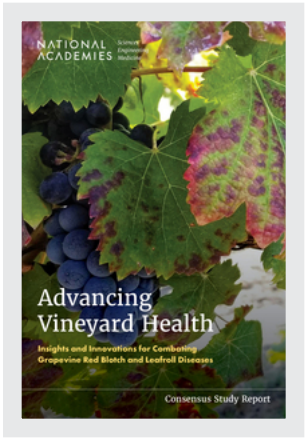

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Committee on Assistance to the California Department of Food and Agriculture Pierce's Disease/Glassy-Winged Sharpshooter Board on Grapevine Viruses and Grapevine Disease Research

Board on Agriculture and Natural Resources

Division on Earth and Life Studies

Consensus Study Report

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COMMITTEE ON ASSISTANCE TO THE CALIFORNIA DEPARTMENT OF FOOD AND AGRICULTURE PIERCE'S DISEASE/GLASSY-WINGED SHARPSHOOTER BOARD ON GRAPEVINE VIRUSES AND GRAPEVINE DISEASE RESEARCH

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Reviewers

This Consensus Study Report was reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise. The purpose of this independent review is to provide candid and critical comments that will assist the National Academies of Sciences, Engineering, and Medicine in making each published report as sound as possible and to ensure that it meets the institutional standards for quality, objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process.

We thank the following individuals for their review of this report:

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Although the reviewers listed above provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations of this report nor did they see the final draft before its release. The review of this report was overseen by **JEFFERY DANGL (NAS)**,[2] University of North Carolina, and **DONALD ORT (NAS)**,[3] University of Illinois. They were responsible for making certain that an independent examination of this report was carried out in accordance with the standards of the National Academies and that all review comments were carefully considered. Responsibility for the final content rests entirely with the authoring committee and the National Academies.

[1] The National Academy of Sciences.
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[3] The National Academy of Sciences.

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Preface

Grapevine red blotch disease (GRBD) and grapevine leafroll disease (GLD) are growing threats to the California wine and wine grape sector, which contributes $73 billion annually to the state's economy. Our committee was charged with analyzing the current state of GRBD and GLD knowledge and identifying key areas where additional research efforts could reduce the spread and economic impacts of these diseases. During visits to wine grape growing regions, we saw firsthand the impact of these diseases on this important crop. Entire fields and even growing regions of wine grapes displayed the characteristic leaf reddening symptoms, providing a striking demonstration of the extent of the problem. Meetings with growers from multiple regions also highlighted the need for new control measures as growers expressed frustration over the rapid spread of these diseases, even in newly planted vineyards, and the resulting loss in quality of the product. Given that these viral diseases not only reduce yields but also affect sugars and other aspects of fruit quality relevant to wine flavor profiles, an additional complication is that, due to the complexity of the processing and aging winemaking involves, it can potentially take years to see the true impact of the disease on the final product.

Control of vector-borne viral diseases like GRBD and GLD is complicated by several factors including the tripartite interaction between the plant host, insect vector, and viral pathogen; the fact that vectors can spread between vineyards and wild areas unhindered; and the lack of effective curative measures for use in the field. The two viruses that are the focus of this study, grapevine red blotch virus (GRBV) and grapevine leafroll-associated virus 3 (GLRaV-3), share some similarities but also have distinct biological features. GLRaV-3 is an ongoing threat to wine grape production in California and globally. An increase in leafroll disease pressure in California was associated with the introduction of the invasive vine mealybug, *Planococcus ficus*, which is an effective vector and has high reproductive capacity, although other mealybugs are effective vectors and have importance in some wine grape production areas of California. While GLD is an existing and increasing threat to California wine grape production, red blotch is a more recently identified virus disease that needs further characterization at the molecular and ecological levels. In contrast to the mealybug vectors of GLRaV-3, the treehopper vector of GRBV appears to have a transient association with wine grapes. Control of both pathosystems requires detailed knowledge of vector biology and strategies for effective areawide pest management. For this report, we have attempted to identify commonalities and areas where control efforts extend to both systems, as well as distinctive features and areas of needed research that require further inquiry and unique interventions.

Our committee's approach to this study included extensive information gathering sessions that involved site visits to vineyards, nurseries, and clean plant centers and meetings with growers, diverse scientists, and extension specialists. We thank the many people that contributed to the report by hosting us, providing space for meetings and tours, or sharing their knowledge. We have acknowledged the expert scientists that addressed the committee's questions in Appendix B.

We owe an enormous thanks to the Study Director, Dr. Camilla Yandoc Ables. Her extensive knowledge of plant pathology, expert guidance, and friendly demeanor enabled the committee to complete the challenging task of addressing the needs for two different pathosystems. Dr. Ables' expert management skills created a supportive and open environment

that enabled the committee to focus on the task. We also thank Samantha Sisanachandeng, Senior Program Assistant, for her assistance with meetings throughout the study and her positive and friendly attitude, which made the work of the committee go smoothly. We thank Robin Schoen, Director of the National Academies Board on Agriculture and Natural Resources for astute and thoughtful advice throughout the study. We also recognize the significant efforts of the California Department of Food and Agriculture Pierce's Disease/Glassy-Winged Sharpshooter (PD/GWSS) Board representative Matthew Kaiser and consultant Kristin Lowe, who answered many committee questions and facilitated necessary meetings.

In closing, we extend an enormous thanks to the members of the committee. The assembled team worked for more than 18 months to address three tasks. They delved into the literature, drew from their own experiences, and explored new scientific realms to document what is known about GRBD and GLD and what might be possible for their effective control. Throughout the study, they gave their time as volunteers, and they all contributed to creating a collegial and supportive environment that made this study an enriching experience scientifically and personally. The committee was motivated by the goal of providing tangible and forward-thinking solutions for these emerging diseases, and this common goal and mutual respect enabled sustained energy and focus during the study. We speak for the committee when we express hope that the science-based and experience-informed findings, conclusions, and recommendations in this report will provide the PD/GWSS Board with a pathway toward controlling vector-borne viruses of grapevines.

Anna E. Whitfield, *Chair*
Alexander V. Karasev, *Vice Chair*
Committee on Assistance to the California
Department of Food and Agriculture Pierce's
Disease/Glassy-Winged Sharpshooter
Board on Grapevine Viruses and Grapevine Disease Research

Acronyms and Abbreviations

AAP	acquisition access period
ACP	Asian citrus psyllid
AWM	areawide pest management
BYV	beet yellows virus
CDFA	California Department of Food and Agriculture
CP	coat protein
CRISPR/Cas12a	clustered regularly interspaced short palindromic repeats (CRISPR)-associated 12a
CTV	Citrus tristeza virus
DMS	differential mobility spectrometry
ELISA	enzyme-linked immunosorbent assay
EN	electronic nose
EPF	entomopathogenic fungi
EVCWG	Emerging Viruses in Cucurbits Working Group
FPS	University of California Davis Foundation Plant Services
GLD	Grapevine leafroll disease
GLRaVs	Grapevine leafroll-associated viruses
GLRaV-3	Grapevine leafroll-associated virus 3
GRBD	Grapevine red blotch disease
GRBV	Grapevine red blotch virus
HLB	Huanglongbing
HSP	heat shock protein
HTS	high throughput sequencing
IAP	inoculation access period
IPC	individual protective cover
IPM	integrated pest management
LAMP	loop-mediated isothermal amplification
NCPN	National Clean Plant Network
ORF	open reading frame
PCR	polymerase chain reaction

PD	Pierce's disease
PD/GWSS Board	Pierce's Disease/Glassy-Winged Sharpshooter Board
QGB	quintuple gene block
RACE	random amplification of complementary DNA ends
RCA	rolling circle amplification
RdRP	RNA-dependent RNA polymerase
RFP	Request for Proposals
RGB	replication gene block
RNAi	RNA interference
RPA	recombinase polymerase amplification
TCAH	three-cornered alfalfa hopper
TSWV	tomato spotted wilt virus
UV-C	ultraviolet light
VOC	volatile organic compound

Glossary

Acquisition access period	Total time that an insect vector has been kept on the infected plant to acquire the virus
Acquisition	The uptake of virus by an insect vector from an infected source
Anthocyanins	Water-soluble compounds (flavonoids) that provide red, magenta, purple, and blue color to the fruit and flowers of many plants
Areawide pest management	An approach for reducing pests by uniformly applying pest mitigation measures over geographical areas instead of using a field-by-field approach
Bayesian Belief Network	A probabilistic graphical model that captures both conditionally dependent and conditionally independent relationships between random variables; it is employed to infer and estimate the likelihood of causal or subsequent events
Biostimulant	Any substance or microorganism applied to plants to enhance nutrition efficiency, abiotic stress tolerance, and crop quality
Circulative, non-propagative transmission	Viral transmission characterized by longer acquisition and inoculation access periods (hours to days) and longer retention time in the body of the vector
Clade	A group of organisms believed to have evolved from a common ancestor
Closterovirus	Genus of phloem-associated RNA viruses in the family *Closteroviridae*
Coat protein	The protective outer shell of a virus particle (also referred to as capsid)
CRISPR	Clustered regularly interspaced short palindromic repeats, a technology used to selectively modify the DNA of living organisms (gene editing)
CRISPR/Cas12a	An RNA-guided endonuclease that forms part of the CRISPR system and is utilized as a genome editing tool (molecular scissor) to selectively modify the DNA of living organisms
Cross-protection	The use of a mild virus strain to infect a plant to protect it from subsequent infection by a more aggressive strain of the same virus that causes severe symptoms/damage
Degree days	Heat units required for crop or insect development
Digital loop-mediated isothermal amplification (dLAMP)	A technique used for sensitive detection of nucleic acid targets in virus diagnosis
Diapause	The period of delayed development in response to adverse environmental conditions

Dimorphic	Condition in which males and females of the same species differ in their morphological characteristics, particularly characteristics that are not directly involved in reproduction
Electronic nose (EN)	An electronic sensing device intended to detect odors or flavors
Enzyme linked immunosorbent assay (ELISA)	A test that detects viral infection through the interaction between antigens (virus protein) and antibodies (blood protein produced in response to an antigen) in a laboratory setting
Endosymbiont	An organism living symbiotically (equal dependency) inside the cells or body of another organism
Etiology	The cause or origin of a disease
Fecundity	The reproductive rate of an organism
Flavonols	A class of flavonoids that serve as building blocks of proanthocyanins that occur in a variety of fruits and vegetables; intake of flavonols is associated with a wide range of health benefits
Geminivirus	A term used to broadly describe members of the *Geminiviridae*, a family of plant viruses that encode their genetic information on a circular genome of single-stranded DNA
Genome editing	A genetic engineering technique in which DNA is deleted, inserted, modified or replaced at site-specific locations in the genome of a living organism
High-throughput sequencing (HTS)	A method involving sequencing multiple DNA molecules in parallel, enabling hundreds of millions of DNA molecules to be sequenced at a time (also referred to as next generation sequencing)
Host factors	The aspects of infectious disease transmission that are inherent in the potential host
Host plant resistance	The inherent ability of a plant to resist infection by pathogens or damage by pests; the mechanisms of resistance to insects are non-preference or anti-xenosis (the host plant produces stimuli that repel pests or fail to produce stimuli that attract pests), antibiosis (the host plant causes injury, death, reduced longevity or reproduction of the pest), and tolerance (the host plant can endure pest damage and yield well despite the damage)
Hyperspectral imaging	A technique that involves the use of an imaging spectrometer (i.e., hyperspectral camera) to collect and process spectral information, allowing for the identification of objects (e.g., infected plants) by analyzing their unique spectral signatures
Imaging spectroscopy	The simultaneous acquisition of spatially co-registered images in many spectrally contiguous bands; this technology includes both hyperspectral imaging and multi-spectral imaging, which differ in the number and the spectra of electromagnetic radiation that each band contains

Immunocapture polymerase chain reaction (IC-PCR)	A virus detection technique that combines serology and nucleic acid amplification by using antibodies to capture viruses out of virus-containing plant extracts as a preparatory step to provide the template for PCR detection, thus resulting in higher virus detection specificity and sensitivity
Incubation period	The time from infection to the first appearance of symptoms
Inoculation access period	The time required for a viruliferous (virus carrying) vector to introduce the virus to a healthy plant
Inoculation	Part of the virus transmission process wherein the virions are delivered by an insect vector (or via other means) to the site of infection
Instar	In arthropods, such as insects, the developmental stage between two successive molts
Interdisciplinary approach	An approach that involves integration of knowledge and methods from different disciplines to create a holistic approach to a problem
Isolate	A virus obtained (isolated) from a single infected host
Latency period	In plants, the interval during the course of a disease between when the plant is infected by a pathogen and when that plant becomes infectious (i.e., becomes source of virus inoculum)
Lateral flow assays	Tests used to detect the presence of a target molecules in a liquid sample without the need for specialized and costly equipment
Long-read sequencing	A DNA sequencing method that produces longer sequence reads (i.e., tens to thousands of kilobases in length); also known as third-generation sequencing
Loop-mediated isothermal amplification (LAMP)	A single-tube technique for DNA amplification that is designed primarily for diagnostics; it involves the formation of magnesium pyrophosphate precipitate as an indicator that amplification has occurred
Mating disruption	An insect pest management technique that uses artificial stimuli (e.g., synthetic sex pheromone) that confuse individuals and disrupt mate location/courtship to block the insect's reproductive cycle
Monopartite	A type of viral particle formed by a single nucleic acid molecule protected by a coat made of proteins (and sometimes also lipids)
Multidisciplinary approach	An approach that involves multiple disciplines working independently to address the same problem
Neonicotinoid	A class of synthetic systemic insecticides derived from nicotine
Non-coding RNAs	Functional RNA molecules that are not translated into proteins
Nymph	An immature stage of an insect that undergoes gradual change until it reaches the adult stage
Open reading frame (ORF)	A portion of a DNA sequence that does not include a stop codon (which functions as a stop signal) and can potentially be translated into a protein

Peptide nucleic acid-locked nucleic acid (PNA-LNA) mediated loop-mediated isothermal amplification (LAMP)	A highly specific method for the detection of low mutant KRAS Q12 and Q13 in a large excess of wild-type DNA
Polymerase chain reaction (PCR)	A temperature-dependent nucleic acid amplification technique used to enzymatically amplify a specific DNA segment in vitro
Polyphagous	Ability of an insect to feed on plants that belong to diverse taxonomic groups
Quantitative polymerase chain reaction (qPCR)	A PCR-based technique (also known as real-time PCR) that allows for monitoring of the amplification of a target DNA segment, thus allowing for its quantification
Random amplification of complementary DNA ends (RACE) assay	An assay that facilitates the amplification of genome segments between a specific internal region and the extremities (5’ or 3’end) of the messenger RNA
Reproductive diapause	A suspension of reproductive functions in adult insects
Resistance (host)	Ability of the plant host to impede or halt the pathogen’s growth and/or development; the ability of the host plant to prevent or reduce damage caused by insect pests. See also host plant resistance
Retention	Part of the virus transmission process wherein the acquired virions are retained at requisite sites within the insect vector
RNA silencing (RNAi)	The process in which RNA molecules are involved in the sequence-specific suppression of gene expression by double-stranded RNA (also referred to as RNAi)
Rolling circle amplification (RCA)	An isothermal enzymatic process in which a short nucleic acid primer is amplified to form a long single-stranded nucleic acid using a circular template and special nucleic acid polymerases
Recombinase polymerase amplification (RPA)	A single-tube isothermal alternative technique to PCR that requires minimal sample preparation and is capable of amplifying as few as 1–10 DNA target copies in less than 20 minutes
Reverse-transcription polymerase chain reaction (RT-PCR)	A technique in which reverse transcriptase enzyme is used to convert RNA to cDNA (i.e., complementary DNA), which is then used as a template for amplification in PCR
Reverse-transcription recombinase polymerase amplification (RT-RPA)	A technique in which a reverse transcriptase enzyme is added to an RPA reaction, enabling it to detect RNA and DNA without the need for a separate step to produce cDNA
Semi-persistent transmission	Mode of transmission wherein plant viruses are retained in the vector foreguts or salivary glands but cannot spread to salivary glands
Serological assay	A test used for identifying viral infections by using antibodies (blood proteins) to specifically react with the antigens (viral proteins) against which the antibodies were produced

Source/sink balance	A conceptual framework for understanding how crop yield is regulated by source activity and sink demand; source organs are photosynthetically active plant parts where carbohydrates (assimilates) originate (typically, sunlit mature leaves but also includes any carbon-exporting organs); sink organs are non-photosynthetic plant parts that do not produce enough assimilates to meet their growth/maintenance requirements (e.g., developing fruits or berries, roots, or immature leaves) and must import assimilates from sources
Squash-blot	A diagnostic technique wherein the tissue of a plant that is suspected to be diseased is crushed onto a membrane (sample), which is then treated with a probe that can bind with the DNA or RNA of the suspect pathogen; subsequent treatment of the bound membrane with other reagents would result in a color reaction if the target pathogen is present or no color reaction if the pathogen is absent in the sample
Tolerance (host)	The ability of the plant host to endure infection by the pathogen or insect infestation without incurring serious damage or yield loss
Transgenic resistance	Resistance to pests, diseases, or environmental stress that is conferred to a plant via genetic engineering (i.e., transferring specific genes from a different species into the plant's genome)
Transtadially	The sequential passage of parasites acquired during one life stage, or stadium, through the molt to the next stage(s) or stadium
Trap crop	A plant that is grown to attract, divert, intercept, and retain pests to reduce damage to the main crop.
Variant	A virus with new mutations (change in genetic sequence)
Vector competence	The ability of a vector to acquire and subsequently transmit a pathogen
Vector population replacement	A strategy for reducing vector competence by replacing existing vectors with genetically modified insects that cannot transmit pathogens
Vector population suppression	A strategy for reducing the insect vector population by releasing sterile males to compete with wild type males for mating
Veraison	The onset of berry ripening in wine grapes when wine grapes change color and start to soften, expand, and become sweet
Virion	A virus particle consisting of an outer protein shell (capsid) and an inner core of nucleic acid (either DNA or RNA)
Virome	The total collection of viruses in and on an organism
Viruliferous	Containing, producing, or conveying a virus
Volatile organic compound (VOC)	An organic substance that easily evaporates at normal temperatures

Summary

Among all cultivated woody perennials, grapevines are known to be infected with the largest number of plant viruses—more than 100 viruses belonging to 21 different families or having similarity to unclassified plant satellite viruses have been reported on *Vitis* germplasm worldwide. Two of these viruses, grapevine red blotch virus (GRBV) and grapevine leafroll-associated virus 3 (GLRaV-3), are considered the primary causal agents of economically important diseases occurring in California and other grape-growing regions: grapevine red blotch disease (GRBD) and grapevine leafroll disease (GLD).

In 2022, the California Department of Food and Agriculture (CDFA) requested the National Academies of Sciences, Engineering, and Medicine to provide guidance to the CDFA Pierce's Disease/Glassy-Winged Sharpshooter (PD/GWSS) Board by convening an ad hoc committee that would conduct three interrelated activities addressing research on GRBV and GLRaV-3. Activity 1 (Review of Proposals Submitted to CDFA PD/GWSS Board) and Activity 2 (Critique of CDFA PD/GWSS Board's Request for Proposals (RFP) and Proposal Selection Process) were completed in 2023. This report addresses Activity 3 (see Box S-1).

BOX S-1
Statement of Task for Activity 3: Review of Current Knowledge on Grapevine Viruses, GRBV and GLRaV-3 Research Outcomes/Gaps and Future Research Approach

The committee will review the state of knowledge about the grapevine red blotch virus (GRBV) and grapevine leafroll associated virus type 3 (GLRaV-3) and the management of diseases they are associated with and develop guidance to the PD/GWSS Board in its efforts to support research that leads to a reduction in the spread of GRBV- and GLRaV-3-associated diseases and their economic impacts.

The committee will examine the scientific literature and gather information from experts, plant health practitioners, and grape growers. The committee will explore recent and current research activities on GRBV and GLRaV-3 and their insect vectors that are funded by the CDFA PD/GWSS Board.

In its review, the committee will identify the following as they relate to GRBV and GLRaV-3:

1. The most significant knowledge gaps in the current understanding of grapevine red blotch disease and grapevine leafroll disease epidemiology;
2. Research areas where significant progress has been/has not been achieved;
3. Research areas that may yield the most promising short- and long-term management solutions;
4. New genetic tools and research platforms that could be used to study grapevine viruses;
5. Opportunities for collaborative research that could accelerate progress in finding grapevine disease management solutions;
6. Other viral pathogen systems (animal and human) that could provide insights or additional research directions; and
7. Opportunities to improve the current CDFA PD/GWSS Board's research review and funding process, and opportunities to draw from a wider range of researchers across various disciplines and fund a wider range of national researchers.

The committee will prepare a consensus report with conclusions from its review, describing what is currently known about GRBV and GLRaV-3; what knowledge is needed to improve management of the diseases caused by these viruses; and the committee's recommendations with respect to a viable approach for supporting research on grapevine viruses.

The committee addressed its task using information gathered from various sources, including published papers, presentations and discussions at webinars and public meetings, and documents provided by CDFA. Based on this information and its deliberations, the committee developed conclusions and recommendations for the consideration of the CDFA PD/GWSS Board and other parties involved in addressing GRBD and GLD. Selected conclusions and recommendations are highlighted in this summary (see Box S-2, S-3, and S-4); full conclusions and recommendations are detailed in the report chapters and in Appendix C.

CURRENT KNOWLEDGE ON GRBD AND GLD AND THEIR ASSOCIATED VIRUSES AND VECTORS

GRBD and GLD occur in red or black- and white-fruited grapevine cultivars, but foliar symptoms vary by cultivar, are less pronounced in white-fruited cultivars, and could be confused with the symptoms of nutritional disorders and other maladies. Because the two diseases cannot be reliably distinguished based on visual inspection alone, definitive diagnosis relies on the detection of GRBV and GLRaVs in the laboratory using nucleic acid-based methods (GRBV and GLRaVs) and serological assays (GLRaVs). Current knowledge about the viruses associated with these diseases and their insect vectors is summarized in Table S-1.

TABLE S-1 Current Knowledge about the Viruses Associated with GRBD and GLD and Their Insect Vectors

Disease	GRBD	GLD
Associated Virus(es)	GRBV	GLRaV-1, -2, -3, -4, -7, and -13
Virus Characteristics	Belongs to genus *Grablovirus*, family *Geminiviridae*; members of this family encode their genetic information on a circular genome of single-stranded DNA	GLD associated GLRaVs belong to three genera in the family *Closteroviridae*; they are composed of monopartite, positive-sense, single-stranded, polycistronic RNA genomes. GLRaVs differ in their genome lengths and in the number and arrangements of their encoded genes
Insect Vectors	Primary insect vector: Three-cornered alfalfa hopper (TCAH; Membracidae); additional insect vectors suspected Mode of virus transmission: Circulative and non-propagative; requires an extended acquisition access period before transmission occurs Host range: Wide; Asteraceae (feeding hosts), Fabaceae (principal breeding hosts) *Vitis* spp. are occasional feeding hosts	Principal vectors of concern (worldwide and in California): Mealybugs (Pseudococcidae) Minor vectors: Soft scales (Coccidae) Mode of virus transmission: Semi-persistent; transmission takes place within a 1-hour acquisition access period and a subsequent 1-hour inoculation access period; no latency period between virus acquisition and transmission
Impact on Grapevine	Interferes with foliar metabolism and metabolite translocation; reduces yield, total soluble solids, and anthocyanin accumulation; alters grape ripening	Reduces grape yield, juice and wine quality, and the productive lifespan of affected vineyards; disrupts photosynthesis and carbohydrate metabolism in symptomatic leaves

KNOWLEDGE GAPS

During the course of the study, the committee identified numerous knowledge gaps and those that need to be addressed sooner are presented in Box S-2 (see Chapter 4 of this report for a full discussion of the knowledge gaps). Box S-2 also contains the high and medium priority research recommendations for addressing knowledge gaps (labeled HP for high priority and MP for medium priority). Research recommended in Chapter 4 would generate information needed for further research/development of other control methods, tools, or strategies.

