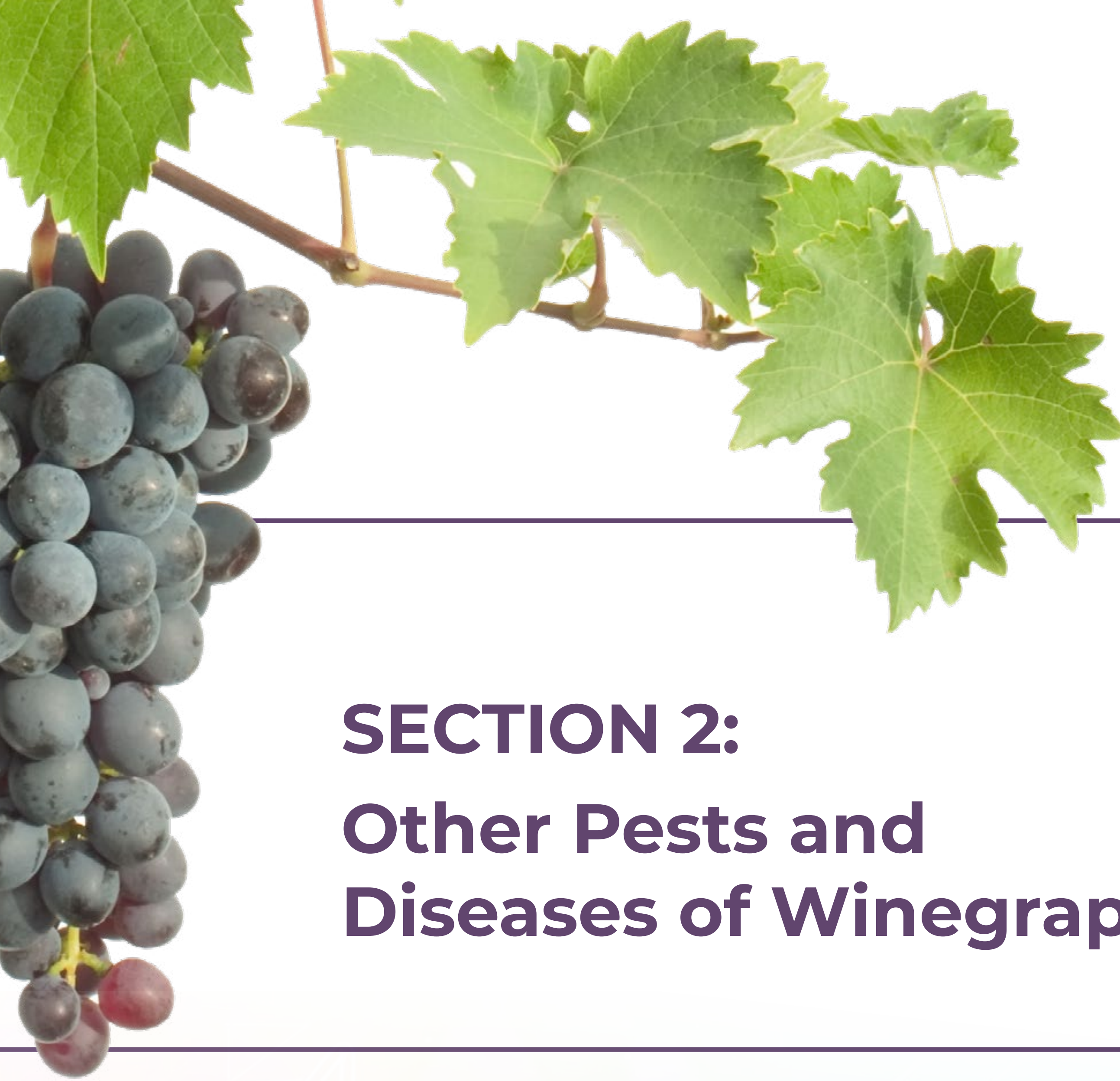
View the full report at bit.ly/3JmuTLU and watch the presentation at youtu.be/jW7-S1abCY8.

Molecular Breeding Support for the Development of Pierce’s Disease Resistant Winegrapes

Project Leader and Cooperators: Andrew Walker, Dario Cantu, Summaira Riaz, and Cecilia Agüero, University of California, Davis

This project provides molecular genetic support to the Pierce's disease (PD) resistant winegrape breeding program by providing mapping and marker development. It also identifies new sources of PD resistance, and studies the genetic diversity of the southwestern United States and Mexican grape species and how they resist PD. We completed greenhouse screening, marker testing and quantitative trait locus (QTL) analysis of breeding populations from 13 new resistance sources.