BOX S-2
Recommended Research to Address Knowledge Gaps to Help with Developing Promising Short- and Long-Term Solutions

GLD Biology, Virus-Host Interactions, and Host Defense Mechanisms

It is generally understood that there is a causal relationship between the presence of GLRaVs and the stronger expression of GLD symptoms in response to GLD in red or black-fruited than in white-fruited grapevine cultivars. The reasons underlying these differences have not been well elucidated. Several knowledge gaps also exist regarding the interactions between GLRaVs and their hosts and host defense mechanisms.

Despite decades of research, knowledge on the genetic and phenotypic complexity of GLD-associated viruses remains limited. Fundamental studies using synthetic biology approaches can be applied to systematically investigate how different GLRaV genotypes influence disease outcomes (Conclusions 4-1, 4-2).

Recommendation 4-1: Support research to generate more information about GLRaV-3 genetic variants that could help guide GLD management.

Recommendation 4-2 (HP): Support foundational research to understand the intrinsic and extrinsic factors contributing to the efficient spread of GLRaV-3, including interactions with other vitiviruses.

Knowledge of the factors required for GLRaV-3 infection and resistance in *Vitis* hosts could create opportunities for developing novel control strategies, but these factors have not been elucidated and the role of non-coding RNAs in grapevine and GLRaV-3 genomes in infection or symptom development also remains unexplored. Further investigations into the extent of GLRaV-3 host range may also generate valuable information that could be exploited for GLD management (Conclusions 4-3, 4-4, 4-5).

Recommendation 4-3 (MP): Support research to identify host factors required for GRLaV-3 infection and resistance in *Vitis* hosts and to investigate the role of non-coding regions of grapevine and GLRaV-3 genomes in infection and symptom development.

Recommendation 4-4: Support research to examine the common and unique responses of red or black- and white-fruited wine grape cultivars to GLRaV-3.

GRBD Biology, Virus-Host Interactions, and Host Defense Mechanisms

GRBV isolates are classified as one of two genetic variants, clade 1 or clade 2. Currently, there is scant evidence regarding the differences between clades in terms of symptom expression, efficiency of transmission by TCAH, or the selection pressures acting on GRBV populations (Conclusion 4-6). Also unknown are any synergistic effects resulting from GRBV co-infection with other viruses, or how mixed infections with other viruses might affect the expression of GRBD symptoms or GRBV fitness.

Recommendation 4-5: Support studies to advance understanding of the epidemiological consequences of GRBV genetic diversity and interactions with other viruses.

There are still gaps in the understanding of the function of the GRBV genome with regard to specific roles of GRBV proteins in plant cells. To date, virions have not been observed in GRBV-infected plants using microscopy, and the lack of a tractable model host that becomes systemically infected with GRBV limits the study of virus gene functions and virus-host interactions (Conclusions 4-7, 4-8).

Recommendation 4-6 (MP): Support research to determine optimal model hosts (e.g., Pixie grapevine and/or herbaceous hosts) to facilitate the study of molecular plant-GRBV interactions and direct research efforts to transfer this knowledge to wine grape cultivars.

Current knowledge about latency and incubation periods after GRBV inoculation, which may vary among grapevine cultivars and under different environmental conditions, is insufficient to inform GRBD management recommendations (Conclusion 4-9).

Recommendation 4-7 (HP): Support research to elucidate latency periods in different cultivars and rootstock-scion combinations, including the time from virus inoculation until vector acquisition, time until symptom expression, and time until the virus is detectable in plant and/or vector tissues.

Effects of Mixed Infections, Environmental Factors, and Rootstock-Scion Interactions

Grapevines can be simultaneously infected with multiple viruses, but how mixed infections affect disease severity and evolution of GRBV and GLRaVs (or GRBD and GLD) has not been thoroughly investigated. The effects of changing climatic conditions and other factors that modulate disease cycles including temperature, humidity, carbon dioxide, ozone, drought, and vineyard management practices on virus-vector-host interactions have not been determined (Conclusions 4-10, 4-11).

Recommendation 4-8: Support research on the effect of mixed infections on GRBV and GLRaV evolution and the diseases they cause, as well as research on the effects of environmental factors, grapevine management practices, and changing climatic conditions on GRBD and GLD virus-vector-host interactions and epidemiology. Industry trends and stakeholder input could be used as a guide for prioritizing scion-rootstock combinations to use in experiments.

A variety of factors including the scion cultivar, genetic background of rootstock, rootstock-scion interactions, virus profile in individual grafted vines, synergistic interactions between co-infecting viruses, and environmental conditions could contribute to the presence and severity of symptoms from GRBD and GLD. Resistant rootstocks along with other control strategies could help to mitigate negative effects of viral diseases in vineyards (Conclusions 4-12, 4-13).

Recommendation 4-9 (MP): Conduct research on the presence and diversity of viral resistance in grapevine rootstocks in virus control strategies.

Recommendation 4-10: Support research to determine the contribution of planting with infected, non-certified vines on virus spread.

Diagnostics and Detection

The lack of affordable diagnostic methods for on-site detection delays timely disease diagnosis and management efforts, allowing the continued spread of GRBV and GLRaVs. There is a need for additional affordable diagnostic tools that can detect GRBV and GLRaV-3 infections early and are suitable for extensive use in commercial vineyards (Conclusion 4-14).

Recommendation 4-11 (HP): Support research to develop any new, simple, and affordable high throughput tests for GRBV and GLRaV-3.

Research to profile plant responses to GRBV and GLRaV-3 (and their vectors) may reveal unique volatile organic compound (VOC) profiles that could establish a basis for the development of hand-held electronic noses or differential mobility spectrometry devices for pathogen detection in the field (Conclusion 4-16).

Recommendation 4-12: Support research to identify VOCs unique to GRBV and GLRaV-3 infection or relevant vector infestations and determine the detection efficiency of VOC-based methods compared with other diagnostic tools.

Remote sensing technology has the potential for remote or in-field diagnosis of GRBD and GLD in individual vines; however, testing the efficacy of this approach will require scalable deployment of remote sensing devices for detection of infected vines in a large-scale area (Conclusion 4-17).

Recommendation 4-13 (HP): Support studies on the use of remote sensing technology to facilitate large-scale and early detection of GRBD and GLD in various tissues of commercial cultivars (including white cultivars) to increase the reliability, specificity, and sensitivity of detection with this technology.

As GRBV and GLRaV-3 continue to evolve in vineyards and non-crop habitats, nucleic acid-based assays used for virus detection will need to be upgraded to enable reliable detection of newly emerged virus variants (Conclusion 4-20).

Recommendation 4-14: Support research to determine the feasibility of using rolling circle amplification or other single-stranded circular DNA detection techniques to help detect GRBV at very low concentrations and for universal GRBV detection.

Recommendation 4-15 (HP): Support research for detecting GRBV and GLRaV-3 with nucleic acid-based methods that can be used in large-scale virus detection in fields.

Currently, the costs associated with sample collection, preparation, and analysis restrict current testing to levels that may not be effective for diagnosing and monitoring virus infected grapevines. Consensus is lacking on the most effective sampling technique and minimum sample size for accurately estimating GRBV and GLRaV-3 prevalence across different vineyard settings, regions, and nursery increase blocks (Conclusion 4-21).

Recommendation 4-16 (HP): Support research evaluating optimal sampling methods and minimum sample size for accurate estimation of GRBV and GLRaV-3 prevalence in vineyards to inform the development of best practices for adopting new technologies and for integrating multiple detection methods to improve accuracy and scale (i.e., using both molecular methods and remote sensing technology).

Standardization and verification by an independent organization(s) are important for enhancing the robustness and reproducibility of diagnostic protocols. To date, laboratory protocols for diagnostic testing of GRBV and GLRaVs have not been standardized (Conclusion 4-23).

Recommendation 4-17 (HP): Support efforts to develop standardized GRBV and GLRaV-3 diagnostic testing protocols that, once verified and certified, could be adopted by all laboratories that provide testing services for nurseries and commercial vineyards.

Vectors

While there are reports about potential additional insect vectors of GRBV, there has not been definitive evidence that other insects in addition to TCAH can transmit GRBV to grapevines (Conclusion 4-25).

Recommendation 4-19 (MP): Support research to identify additional vectors of GRBV using rigorous experimental approaches.

There are gaps in the understanding of GLRaV-3 transmission, particularly about the role of different vector species and their distribution in California; the mechanisms of GLRaV-3 acquisition and transmission; the transmission efficiency of diverse GLRaV-3 isolates; the acquisition, retention, and inoculation periods of all vector species; and how environmental factors influence GLRaV-3 transmission dynamics (Conclusion 4-26).

Recommendation 4-20 (HP): Support research on the mechanisms and timing of acquisition, retention, and transmission of all GLRaV vector species, as well as the influence of environmental conditions and host genotype on GLRaV transmission dynamics.

Additional knowledge gaps for GRBV and GLRaVs include the mechanisms of vector-virus interactions, the effect of the environment on epidemiology, and how mixed infections with multiple viruses might impact transmission. The time required to acquire and transmit these viruses has been examined, but virus localization in the vectors has not been confirmed, and the precise viral retention sites have not been thoroughly characterized; knowledge of these factors would improve understanding of the mode of transmission for GRBV or GLRaV-3. In addition, the roles of vector endosymbionts, genes, proteins, and metabolites mediating transmission have not been studied for GRBV or GLRaVs; this information is needed to understand transmission dynamics and to develop novel tools for disrupting transmission for the management of GLD (Conclusions 4-27, 4-28).

Recommendation 4-21: Support studies to identify interactions between GRBV and GLRaVs and their vectors that are required for transmission, as well as studies to identify genes, proteins, and metabolites involved in virus transmission to develop control strategies based on interference of virus-vector interactions.

Vector Plant Preference and Behavior Manipulation by GRBV and GLRaVs

GRBV and GLRaV-3 have only been reported to occur on *Vitis* and non-cultivated grapevines, but the relative contributions of different host species or varieties in GRBV or GLRaV-3 spread are not known and comprehensive studies to understand host plant utilization and preferences of vectors have not been completed. In addition, vector behavior might change in response to plant infection by GRBV and GLRaV-3 (i.e., changes in insect behavior mediated through the host plant), which may affect the settling, feeding, fitness, and dispersal behavior of the vectors (Conclusions 4-29, 4-30, 4-31).

Recommendation 4-22 (MP): Support research on virus-vector-host interactions to determine how the different species or varieties of *Vitis* and non-cultivated grapevines contribute to virus spread, as well as how GRBV or GLRaV-3 infection of the host can alter vector behavior.

Recommendation 4-23 (MP): Support research to broaden the understanding of complex interactions among the virus, vector, and host to enable the development of models of disease spread and strategies to prevent disease transmission

There are knowledge gaps regarding TCAH overwintering behavior, seasonal GRBV spread to grapevines, and differences among distinct grapevine-growing regions in California. Population models may help predict TCAH generation development associated with TCAH movement into vineyards; models may need to include information other than temperature to accurately predict population development and movement behavior (Conclusions 4-32, 4-33).

Recommendation 4-24 (MP): Support research on the seasonal virus spread of GRBV by TCAH, focusing on year-long TCAH abundance and overwintering behavior throughout California.

RESEARCH AND ACTIONS THAT MAY YIELD THE MOST PROMISING MANAGEMENT SOLUTIONS

To sustain the wine grape industry, improvements in short-term "stopgap" measures are needed as research to develop longer-term solutions is pursued. Stopgap measures include the use of vines free of GLRaVs and GRBV to help prevent the introduction of GLRaVs and GRBV into vineyards, removal of infected plants (roguing), and vector management to reduce virus transmission and spread. The high- (HP) and medium-priority (MP) recommended actions and research (Box S-3) are meant to increase the efficacy of practices and tactics for GLD and GRBD management in the short and long term.

BOX S-3
Recommended Actions and Research for Improving GLD and GRBD Management

The recommended research and actions in the following sections would contribute to GRBD and GLD management in the short term.

Clean Plants

Recommendation 5-1 (HP): Encourage the adoption and implementation of higher sanitary standards in registered mother blocks using robust, state-of-the-art, sensitive, and reliable diagnostic methods; and roguing of infected vines to maintain disease-free stock and provide clean planting materials for growers.

Roguing Infected Vines

Recommendation 5-2 (HP): Support research to develop optimal roguing and replanting schemes and techniques to manage GLD and GRBD, and to facilitate their implementation by growers.

Vector Management

Recommendation 5-3 (HP): Support research to determine the optimal conditions for the application of systemic insecticides to achieve better mealybug control.

Recommendation 5-4 (HP): Develop and implement insecticide resistance management programs and support research to develop new active ingredients for mealybug management, including by evaluating the efficacy of natural products such as plant essential oils, that could provide additional options for both organic and conventional vineyards.

Recommendation 5-5 (HP): Support research to determine the optimum conditions for the application of insecticides to achieve better TCAH control and to establish economic or action thresholds to guide insecticide application programs.

Recommendation 5-6 (HP): Support research to generate information needed for improving the efficacy of mating disruption for mealybug control and to determine the benefits (economic and otherwise) of employing this technique as part of an integrated approach to manage insect vectors in grapevines.

Recommendation 5-8 (MP): Support research to determine the costs and benefits of removing vegetation that harbors TCAH in and around vineyards and the use of trap crops to inform grower decision-making regarding the employment of these methods for managing TCAH in vineyards.

Sanitation

Recommendation 5-10 (HP): Support research to determine the most effective and practical farm and worker equipment sanitation measures and harvesting and pruning strategies that can help minimize the spread of insect vectors.

Physical Barriers

Recommendation 5-11 (MP): Support research to evaluate the efficacy of physical barriers in deterring TCAH movement from natural or vineyard-adjacent habitats to vineyards.

Recommendation 5-12 (MP): Support research to evaluate the efficacy of reflective mulches in reducing the abundance of insect vectors in vineyards and research on improving the longevity and durability of reflective mulches.

Areawide Pest Management

Recommendation 5-13 (HP): Support efforts to develop areawide GLD and GRBD vector management programs for regions of California with different threat levels from these diseases, along with activities to encourage grower participation in these programs.

The recommended research and actions in the following section would contribute to GRBD and GLD management in the long term.

Host Plant Resistance

Recommendation 5-14 (HP): Support research using traditional and bioengineering approaches for developing GLD and GRBD resistance; when conducting resistance screening assays, the biological vector should be used as much as possible.

Recommendation 5-17 (HP): Establish multidisciplinary and trans-institutional collaborations to enhance synergies in pursuing bioengineering approaches, such as RNAi-mediated resistance and CRISPR/Cas-based genome-editing technologies, as an alternative to traditional breeding for resistance against GLD and GRBD.

CONSIDERATIONS FOR FUTURE RESEARCH ON GRAPEVINE VIRUSES AND DISEASES

The committee also developed recommendations regarding future considerations for the CDFA PD/GWSS Board as it continues to support research to develop viable solutions to virus diseases that threaten vineyard health and the sustainability of the California wine grape industry. The high- and medium-priority recommendations are presented in Box S-4 and labeled HP and MP.

BOX S-4
Future Considerations

New Genetic Tools and Research Platforms

Genetic pest management strategies, in which the insect vector is modified rather than the plant, could aid disease control via vector population replacement and/or suppression. The biology of mealybugs makes them good targets for genetic pest management.

Recommendation 6-1: Support basic research to enable genetic pest management strategies for GLD and GRBD vectors and support modeling and sociological research to predict whether these strategies will be effective in the field and be accepted by consumers.

Approaches Used in Other Pathosystems

RNA interference (RNAi) has the potential for use in managing insect vectors. RNAi biopesticides should have narrow activity based on target-specific double-stranded RNA (dsRNA) that will trigger RNAi suppression only in the targeted organism and no activity in other insects. Genetically engineered plants expressing dsRNA may more effectively manage mealybugs and other insects that reside under bark where it is hard to contact them with insecticide sprays.

Recommendation 6-2 (MP): Consider supporting interdisciplinary research teams to advance RNAi research for the suppression of vectors in vineyards.

Trunk injection, which has been shown to be effective in controlling vasculature diseases and insect pests in other tree crops suggests the approach could be applicable to the grapevine industry in California. Studies conducted in European vineyards have demonstrated the potential to control esca disease complex by injecting fungicides and chemicals into the grapevine trunk. This approach could help with vector or disease management in the medium or long term.

Recommendation 6-4 (MP): Consider supporting research to investigate the potential utility of trunk injection to control vectors with various pesticides (including new approaches such as RNAi and nanobodies) in grapevines.

Insect population models and disease risk models have been valuable tools for stakeholders to understand pest risk, production practices that mitigate risk, and to identify critical windows of time for scouting and management activities. These models could help with vector and disease management in the short term.

Recommendation 6-5 (HP): Fund research that will lead to the development of publicly available, regionally relevant insect population models and disease risk models that can be used to guide local and areawide management activities for GLD and GRBD.

Engaging a Wider Range of Researchers

Researchers who are not familiar with the PD/GWSS Board research and outreach grants may not be aware that this program also funds research on other grapevine pests and diseases, such as GLD and GRBD. Allocating specific funding for early and mid-career scientists may help expand the pool of researchers working on grapevine virus diseases. Inviting researchers to address specific knowledge gaps may increase the pool of interested researchers.

Recommendation 6-6 (HP): To draw in diverse researchers, consider changing the name of the PD/GWSS Board research and outreach grants to accurately reflect the scope of its RFPs, which include multiple grapevine virus diseases and their insect vectors.

Recommendation 6-8 (MP): Consider offering specific funding for early and mid-career researchers to encourage engagement in grapevine virus diseases research and build a network of scientists to address long-term questions.

Recommendation 6-9 (HP): Consider developing additional funding mechanisms to address particular needs for GLD or GRBD research, such as through inviting specific researchers to address particular knowledge gaps or accepting off-cycle proposals for projects that have potential to generate information for dramatically improving GLD and GRBD management.

Longer Term and Replicated Studies

The study of complex systems such as vector-borne diseases in perennial crops may take longer than three years and more funding to accurately describe disease biology and inform recommendations for disease and vector management.

Recommendation 6-10 (HP): Consider funding longer-term projects (lasting more than three years) such as studies that advance control recommendations, translational research, and projects that integrate economic and societal impacts.

Another important issue is replicability of results. Collaborative research proposals provide a mechanism to support multiple research teams addressing the same research questions.

Recommendation 6-11 (HP): Consider funding research to replicate experimental results in more than one location and with different research teams to obtain more robust and reliable insights.

Recommendation 6-12: Consider new ways to leverage available funds using different proposal and award structures to encourage collaboration.

Knowledge Sharing and Collaborative Research

Greater sharing and integration of research findings could be facilitated by the establishment of a dedicated working group and/or through expanded opportunities for researchers to interact and share ideas at in-person meetings.

Recommendation 6-14 (MP): As an alternative to the annual Pierce's disease symposium, consider coordinating with other organizations to hold sessions on GLD and GRBD at events such as the annual conference of the American Society for Enology and Viticulture and the Unified Wine and Grape Symposium. These sessions could also serve as a platform to facilitate new collaborations involving scientists working on other grape diseases or working in other wine grape producing regions.

Recommendation 6-16 (HP): Explore the feasibility of creating a working group, supported by the wine grape industry and funded by another entity, that can facilitate information sharing and foster collaboration among GLD and GRBD researchers.

Education and Outreach

A lack of communication and knowledge dissemination contributes to the non-adoption of GLD and GRBD management practices; this underscores the importance of having more effective educational and outreach strategies as the knowledge of GLD and GRBD advances.

Recommendation 6-18 (HP): Provide opportunities for funded researchers to share findings and recommendations regarding grapevine viruses via a dedicated website or a virtual town hall that facilitates interactive discussions about GLD and GRBD among researchers, extension agents, and growers.

Successful control of vector-borne diseases control does not rely solely on understanding the pathosystem and devising strategies to control the pathogen or its vector, it also relies on what growers decide to do. Social science research has shown that social networks play an important role in learning and in the adoption of innovations.

Recommendation 6-19 (HP): Support research to better understand the sociological aspects of managing vector-borne diseases through collective action (i.e., areawide pest management) and find ways to increase grower participation in areawide pest management programs.

Recommendation 6-20 (HP): Support research on understanding and improving the flow of information across grower social networks and on outreach efforts to understand the drivers and barriers to successful adoption of GLD and GBRD management practices.

1
Introduction

Grapevine (*Vitis* spp.) is one of the most widely cultivated crops in the world; *Vitis* species are grown for wine, fresh fruit, raisin, and juice. Grapes (table, raisin, and wine) rank second among California's top 10 agricultural commodities, valued at $5.54 billion for the 2022 crop year (CDFA, 2024). California is the number one wine-producing state in the United States; in 2022, California accounted for about 80% (599,557,535 gallons) of the total U.S. wine production (752,077,206) (Wine Institute, n.d.). Annually, the California wine and wine grape sector and allied businesses contribute $73 billion to the state's economy and $170.5 billion to the U.S. economy (Wine Institute, 2022).

There are 904,000 grape-bearing acres (365,836 hectares) in the United States that produce grapes at an average of 6.69 tons/acre (16.53 tons/ha) (Ag Marketing Resource Center, 2023). In California, where most of the U.S. grape production occurs, the total grape acreage in 2021 was 881,000 acres (356,528 ha), of which 615,000 acres (248,882 ha) produced wine-type grapes, 127,000 acres (51,396 ha) produced table-type grapes, and 138,000 acres (55,847 ha) produced raisin-type grapes (CDFA, 2022). Among the grape-growing regions of California (Figure 1-1), the North Coast, Central Coast, North San Joaquin Valley, South San Joaquin Valley (four counties), and San Bernardino are the major wine grape producing areas (based on data from CDFA, 2006). Table and raisin grape production is concentrated in the southern San Joaquin Valley (Alston et al., 2020). Census data indicate that there were 11,812 grape-growing farms in California as of 2017 (USDA/NASS, 2017) with larger farms (average of 140 acres or 56.7 ha)) located in the San Joaquin Valley and smaller farms (average of 36 acres or 14.6 ha)) located in the coastal regions (Alston et al., 2020). California wine grape growing regions vary in terms of climate, terrain, soil types, mixture of grape varieties grown, and the quantity and quality of grapes and wine produced (Alston et al., 2020). Vineyards across these regions also differ in age and employ different management practices.

GRAPEVINE VIRUSES AND DISEASES

Among all cultivated woody perennials, grapevines are known to be infected with the largest number of viruses (Naidu et al., 2014; Xiao et al., 2018). To date, more than 100 viruses belonging to 21 different families or having similarity to unclassified plant satellite viruses have been reported on *Vitis* germplasm worldwide (Fuchs, 2023 and cited references). Viruses of grapevines have been grouped into four categories based on the disease they cause or symptoms they are associated with: 1) degeneration or decline disease complex; 2) leafroll disease complex; 3) rugose wood complex; and 4) fleck disease complex (Naidu et al., 2014).