View the full report at bit.ly/3rQ81fi.



SECTION 2:
Other Pests and Diseases of Winegrapes

Monitoring Grapevine Red Blotch Virus at Russell Ranch Foundation Vineyard

Project Leaders and Cooperators: Maher Al Rwahnih, Neil McRoberts, Deborah Golino, and Vicki Klaassen, University of California, Davis; Kent Daane, University of California, Berkeley; and Houston Wilson, University of California, Riverside

Establishing new vineyards with virus-tested, certified planting material will continue to be an essential component of Grapevine Red Blotch Virus (GRBV) control. However, the introduction of GRBV at Russell Ranch from outside sources demonstrates that starting with clean planting material may not be enough to stop GRBV from entering vineyards at some point. Once GRBV has been introduced into vineyards, our work indicates that spread can be rapid with annual rates up to 18%.

GRBV distribution within relatively newly infected vines is highly uneven, making it difficult to detect. These vines almost certainly contribute to false negative test results in any given year's testing and could serve as inoculum sources for transmission that same year if a vector is present. Determining how quickly these infections can be reliably detected requires field transmission experiments with a known vector.

We hope to have a more accurate estimate of annual spread rates in areas of highly aggregated GRBV infections as we continue to test the sentinel vines. To date, we have not detected GRBV in these vines. However, during sampling in October and November 2021 we did notice girdled petioles, which indicates that threecornered alfalfa hopper is present and feeding on these vines.

While it's clear that GRBV has continued to spread at Russell Ranch, we did not gain any specific insights on a vector from this work. Spatial-temporal analyses for 2017-2020 indicates that spread is occurring via a vector that is more mobile than mealybugs.

View the full report at bit.ly/3r2EbJv.

Genomics Resources for Identification, Tracking, Surveillance, and Pest Management of Vine Mealybug in Vineyards

Project Leaders and Cooperators: Lindsey Burbank, Rachel Naegele, and Mark Sisterson, United States Department of Agriculture, Agricultural Research Service; Dario Cantu, University of California, Davis; and Kent Daane, University of California, Berkeley

Continued use of chemical control for insect pests such as mealybugs is likely to lead to 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 development of new pest control technologies. 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 prevalence of insecticide resistance, and develop new pest control technologies based on novel genetic targets.

A high-quality vine mealybug reference genome was created from single insect DNA extraction. Work is ongoing to annotate the reference genome using RNA sequence 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.

View the full report at bit.ly/33euhpq.

A Study on the Impact of Individual and Mixed Leafroll Infections on the Metabolism of Ripening Wine Grape Berries

Project Leader and Cooperators: Dario Cantu, Susan Ebeler, Maher Al Rwahnih, Deborah Golino, Amanda Vondras, Larry Lerno, Melanie Massonnet, Andrea Minio, Adib Rowhani, Dingren Liang, Jadran Garcia, Daniela Quiroz, and Rosa Figueroa-Balderas, University of California, Davis

This study provides novel insight into and generated novel hypotheses concerning the regulation of responses in grapevine elicited by Grapevine Leafroll-Associated Viruses (GLRaVs). In conducting the study in two consecutive years, the authors report the reproducible responses to GLRaVs. This work alone is insufficient to recommend the use of one rootstock or another, but the disparity in sensitivity and symptom severity observed in berries from Cabernet Franc vines grafted to different rootstocks suggests that rootstock selection should be further explored as a strategy to mitigate some of the negative consequences of leafroll virus infections, should vectors of the virus encroach upon a vineyard.

View the full report at piercesdisease.cdfa.ca.gov/.

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 L. Cooper, Malcolm B. Hobbs, and Larry Bettiga, University of California Cooperative Extension; Stephanie Bolton, Lodi Winegrape Commission; and Michelle A. Moyer, Washington State University

Grapevine Leafroll Disease and Grapevine Red Blotch Disease are consequential viral diseases of grapevine that are actively managed by wine grape industry professionals in the western United States. Uptake and implementation of management practices varies among growers and across regions. To understand why, we conducted a survey and interviews with growers in California and Washington and collected feedback from decision-makers on educational resources.

Economic, technical, and social-behavioral factors influence the adoption of management practices for grapevine leafroll and red blotch diseases. The cost of practices—in time, labor, and outlay—are important considerations, as are production demands and salability of product. Specifically, yield, quality and grape pricing were contributing factors. 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 adoption of management practices.

View the full report at bit.ly/3GFYbR7.

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, University of California Cooperative Extension; Tom Shapland, Tule Technologies; Rodrigo P.P. Almeida, University of California, Berkeley; Kar Mun Chooi, The New Zealand Institute for Plant and Food Research; and Keith L. Perry, Cornell University

Since Grapevine Leafroll Disease (GLD) and Grapevine Red Blotch Disease (GRBD) are incurable, mitigation efforts to reduce spread include (a) sourcing virus-screened plant material; (b) removing diseased vines individually (roguing) or redeveloping high-incidence blocks; and (c) reducing vector populations. Successful roguing requires accurate identification of diseased vines, which can be challenging when symptoms are confusing, asynchronous, or absent (such as in white-berried cultivars).

This project seeks to increase the accuracy of visual assessments and improve vine removal efforts using artificial intelligence (AI) and an “in-house” assay. We will also address fundamental questions of GRBD ecology by harnessing the power of grower-collected data. Regional monitoring of the disease and vector, combined with network-based learning, will address uncertainties in GRBD epidemiology and management. Educational opportunities will include field days, workshops, seminars, and networking groups. Educational products will include handouts and instructional videos. This project will advance the use of emerging technologies to identify diseased vines and support grower-coordinated efforts to reduce the economic and environmental impacts of GLD and GRBD for the grape industry.

Visit the project website at bit.ly/ucce-red-blotch and view the full report at bit.ly/3HLLLbS.

Developing a GMO-Free RNA Interference Approach to Mitigate Red Blotch Negative Impacts on Grape Berry Ripening

Project Leader and Cooperators: Laurent Deluc and Jeffrey Nason, Oregon State University; Keith Lloyd Perry, Cornell University; Robert Martin, United States Department of Agriculture; and Denise Dewey, Oregon Wine Research Institute

RNA interference (RNAi) is a conserved biological response across living organisms (animal or plant cells) initiated by the presence of double-stranded RNA molecules from various pathogens, including viruses. The RNAi mechanism initiated in the plants will lead to a cascade of molecular events that are meant to repress the activity of the virus and its propagation within the plant. Once infected, the plants will recognize and produce specific nucleic regions of the viral genome to activate the RNA silencing machinery. These regions are named "hot spots."

The main goal of this project is to identify these "hot spots" of the grapevine red blotch virus. In the long-term, this knowledge could help develop innovative technology tools like ectopic RNA molecule application in vineyards to mimic the virus's presence and make the plants immune or "primed" to further infections like a vaccine will do. The team expects to have all the data necessary by the end of winter and to have the "hot spots" identified by the end of spring 2022.

View the full report at bit.ly/3Jnejc8.

Ecology of Grapevine Red Blotch Virus

Project Leaders and Cooperators: Marc Fuchs and Keith Perry, Cornell University and Deborah Golino, University of California, Foundation Plant Services

Grapevine Red Blotch Virus (GRBV), the causal agent of Grapevine Red Blotch Disease, is transmitted by grafting and by the threecornered alfalfa hopper, although this insect is not a pest of grape. Our research shows the threecornered alfalfa hopper's active role in transmitting GRBV in the vineyard. We documented 10 days of insect exposure to infected grapevines are necessary for the virus to be transmissible to healthy grapevines. We then revealed transmission of red blotch virus from and to free-living grapevines by the threecornered alfalfa hopper. Next, the ability of the threecornered alfalfa hopper at transmitting GRBV in the vineyard was shown as early as three-months post-exposure of insects carrying the virus to healthy grapevines in an experimental vineyard. Test vines that became infected via vector-mediated inoculation are continuously monitored, as they have not exhibited disease symptoms yet.

Additionally, an accurate, cheap, and user-friendly GRBV diagnostic assay was developed and validated with winegrape growers in Napa and Sonoma counties. Some of these growers have adopted the assay for on-site diagnosis to determine GRBV incidence in vineyards. Research progress and information on disease ecology was communicated to grower communities.

View the full report at bit.ly/3Bicsmc.