Grapevine Leafroll Disease

Grapevine leafroll disease (GLD) occurs in all grape-growing regions in the world and causes the most economic damage among all virus and virus-like diseases of grapevine (Martelli, 2000; Freeborough and Burger, 2006; Nimmo-Bell, 2006; Naidu et al., 2008). Several viruses are associated with GLD, and they are collectively referred to as grapevine leafroll-associated viruses or GLRaVs. Among the GLRaVs, grapevine leafroll-associated virus 3 (GLRaV-3) is considered as the primary causal agent of GLD (Maree et al., 2013). In 1992, using serological testing techniques, GLRaVs were detected for the first time in previously healthy vines at the Foundation Plant Services vineyard of the University of California, Davis (Rowhani and Golino, 1995). Symptoms of GLD vary between grape cultivars and within the same cultivars. Leaves of infected red grape cultivars have red or reddish-purple discolorations in the

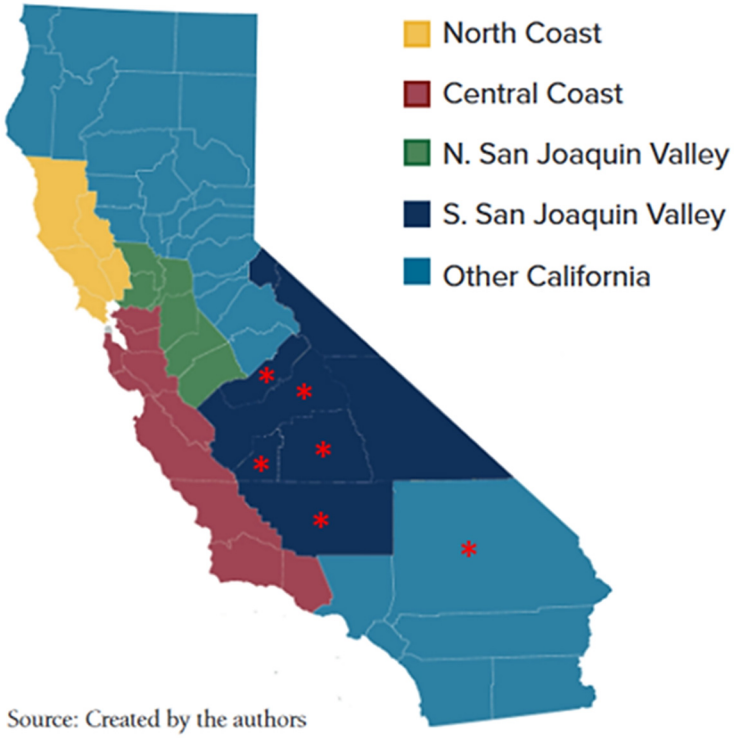

FIGURE 1-1 Grape-growing regions of California.
NOTE: North Coast, Central Coast, North San Joaquin Valley, and counties marked with asterisks within South San Joaquin Valley and Other California are the major wine grape producing regions (based on data from CDFA Final Grape Crush Report 2006).
SOURCE: Adapted from Alston et al. (2020).

inter-veinal areas while tissue on either side of the veins appears green. Leaves of infected white grape cultivars exhibit mild yellowing or chlorotic mottling in the inter-veinal areas, but these symptoms are not pronounced and may not be apparent in many white grape cultivars (Naidu et al., 2014). In both the red or black and white grape cultivars, disease symptoms may become apparent in early to mid-summer (i.e., during post-veraison) and leaf margins of symptomatic leaves usually roll downward toward the end of the season (Martelli et al., 2014; Naidu et al., 2014).

Grapevine Red Blotch Disease

In 2008, disease symptoms that somewhat resembled GLD were observed in a Cabernet Sauvignon vineyard in a research station in Oakville, California (Calvi, 2011). Disease symptoms suggestive of GLD were also observed in a Cabernet franc vineyard in New York, and the viral nature of this disease was determined in 2011. In 2012, research groups in California and New York proposed to call the virus associated with this disease (referred to as grapevine red blotch disease or GRBD) as grapevine red blotch-associated virus, and eventually grapevine red blotch virus (GRBV) (Sudarshana et al., 2015; Yepes et al., 2018). GRBD causes delayed fruit ripening, reduced fruit quality and yield, and effects on wine composition (Blanco-Ulate et al., 2017; Cieniewicz et al., 2017; Martínez-Lüscher et al., 2019; Cauduro Girardello et al., 2020; Rumbaugh et al., 2021). Since the discovery of GRBV in North America, the virus has been detected in grapevines in other countries, including South Korea (Lim et al., 2016), Switzerland (Reynard et al., 2018), India (Marwal et al., 2019), Argentina (Luna et al., 2019), Italy (Bertazzon et al., 2021), France (Reynard et al., 2022), Australia (Kaur et al., 2023), and Iran (Gholampour et al., 2024).

CDFA SUPPORT FOR GRAPEVINE DISEASES AND PESTS RESEARCH

In 2001, an annual Pierce's disease (PD) wine grape assessment was established to fund research and other related activities on PD and its vector, the glassy-winged sharpshooter (GWSS). The California Department of Food and Agriculture (CDFA) Pierce's Disease/Glassy-Winged Sharpshooter (PD/GWSS) Board, which is composed of representatives from the California wine grape industry, was also established in that year and since then has been providing recommendations to the CDFA Secretary on the use of funds collected under the PD/GWSS wine grape assessment. The annual assessment rate, which is set by the PD/GWSS Board, has averaged $1.35 per $1,000 of value since 2001 and has collected $83.1 million since 2001 (Kaiser, 2023; California Ag Network, 2023). The rate for the 2024 harvest is $1.25 (CDFA, 2024). Every five years, the PD/GWSS Board and the wine grape assessment are subject to a referendum of growers who pay the assessment (M. Kaiser, personal communication, October 4, 2024). The PD/GWSS Board Research and Outreach Program issues a Request for Proposals (RFP) and accepts proposals each year between December 1 – January 31.[1] The process by which CDFA selects proposals to fund is illustrated in Figure 1-2.

A total of $43.5 million has been allocated for PD and GWSS research and outreach activities since 2001, while a total of $14.2 million has been allocated for research and outreach activities on other grapevine pests and diseases (e.g., European grapevine moth, brown marmorated stink bug, GRBV, GLRaV, and mealybugs) since 2010.[2] From July 2015 through July 2023, the CDFA PD/GWSS Board funded a total of 60 grants: 28 on GRBV; 13 on GLRaV; 10 on vine mealybug *Planococcus ficus* Signoret, which is a vector of GLRaV-3; and 9 on multiple viruses. Several GLRV projects include mealybugs and one GRBV project included PD (Kaiser, 2023). To date, CDFA has funded a total of 72 grants on grapevine viruses and their vectors (M. Kaiser, personal communication, August 12, 2024). Information about CDFA-funded research projects, project summaries, and year-end progress reports are available at the CDFA website.[3]

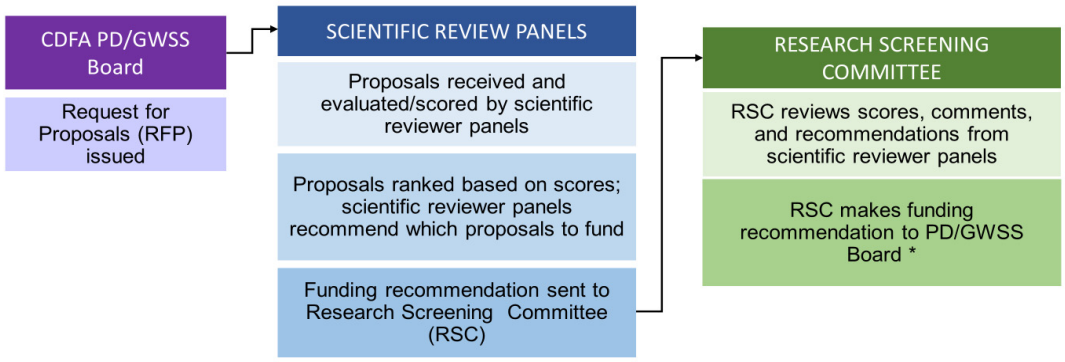

FIGURE 1-2 CDFA proposal funding process.
NOTE: *Factors considered by the RSC when making a recommendation to the PD/GWSS Board include scores, reviewer comments, industry and research priorities, program history, history of the project and researchers who submitted the project proposal, and the amount of funding being recommended in relation to the PD/GWSS Board's overall budget.
SOURCE: Created with information provided by CDFA representatives (M. Kaiser and K. Lowe, personal communication, July 21, 2023).

[1] The RFP is issued via https://www.cdfa.ca.gov/pdcp/grants/.
[2] Information obtained from Funding Research to Safeguard California Winegrapes PDGWSS Board Fact Sheet_5-29-24.
[3] See https://www.cdfa.ca.gov/pdcp/research.html.

STUDY ORIGIN

In 2022, CDFA requested the National Academies of Sciences, Engineering, and Medicine to provide guidance to the CDFA PD/GWSS Board on grapevine viruses and grapevine diseases research by convening an ad hoc committee that would conduct a study with three interrelated activities that address research on GRBV and GLRaV-3, the diseases they are associated with, and their insect vectors. The committee's charge is provided in Box 1-1. Activities 1 and 2 were conducted from April to June 2023; the *Report to the California Department of Food and Agriculture Pierce's Disease/Glassy-Winged Sharpshooter Board on the Review of Research Proposals on Grapevine Virus Diseases and their Vectors*[4] was released on May 4, 2023 and *A Critique of the California Department of Food and Agriculture Pierce's Disease/Glassy-Winged Sharpshooter Board's Request for Proposals* was released on July 5, 2023.[5] This report addresses Activity 3 (Review of Current Knowledge on Grapevine Viruses, GRBV and GLRaV-3 Research Outcomes/Gaps and Future Research Approach) in the Statement of Task.

BOX 1-1
Statement of Task

At the request of the California Department of Food and Agriculture (CDFA), the National Academies of Sciences, Engineering, and Medicine will convene an ad hoc committee to provide guidance on grapevine disease research to the CDFA Pierce's Disease/Glassy-Winged Sharpshooter (PD/GWSS) Board. The committee will carry out three interrelated activities.

Activity 1: Review of Proposals Submitted to CDFA PD/GWSS Board

The committee will evaluate research proposals submitted to the PD/GWSS Board in response to a Request for Proposals (RFP). Specifically, the committee will evaluate research proposals on the grapevine red blotch virus (GRBV) and the grapevine leafroll associated virus type-3 (GLRaV-3), their insect vectors, and the diseases they are associated with, using the proposal evaluation and selection criteria developed by the PD/GWSS. The committee will prepare a brief document that describes the proposal evaluation process and a list of projects recommended by the committee for funding, for the board's consideration. A non-public appendix will contain detailed evaluations of individual proposals.

Activity 2: Critique of CDFA PD/GWSS Board's Request for Proposals (RFP) and Proposal Selection Process

The committee will provide a review of and recommendations for improving the PD/GWSS Board's RFP and its proposal evaluation and selection process.

Activity 3: Review of Current Knowledge on Grapevine Viruses, GRBV and GLRaV-3 Research Outcomes/Gaps and Future Research Approach

The committee will review the state of knowledge about the grapevine red blotch virus (GRBV) and grapevine leafroll associated virus type 3 (GLRaV-3) and the management of diseases they are associated with and develop guidance to the PD/GWSS Board in its efforts to support research that leads to a reduction in the spread of GRBV- and GLRaV-3-associated diseases and their economic impacts.

The committee will examine the scientific literature and gather information from experts, plant health practitioners, and grape growers. The committee will explore recent and current research activities on GRBV and GLRaV3 and their insect vectors that are funded by the CDFA PD/GWSS Board.

continued

[4] Available at https://nap.nationalacademies.org/catalog/27145/a-critique-of-the-california-department-of-food-and-agriculture-pierces-diseaseglassy-winged-sharpshooter-boards-request-for-proposals.
[5] Available at https://nap.nationalacademies.org/catalog/27145/a-critique-of-the-california-department-of-food-and-agriculture-pierces-diseaseglassy-winged-sharpshooter-boards-request-for-proposals.

BOX 1-1 *continued*

In its review, the committee will identify the following as they relate to GRBV and GLRaV-3:

1. The most significant knowledge gaps in the current understanding of grapevine red blotch disease and grapevine leafroll disease epidemiology;
2. Research areas where significant progress has been/has not been achieved;
3. Research areas that may yield the most promising short- and long-term management solutions;
4. New genetic tools and research platforms that could be used to study grapevine viruses;
5. Opportunities for collaborative research that could accelerate progress in finding grapevine disease management solutions;
6. Other viral pathogen systems (animal and human) that could provide insights or additional research directions; and
7. Opportunities to improve the current CDFA PD/GWSS Board's research review and funding process, and opportunities to draw from a wider range of researchers across various disciplines and fund a wider range of national researchers.

The committee will prepare a consensus report with conclusions from its review, describing what is currently known about GRBV and GLRaV-3; what knowledge is needed to improve management of the diseases caused by these viruses; and the committee's recommendations with respect to a viable approach for supporting research on grapevine viruses.

COMMITTEE'S APPROACH TO ITS CHARGE

To address its charge under Activity 3, the committee gathered information and deliberated from June 30, 2023, to August 12, 2024, holding a total of 14 closed meetings and 6 public meetings. The committee held a virtual public meeting with CDFA representatives on June 30, 2023, to clarify the committee's task and CDFA's expectations from the study's Activity 3. On October 12-13, 2023, the committee convened in Prosser, Washington to visit a grapevine nursery, the Washington State University Irrigated Agriculture Research and Extension Center, commercial vineyards infected with GRBD and GLD, a winery, and the Ste. Michelle Wine Estates Washington State University Wine Science Center. The committee also met with the Wine Research Advisory Committee of the Washington State Wine Commission to hear their perspective on GRBD and GLD.[6] The committee held a hybrid public meeting on March 4-5 in Davis, California, and visited the University of California, Davis Foundation Plant Services diagnostic laboratory, meristem-tip culture laboratory, greenhouse, and nursery areas. On May 8-9, the committee held an in-person meeting in Washington, DC. Information-gathering activities during the public meetings featured presentations and question-and-answer sessions with invited speakers; discussions with CDFA PD/GWSS Board members, wine grape growers, pest control advisors, farm and integrated pest management advisors, and nursery operators; and virtual question-and-answer sessions based on pre-recorded presentations from invited speakers. All open sessions were livestreamed[7] on the study website.[8] Open session agendas, recordings, and some materials presented by invited speakers are available at the study website. All open session agendas are also provided in Appendix B of this report.

The committee's review of current knowledge, research outcomes, knowledge gaps, and future research approaches relevant to GRBV and GLRaV-3 was conducted using information from many sources, including published papers, presentations at open sessions and webinars, pre-recorded

[6] This meeting was an open session but was not livestreamed because no members of the public registered to attend remotely.
[7] Except for open sessions where no members of the public registered to attend remotely or attended in person.
[8] Available at https://www.nationalacademies.org/our-work/assistance-to-the-california-department-of-food-and-agriculture-pierces-diseaseglassy-winged-sharp-shooter-board-on-grapevine-viruses-and-grapevine-disease-research.

presentations, question-and-answer session discussions, and documents provided by CDFA[9] and members of the public. All documents received from third parties were added to the study's public access file, which is available on request from the National Academies' Public Access Records Office.[10]

SCOPE AND ORGANIZATION OF THE REPORT

Over the course of several months, the committee drafted a report in response to the statement of task. This report comprises six chapters. This chapter (Chapter 1) provides the general background for the study and the committee's statement of task and explains how the committee approached its charge. Chapter 2 and 3 provide information on the current state of knowledge on GRBD and GLD, respectively. These two chapters provide general information about GRBD and GLD symptoms, the viruses associated with these diseases and their vectors, and currently available diagnostic methods. These chapters also provide an overview of how each disease is managed, although they are not meant to provide a comprehensive discussion of disease management tactics. Chapter 4 discusses the significant knowledge gaps in GRBD and GLD. Chapter 5 identifies actions and research strategies that could yield promising management solutions, and Chapter 6 discusses new tools and research approaches, as well as insights to help improve the PD/GWSS Board's research program on GRBD and GLD. Although the committee touched on and emphasized the importance of clean virus-free planting materials, this report does not include an in-depth review of the California certification program, as this was not part of the committee's charge.

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[10] Requests can be directed to PARO@nas.edu.

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2
Current Knowledge on Grapevine Red Blotch Disease

Grapevine red blotch disease (GRBD) was initially referred to as "red-leaf disease" on *Vitis vinifera* cv. Cabernet Sauvignon in California. It was first described in a master's thesis (Calvi, 2011), which reported that grapevines were observed to have foliar symptoms similar to leafroll disease—with documented effects of a suspected viral pathogen both on leaves and fruit—but tested negative for all known viruses. Around the same time, a circular DNA virus was discovered in a declining *V. vinifera* cv. Cabernet franc vineyard in New York and tentatively named grapevine cabernet franc-associated virus (Krenz et al., 2012). A similar virus was then discovered in California, found to be associated with red blotch (i.e., red leaf disease) symptoms, and tentatively named grapevine red blotch-associated virus (Al Rwahnih et al., 2013), while a similar virus was also found in Washington State and tentatively named grapevine red leaf-associated virus (Poojari et al., 2013).

Since the aforementioned viruses were determined to have similar genomes, the scientific community agreed to refer to them as grapevine red blotch-associated virus until Koch's postulates were satisfied demonstrating a causal relationship between the virus and the disease (Yepes et al., 2018; see Box 2-1), at which point the name was changed to grapevine red blotch virus (GRBV).

SYMPTOMS

GRBD foliar symptoms manifest differently in *V. vinifera* depending on the cultivar. Foliar symptoms vary among red or black-fruited cultivars (e.g., Pinot noir, Cabernet Sauvignon, Cabernet franc, Syrah), but typically appear on older leaves as red blotches on the leaf blade which eventually may coalesce to cover the whole leaf, and the leaf may prematurely senesce (see Figure 2-1). In white-fruited cultivars (e.g., Chardonnay and Sauvignon blanc) symptoms are less conspicuous, but chlorosis and leaf curling may occur (see Figure 2-1). Foliar symptoms are similar to other problems such as nutritional disorders, mite damage, and leafroll disease. Thus, using symptomatology for diagnosis is not reliable.

The economic impact of GRBV is dependent on several factors including geographic location, initial infection incidence, cultivar, and price penalty for low-quality fruit. The impact of GRBV was estimated to range from $2,213 ($2,810.61)[1] per hectare in eastern Washington (with a low infection rate and low-price penalty) to $68,548 ($87,059.20)[12] per hectare in California's Napa Valley when infection rates and price penalties are high (Ricketts et al., 2017). These findings and subsequent studies underscore the importance of reducing GRBV inoculum sources (Ricketts et al., 2017; Cieniewicz et al., 2020a; Fuchs et al., 2021; Hobbs et al., 2022).

CAUSAL VIRUS

GRBV (species *Grablovirus vitis*, genus *Grablovirus*, family *Geminiviridae*) was the first geminivirus discovered in grapevine. Grapevine red blotch virus was the first member of a new genus in the *Geminiviridae*, called *Grablovirus* (Varsani et al., 2017), in which there are now three ratified members: grapevine red blotch virus, wild vitis latent virus, and prunus latent virus (Varsani et al., 2017; Al Rwahnih et al., 2018; Perry et al., 2018). Evolutionary analyses of the available full genomes of GRBV demonstrate two major clades of GRBV (see Figure 2-2); of these, clade 1 has higher genomic variability but clade 2 contains more isolates (Krenz et al., 2014; Cieniewicz et al., 2020a; Thompson, 2022). More genetic variation was discovered when additional GRBV isolates were collected from a

[1] Adjusted for inflation using the Consumer Price Index (CPI) from the U.S. Bureau of Labor Statistics.

BOX 2-1
Establishing a Causal Relationship of GRBV in Red Blotch Disease

Demonstrating virus disease etiology is important to ensure that management efforts are focused on the correct agent. Using Koch's postulates, researchers demonstrated that GRBV is the causal agent for GRBD.

Koch's Postulates

In the late 19th century, microbiologist and physician Robert Koch formulated guidelines for establishing the causal agents of diseases (referred to as Koch's postulates). Koch's foundational studies on disease etiology established the causal agents of diseases for anthrax, cholera, and tuberculosis, and were later applied to numerous other diseases of animals and eventually plant diseases. Briefly, the postulates state that:

1. The agent must be consistently found associated with a disease phenotype, i.e., present with disease and absent from healthy phenotypes.
2. The agent can be isolated from the diseased organism and identified for its intrinsic properties.
3. When the agent is inoculated from the pure culture into a healthy organism, the same disease phenotype is observed.
4. The same agent (identified for its intrinsic properties) can be re-isolated from the disease organism.

It is challenging to fulfill Koch's postulates to infer virus disease etiology because viruses cannot be isolated in pure cultures. Virus disease etiology has been further complicated by the advent of high throughput sequencing and discoveries that mixed virus infections are common in nature. One tactic to isolate a plant virus in pure culture is to propagate a complementary DNA (cDNA) copy of the viral genome in *Agrobacterium tumefaciens* as an infectious clone.

Fulfilling Koch's Postulates for GRBD [a]

To resolve the GRBD etiology, clones of the GRBV genome were generated by amplification of partial tandem repeats using clade 1 and clade 2 isolates as references. The GRBV clones were mobilized in *A. tumefaciens* and then agro-inoculated in virus-negative grapevine (*Vitis vinifera* and rootstocks) plantlets in tissue culture via vacuum-assisted agroinoculation and agropricking, i.e., using a sterile metal pin to deliver cultures directly to the phloem. This study demonstrated that GRBD symptoms develop in the GRBV-inoculated *V. vinifera* cultivars in tandem with detection of actively replicating GRBV, in some cases requiring a dormancy period before symptom development. Although most of the rootstocks did not show GRBD symptoms, GRBV was detected in the agro-inoculated rootstocks, thus fulfilling Koch's postulates.

[a] Yepes et al., 2018.

recently released interspecific hybrid cultivar 'Blanc du Soleil' and formed a distinct sub-clade within clade 2 (Ouro-Djobo et al., 2023), a finding suggesting that much remains to be discovered about GRBV diversity. In addition to genomic variability introduced by the accumulation of mutations, recombination among GRBV isolates has been reported in several studies (Krenz et al., 2014; Perry et al., 2016; Cieniewicz et al., 2018a; Thompson, 2022; Ouro-Djobo et al., 2023). Currently, no biological significance regarding different clades of GRBV has been described, though infectious clones based on isolates from both clades have been generated (Yepes et al., 2018).

EPIDEMIOLOGY AND VECTOR(S)

GRBV is distributed in nearly all viticultural regions of the United States (Krenz et al., 2014; Adiputra et al., 2019; Brannen et al., 2018; Yao et al., 2018; Jones and Nita, 2019; Schoelz et al., 2021; Thompson et al., 2019; Hoffmann et al., 2020; Hu et al., 2021; Hu, 2022; Soltani et al., 2020). In North

America, GRBV is also widespread in Canada (Poojari et al., 2017, 2020; Xiao et al., 2018; Kahl et al., 2022) and Mexico (Gasperin-Bulbarela et al., 2019). Outside of North America GRBV has been detected in South Korea (Lim et al., 2016), Switzerland (Reynard et al., 2018, 2022), India (Marwal et al., 2019), Argentina (Luna et al., 2019), and Italy (Bertazzon et al., 2021). Many of these GRBV detections have been isolated events, often in germplasm collections in which the material can be traced back to a U.S. origin. However, some detections, e.g., the recent detections of GRBV in Italy in the Italian accession 'Incrocio Dalmasso VIII-5' cannot be explained by a North American origin (Bertazzon et al., 2021). So far, it is not known if GRBV occurs in vineyards in Europe (Reynard et al., 2022).

Evidence compiled thus far suggests that GRBV may have a North American origin, potentially having originated in wild grapevines and diverged from the ancestral wild Vitis latent virus prior to the cultivation of *V. vinifera* in North America (Cieniewicz et al., 2020a; Reynard et al., 2022; Thompson, 2022). GRBV was detected in archival grapevine material maintained in a herbarium sample collected from Sonoma County in 1940, suggesting it has been in commercial wine grape production in this region for at least 80 years (Al Rwahnih et al., 2015).

Wild *Vitis* reservoirs include free-living *Vitis* spp. and hybrids of *V. vinifera* and *V. californica,* all of which are naturally occurring in vineyard ecosystems (Krenz et al., 2014; Bahder et al., 2016a; Perry et al., 2016; Cieniewicz et al., 2018a; Achala et al., 2022; Wilson et al., 2022). Bahder et al. (2016a) reported GRBV detection on wild *Rubus* spp., but only transiently, which suggested that wild *Rubus* is not a systemic host of GRBV. Some experimental hosts have been identified as a result of agro-inoculation of GRBV infectious clones, including snap bean (*Phaseolus vulgaris*) and *Nicotiana benthamiana* (Flasco et al., 2021). GRBV has not been found in any of the field-collected herbaceous weed or cover crop species tested to date (Cieniewicz et al., 2019; Wilson et al., 2022). The only confirmed natural, systemic hosts of GRBV are *Vitis* spp., interspecific hybrids (Cieniewicz et al., 2020a), and muscadines (Soltani et al., 2020).

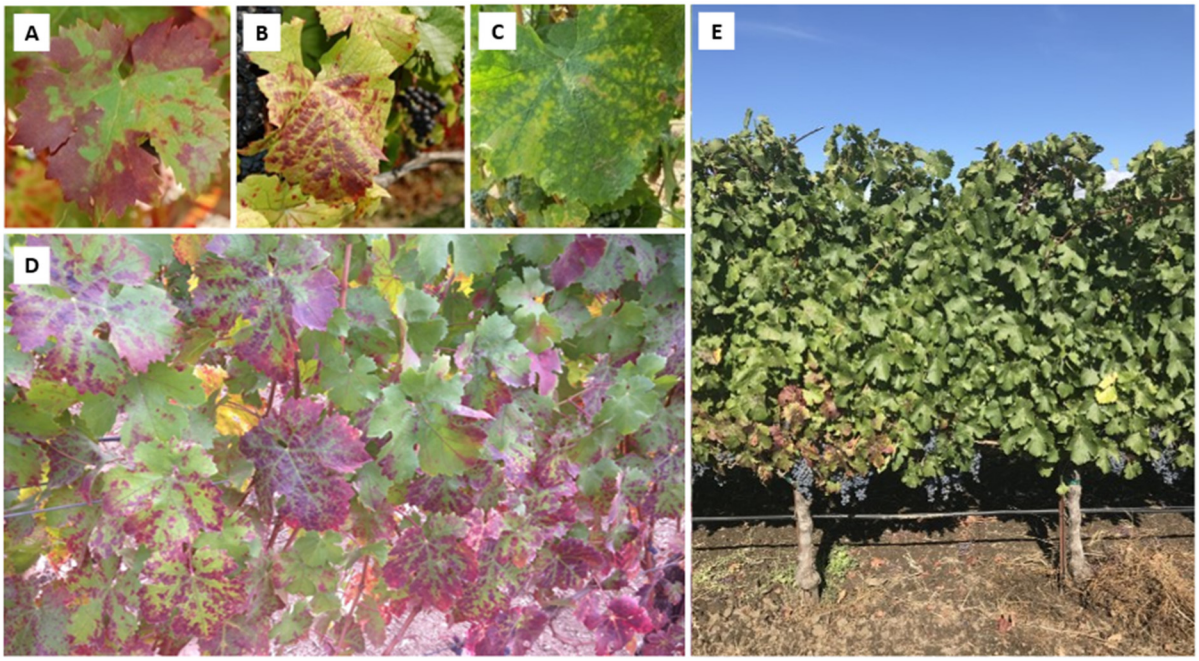

FIGURE 2-1 Foliar symptoms of grapevine red blotch virus infection in *V. vinifera* cv. (A) Syrah, (B) Pinot noir, (C) Chardonnay, (D) Cabernet franc. In (E) a GRBV-infected vine (left) is shown compared to a GRBV-negative vine (right) in a Cabernet franc vineyard in Napa County, California.
SOURCES: Marc Fuchs, Cornell University (A, B, C) and Elizabeth Cieniewicz, Clemson University (D, E).

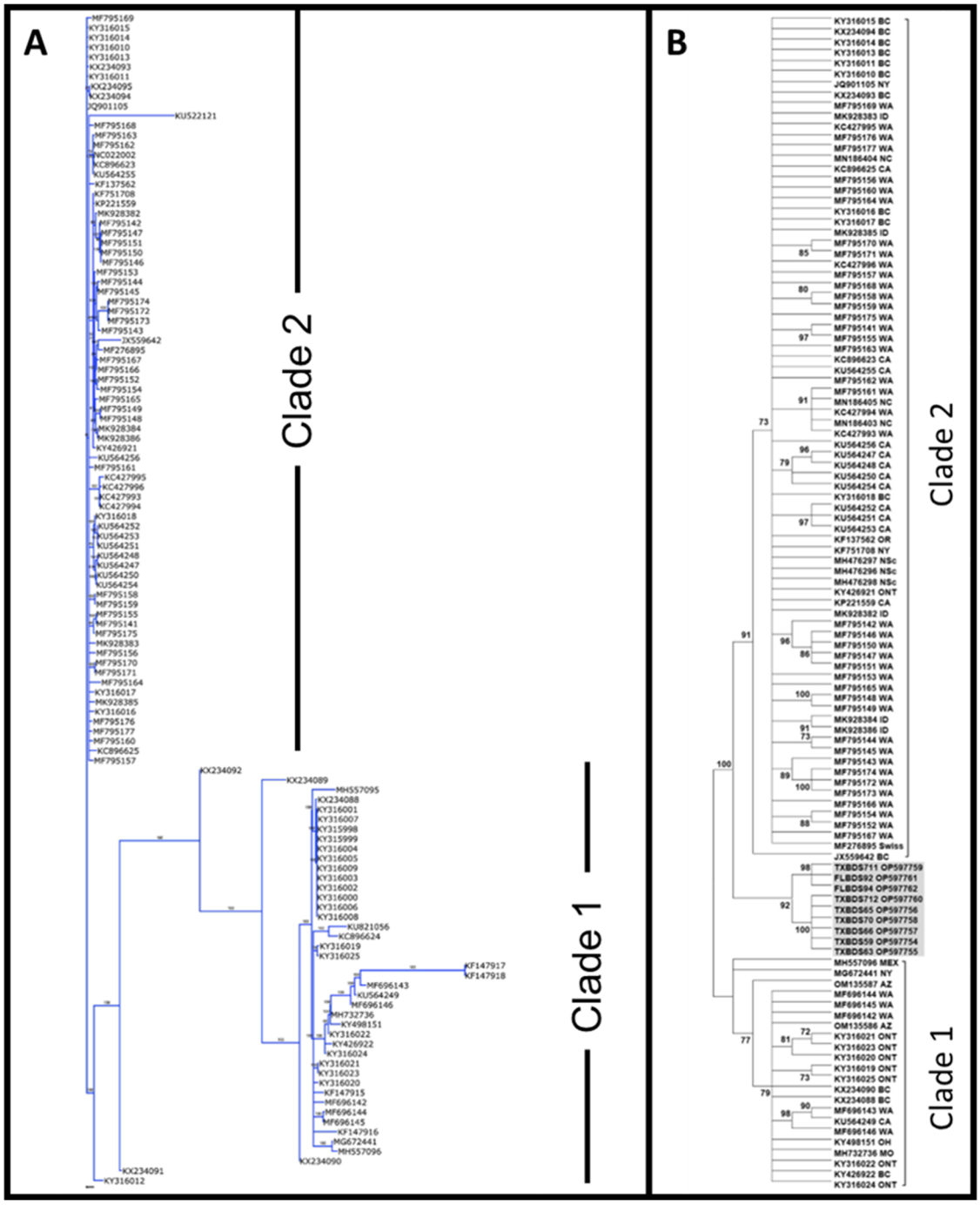

FIGURE 2-2 Phylogenetic analyses of GRBV full genome isolates adapted from (A) Cieniewicz et al. (2020a) and (B) Ouro-Djobo et al. (2023). Both phylogenies demonstrate two distinct lineages of GRBV isolates, in which most sequences are in clade 2 and remaining isolates are in clade 1. A divergent lineage of GRBV isolates from an interspecific hybrid 'Blanc du Soleil' was recently reported to cluster in clade 2 (highlighted gray in B, Ouro-Djobo et al., 2023). Recombination among isolates has been suggested in several studies. SOURCE: Adapted from Cieniewicz, E. J., W. Qiu, P. Saldarelli, and M. Fuchs. 2020a. Believing is seeing: Lessons from emerging viruses in grapevine. Journal of Plant Pathology 102:619-632; Ouro-Djobo et al., 2023, Molecular characterization of divergent isolates of grapevine red blotch virus from Blanc du Soleil, an interspecific hybrid white grapevine cultivar, PhytoFrontiers 3(2):290-295.

GRBV is efficiently transmitted through vegetative propagation (i.e., grafting and propagation of cuttings), and by treehopper insect vectors (Hemiptera: Membracidae). As with all grapevine viruses, GRBV is not transmitted mechanically via equipment. In the United States, the dynamics of secondary spread of GRBV vary by region (Cieniewicz et al., 2017b, 2019; Dalton et al., 2019; Achala et al., 2022) and even between neighboring vineyards (Cieniewicz et al., 2019; Flasco et al., 2023a). Epidemiological studies provide support that GRBV is spread by a Hemipteran vector in northern California and Oregon (Cieniewicz et al., 2017b, 2018b; Dalton et al., 2019; Achala et al., 2022), whereas GRBV spread was not apparent in New York (Cieniewicz et al., 2019) nor in the Niagara region of Canada (Vu et al., 2023). Spatiotemporal spread of GRBV has not been explored in other regions.

The primary treehopper species implicated in the spread of GRBV in the western United States based on transmission studies and abundance in California vineyards is *Spissistilus festinus* Say, the three-cornered alfalfa hopper (TCAH) (see Figure 2-3). In Oregon, secondary spread of GRBV has been observed in some vineyards where the TCAH was present but spread was also observed at sites where TCAH was not found (Dalton et al., 2019). These authors did find *Tortistilus* spp. at sites where *S. festinus* was absent but spread of GRBV was occurring, suggesting a need to determine whether *Tortistilus* spp. are also vectors of GRBV (Dalton et al., 2019). However, *Tortistilus* spp. are unlikely to be major vectors in California based on testing of specimens caught from 102 vineyards in the Napa Valley (Hoyle et al., 2024). Another study in Oregon vineyards demonstrated spread of GRBV in areas where the presence of *S. festinus* (and other potential vectors) was noted, but no sampling of vectors was performed (Achala et al., 2022). To date, the only vector with confirmed epidemiological relevance is *S. festinus*.

TCAH have been shown to start testing positive for GRBV in vineyards during June in the northern hemisphere (Cieniewicz et al., 2018b). The concentration of GRBV in grapevines increases over the course of the growing season (Setiono et al., 2018) and could increase acquisition of GRBV in the summer months when TCAH uses *Vitis* spp. as a feeding host, increasing the risk of spread.

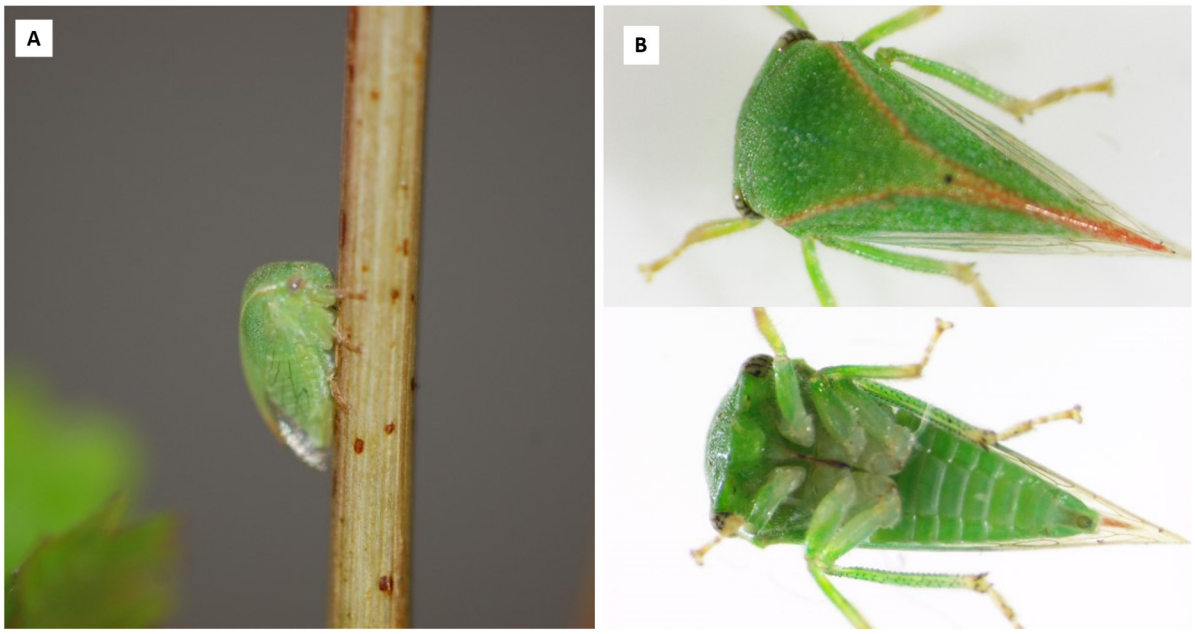

FIGURE 2-3 Adult *S. festinus* female (A) and male (B, top and bottom).
SOURCE: Elizabeth Cieniewicz, Clemson University.

TCAH may be absent at locations where GRBV spread is apparently occurring, suggesting the possibility of additional vector species (Dalton et al., 2019). Initial surveys have tested other Hemipteran species collected from vineyards in North America for acquisition of GRBV. To date, several species of treehoppers in the family Membracidae have been proposed to transmit GRBV, but few others have been tested for vector competence (Flasco et al., 2023d). Other Hemipteran species collected from California vineyards have tested positive for GRBV ingestion, including the leafhoppers (Cicadellidae) *Erythroneura elegantula* Osborn and *Erythroneura variabilis* Beamer, *Caladonus coquilletti* Van Duzee, *Colladonus reductus* Van Duzee, *Osbornellus borealis* DeLong & Mohr, *Scaphytopius graneticus* Ball, *Aceratagallia* spp., *Acinopterus angulatus* Lawson, *Colladonus* sp., and *Empoasca* spp., planthoppers *Melanoliarus* sp. (Cixiidae), an unknown species from the family Delphacidae, and unknown species from the family Aphididae (Bahder et al., 2016b; Cieniewicz et al., 2018b; Wilson et al., 2022). Outside of California *Entylia carinata* Forster, *Enchenopa bionata* Say, *Stictocephala basalis* Walker, and *S. bisonia* Kopp and Yonke were shown to ingest GRBV (Kahl et al., 2021; LaFond et al., 2022). Of all these species shown to ingest GRBV, only six have been tested for vector competence. In California *Erythroneura ziczac* Walsh, the Virginia creeper leafhopper, has been found to ingest GRBV (Bahder et al., 2016b) but vector status remains unclear because transmission was only reported in one (Poojari et al., 2013) of two studies (Bahder et al., 2016b). Treehoppers collected in the Okanagan and Similkameen valleys of British Columbia, *S. basalis* and *S. bisonia* (Membracidae), were shown to transmit GRBV to artificial diet in the laboratory (Kahl et al., 2021). In Missouri, treehoppers *E. carinata* and *E. bionata* were shown to acquire and transmit GRBV to grapevines; *E. carinata* was the second most collected species in four vineyards sampled, whereas *E. bionata* was rarely observed (LaFond et al., 2022). *E. elegantula* and *E. variabilis* were not found to transmit GRBV, but the acquisition and inoculation times used in the study were much shorter than those characterized as necessary for TCAH to acquire and transmit GRBV (Flasco et al., 2021).

Many species shown to ingest GRBV have not been tested for vector competence to transmit GRBV, and many are not good candidates for testing due to their overall low abundance or low detection of GRBV in individuals tested. While studies have detected GRBV in Hemipterans from multiple families, reports of vector transmission of plant viruses to date have shown a high degree of specificity for species of viruses being transmitted by one or few species of insects from a single insect family (Nault, 1997), and so far, vector competence has been confirmed in multiple studies for members of Membracidae. According to Flasco et al. (2023d), this does not rule out the possibility that closely related species (i.e., plant hoppers) are GRBV vectors, but experiments testing transmission efficiency using long-duration acquisition access periods and inoculation access periods are required to confirm vector status of all species. Ensuring that appropriate controls are used and that experiments are not confounded by virions present in honeydew excreted by insects in transmission assays can help avoid false classification of insects as vectors of GRBV (Flasco et al., 2023d).

The seasonal dynamics and ecology of the potential Hemipteran vectors are not well understood. Few studies have included data on the seasonal dynamics, distributions, or acquisition of GRBV for *E. elegantula*, *C. coquilletti*, *Colladonus* spp., *Scaphytopius graneticus*, *Scaphytopius* spp., *Melanoliarus* sp., *Colladonus reductus*, and *Osbornellus borealis* (Cieniewicz et al., 2018b; Wilson et al., 2020, 2022; Billings et al., 2021). These potential vector species may differ from TCAH in important aspects such as abundance in vineyards, timing of GRBV acquisition, seasonal population dynamics, and transmission abilities and efficiencies. The differences in the ecology and vector competence of different species will need to be understood if additional vectors are identified because these factors will influence the development of vector management strategies.

PATHOGEN-VECTOR INTERACTIONS

The TCAH transmits GRBV in a circulative and non-propagative manner and requires an extended acquisition access period before transmission occurs; 10 days of feeding on infected grapevines is required for GRBV to be acquired and circulate through the insect (Flasco et al., 2021). TCAH can

transmit isolates of GRBV from the two primary phylogenetic clades reported in the United States, and vineyards containing both GRBV clade 1-infected vines and clade 2-infected vines have been reported (Flasco et al., 2023a). Efficiency of GRBV transmission by the TCAH is generally low (Bahder et al., 2016b; Flasco et al., 2021; Hoyle et al., 2022) but transmission to grapevines in the vineyard has been demonstrated (Flasco et al., 2023b) and GRBV spread in a California vineyard was positively associated with viruliferous TCAH (Cieniewicz et al., 2018b, 2019).

When feeding on leaf petioles and green shoots of grapevines, TCAH and other treehoppers cause girdles on grapevine tissue (see Figure 2-4) that act as nutrient sinks benefitting the insect (Smith, 2013; Preto et al., 2018a). These girdles are also believed to negatively influence infection and localization of the virus after transmission (Flasco et al., 2023b). Transmission experiments conducted in vineyards were more successful when two vector individuals were used to transmit GRBV to individual leaves than when 10-12 individuals were used to transmit GRBV to half- or whole shoots (Flasco et al., 2023b).

VECTOR–HOST INTERACTIONS

Research has revealed two distinct genotypes of TCAH in the United States that are differentiated by geography and not host plant, with samples from California and Arizona comprising one genetic group and individuals collected from Alabama, Mississippi, Georgia, North Carolina, and Virginia comprising the other (Cieniewicz et al., 2020b). Populations of each genotype were reared separately and shown to be reproductively compatible as a result of reciprocal male-female crosses from each genotype, with subtle morphological differences in the resulting progeny (Flasco and Fuchs, 2023). The mitochondrial cytochrome oxidase subunit 1 (COI) gene sequence in the resulting progeny was consistent with the maternal parent genotype in each cross, as expected for a mitochondrial gene (Flasco and Fuchs, 2023). Notably, this study also revealed that TCAH of the southeastern United States genotype transmit GRBV at a higher efficiency than the California genotype, highlighting the need to study GRBV ecology in the southeastern United States (Flasco and Fuchs, 2023).

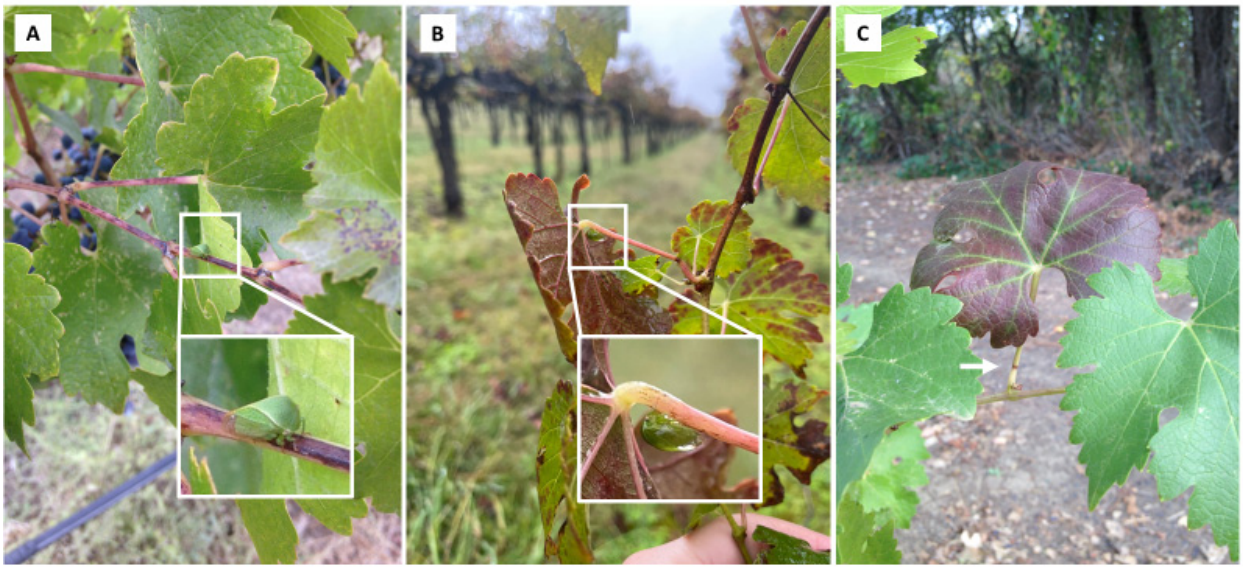

FIGURE 2-4 Adult *S. festinus* in vineyards in California (A) and Georgia (B) with close-up views in windows. Girdle damage (B and C) resulting in foliar reddening can be caused by *S. festinus* feeding. SOURCES: Elizabeth Cieniewicz, Clemson University (A, B) and reproduced from Cieniewicz et al. (2017a, Figure 14.5) (C).

Research on hosts of GRBV, feeding and reproductive hosts of TCAH, seasonal dynamics, and dispersal of hemipterans in vineyards has revealed important information about factors influencing the spread of GRBV. TCAH has been found to feed on and girdle leaf petioles and green shoots of grapevines (Smith, 2013; Preto et al., 2018b) and lay eggs in *V. vinifera*, but nymphs cannot complete development on grapevines and TCAH does not survive on dormant wood (see Figure 2-5) (Preto et al., 2018b). The plant host range of the TCAH vector is wide; species in the family Asteraceae are preferred feeding hosts and species in the family Fabaceae are primary breeding hosts (Newsom et al., 1983; Preto et al., 2018a; Hoyle et al., 2023). This means that TCAH are utilizing *Vitis* spp. as occasional feeding hosts as they move through the environment, or when other preferred hosts are limited or absent, and that acquisition and transmission of GRBV likely occurs during periods where primary feeding hosts are scarce. TCAH adults from overwintering generations have been collected before budbreak from groundcover of vineyards. They are believed to complete one to two generations in California vineyards. TCAH and GRBV tend to be aggregated along field edges, with spread appearing localized and extending into vineyards over time in most study locations (Cieniewicz et al., 2017b; Dalton et al., 2019; Preto et al., 2019; Flasco et al., 2023a), but TCAH abundance and girdling are not always greater at field edges or locations adjacent to riparian habitats (Wilson et al., 2020). Populations of TCAH are believed to move into vineyards from riparian habitats or other natural habitats near vineyards, whereas fabaceous cover crops and weeds support TCAH populations and may facilitate TCAH spread throughout vineyards (Cieniewicz et al., 2017a; Preto et al., 2019; Kron and Sisterson, 2020a; Wilson et al., 2020; Sisterson et al., 2023). The first in-field generation of adults and immatures collected on grapevines was observed to coincide with anthesis, when the flower is fully open and ready to be pollinated (Preto et al., 2019). In California, girdling of grapevines was first observed in June or July, when TCAH are relatively abundant; however, after vegetation on the vineyard floor dried in August, populations of TCAH captured decreased, but girdling increased (Cieniewicz et al., 2018b; Preto et al., 2019; Wilson et al., 2020) and continued until early November (Preto et al., 2019). In a laboratory flight mill study, males were observed to fly longer and farther than females with an average of 570.2 m flights compared to 239.6 m flights of females (Antolínez et al., 2023). Age also influenced flight duration and distance of TCAH; males aged 8-21 days old and females aged 15-21 days old flew longer and farther than individuals aged 2-7 days old. There were no differences in the number of flights, time to first flight, or percentage of individuals engaging in flight between the different sexes or age groups. Under natural conditions TCAH may be influenced by temperature, wind, barometric pressure, plant-host associated cues, and biological factors, but this information helps to provide information that can guide future field studies on insect dispersal in the landscape among available host plants.