Grapevine Fanleaf Virus-Host Interactions for Disease Symptom Development

Project Leader and Cooperator: Marc Fuchs, Cornell University and Deborah Golino, University of California, Foundation Plant Services

Grapevine Fanleaf Virus (GFLV) causes fanleaf degeneration, one of the most detrimental virus diseases of grapevine. This virus is managed in diseased vineyards through the use of rootstocks that are resistant to *Xiphinema index*, the ectoparasitic nematode vector 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 get infected eventually. Therefore, there is a need to explore innovative disease management strategies. We will investigate how GFLV interacts with its plant hosts and how this knowledge could lead to the development of new disease management options by altering the expression of key plant genes that might be indispensable for the virus to complete its infectious cycle.

View the full report at piercesdisease.cdfa.ca.gov/.

Resistance to Grapevine Leafroll Virus 3 and its Major Mealybug Vectors

Project Leaders and Cooperators: Marc Fuchs and Greg Loeb, Cornell University and Deborah Golino, University of California, Foundation Plant Services

The objective of our research is to develop grapevines resistant to Grapevine Leafroll-Associated Virus 3 (GLRaV-3), the dominant leafroll virus in diseased vineyards, and to the grape mealybug and the vine mealybug, the two most important vectors of GLRaV-3 in California vineyards, using RNA interference (RNAi).

Preliminary results showed that mortality of grape mealybug nymphs significantly increased after feeding on an artificial diet supplemented with double strand RNA (dsRNA) contructs against mealybug genes. Predictive modeling suggested that one of the dsRNA constructs against the grape mealybug is unlikely to provide any cross-reactivity against the vine mealybug. Therefore, we engineered a new dsRNA construct against the counterpart gut gene of the vine mealybug. This new RNAi construct was identified and characterized from dissected gut tissue of vine mealybugs established on winter squash in a growth chamber. The integrity of this new dsRNA construct was validated. It will be used in combination with the three dsRNA constructs from the grape mealybug for optimal RNAi efficacy.

For resistance to GLRaV-3, target dsRNA constructs were developed and used in grape transformation experiments for the recovery of stable transformants. Some transgenic rootstock 110R and 101-14 and *Vitis vinifera* cultivars Cabernet franc and Pinot noir were obtained and characterized. Transformation efforts are pursued by using stacked dsRNA constructs against the mealybugs and GLRaV-3, and by directing their expression in the phloem tissue using a tissue specific plant promoter. The production of grape plants stably transformed with dsRNA constructs against GLRaV-3 and the two mealybug pests is advancing.

View the full report at bit.ly/3HRqEF9.

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

This project will provide new important information and help with development of contemporary strategies and management approaches for the Grapevine Leafroll Disease and could be directly applicable against other important grapevine viruses such as Grapevine Red Blotch Virus. We will use grapevine virus-based RNA interference (RNAi) approach to target Grapevine Leafroll-Associated Viruses (GLRaVs). We have successfully developed a vacuum infiltration method to efficiently deliver DNA expression plasmids and viral infectious clones into grapevine plants. We have successfully developed two grapevine virus infectious clones, grapevine geminivirus A and grapevine virus A, that give asymptomatic infections in greenhouse grown grapevines. We will use these two viral vectors to deliver and enhance the RNAi efficacy in grapevine rootstocks and scions against GLRaVs. After identifying the best insertion sites in our infectious clones, we will start to construct RNAi inducers targeting grapevine leafroll viruses and test for efficacies.

View the full report at bit.ly/3rJTa5P.

Investigating the Impact of Grapevine Red Blotch Virus 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

Our research indicates that Grapevine Red Blotch Virus (GRBV) causes a delay in ripening events in grapes, leading to significant decreases in sugar accumulation, color development, and aroma compound accumulation. In addition, our work has indicated that GRBV affects key metabolic pathways that are responsible for the production of compounds (phenolics) that are important to the color, flavor, and mouthfeel of a final wine. 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.

Our current research indicates that grapes infected with GRBV have significantly higher quantities of pectin (acidic heteropolysaccharide groups located in the cell wall) and soluble proteins. Previous studies have shown that higher levels of pectin and soluble pathogenesis-related (PR) proteins result in binding reactions to phenolic compounds such as tannin. Therefore, studying the impact of GRBV on PR proteins, cell wall composition, and cell wall enzymatic processes will 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 bit.ly/33T9S2T.