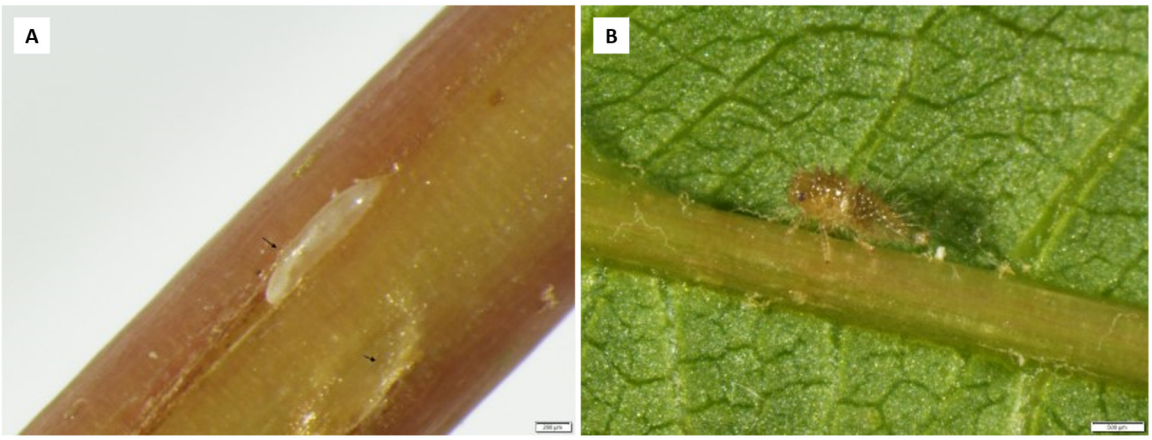

FIGURE 2-5 *S. festinus* egg oviposited in a grapevine petiole (A) and the first instar nymph hatched from the egg (B). The nymph did not survive past the second instar stage on grapevine.
SOURCE: Elizabeth Cieniewicz, Clemson University.

HOST–PATHOGEN INTERACTIONS AND HOST DEFENSE MECHANISMS

Since geminiviruses rely on host DNA replication machinery, geminiviruses reprogram the cell cycle in order to make the DNA replication machinery available (Hanley-Bowdoin et al., 2013). RNA silencing is an antiviral strategy that is highly conserved among plants; thus, many viruses have evolved mechanisms to suppress RNA silencing by interfering with one or more parts of the RNA silencing pathway (Incarbone and Dunoyer, 2013; Pumplin and Voinnet, 2013; Csorba and Burgyan, 2016). Geminiviruses are subject to both transcriptional gene silencing and post-transcriptional gene silencing. Therefore, many geminiviruses have viral suppressors of RNA silencing that overcome methylation (transcriptional gene silencing) and also that interfere with small RNA signaling (post transcriptional gene silencing) (Bisaro, 2006; Hanley-Bowdoin et al., 2013). So far, for GRBV, a single study has reported open reading frames (ORFs) C2 and V2 as having silencing suppressor activity, but the specific functions of these genes in terms of their interference with silencing are not yet known (Weligodage et al., 2023).

The GRBV genome is comprised of a single molecule of circular, single-stranded DNA (see Figure 2-6), approximately 3.2 Kb (Krenz et al., 2012). There are seven putative ORFs, four in the viral orientation and three in the complementary orientation, for which expression is temporally regulated based on the virus infection cycle (Krenz et al., 2012; Al Rwahnih et al., 2013; Vargas-Ascencio et al., 2019). The complementary sense (c-sense) ORFs encode the early proteins, i.e., those involved in genome replication, and the viral sense (v-sense) ORFs encode the late proteins, i.e., the structural proteins such as the putative coat protein (CP) and movement protein. The v-sense and c-sense ORFs are separated by a short intergenic region and a long intergenic region but overlap within their respective groups. The first six ORFs (three overlapping ORFs each in v-sense and c-sense) were initially predicted by *in silico* analyses, analogous to other geminiviruses (Al Rwahnih et al., 2013; Krenz et al., 2014). Expression of these six ORFs was later confirmed with additional evidence for a small seventh ORF, named ORF V0, upstream of V2, discovered by RNA sequencing (Vargas-Ascencio et al., 2019) (see Figure 2-6). The Rep protein is translated as a result of a messenger RNA (mRNA) splicing event that fuses ORFs C1 and C2, which has been observed in GRBV and in other geminiviruses (Nash et al., 2011). Splicing in the v-sense ORFs has only been observed in the capulaviruses, mastreviruses, and now the grabloviruses in the *Geminiviridae* (Vargas-Ascencio et al., 2019). The V2 and V3 ORFs may have a role in movement (Guo et al., 2015). Vargas-Ascencio et al. (2019) propose the v-sense splicing event, which was confirmed by RNA sequencing, to result in the V2 ORF being out of frame, thus resulting in higher downstream expression of V1 (encoding the CP), which would be consistent with mechanisms proposed for mastreviruses. The putative function of the V0 is still unknown, but the sequence is highly conserved among at least 74 grablovirus sequences, and therefore V0 is likely to have an important biological function. Expression of the v-sense ORFs, including the CP (V1), is still not well understood and attempts to visualize viral particles have failed repeatedly (Vargas-Ascencio et al., 2019), suggesting the importance of an amenable model host in order to more effectively study GRBV gene expression.

To date, it has not been possible to visualize GRBV particles, although a twinned icosahedral virion structure is predicted based on homology to other geminiviruses (Zhang et al., 2001; Hipp et al., 2017; Hesketh et al., 2018). However, GRBV protein products from ORFs V1 (putative CP) and V2 (putatively involved in movement) were detected in grapevine petioles and leaves using mass spectrometry, with CP detection six times higher in petioles compared to leaves (Buchs et al., 2018). The same study noted upregulation of flavonoid biosynthesis proteins in GRBV-infected grapevines, suggesting the activation of plant defense against GRBV (Buchs et al., 2018). Wallis and Sudarshana (2016) also observed upregulation of amino acids involved in plant defense in GRBV-infected Cabernet Sauvignon and Cabernet franc both before and after symptom development. They also suggested that shifts in vine physiology responses to GRBV could be related to defenses activated against other stresses (Wallis and Sudarshana, 2016).

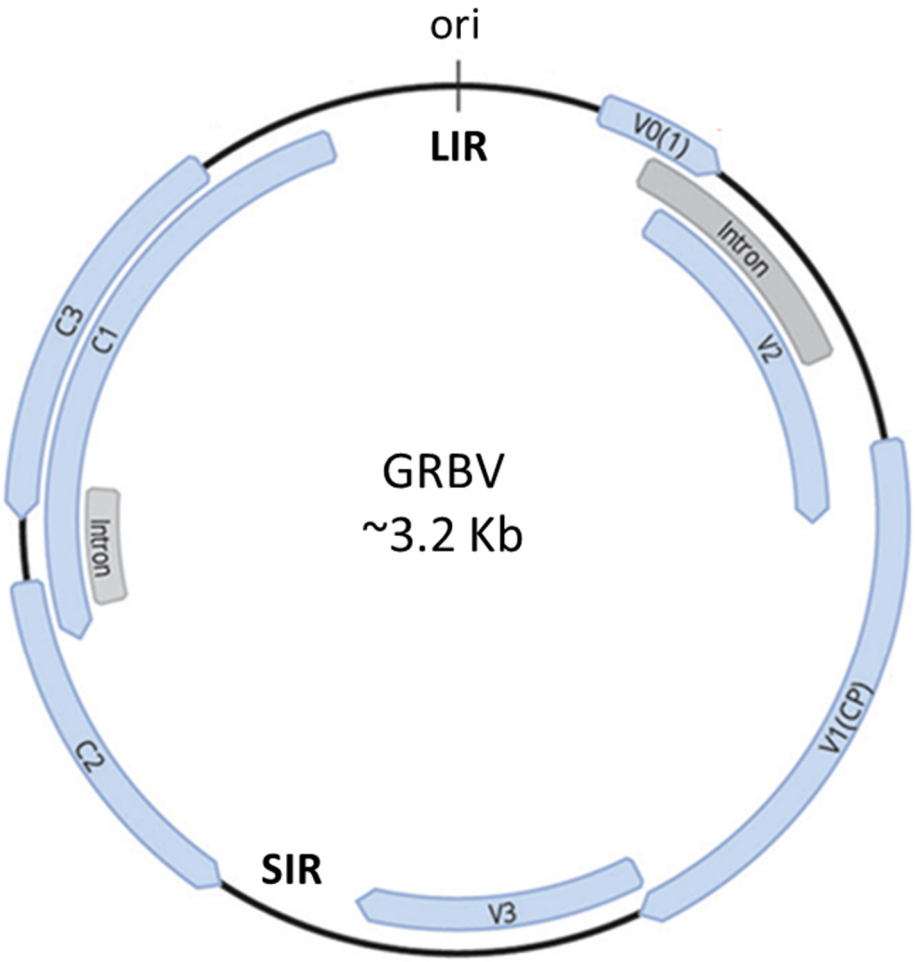

FIGURE 2-6 GRBV genome, with ORFs marked in blue. Also shown are the locations of the origin of replication (ori), the long intergenic region (LIR), and the short intergenic region (SIR). Numerous genome sequences have been reported, and genome length is approximately 3.2 Kb, with some variation.
SOURCE: Adapted from Thompson (2022). Reprinted from the Journal of General Virology, Microbiology Society.

GRBD effects on vine physiology, fruit characteristics, and wine attributes vary by cultivar (Rumbaugh et al., 2021a) and also by vintage and rootstock (Wallis, 2022). GRBV interferes with foliar metabolism and metabolite translocation (Wallis and Sudarshana, 2016; Martínez-Lüscher et al., 2019; Levin and Achala, 2020), reduces pruning weight (Reynard et al., 2018; Bowen et al., 2020), reduces total soluble solids and anthocyanin accumulation (Calvi, 2011; Girardello et al., 2019; Lee et al., 2021), and alters grape ripening (see Figure 2-7) by interfering with hormone pathways (Blanco-Ulate et al., 2017; Rumbaugh et al., 2022). As effects on fruit directly impact wine attributes, some studies have described GRBV effects on wine such as reduced ethanol in Chardonnay (Girardello et al., 2020a) and Merlot (Girardello et al., 2020b). GRBV also alters grape skin cell wall composition, reducing the extractability of phenols in infected vines (Rumbaugh et al., 2023). Changes in the chemical profiles of wines made from GRBV-infected fruit reflect sensory attributes (e.g., mouthfeel and astringency), as well (Girardello et al., 2020a,b). GRBV effects on fruit and wine composition are especially problematic for a value-added fruit crop like grapevine. Understanding the mechanisms underlying the impacts of GRBV on fruit qualities could aid in developing potential mitigation strategies.

FIGURE 2-7 Impacts on fruit color development due to altered ripening of a GRBV-infected Cabernet franc vine (left) compared to GRBV-negative vines (right) in a vineyard on Long Island, New York.
SOURCE: Alice Wise, Cornell Cooperative Extension, Suffolk County.

DIAGNOSTICS

Effective management of GRBD would involve the implementation of a comprehensive strategy that includes the use of certified disease-free planting material, regular monitoring by early detection, prompt removal of infected vines, strict quarantine measures, control of vectors (once identified), and the best viticultural practices (Sudarshana et al., 2015; Cieniewicz et al., 2017a; Meng et al., 2017). Early, sensitive, and reliable detection methods are paramount for disease management (Sudarshana et al., 2015). Various diagnostic techniques have been developed for GRBV detection, each with distinct attributes in terms of sensitivity, specificity, cost, and applicability (Krenz et al., 2014; Li et al., 2019; Romero Romero et al., 2019). This section discusses these available diagnostic techniques and strategies for their use.

Detection of GRBV by PCR and qPCR

Since the advent of polymerase chain reaction (PCR) in the late 1980s, PCR has been widely used in the detection of grapevine viruses and evolved into the gold standard for nucleic acid amplification techniques (Mullis et al., 1986; Rowhani et al., 1993; Gambino, 2015). PCR was the first method developed for detecting GRBV (Krenz et al., 2014). Two sets of primers were designed in the viral genome encoding the CP and replicase genes, and a pair of primers was also designed to amplify a fragment of the 16S ribosomal RNA (rRNA) gene as an internal PCR control, resulting in a triplex PCR assay used for a GRBV survey in the United States (Krenz et al., 2014). Later, Setiono et al. (2018) developed a quantitative PCR (qPCR) using a set of primers targeting the replicase gene in GRBV and the

grapevine actin gene to find the best time and tissues for collecting samples for detection. Although the quantity of GRBV is measured by qPCR, conventional PCR and qPCR have comparable sensitivity in detecting GRBV (Krenz et al., 2014; Setiono et al., 2018).

Detection of GRBV by LAMP

Loop-mediated isothermal amplification (LAMP) has been used to detect many plant viruses (Bhat et al., 2022). LAMP amplifies a target DNA fragment to detectable levels by using Bst DNA polymerase from *Bacillus stearothermophilus* and a set of four or six primers within a short time. The amplified DNA fragments can be detected directly by the color change of the reaction or indirectly by gel electrophoresis or lateral flow assay. An improved "pin-prick" LAMP method was developed to detect GRBV (Romero Romero et al., 2019). In this method, no DNA extraction is required; instead, the template for the LAMP is made by stabbing grapevine leaves or petioles three times with a 10 µl pipette tip and mixing trace amounts of tissues with sterile water. After the LAMP reagents and primers are added to the template, the reaction is performed at 65°C for 35 minutes, and the color change from pink to yellow indicates the presence of GRBV in the sample. The pin-prick GRBV LAMP method is 10,000 times more sensitive, costs less, and takes less time than conventional PCR. Leaves, petioles, and dormant budwood tissues can be pricked by the pipette tips for preparing the template. Although it is complicated to design the three sets of primers, online programs are now available for designing the primers, such as PrimerExplorer,[2] and primer sets are available for the detection of GRBV by LAMP (Romero Romero et al., 2019). In a recent study, pin-prick GRBV LAMP was shown to be a reliable, cost-effective, and rapid assay for detecting GRBV in the late developmental stages of grapevines (DeShields and Achala, 2023).

Detection of GRBV by RPA

Recombinase polymerase amplification (RPA) is another isothermal procedure for amplifying a target DNA fragment under a constant temperature in a short time. RPA has been commercially developed by TwistDx. It requires recombinase, DNA polymerase, single-strand binding protein, and a pair of primers specific to the target DNA sequences. A modified RPA assay, AmplifyRP Acceler8, has been developed for detecting GRBV (Li et al., 2017). In this assay, the primers and probes were designed in the CP and replicase region of the GRBV genome, and crude leaf extract was used directly in the reaction. The AmplifyRP Acceler8 was demonstrated to be consistent with PCR in detecting GRBV and is 100 times more sensitive than conventional PCR. Since RPA reagents are delivered in a pellet form under normal temperatures, crude tissue extracts are used, and the reaction is performed at 37°C for 20 minutes. RPA is considered to be a practical method for the onsite detection of GRBV in vineyards.

Detection of GRBV by RCA

Rolling circle amplification (RCA) is an isothermal enzymatic process to amplify circular DNA molecules. It relies on a DNA or RNA polymerase, with Φ29 DNA polymerase being one of the commonly used enzymes in this technique. RCA is particularly useful for various molecular biology and diagnostics applications because it generates long single-stranded DNA (ssDNA) or RNA molecules from a circular template (Gu et al., 2018). Previously, RCA was used to obtain the complete GRBV genome (Al Rwahnih et al., 2013; Krenz et al. 2014; Thompson, 2022) and to create GRBV infectious clones (Yepes et al., 2018). Although RCA has been used to amplify the whole genome of GRBV, it has not been developed for diagnosing GRBV. The application of RCA in diagnostics could be further studied to potentially provide an additional method to detect GRBV.

[2] See https://primerexplorer.jp/e/.

Detection of GRBV by CRISPR/Cas12a-Based Assay

Clustered regularly interspaced short palindromic repeats (CRISPR-)-associated 12a (CRISPR/Cas12a) was first developed for detecting human viruses (Chen et al., 2018) and is now used in the onsite detection of plant DNA viruses (Bhat et al., 2022). This assay is performed on a DNA fragment of a virus that is initially amplified by PCR, LAMP, or RPA. In this method, a single guide RNA is designed to bind to the amplified viral DNA fragments where it guides Cas12a to cut the target DNA and the single-stranded probe DNA molecules by the indiscriminate DNAase of Cas12a. The degraded products can be visually detected by a color change or lateral flow assay. A plasmonic CRISPR/Cas12a assay has been developed to detect GRBV visually by observing the color change in the reaction tubes (Li et al., 2019). This method still requires the extraction of total nucleic acids from grapevine tissues, but it can be improved by using RPA to amplify the target DNA fragments of GRBV first and then applying the plasmonic CRISPR/Cas12a assay.

Detection of GRBV by Hyperspectral Imaging

Hyperspectral imaging, which can be performed remotely by mounting hyperspectral cameras on unmanned aerial vehicles, represents a promising avenue for the advancement of virus disease scouting (Moghadam et al., 2017; Nguyen et al., 2021; Peng et al., 2022); however, the exploration of this technology in the context of virus-infected vines, specifically targeting GRBV, has been relatively limited (Reynolds et al., 2018). Within the visible region of the electromagnetic spectrum, the most discriminative wavelengths for predicting virus presence primarily reside in the red and orange regions, corresponding to anthocyanin presence and wavelengths associated with the absorption characteristics of chlorophyll and carotenoids (Sawyer et al., 2023). Further research could help enhance the precision of virus prediction methodologies with hyperspectral imaging. Particularly noteworthy is the consideration of extending the analysis to a broader electromagnetic spectrum range, a strategy for effectively evaluating and classifying vines that pose greater challenges regarding virus diagnosis.

Strategies for the Use of Available GRBV Diagnostic Techniques

The selection of the most suitable detection method depends on various factors, including available resources, required sensitivity, and the nature and number of the samples being analyzed. Overall, isothermal amplification methods such as LAMP and RPA provide accessible, cost-effective, and time-efficient options for routine GRBV testing (Li et al., 2017; Romero Romero et al., 2019). These methods are enhanced by using pin-pricked DNA extraction and crude tissue extract to expedite the diagnostic process. However, the sensitivity and specificity of all diagnostic tests are significantly influenced by factors such as the timing of sample collection and the type of tissue examined. For example, qPCR, LAMP, and endpoint PCR achieve their highest sensitivity when used to test basal and middle leaf samples (DeShields and Achala, 2023). During specific phenological stages, such as fruit set and veraison, qPCR exhibits a sensitivity of 98 percent, while LAMP demonstrates sensitivity values of 49 percent and 78 percent from basal leaf samples during the same stages, respectively. At the harvest and dormancy stage, qPCR, LAMP, and PCR exhibit 100 percent sensitivity in basal and middle leaf or dormant cane samples (DeShields and Achala, 2023).

Determining the optimal sample number is an important consideration for comprehensive diagnostics. Research suggests that four tissue samples per vine is the optimal number for effectively discriminating between GRBV-positive and GRBV-negative vines (DeShields and Achala, 2023). Another sampling method for minimizing false negatives in diagnostic assays is a composite sampling of petiole tissue from older leaves at the base of the vine with three evenly distributed excisions (Reynard et al., 2018; Setiono et al., 2018). Considering that GRBV titer varies among cultivars and even from cordon to cordon in a single vine, it is critical to determine which tissues shall be sampled and the dates for sampling. A recent study reported that GRBV titer is consistently high in infected grapevines in different

vineyards in three states in June (Flasco et al., 2023c), suggesting that sampling tissues for diagnostics in June will reduce incidences of false negative results. Kahl et al. (2022) also found a low rate of false negatives when basal leaves were sampled in summer months.

Visual determination of GRBD, as would be used by practitioners when scouting vineyards, is most effectively done when symptom expression peaks prior to leaf fall. However, symptom onset is variable between sites, cultivars, and vines of different ages, and can be confounded with other biotic and abiotic stressors (Adiputra et al., 2019; Rohrs et al., 2023), undermining the utility of visual inspection in facilitating the accurate and timely detection of GRBV.

Emerging technologies like plasmonic CRISPR Cas12a assays have shown improved sensitivity, offering promising options for GRBV detection (Li et al., 2019). Other methods that can be employed to detect low-titer plant viruses include serological methods, exemplified by immunocapture PCR (IC-PCR), which enhance sensitivity by combining immunocapture with PCR (Mulholland, 2009); digital LAMP (dLAMP), a digital variation of LAMP that enables highly sensitive quantification of viral nucleic acids (Panno et al., 2020); and high throughput sequencing (HTS), which can identify known and novel viruses at low titers with metagenomic sequencing (Boonham et al., 2014; Massart et al., 2014).

In general, the choice of a diagnostic technique should be guided by factors such as the number of samples requiring testing, the nature of the plants (i.e., foundation stock, nursery stock, germplasm, commercial vineyards, or a source of budwood), the phenological stage of grapevines, available resources, the urgency of diagnosis, and the desired level of sensitivity. Merging cost-effective methods with advancements in molecular diagnostics can enhance accessibility and reliability in GRBV detection and visual assessment, thereby contributing to more effective disease management.

MANAGEMENT

GRBD poses a significant threat to the viticulture industry with the potential for substantial economic losses (Al Rwahnih et al., 2013; Sudarshana et al., 2015; Cieniewicz et al., 2017a). The effective management of this disease would involve a comprehensive strategy (see Figure 2-8) that includes the use of certified virus-tested planting material, regular monitoring and early detection, prompt removal of infected vines, strict quarantine measures, control of vectors, and best viticultural practices (Sudarshana et al., 2015; Cieniewicz et al., 2017a; Meng et al., 2017). However, given that GRBV is a pathogen that has been recognized only recently, tactics for management of GRBV are still in the early stages of development and refinement.

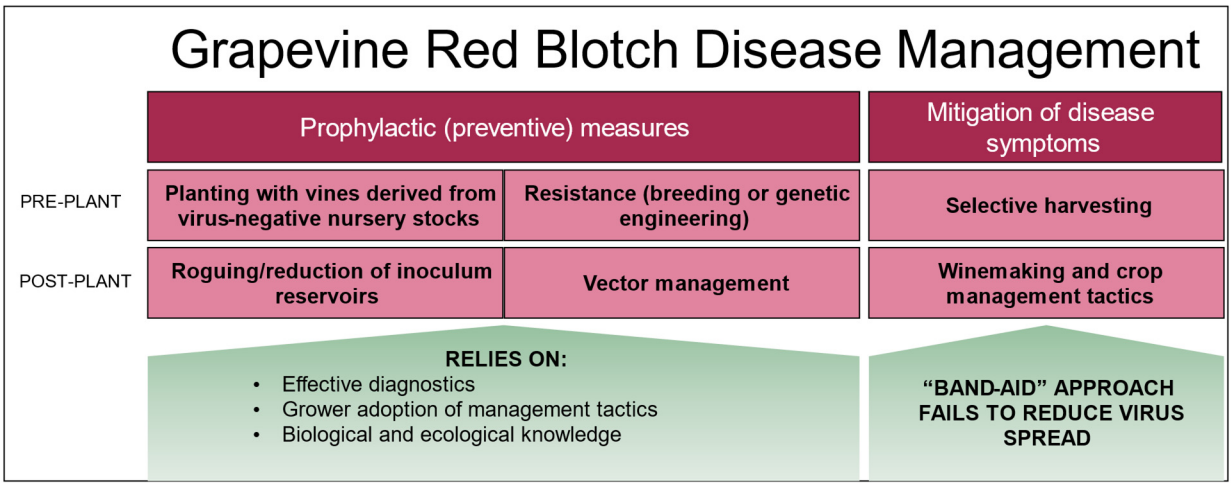

FIGURE 2-8 Schematic description of GRBD management strategies.

Management of viral grapevine diseases relies mainly on prophylactic (preventive) measures because viral infections are impossible to cure once they are established in the vineyard. Although some management strategies aim to mitigate symptoms, there is limited evidence of the effectiveness of such methods and there is a risk that they could inadvertently promote virus inoculum accumulation in vineyards. Thus, prophylactic methods to prevent infection are considered to have the highest chance of success. These management strategies can be divided into those that may be employed before planting (pre-plant) and those that may be employed after planting (post-plant) and are described in the following sections.

Pre-Plant Management

Clean Plant Programs

GRBV can be spread through infected propagation material (Sudarshana et al., 2015). This route of introduction, as opposed to vector transmission, is the predominant way by which GRBV is introduced into new areas and points to the need for clean plant programs that reduce the risk of spread of the virus (Poojari et al., 2017; Fuchs et al., 2021). One such program is the National Clean Plant Network (NCPN; see Box 2-2), which includes six Clean Plant Centers across the United States that focus on the propagation of clean grapevines (i.e., those derived from stocks that have tested negative for certain identified viruses).[3] Although activities vary across centers, as a whole the NCPN-Grapes centers import grapevine accessions under quarantine conditions, conduct diagnostics and virus elimination therapies, and maintain foundation collections to ultimately distribute clean plant materials to nurseries for further commercial propagation (see Box 2-2). Clean plants can also be certified[4] by state departments of agriculture in some states; the largest of these certification programs for grapevine is the California Grapevine Registration and Certification program, which was established in the 1950s and recently added GRBV to its list of pathogens of concern in response to grower interest.[5] The use of clean (i.e., derived from virus-negative stocks) plants is voluntary, but the high quality of nursery stock developed through clean plant programs and the resulting savings in management costs have provided an incentive for growers to make use of them (Arnold et al., 2019).

Studies demonstrate that certification programs are a cost-effective means of mitigating the impact of grapevine diseases. In one analysis, the value of using certified nursery stock was estimated at over $20 million annually for the mitigation of grapevine leafroll-associated virus 3 alone, substantially outweighing the costs of the certification program (Fuller et al., 2019). In light of estimates that GRBD could cost Napa County growers approximately $34,000 per acre over the 25-year lifetime of a vineyard (2023 dollars) (Ricketts et al., 2017)—potentially resulting in hundreds of millions of dollars in lost revenue and management costs for individual growing areas—incorporating GRBV testing into certification programs is likely to pay off.

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[3] See https://www.nationalcleanplantnetwork.org/grapes-1.

[4] It is important to distinguish between how the terms “clean” and “certified” are used in this report in reference to grapevine planting material. Throughout this report, both terms indicate that steps have been taken to minimize the likelihood that the material is infected with an economically important virus (e.g., GRBV or GLRaV-3); both clean and certified planting materials are tested for target viruses and maintained under conditions that minimize the risk of infection. However, each term has a specific meaning reflecting the context in which these steps are taken. In the case of “clean” plants, Grape Clean Plant Centers within the National Clean Plant Network determine what viruses to test for, what testing methods to use, and what protocols to use to minimize the risk of infection. In the case of “certified” or “certified clean” plants, certification programs administered by state departments of agriculture set rules that determine what viruses to test for, what testing methods to use, and what protocols to use to minimize the risk of infection. Because requirements may vary among clean plant centers and different state certification programs, “clean” and “certified” plants may be subject to different standards and practices.

[5] https://www.cdfa.ca.gov/plant/pe/nsc/docs/regs/ccr_3024_grapevine.pdf.

BOX 2-2
Production and Distribution of Clean Grapevines in the United States

The National Clean Plant Network (NCPN), foundation collections, and state certification programs provide processes and materials that help to prevent the spread of GRBD and other grapevine diseases with the goal of ensuring rootstocks or scions are derived from virus-negative sources before they are introduced to a vineyard.

National Clean Plant Network and NCPN-Grapes

The mission of the NCPN is summarized as "healthy agriculture through clean plants" with a vision of "safeguarding and supporting specialty crops by providing a sustainable source of clean plant material through innovation, collaboration, translational science, and outreach." There are currently 47 collaborating programs at 35 clean plant centers in 20 U.S. states. The NCPN was founded in 2008, originally supporting only fruit trees and grapevines, and has since grown to include seven major crop groups: fruit trees, grapevines, citrus, berries, hops, sweet potatoes, and roses. In general, the clean plant centers work with one or more NCPN crops and conduct diagnostics for graft-transmissible pathogens; pathogen elimination therapies; and maintenance, production, and distribution of clean plants in foundation collections.

NCPN-Grapes is a collaborative endeavor that includes clean plant center directors, industry members (growers and nurseries), extension associates, and federal and state regulators. NCPN-Grapes includes six centers as of 2024 and is headquartered at Foundation Plant Services at the University of California, Davis. Other clean plant centers supporting grapes include the Clean Plant Center Northwest at Washington State University, the Midwest Center of NCPN-Grapes at Missouri State University, the Eastern NCPN-Grapes Center at Cornell University, the Micropropagation and Repository Unit at North Carolina State University, and the Center for Viticulture and Small Fruit Research at Florida A&M University.

Grapevine Foundation Collections

Most clean plant centers maintain foundation (i.e., Generation 1 or G1) collections, which are collections of commercially relevant accessions of grapevine that have tested negative for known pathogens, are true-to-variety, and are maintained under conditions that minimize the risk of re-infection. Foundation collections are highly valued and vitally important to the preservation of clean plant material. Foundation Plant Services at the University of California, Davis recently moved its entire grapevine foundation collection indoors to a new $5.25 million greenhouse in response to GRBV pressure in its previous open-field foundation vineyard.

State Certification Programs

Clean plant centers produce clean plants and maintain foundation collections; however, they do not certify any plants. Certification is administered by state departments of agriculture. For example, in California, the California Department of Food and Agriculture administers the Grapevine Registration and Certification Program. Currently only five states have certification programs for grapevines: California, Missouri, New York, Oregon, and Washington. Since grape growers in many other states source material from nurseries in these states, the impact of these state certification programs extends beyond those particular states.

Foundation plants are maintained in G1 blocks at clean plant centers. Material that is propagated from foundation stock and established at nurseries can be certified by the states in registered increase blocks (G2 to G4). Plants from the increase blocks represent registered stock, which are propagated and sold to growers as certified planting material. Each classification within the certification scheme is subject to specific regulations on pathogens to be tested for and how often testing needs to occur, as well as when and how material can be propagated. It is important to note that "certified" does not equate to "clean" or "virus-negative."

Genetic Resistance

Host plant resistance to insect vectors of plant viruses can dramatically affect the spread of disease within a crop (Kennedy, 1976), and plant tolerance to vectors or diseases can reduce the negative impacts of a vector or viral infection on plant health or yield. Routes of resistance can include non-

preference (antixenosis), in which traits make the plant unattractive to the vector or do not provide appropriate stimuli to attract the vector; antibiosis, in which plant traits incapacitate or kill a vector; and tolerance, in which plant traits reduce the impact of a vector or infection. While resistance to a vector can be an important management tactic, there is also a risk that it can amplify disease spread depending on the form of the resistance and the dynamics of the pathosystem. No sources of genetic resistance to or tolerance of GRBV have been identified for grapevine, nor have traits conferring resistance to the insect vector TCAH been demonstrated in grapevine. Pre-plant options for GRBD management are therefore currently limited to the use of planting material derived from virus-negative sources, and at this point that is ultimately the responsibility of the customer in California.

Post-Plant Management

Roguing

Once established in a geographic region, GRBV can spread to uninfected plants from infected plants within the same vineyard, from neighboring vineyards, or from wild *Vitis* (Bahder et al., 2016a; Cieniewicz et al., 2017b, 2019). The route of pathogen spread within vineyards is unclear and there is mixed evidence regarding the prevalence and impact of this type of spread. Studies conducted in northern California and southern Oregon have shown evidence of secondary spread within vineyards from infected vines (Cieniewicz et al., 2017b; Dalton et al., 2019); however, secondary spread within vineyards has not been observed in other areas, such as New York (Cieniewicz et al., 2019). The lack of secondary spread in New York has been attributed in part to the absence of populations of TCAH in the site surveyed and underscores the role of infected propagation materials as a means of viral spread (Cieniewicz et al., 2019).

When GRBV is detected in a vineyard, current management guidelines recommend roguing (removing) infected vines. Roguing individual infected vines and replanting them with vines derived from virus-negative sources appears to be economically viable when relatively few vines are infected, defined as less than 30 percent of the vineyard (Ricketts et al., 2017). When more vines are infected, replanting the entire vineyard may be warranted and more cost effective in the long term. Roguing infected vines significantly reduces the spread of the virus in vineyards where TCAH is known to occur (Achala et al., 2022). Frequent scouting for infected vines is important for roguing practices to be effective as a cultural management tactic. However, accurate and reliable molecular diagnostics are also critical as symptoms may not be readily apparent or may appear similar to those of other diseases, mite damage, or nutrient deficiencies. The likelihood of grower adoption of these practices increases with their knowledge regarding GRBD. Growers who have personally experienced losses on their farms are most likely to implement management programs (Hobbs et al., 2022).

Vector Management

Because of knowledge gaps regarding TCAH, other potential vectors, vector-virus interactions, and interactions between GRBV and grapevines, best practices for vector management are currently not well established. Practices that have been considered include habitat management, insecticide application, and biological control.

Habitat management within vineyards may be important for disease management. Vineyards are diverse agroecosystems that are often populated with large numbers of plants beyond grapevines that may be reproductive hosts or adult feeding hosts for TCAH. These non-grapevine TCAH hosts may also be hosts of GRBV, or they may simply serve as green bridges that facilitate vector movement to grapevines. Discing groundcovers (the rows between vine rows) can reduce numbers of TCAH captured on yellow sticky cards placed in the vine canopy. This practice likely operates by spatially segregating more preferred TCAH hosts from grapevines, thus decreasing the likelihood of TCAH reaching grapevines (Billings et al., 2021).

Insecticide management of TCAH is not currently a recommended management practice until more is known about virus transmission and seasonal population dynamics of the vector(s). The initial response of growers to the emergence of a novel insect-transmitted plant pathogen has often been intensive insecticide applications in efforts to control the vector (Cho et al., 1989; Culbreath et al., 2003; Alvarez et al., 2016; Wenninger and Rashed, 2024). However, reliance on intensive insecticide use rarely provides effective, sustainable disease management, and comprehensive integrated approaches are often ultimately more effective. TCAH is recognized as an occasional, minor pest of annual crops, such as soybeans and peanuts, and short-lived perennials, such as alfalfa, where the feeding by late instar nymphs can cause girdling of stems resulting in stand loss (Andersen et al., 2002; Beyer et al., 2017). It is not known to be a vector for other plant pathogens besides GRBV. Significant knowledge gaps in the GRBV pathosystem, especially regarding the ecology and population dynamics of the vector(s), preclude definitive recommendations for the role of insecticides in management programs (Cieniewicz et al., 2017a).

Little is known about the potential for biological control of TCAH. A fungal pathogen, *Erynia delphacis*, has been identified infecting TCAH in the southeastern United States (Miller and Harper, 1987). Although the fungus was found to be highly virulent against TCAH, it is not host specific, and it may not be as virulent in drier climates with greater ultraviolet radiation (Quesada-Moraga et al., 2023). TCAH is susceptible to a number of insect predators and parasitoids. Kron and Sisterson (2020b) evaluated six commercially available predatory insects against nymphal and adult TCAH. They found only *Hippodamia convergens* adults (Coleoptera: Coccinellidae) and *Chrysoperla rufilabris* larvae (Neuroptera: Chrysopidae) were effective predators of TCAH. Medal et al. (1997) found that *Geocoris punctipes* and *Nabis roseipennis* favored preying on TCAH, even in the presence of alternative prey. These predatory insects were most likely to prey on younger nymphs, so promoting their populations on reproductive hosts rather than directly on grapevines would be the most effective use.

Mitigation of GRBD Effects

GRBD is known to affect fruit and wine quality (Girardello et al., 2019; Pereira et al., 2021; Rumbaugh et al., 2021a). Virus infection reduces berry weight and alters the level of total soluble solids and profiles of primary and secondary phytochemicals, including phenolics. Research is ongoing to identify mechanisms to compensate for these effects. Supplemental irrigation can compensate for berry weight but does not provide consistent recovery of anthocyanins (Copp and Levin, 2021; Copp et al., 2022). At present, available crop management practices do not seem sufficient to overcome the adverse effects of GRBD (Copp et al., 2022; Kurtural et al., 2023). Delayed harvest of fruit from GRBV-infected vines can mitigate some of the effects on specific wine aroma compounds, but whether this practice is practical in a winemaking setting has not been determined (Rumbaugh et al., 2021b; Girardello et al., 2024). Ultimately, any tactics aimed at mitigating the effects of GRBD will not address the drivers of GRBV inoculum in vineyards, potentially exacerbating the problem of virus spread. For these reasons, Fuchs (2024) recommends focusing primarily on reducing virus inoculum in vineyards and reducing secondary spread of GRBV.

Factors Impacting Effective Management

GRBD management tactics at all levels rely on effective and affordable diagnostics, knowledge and adoption of strategies by growers, and credible evidence of the biological and ecological drivers of GRBV. Emphasizing grower knowledge acquisition and developing management strategies with economic feasibility in mind will improve management in practice (Hobbs et al., 2022). Fuchs (2020) highlights the importance of bridging the communication gap between researchers and growers (i.e., decision-makers) in order to promote adoption of effective management strategies. Also, perceptions of wine grape crop quality may differ between winemakers and vineyard managers, which is another highlighted gap (Fuchs, 2020). Fuchs (2020) recommends applying a premium for clean, certified vines to

increase confidence in the quality of certified nursery material and incentivize the use of this material. Economic feasibility is a major factor in the adoption of management strategies and is a message that resonates with growers (Fuchs, 2020; Hobbs et al., 2022, 2023). Most of the management strategies that have been demonstrated to be effective in managing GRBV and other grapevine viruses are relatively simple, but they are not widely adopted, likely due to deficiencies in communication and knowledge dissemination that resonates with the decision-makers (Fuchs, 2020).

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3
Current Knowledge on Grapevine Leafroll Disease

Among the viral diseases affecting grapevine, grapevine leafroll disease (GLD) is the most widespread, occurring wherever grapes are grown (Maree et al., 2013; Naidu et al., 2014), in all types of climates, and in all grapevine varieties. It is also considered the most economically important, with documented negative impacts on grape yield, juice and wine quality, and productive lifespan of affected vineyards (Almeida et al., 2013; Naidu et al., 2014, 2015; Alabi et al., 2016).

As discussed in a historical account by Maree et al. (2013), it is unclear exactly when GLD was first recognized as an infectious disease, although it likely originated in Europe, the Mediterranean basin, the Near East, or the Caucasus region since these geographical regions are where grapevines were first domesticated (Dong et al., 2023). From there, the disease likely spread via human-mediated distribution of infected grapevine cuttings (Maree et al., 2013 and cited references), probably through vegetative cuttings collected during dormancy when grapevine is devoid of foliage and GLD symptoms are not apparent.

The deciphering of GLD etiology and biology was (and continues to be) a long and arduous process. Although it was recognized as a malady of grapevine since at least the 1800s, it was not until the 1930s that the graft-transmissible nature of GLD was documented (Scheu, 1935). In the 1970s, closterovirus-like virions were found in transmission electron microscopy studies of GLD-affected tissues (Namba et al., 1979; Faoro et al., 1981; Castellano et al., 1983), and in the 1980s scientists demonstrated the transmission of GLD by mealybugs (Engelbrecht and Kasdorf, 1990). The latter two developments strengthened the hypothesis that GLD is a viral disease whose etiological agent(s) likely reside within the phloem tissues.

SYMPTOMS

Grapevine is a deciduous, woody perennial plant that goes through phases of vegetative growth, reproductive growth, and dormancy, with the timing and duration of each of these three phases varying across different regions and climates. All grapevine species can be classified broadly into two categories based on the color of the berry skin at maturity: red or black-fruited cultivars have reddish-purple berry skin that is conferred by the pigment anthocyanin, whereas white-fruited cultivars have green or golden berry skin (Walker et al., 2007). Vines affected by GLD contain detectable levels of grapevine leafroll-associated viruses (GLRaVs) throughout the year, but visual foliar GLD symptoms only begin to become apparent on affected vines around the middle of the reproductive growth phase that coincides with the onset of berry maturation or veraison (Naidu et al., 2014). The leaves remain symptomatic until they fall, following which the vine goes into dormancy. This pattern continues through each seasonal cycle for the lifespan of the infected grapevine, with symptoms absent during each subsequent vegetative growth stage and then re-emerging during veraison.

Foliar discoloration of grapevine leaves due to GLD is more apparent in red or black-fruited than white-fruited cultivars (Naidu et al., 2014). In most red or black-fruited grapevine varieties, the classic foliar symptoms of GLD consist of red to purple coloration of the leaf areas between the veins, which typically develops first on the lower canopy mature leaves and then gradually expands to the upper canopy leaves, while uninfected vines of the same cultivar and age show no such coloration. The main veins on the discolored leaves remain green. GLD symptoms in white-fruited cultivars are much more subtle. In these grapevines, the interveinal areas of infected plants may become mildly chlorotic; however, this disease phenotype is not consistent across white-fruited cultivars and, even when present, may be confused with nutrient deficiency symptoms (see Figure 3-1). Hence, whereas classic GLD symptoms in red or black-fruited cultivars are relatively reliable signs of the likely occurrence of one or

more of the GLRaVs in the vine, these symptoms may be less reliable for white-fruited cultivars. Other factors such as virus species and/or strain type, cultivar differences, and virus co-infections may also influence the expression of symptoms or lack thereof. In addition, stresses such as damage caused by mites and drought may mask foliar virus symptoms.

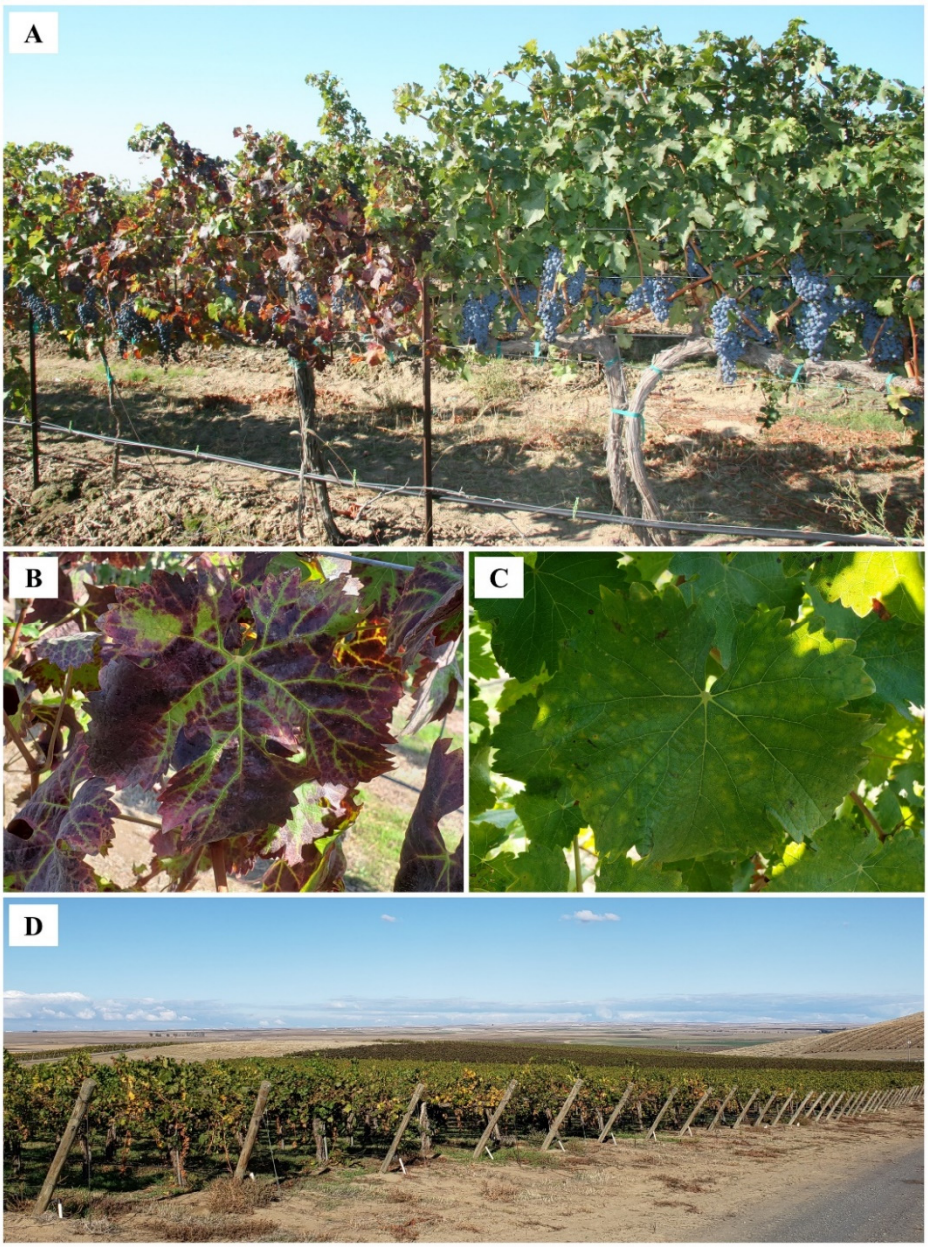

FIGURE 3-1 GLD symptoms on a black-fruited *Vitis vinifera* cv. Cabernet Sauvignon (A: left) relative to an adjacent non-symptomatic vine of the same cultivar (A: right). Close-up photo of GLD-induced interveinal reddening on leaf of symptomatic, black-fruited *V. vinifera* cv. Cabernet franc (B) and foliar interveinal chlorosis on white-fruited cv. Chardonnay (C). Field view of a GLD-affected vineyard showing infected vines of white-fruited cv. Chardonnay (foreground) and black-fruited cv. Merlot (background) grapevines (D). SOURCE: Naidu A. Rayapati, Washington State University IAREC, Prosser, WA; Olufemi J. Alabi, Texas A&M AgriLife Research and Extension Center, Weslaco, Texas.

In advanced stages of the disease, mature leaves of GLD-affected grapevine typically display downward rolling of the leaf margins regardless of the berry color type (see Figure 3-2). Although the incubation period (i.e., time from infection to the first appearance) of the downward leafroll phenotype is not clear, the consistency of this symptom as an eventual outcome of infection by GLD-associated viruses likely informed its choice as the descriptor that typifies the disease. It is worth noting that grapevine red blotch virus (GRBV) may also induce leaf reddening in red or black-fruited grapevine cultivars, but in the case of grapevine red blotch disease (GRBD), the pattern of coloration is blotchy, the leaves do not show green vein banding, and the leaves do not display downward rolling in most cases. That said, there is significant overlap in symptoms of foliar coloration between GLD and GRBD (Adiputra et al., 2019); hence, visual observation of symptoms may be unreliable for their accurate diagnosis.