Effects of Grapevine Red Blotch Disease on Flavor and Flavor Precursor Formation in the Grape and on Wine Quality

Project Leaders and Cooperators: Michael Qian, Alexander D. Levin, James Osborne, Elizabeth Tomasino, and Achala KC, Oregon State University; Michael Moore, Quail Run Vineyards; and Randy Gold, Proprietor, Pacific Crest Vineyard Services

Two irrigation treatment main plots were randomized in two blocks of fields and characterized by varying water application rates (wet and dry) on both red blotch infected (RB+) and non-infected (RB-) Pinot noir grapevines. A wet treatment was irrigated at 100% estimated crop evapotranspiration (ETc) and dry treatment was irrigated at 66% ETc. The impact of grapevine red blotch disease on grape and wine quality was studied across three years. Pinot noir grapes were collected during berry ripening until the harvest (one week after harvest in 2019 and 2020) from RB+ and RB- grapevines. Wines were made from Pinot noir grapes with treatments included D+ (dry treatment on RB+ grapevines), D- (dry treatment on RB- grapevines), W+ (wet treatment on RB+ grapevines), and W- (wet treatment on RB- grapevines) conducted through 2018 to 2020.

Berry maturity parameters, berry free and bound form C13-norisoprenoids, wine anthocyanins, phenolics, and flavor profiles were investigated. The results indicated that infected grapes had a lower level of total soluble solids at harvest. Wines made from infected grapes showed lower total phenolic content compared to wine from non-infected grapes. Certain volatile compounds can be affected by both the health status and irrigation treatments of grapevine. Wet treatment may enhance the levels of some volatile compounds in RB+ wines based on the result shown in 2019 and 2020. The patterns were not consistent throughout the years, which suggested that vintage was also an important factor in the volatile wine profile.

View the full report at bit.ly/3BhPnzW.

Structure-Function Studies on Grapevine Red Blotch Virus to Elucidate Disease Etiology

Project Leaders and Cooperators: Christopher D. Rock and Sunitha Sukumaran, Texas Tech University; David Tricoli, University of California, Davis; Rhonda Smith, University of California Cooperative Extension; and Achala KC, Oregon State University

This project aims to establish proof of concept that genetic modification of genes involved in Grapevine Red Blotch Virus (GRBV) expression may change the disease etiology. The research targets diffusible signals (viral suppressor proteins) and host target small RNAs directed to GRBV C2 and V2 genes, and aims to develop chemical inducers of host resistance. It is likely that microRNA and trans-acting small interfering RNAs generated from hairpin viral suppressor targets operate systemically by moving through vasculature. If proven, this research has applications to develop genetically engineered grapevine rootstocks for GRBV resistance to use with non-genetically modified organism grafted scions.

View the full report at bit.ly/3JwsJXv.

Improved Understanding of Virus Transmission of Grapevine Red Blotch Virus

Project Leader: Vaughn Walton, Professor, Oregon State University

It was previously believed that treehoppers are the key insect species transmitting Grapevine Red Blotch Virus (GRBV). Our work was conducted with extreme rigor, ensuring that there was no cross contamination of insect tissue or feces on plant tissue, possible sources of contamination and false positive tests. Our data illustrate that it is possible for the threecornered alfalfa hopper to vector GRBV, but that this transmission happens in rare cases. None of the other tested treehopper species were able to vector GRBV to grapevines. These data therefore point to the fact that there has to be other arthropod species possibly vectoring GRBV.

View the full report at bit.ly/36dSBJk.

Biology and Role of Treehoppers in Grapevine Red Blotch Disease

Project Leaders and Cooperators: Frank G. Zalom, Mysore R. Sudarshana, Kaan Kurtural, Cristian Olaya, Teresa Erickson and Michael Bollinger, University of California, Davis; Kent Daane, University of California, Berkeley; Rhonda Smith, Cindy Kron, Lynn Wunderlich, and Cindy Preto (Kron), University of California Cooperative Extension; and Vaughn Walton, Oregon State University

The results of this project added significant new knowledge towards better understanding the role of the threecornered alfalfa hopper and other vineyard treehoppers in the epidemiology of Grapevine Red Blotch Virus (GRBV), including management of virus spread, by determining feeding on grapevines seasonally and their phenology in relation to cover crops and non-crop vegetation in and around vineyards. Possible transmission by other treehoppers, planthoppers, and phloem-feeding leafhoppers found in vineyards where GRBV is spreading has also been studied. This essential information will contribute to the management of grapevine red blotch disease by cultural methods such as reducing plant hosts favorable to sustaining vector populations or precise treatment timings based on treehopper biology in vineyards, and when transmission is most likely to occur.

View the full report at bit.ly/3LwxguF.