FIGURE 3-2 Classic downward rolling of leaf margins due to GLD in *V. vinifera* black-fruited cv. Cabernet franc (A) and white-fruited cv. Chardonnay (B).
SOURCE: Olufemi J. Alabi, Texas A&M AgriLife Research and Extension Center, Weslaco, Texas.

While the described leaf discoloration and leafroll symptoms are generally consistent for commonly grown *V. vinifera* grapevine cultivars, symptomology is less consistent for other *Vitis* species. For instance, some non-*vinifera* grapevines with dark-colored berry skins such as juice grapes (e.g., *V. labruscana* 'Concord'), muscadine grapes (*M. rotundifolia*), and rootstocks (e.g., *V. riparia*, *V. rupestris*, *V. berlandieri*, *V. champini*, and their hybrids) often remain symptomless throughout their growth phases even when infected with GLRaVs (Naidu et al., 2014). Some GLRaVs and/or their strains may also occur in *V. vinifera* vines as symptomless infections (Martelli et al., 2012; Poojari et al., 2013), and GLD symptomology may differ between specific virus genotypes (Chooi et al., 2022). Given the complexity and inconsistency of GLD symptomology, it is therefore important to be cautious when interpreting symptoms to inform diagnosis of the disease and its associated viruses.

IMPACT

Most of the documented impact of GLD comes from studies conducted using red or black-fruited *V. vinifera* cultivars since their unique symptomatology lends itself to the proper selection of experimental vines to be used for such studies. Also, such studies have routinely been conducted with vines confirmed positive for grapevine leafroll-associated virus 3 (GLRaV-3), the most widely distributed of the GLD-associated viruses (Maree et al., 2013). Results from these studies show that GLD perturbates photosynthesis and carbohydrate metabolism in symptomatic leaves with significant reductions in both physiological parameters occurring during the post-veraison stage, which coincides with the expression of foliar GLD symptoms (Bertamini and Nedunchezhian, 2002; Sampol et al., 2003; Basso et al., 2010; Gutha et al., 2012; Moutinho-Pereira et al., 2012). GLRaV-3 can also affect the source/sink balance during the post-veraison stage of berry development by interfering with the berry maturation process via altering the expression of genes involved in the biosynthesis of anthocyanin and sugar metabolism (Vega et al., 2011). Enhanced expression of key genes involved in the biosynthesis of flavonols was detected in GLD symptomatic Merlot leaves, which also were found to accumulate anthocyanin compounds that should typically accrue predominantly in berry skins (Gutha et al., 2012). This probably explains the classic interveinal reddening symptoms that are displayed in red or black-fruited grapevine cultivars with GLD during the post-veraison stage.

Studies have also documented GLD associated yield penalties, including reduction in berry cluster numbers and weights, uneven coloration of berries, reduced total soluble solid content of berries, and detrimental alterations to other fruit juice chemistry parameters (Cabaleiro et al., 1999; Borgo et al., 2003; Komar et al., 2007; Mannini et al., 2012; Alabi et al., 2016). GLD impacts on berry chemistry have been found to translate into negative impacts on various wine quality attributes (Mannini et al., 1998; Legorburu et al., 2009; Alabi et al., 2016) to such an extent that consumers could perceive GLD effects during sensory evaluations (Alabi et al., 2016). At the grower level, GLD can be detrimental to vineyard profitability. Annual average GLD-associated economic loss estimates derived from data from cv. Merlot vines in New Zealand (Nimmo-Bell, 2006), Cabernet Sauvignon vines in South Africa (Freeborough and Burger, 2008), and Cabernet franc vines in New York (Atallah et al., 2012) range from $972 ($1,322)[1] to $2,117 ($2,880)[1] per hectare. GLD and its associated viruses also hinder the free, fast, and cost-effective exchange and movement of grapevine vegetative cuttings owing to the need to comply with phytosanitary regulations.

CAUSAL (OR ASSOCIATED) VIRUSES

Among viral plant diseases, GLD is unique in the complexity of its etiology. Most well-studied viral diseases in plants are caused by single virus species, although there are often many sequence variants or quasispecies of these viruses found in host plants (Domingo et al., 2006). By contrast, several distinct but taxonomically related virus species have been documented in GLD-affected grapevines, each of which may also have divergent strains and/or molecular variants. Collectively, viruses characterized from GLD-affected vines and linked to the disease are called grapevine leafroll-associated viruses (Martelli, 2000; Martelli et al., 2012). The word "associated" is contained in their species name because the classical set of rules guiding the decision to declare a pathogen as the causal agent of a disease, known as Koch's postulates (Loeffler, 1884; Brock, 1999; see Box 2-1 in Chapter 2), is yet to be completed for GLRaVs. To elucidate the etiological role of GLRaV-3 in GLD, Jarugula et al. (2018) developed complementary DNA (cDNA) clones of the virus and demonstrated its infectivity in *Nicotiana benthamiana*. More recently, Li et al. (2023) also reported the construction of an infectious GLRaV-3 clone which was successfully inoculated into virus-free grapevine plantlets via agro-infiltration (Shabanian et al., 2023) to reproduce GLD symptoms. If this report from Li et al. (2023) is independently

[1] Adjusted for inflation using the Consumer Price Index (CPI) from the U.S. Bureau of Labor Statistics.

validated by other investigators, Koch's postulates would be fulfilled, confirming GLRaV-3 as a causal agent of GLD and opening up a wider discussion on possible change to the names of GLRaVs.

Maree et al. (2013) provided a detailed historical account of the discovery of GLRaVs. The first two GLRaVs to be identified from symptomatic GLD-affected grapevine were determined to be morphologically similar but serologically distinct; these viruses were named GLRaV-1 (Gugerli et al.,1984) and GLRaV-2 (Zimmermann et al., 1990). Next to be discovered was GLRaV-3 (Zee et al., 1987), followed by subsequent reports of additional morphologically similar but serologically and/or molecularly distinct closteroviruses from GLD-affected vines which were sequentially named with number suffixes denoting their order of discovery. Various research groups reported the discovery of additional GLRaVs (Hu et al., 1990; Zimmermann et al., 1990; Walter and Zimmermann, 1991; Gugerli and Ramel, 1993; Choueiri et al., 1996; Gugerli et al., 1997; Alkowni et al., 2004; Maliogka et al., 2009; Abou Ghanem-Sabanadzovic et al., 2010), largely based on sequence-based taxonomic criteria such as the heat shock protein homology 70 (HSP70h) and the coat protein (CP) gene using thresholds specified by the *Closteroviridae* study group of the International Committee on the Taxonomy of Viruses.

As complete genomes of GLRaVs became available, revealing common genome length and architecture among some viruses that had been previously designated as distinct species, the taxonomy of GLRaVs was revised to encompass not only their biological and serological properties, but also their genome characteristics (Martelli et al., 2012). This effort led to the current recognition of six distinct GLRaVs assigned into three genera in the family *Closteroviridae* (Martelli et al., 2012). These six viruses are grapevine leafroll-associated virus 1 (GLRaV-1; *Ampelovirus univitis*), grapevine leafroll-associated virus 2 (GLRaV-2; *Closterovirus vitis*), grapevine leafroll-associated virus 3 (GLRaV-3; *Ampelovirus trivitis*), grapevine leafroll-associated virus 4 (GLRaV-4; *Ampelovirus tetravitis*), grapevine leafroll-associated virus 7 (GLRaV-7; *Velarivirus septemvitis*), and grapevine leafroll-associated virus 13 (GLRaV-13; *Ampelovirus tredecimvitis*). GLRaV-1, GLRaV-3, GLRaV-4, and GLRaV-13 belong to the genus *Ampelovirus*, GLRaV-2 belongs to the genus *Closterovirus*, and GLRaV-7 is in the genus *Velarivirus*. More rigorous studies are warranted to better understand the role of GLRaV-7 variants in producing leafroll symptoms since the virus can be detected in both symptomatic and non-symptomatic vines and often in mixed infection with other viruses (Choueiri et al., 1996; Avgelis and Boscia, 2001; Reynard et al., 2015; Al Rwahnih et al., 2017). GLRaV-3 is the most prevalent of these viruses and is also considered the most economically damaging (Naidu et al., 2015); hence, GLRaV-3 is a primary focus of this report.

All GLRaVs are composed of monopartite, positive-sense, single-stranded, polycistronic RNA genomes, but they differ in their genome lengths and in the number and arrangements of their encoded genes (see Figure 3-3). Although there is some variation between studies and isolates, in general, GLRaV-1 and GLRaV-3 have been shown to have the largest genomes, GLRaV-4 strains have the smallest genomes, and the rest have intermediate genome sizes. The size variations among *Closteroviridae* viruses result from various modification events during viral replication such as sequence deletion, sequence acquisition from other sources, genome bipartition, and gene duplication (Martelli et al., 2012).

The genome size differences are also reflected in the number of open reading frames (ORFs) that are encoded by the GLRaVs. However, regardless of their genome size differences and the number of ORFs, GLRaVs carry two main conserved gene block segments across the species. These are the replication gene block (RGB), which contains two N-terminal ORFs that function in genome replication, and the quintuple gene block (QGB), which contains a set of five genes of varied functions toward the C-terminus. Only GLRaV-4 lacks the QGB. Apart from these conserved genes, some GLRaVs encode additional genes toward their C-terminus; some of these have known functions while others are putative genes. The evolutionary basis for such genome complexity is not well understood and could be further elucidated through future research.

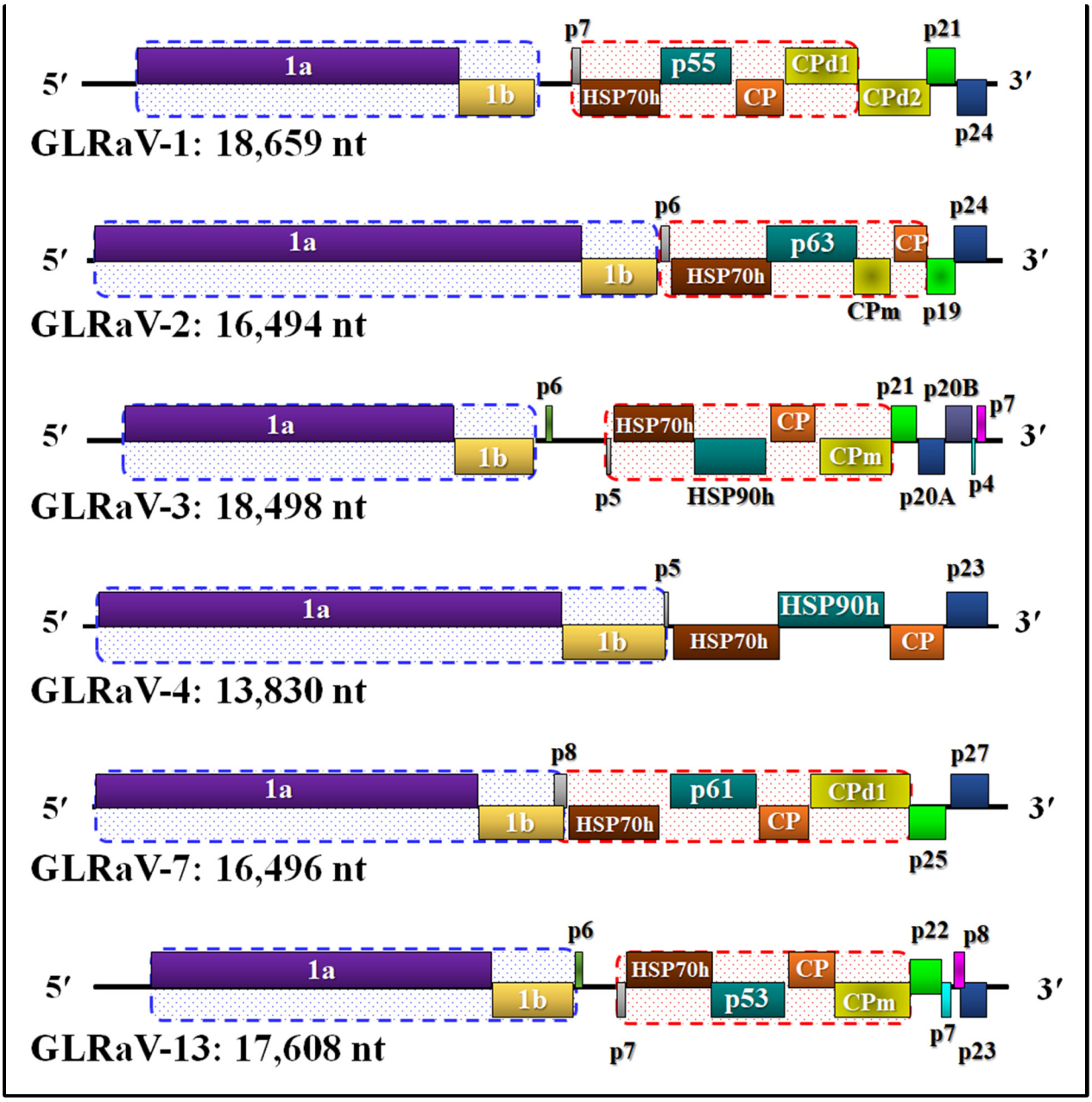

FIGURE 3-3 A depiction of the genome organization of GLRaVs showing differences in their typical lengths as well as the number and arrangement of their encoded genes. The different GLRaVs have two conserved gene blocks in common: replication gene block (blue patterned box) and quintuple gene block (red patterned box). The genome sequences used to represent the different GLRaVs include GenBank accessions NC_016509 (GLRaV-1), NC_007448 (GLRaV-2), EU259806 (GLRaV-3), NC_016416 (GLRaV-4), JN383343 (GLRaV-7), and NC_029783 (GLRaV-13).
NOTE: Details of the functions of the encoded genes can be gleaned from Naidu et al. (2015) and Song et al. (2021).
SOURCE: Olufemi J. Alabi, Texas A&M AgriLife Research and Extension Center, Weslaco, Texas.

Given that GLRaV-3 was a main focus of this study, a query of the National Center for Biotechnology Information GenBank database was conducted to examine the GLRaV-3 genome in greater detail. This search returned 81 hits for complete GLRaV-3 genomes, which ranged in length from 17,919 to 18,785 nucleotides (as of 08/08/2024). Notably, the 5′ and 3′ extremities have only been experimentally verified for a few of these GLRaV-3 isolates via random amplification of complementary DNA ends (RACE) assays. Each of these genomes contain 12 ORFs and some GLRaV-3 isolates may also encode an additional ORF2 (p6) proximal to the RGB and before the QGB. The GLRaV-3 ORFs are numerically named with the RGB ORFs designated as 1a and 1b, a non-conserved ORF2, the QGB ORFs 3-7, and the ORFs 8-12. In analogy with monopartite closteroviruses, ORFs 1a and 1b of GLRaV-3 function in replication, ORF2 has unknown functions, ORF3 encodes a small transmembrane protein and may function in cell-to-cell movement, ORF4 encodes the HSP70 likely serving as a molecular chaperone for plasmodesmata targeting and cell-to-cell movement, ORF5 (p55) has unknown functions, ORF6 encodes the CP for virion encapsidation, and ORF7 encodes the minor capsid protein (CPm) that may be a component of the virion tail (Agranovsky et al., 1995; Satyanarayana et al., 2004; Naidu et al., 2015). The functions of ORFs 8-12 are not clearly understood, although recent studies have shown that ORFs 8-10 could function as RNA silencing suppressors to counteract the grapevine RNA interference (RNAi) defense (Reed et al., 2003; Lu et al., 2004; Chiba et al., 2006).

VECTORS

Propagation of infected planting material is the primary mechanism for GLD spread, and spatial patterns suggest that the disease typically emanates from a focal point source of insect infestation (Cabaleiro and Segura, 1997; Cabaleiro et al., 2008; Arnold et al., 2017). Vectors of GLRaVs are classified in the order Hemiptera and fall into the superfamily Coccoidea, comprising approximately 8,000 species. Within this superfamily, mealybugs (family Pseudococcidae) and soft scales (family Coccidae) are demonstrated vectors for GLRaVs (Tsai et al., 2010; Le Maguet et al., 2012; Blaisdell et al., 2015; Herrbach et al., 2017). GLRaV-3 transmission by a mealybug, *Planococcus ficus* Signoret, was first documented in 1980 (Engelbrecht and Kasdorf, 1990), followed by reports of transmission by additional mealybug and soft-scale species (Belli et al., 1994; Cabaleiro and Segura, 1997). Almeida et al. (2013) reported that among some of the primary wine grape growing regions of the world, *Pl. ficus* and *Pseudococcus calceolariae* Maskell appear to be the most important vectors but noted that all mealybugs and soft scales that feed on wine grapes should be viewed as potential vectors. Insects considered capable of transmitting GLRaVs essentially encompass all common mealybugs and soft scales found worldwide in regions where GLD is present. In addition to *Pl. ficus* and *Ps. calceolariae*, these include the mealybugs *Pseudococcus maritimus* Ehrhorn (grape mealybug), *Pseudococcus viburni* Signoret (obscure mealybug), *Pseudococcus longispinus* Targioni-Tozzetti (longtailed mealybug), *Ferrisia gilli* Gullan (Gill's mealybug) (Jones and Nita, 2020), *Pseudococcus comstocki* Kuwana (Comstock mealybug), *Planococcus citri* Risso (citrus mealybug), *Phenacoccus aceris* Signoret (apple mealybug), and *Heliococcus bohemicus* Sulc (bohemian mealybug) (as reviewed in Daane et al., 2012; Herrbach et al., 2013), as well as soft scales such as *Pulvinaria vitis* L. (woolly vine scale), *Parthenolecanium corni* Bouché (European fruit lecanium scale), *Ceroplastes rusci* L. (fig wax scale), *Neopulvinaria innumerabilis* Rathvon (cottony maple scale), *Coccus longulus* Douglas (long brown scale), *Parasaissetia nigra* Nietner (black scale), and *Saissetia* sp. (Belli et al., 1994; Mahfoudhi et al., 2009; Le Maguet, 2012; Herrbach et al., 2013; Krüger and Douglas, 2013).

GLRaV-3 is the primary virus that causes GLD in the United States. However, multiple viruses causing GLD can coexist and can be transmitted by the same insect vector. There is no indication of strict vector-virus species specificity in transmission (Tsai et al., 2010). *Pl. ficus* can transmit at least five different GLRaVs, although transmission efficiency varies. To date, all tested grape-associated mealybug species can transmit various GLRaV species (Tsai et al., 2010; Le Maguet et al., 2012). Thus, all mealybugs inhabiting grapevines are presumed to be GLRaV vectors unless proven otherwise. Fewer studies have investigated GLRaV transmission by soft scales, as compared with mealybugs (Bahder et al.,

2013), but two, *Pu. vitis* and *Pa. corni*, are known to be competent (Hommay et al., 2009; Bahder et al., 2013). The grape mealybug, *Ps. maritimus* and *Pa. corni* are both competent vectors of GLRaV-3 with established populations in North American vineyards (Bahder et al., 2013). Although vector efficiency differs between these, Bahder et al. (2013) demonstrated that multidirectional transmission could occur between *Vitis* species by both insects (interspecific transmission).

Four mealybug species are commonly found in California vineyards: *Pl. ficus*, *Ps. longispinus*, *Ps. maritimus*, and *Ps. viburni*. The vine mealybug, *Pl. ficus,* causes the most damage to wine and table grapes. It is distributed throughout many wine grape growing regions of the world, occurring in more than 47 countries (Ji et al., 2020). The insect's native range is not clear, although Israel is postulated as the origin of populations in North and South America, Europe, and South Africa (Daane et al., 2018). There have been few studies of host plant preference and suitability for mealybug species associated with grapes in California. Although grapevines are a preferred host for *Pl. ficus,* the species is polyphagous and invades host plant species in more than 31 genera from 25 families (Almeida et al., 2013; García Morales et al., 2016). In addition to causing damage to grapes, *Pl. ficus* also affects weedy and agricultural plants such as fig, quince, mangos, tomatoes, beets, and avocados. Recently, Correa et al. (2023) revisited the taxonomy of previously synonymized as *Pl. ficus* specimens from different regions of the world and delineated them into two species: *Pl. ficus* (Signoret) s.str. and *Pl. vitis* (Niedielski) based on morphological and molecular analyses. The specimens from eastern Mediterranean and California were reclassified as *Pl. vitis* (Niedielski).

Nymph and adult mealybugs feed on the phloem sap from all parts of host plants, including the roots, leaves, fruits, and trunks. To extract sap, the insects insert a needle-like feeding structure called a stylet into plant vascular tissues. If the grapevine is infected with GLRaV, the virus particles are ingested along with the sap during this process. During feeding, mealybugs excrete honeydew that is high in carbohydrates. As honeydew is flicked away from the insect, it accumulates on the surrounding leaves and plant where it serves as a substrate for the growth of sooty mold, which reduces host photosynthesis. Accumulation of high concentrations of sooty mold causes cosmetic damage that reduces fruit marketability and may form a hard waxy layer on the infested plant. Extensive infestations of grapevine mealybug can lead to premature leaf shedding and the gradual weakening of vines when infestations occur in consecutive years. Over time, excessive feeding damage can cause defoliation and vine death. Certain mealybug species, including *Ps. calceolariae* and *Pl. ficus*, often establish a segment of their population on vine roots (Walton and Pringle, 2004; Bell et al., 2009), and these insects can dwell underground. In California, root colonization appears to be limited to regions with sandy soils and/or extreme heat and climate change could influence the geographical range and ecology of this vector (Ji et al., 2020). This situation poses a significant challenge during replanting because even after the vine is removed, residual roots can remain viable for extended periods, providing sustenance for GLRaVs and mealybugs, thereby acting as a conduit from the previously infested vineyard to the new replanted vines (Pietersen, 2006).

Mealybugs are the primary vectors of GLRaVs in California wine grape production systems. Diverse mealybugs are known pests of wine grapes in California, and based on current studies, the transmission of these viruses appears to be somewhat non-specific. Mealybugs present in California and shown to transmit various GLRaVs using local virus isolates and insect populations include *Pl. ficus*, *Ps. longispinus*, *Pl. citri*, *Ps. viburni*, *Ps. maritimus*, and *F. gilli* (Golino et al., 2002; Tsai et al., 2010; Wistrom et al., 2016). Due to the reproductive capacity and the generation time (number of generations per year) of *Pl. ficus*, it is the primary vector of concern in most wine grape production areas of California (Daane, 2024). Other species of concern in North Coast and San Joaquin Valley vineyards are the grape and obscure mealybugs; in Central Coast vineyards, obscure and longtailed mealybugs can cause damage, and longtailed mealybugs may also occur in the Coachella Valley. Soft scale insects are present as pests in California vineyards, but thus far, no transmission assays have been reported for grapevine scale insects collected from California. In Washington State, Bahder et al. (2013) demonstrated that European fruit lecanium scale, *Pa. corni*, could transmit GLRaV-3 with low efficiency.

Table 3-1 summarizes key features of known mealybug GLRaV vectors in California. The mealybug life cycle (see Figure 3-4) is sexually dimorphic, and features of the insects' reproduction and ecology vary depending on the environment. Some mealybug vectors of GLRaVs produce eggs that are deposited and hatch later (information about number of generations is provided in Table 3-1), while others produce eggs that develop in the female and hatch within or immediately after release. Females can be highly fecund, producing 100-200 eggs in a 10-12-day period. Female mealybugs have 2-3 larval instars, with the 1st instar nymph referred to as the crawler phase. Male mealybugs have 3-4 larval instars and then a prepupal or cocoon stage pupal stage. The newly hatched mealybug nymphs (crawlers) are the most mobile developmental stage and considered to be the most important in transmission and spread of GLRaVs. The female insects become less mobile as they mature. Mature male mealybugs are small in size and have wings, but they are rarely seen and do not feed on plants because of non-functional mouthparts. The number of generations produced per year varies depending on environmental conditions and species-specific differences; all life stages may be present throughout the year for most mealybug species, and they may have more generations and longer periods of activity.

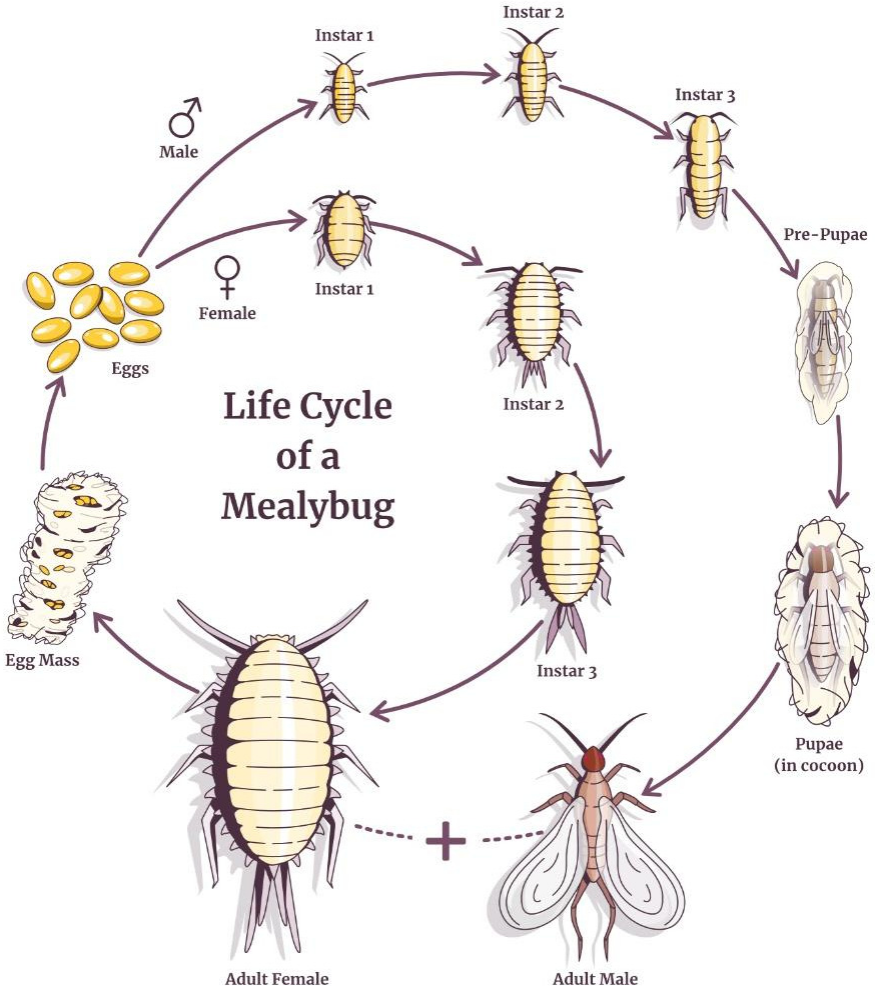

FIGURE 3-4 General mealybug life cycle.

TABLE 3-1 Vectors of GLRaVs in California

Common name	Scientific name	Regions of importance/distribution	Life cycle	Oviposition	Virus transmission (virus species)[a]	References
Grape mealybug	*Pseudococcus maritimus*	North Coast, San Joaquin Valley, Central Coast (Monterey and Sta. Cruz County) and the Foothills	Two generations each year; overwinters as an egg or crawler under loose bark, in cordons, or along upper portions of the vine trunk	Eggs deposited within an egg sac	GLRaV-3	Golino et al. (2002); Bahder et al. (2013)
Obscure mealybug	*Pseudococcus viburni*	North Coast, San Joaquin Valley, Central Coast	Multiple overlapping generations with no diapause over the winter; all life stages present on vines year-round; may overwinter under bark of the trunk, cordons, and spurs	Eggs deposited within an egg sac	GLRaV-3, GLRaV-5[a]	Golino et al. (2002)
Longtailed mealybug	*Pseudococcus longispinus*	Central Coast, Coachella Valley	Multiple overlapping generations with no diapause over the winter; all life stages present on vines year-round	Give birth to live crawlers	GLRaV-3, GLRaV-5,[a] GLRaV-9[a]	Cabaleiro and Segura (1997); Golino et al. (2002); Tsai et al. (2010)
Vine mealybug	*Planococcus ficus*	Established in at least 17 California counties across Coachella Valley, San Joaquin Valley, foothills of the Sierra Nevada, Central Coast, North Coast	More sensitive to cold temperatures than grape mealybug; 2-3 generations per year in coastal regions and 5-7 in warmer regions (e.g., lower San Joaquin Valley); no diapause during the winter; all or most life stages present on vines year-round depending on region	Eggs deposited within an egg sac	GLRaV-1, GLRaV-3, GLRaV-4, GLRaV-5,[a] GLRaV-9[a]	Golino et al. (2002); Tsai et al. (2008); Petersen and Charles (1997)
Citrus mealybug	*Planococcus citri*	Widespread distribution in California, except North Coast; polyphagous	Capable of multiple generations depending on temperature; at least 4-5 overlapping generations per year in California; overwinter as eggs; all or most life stages usually present	Eggs deposited within an egg sac	GLRaV-3, GLRaV-5[a]	Cabaleiro and Segura (1997); Cocco et al. (2018); Golino et al. (2002)
Gill's mealybug	*Ferrisia gilli*	Recently established on grapes in El Dorado County; present in Lake County; found on pistachios in the Southern San Joaquin Valley but not known to be widespread on grapes in other areas of the state	Two to three generations per year; overwinter as nymphs under bark, in crevices, and several inches below the soil line (not observed to feed when overwintering)	Give birth to live crawlers	GLRaV-3	Wistrom et al. (2016); Jones and Nita (2020); Gullan et al. (2003); UCCE (n.d.); Haviland et al. (2006)
European fruit lecanium scale	*Parthenolecanium corni*	Found to be a vector in Washington State; present in California	Develop through three life stages (egg, nymph, and adult); produce one generation per year	Eggs produced under adult female body	GLRaV-1, GLRaV-3	Martelli (2000); Hommay et al. (2009); Bahder et al. (2013)

continued

TABLE 3-1 *continued*

Common name	Scientific name	Regions of importance/distribution	Life cycle	Oviposition	Virus transmission (virus species)[a]	References
Cottony maple scale	*Neopulvinaria innumerabilis*	Present in California	Develop through three life stages (egg, nymph and adult); produce one generation per year	Eggs produced under adult female body	GLRaV-1, GLRaV-3	Martelli (2000); Zorloni and Prati (2006)

[a] GLRaV-5 and GLRaV-9 have been reclassified as GLRaV-4 strains (Adiputra et al., 2019).

PATHOGEN–VECTOR INTERACTIONS

The characteristics of GLRaV transmission based on timing of acquisition, latent period, and inoculation are consistent with a semi-persistent mode of transmission (Tsai et al., 2008), which is generally characterized by acquisition periods of hours to days and similar timing for inoculation (reviewed in Ng and Falk, 2006). Acquisition and inoculation of semi-persistent viruses involves phloem feeding, which takes time as most hemipterans must feed for 30 minutes to several hours to reach the phloem tissue (Moreno et al., 2012). GLRaVs have been presumed to be transmitted in a semi-persistent manner because of their classification in the family *Closteroviridae*. All viruses in this family that have been thoroughly characterized for virus transmission characteristics show clear signatures of semi-persistent transmission with regard to timing and virus retention sites in the vectors. For example, the whitefly-borne criniviruses and aphid-borne closteroviruses are transmitted semi-persistently and bind to the insect foregut (classified as externally borne) (Costa and Grant, 1951; Raccah et al., 1976; Tian et al., 1999; Chen et al., 2011; Killiny et al., 2016). For GLRaVs, the localization of viral particles within the vector is not yet clear but transmission characteristics are consistent with a non-circulative, externally borne virus. GLRaV virus particles appear to bind to the insect exoskeleton but not traverse membrane barriers or replicate, a feature they share with known non-persistent and semi-persistent transmitted viruses. As insects ingest phloem sap from plants infected with GLRaV-3, virus particles are retained in the foregut up to four days, after which insects molt and GLRaV-3 and infectivity are lost (Tsai et al., 2008). It is hypothesized that virus particles are shed along with the exoskeleton during insect molts; thus, GLRaVs are not transstadially passaged, and insects are thought to lose infectivity after a molt.

Transmission of GLRaV-3 takes place within a 1-hour acquisition access period (AAP) and a subsequent 1-hour inoculation access period (IAP), with apparently no latency period between virus acquisition and transmission (Tsai et al., 2008). However, a study conducted in South Africa by Krüger et al. (2015) determined that an AAP and IAP of 15 minutes each was sufficient for *Pl. ficus* to acquire and transmit GLRaV-3. The differences observed between these studies may be attributed to differences in GLRaV-3 isolates, their titers in source plants, the sensitivity of virus detection methods, and non-uniform experimental conditions. Although all grape-associated mealybug species appear to be competent vectors of GLRaV species in laboratory assays, transmission efficiency varies across vector species (Blaisdell et al., 2015; Wistrom et al., 2016). Transmission efficiency increases with the amount of time spent feeding, up to 24 hours, with efficiency peaking at around 10 percent daily per individual under controlled laboratory conditions for *Pl. ficus* (Tsai et al., 2008). Prator and Almedia (2020) observed two virus-binding sites, the stylet and the cibarium (the space in front of the true mouth cavity in which the food of an insect is chewed), for GLRaV-3 in *Pl. ficus* mouthparts of insects fed on purified virus solutions and infected plant cuttings. Overall, the transmission efficiency in these experiments was low, ranging from 0.5 percent to 12.7 percent and the number of insect stylets and cibaria that exhibited detectable virus was also low, ranging from 2.7 percent to 4.8 percent depending on the source of the virus.

Vector species with a greater number of generations per year or higher fecundity levels present a more significant transmission risk. It is possible to find all life stages of *Pl. ficus* year-round on grapevines. The vine mealybug exhibits 4-7 generations annually, depending on temperature and geographic location. In coastal California vineyards, there are roughly one, two, three, and four annual generations of *Pa. corni*, *Ps. maritimus, Ps. viburni*, and *Pl. ficus*, respectively (Geiger and Daane, 2001; Gutierrez et al., 2008; Varela et al., 2013). All life stages of mealybugs and soft scales may have the capacity to transmit GLRaV-3, but nymphs are more proficient (Petersen and Charles, 1997; Tsai et al., 2008). Early instar nymphs are the primary dispersal stage because they are more active than adults. In addition, females and nymphs are primarily responsible for virus transmission because adult males do not feed. The spread of GLRaV transmission occurs over short distances corresponding to the movement pattern of vector insects. Females and immature instars, which lack wings, must crawl between hosts to colonize and inoculate new plants. Long-distance spread is likely associated with physical movement of insects via wind, clothing, or farm equipment (Haviland et al., 2005; Tsai et al., 2010; Daane et al., 2012; Almeida et al., 2013).

Interestingly, virus-encoded functions responsible for the vector transmission have not yet been assigned or mapped to specific genes or ORFs in GLRaV-3, possibly due to challenges associated with such experiments. However, the same genes involved in systemic spread of closteroviruses may be involved in vector transmission, similar to the crinivirus or potyvirus transmission (Torrance et al., 2006; Chen et al., 2011). For instance, the CPm protein was demonstrated to be involved in whitefly transmission of lettuce infectious yellows virus, a bipartite crinivirus, binding specific receptors in the foregut of the insect and facilitating virion retention (Chen et al., 2011; Ng et al., 2021). A connection between vector specificity of closterovirids and amino acid sequences of the most conserved protein motifs, i.e., RNA-dependent RNA polymerase (RdRP), helicase, and HSP70, was revealed in phylogenetic analyses that produced separate lineages of viruses transmitted by aphids (genus *Closterovirus*), whiteflies (*Crinivirus*), and mealybugs and scales (*Ampelovirus*) (see Karasev, 2000). While not implying a direct involvement of these conserved domains in insect transmission, this phylogenetic distinction suggests a powerful effect of the vector shaping closterovirus evolution, more powerful than a host plant influence (Karasev, 2000).

HOST–PATHOGEN INTERACTIONS AND HOST DEFENSE MECHANISMS

Just like other closterovirids, GLRaV-3 and other GLRaVs display clear tissue tropism and are phloem-limited in infected plants (Lesemann, 1988; Ng and Zhou, 2015). In early electron microscopy studies, closteroviruses were found to induce characteristic membrane vesicles in infected cells and this feature was even considered a diagnostic mark of closteroviruses at the dawn of virus taxonomy (Esau, 1960; Esau and Hoefert, 1971; Lesemann, 1988). This association with the phloem leads to symptoms induced by closteroviruses that often affect phloem tissue, veins, cambium, and resemble nutritional deficiencies, and it may also be responsible for the low concentration of the viruses in infected plants, one factor that makes closterovirus detection challenging (Lesemann, 1988; Wisler et al., 1998; Sun and Folimonova, 2022).

In the infected plant, GLRaV-3 induces multiple 3’ co-terminal sub-genomic RNAs (sgRNAs) (Hu et al., 1990; Rezaian et al., 1991; Saldarelli et al., 1994; Ling et al., 1998), which are believed to be translated into the proteins encoded by the ORFs downstream of the replication-associated ORFs 1a and 1b (Ling et al., 2004; Jarugula et al., 2010, 2018; Maree et al., 2010). Assignments of the functional activity for the GLRaV-3 encoded proteins (Ling et al., 1998, 2004; Burger et al., 2017) are largely based on conserved protein motifs and comparisons to other closterovirus model systems, such as beet yellows virus (BYV) and citrus tristeza virus (CTV), where genetic systems based on infectious cDNA clones were better developed and functions of many virus-encoded protein products were established experimentally (see Dolja et al., 2006; Folimonova, 2020).

Replication-associated functions have been assigned to the protein products encoded by ORFs 1a and 1b with easily identifiable conserved domains of RdRP, helicase, and methyltransferase; the ORF 1a product also contains a leader papain-like protease (L-Pro) domain close to its N-terminus (Ling et al., 2004). This same ORF 1a-encoded protein also contains an AlkB conserved domain, located between methyltransferase and helicase domains; it is often found in viruses infecting woody plants and is implicated in RNA demethylation related to the RNA damage repair (Maree et al., 2008; van den Born et al., 2008). L-Pro domain encoded by ORF 1a has been implicated in RNA accumulation, virus invasiveness, and systemic spread in BYV and CTV (Dolja et al., 2006; Kang et al., 2018) and in superinfection exclusion in CTV (Atallah et al., 2016).

The functional activity for p6, downstream of ORF 1b, has not been established or assigned, and its expression in infected plants is uncertain (Maree et al., 2015). The p5 ORF encodes a small hydrophobic protein similar to an analogous protein expressed by BYV and shown to target endoplasmic reticulum membrane, facilitating cell-to-cell movement of BYV (Peremyslov et al., 2004a). The HSP70 homolog protein which, together with the downstream p55 protein, is involved in cell-to-cell movement and virion assembly, forming a peculiar “tail” structure including also the major CP and CPm

(Agranovsky et al., 1995; Tian et al., 1999; Alzhanova et al., 2000, 2001; Satyanarayana et al., 2000, 2004; Peremyslov et al., 2004b).

GLRaV-3 genetic variability studies have typically been conducted using partial sequences of a few taxonomically informative genes such as RdRP, HSP70h, and CP (Maree et al., 2013 and cited references), although a few more recent studies have addressed the same topic using complete or nearly complete viral genomes (Diaz-Lara et al., 2018). Together, these studies have revealed that GLRaV-3 comprises a complex of genetic variants in several phylogenetic groups which may diverge from each other by as much as 30 percent in the whole genome nucleotide sequence (Thompson et al., 2019). Researchers have currently identified up to eight distinct phylogroups of the virus across different geographical regions of the world based on complete CP and genome-length sequences of GLRaV-3 (Maree et al., 2013; Diaz-Lara et al., 2018; Thompson et al., 2019), designated with Roman numerals as groups I, II, III, V, VI, VII, IX, and X. Apart from the identified phylogroups, a few GLRaV-3 isolates appear to be divergent in that they do not cluster into any of the identified clades (Diaz-Lara et al., 2018; Thompson et al., 2019).

The tremendous genetic diversity of GLRaV-3 leads to potential challenges in virus detection and diagnosis, and GLD management strategies. Diaz-Lara et al. (2018) identified only a short area in the 3'-terminal, untranslated region of the GLRaV-3 genome suitable as a "universal" target for detection of all genetic variants of GLRaV-3 by reverse transcription polymerase chain reaction (RT-PCR). Genetic diversity assessments conducted for other major GLRaVs, including GLRaV-1 (Alabi et al., 2011; Fan et al., 2015; Donda et al., 2017), GLRaV-2 (Jarugula et al., 2010), and GLRaV-4 (Rubio et al., 2013; Adiputra et al., 2019), have revealed that grapevines harbor complex populations of genetic variants of GLRaVs due to multiple factors, which are extensively discussed in Naidu et al. (2015). It is also important to recognize that virus isolates included in genetic diversity studies of the different GLRaVs mainly emanated from *V. vinifera* vines; as a result, the extent of the complexity of natural populations of GLRaVs may be underestimated. Although the biological significance of such genetic variability is poorly understood, it may have implications for accurate and reliable diagnosis in clean plant programs.

DIAGNOSTICS

Given the negative impacts of GLD, it is imperative to conduct regular monitoring to identify grapevines affected by the disease. Tools for early and accurate identification of GLD and its associated viruses enable critical disease control measures such as removing affected vines, controlling vectors and the movement of agricultural machinery, treating instruments, and replanting with certified vines (Fuchs, 2020; Javaran et al., 2023a). This section examines the diverse diagnostic methods employed for detecting GLD with a particular focus on GLRaV-3 (see Figure 3-5), since it is understood to be the most prevalent and most economically damaging virus. However, it is important to recognize that various technical protocols for virus detection may be more or less sensitive to the genetic diversity of the GLRaVs beyond GLRaV-3, which needs to be taken into account when utilizing these methods to inform management strategies.

Detection of GLD by Optical-Based Diagnostic Methods

Visual inspection of grapevines has historically been a mainstay of GLD detection. Recently, hyperspectral imaging has emerged as a cutting-edge technology to enhance optical-based diagnosis and facilitate early detection (MacDonald et al., 2016; Mahlein, 2016; Galvan et al., 2023). This technology uses spectral sensors to capture the electromagnetic spectrum reflected by plants, allowing for the identification of distinct spectral patterns associated with infected and non-infected leaves. While hyperspectral imaging shows promise in monitoring the dynamic development of symptoms (Bendel et al., 2020), it is still in the early stages of validation and remains sensitive to environmental factors, such as daylight intensity. Although this technology has demonstrated effectiveness in detecting GLD

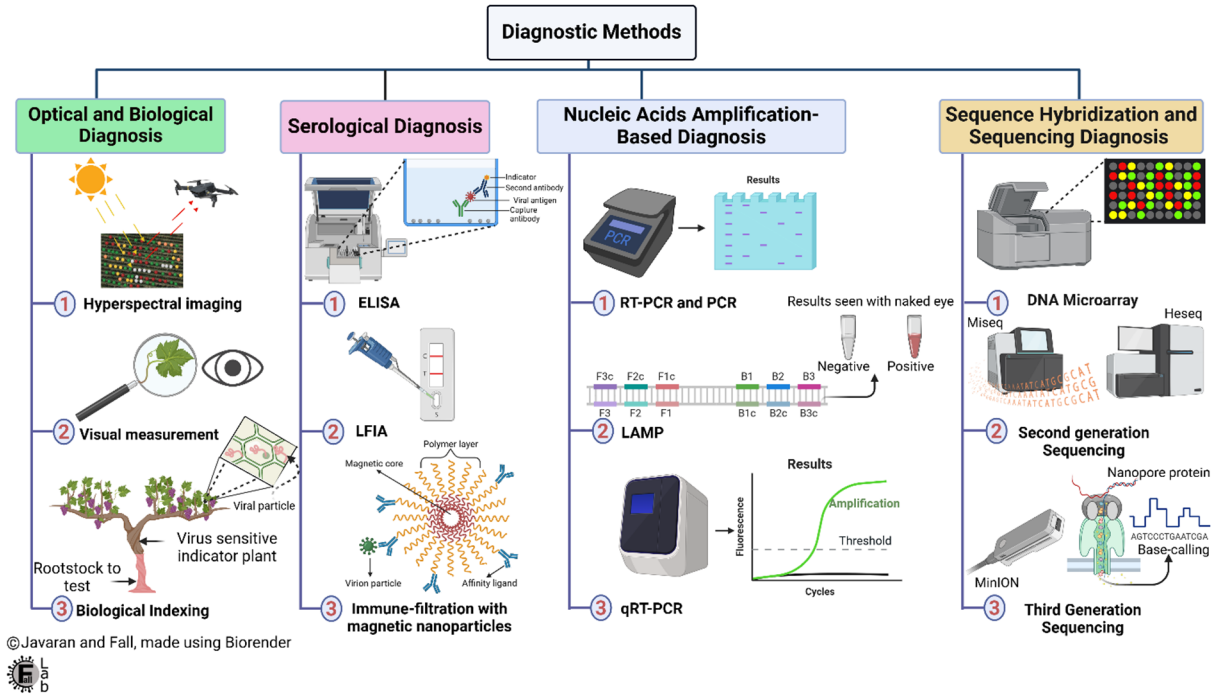

FIGURE 3-5 Graphical representation of the diagnostic methods currently available for the detection of GLRaV-3.
SOURCE: Mamadou L. Fall, Agriculture and Agri-Food Canada.

symptoms in both red or black- and white-fruited cultivars, further validation is needed to establish its reliability as a practical diagnostic tool (Naidu et al., 2009; MacDonald et al., 2016; Sinha et al., 2019; Bendel et al., 2020; Gao et al., 2020; Junges et al., 2020; Galvan et al., 2023). Recent studies indicate that asymptomatic GLRaV-3-infected vines exhibit the most distinctive spectral differences, enabling reliable differentiation of non-infected vines and symptomatic vines (Galvan et al., 2023), highlighting the potential of this method to detect early-stage infection.

Detection of GLRaVs by Biological Indexing

Biological indexing involves the use of sensitive indicator plants to detect viruses in infected plants. While the use of this diagnostic method has declined in recent years in favor of molecular and serological methods, it still holds value for studying newly discovered viruses for which nucleotide sequences and antibodies are unavailable, precluding their molecular and serological identification, respectively (Zherdev et al., 2018). Biological indexing is also invaluable for studying the etiology and biology of newly detected viruses. There are two distinct approaches to detect grapevine viruses using indicator plants: sap inoculation and bud grafting. Sap inoculation, which involves the use of the sap and juice from infected plants, is suitable for detecting mechanically transmissible viruses. Compared with woody plants like grapevine, virus detection with this method can be accomplished more rapidly when using herbaceous indicator plants like *Chenopodium quinoa*, *Chenopodium amaranticolor*, *Cucumis sativus*, and *Nicotiana* species (Javaran et al., 2023b). Because GLRaV-3 is not transmitted by mechanical equipment or pruning, sap inoculation is not suitable for its detection. Bud grafting is a biological indexing procedure that is used to detect graft-transmissible viruses like GLRaV-3, but it is a very time-consuming technique. Common grapevine varieties used as indicators include *V. vinifera* cvs. Barbera, Cabernet franc, Cabernet Sauvignon, Gamay, Mission, and Pinot noir (Rowhani et al., 2017). Using conventional methods, bud grafting may take anywhere from 16 months to three years to complete.

Alternatively, a time-efficient *in vitro* biological indexing method has been developed, which takes only 4-12 weeks from grafting to the expression of symptoms (Cui et al., 2015; Hao et al., 2021). While biological indexing can indicate the involvement of an infectious agent in the tested material, the technique is insufficient for specific detection of viruses involved in the GLD complex due to their overlapping symptomatology.

Detection of GLRaVs by Serological Methods

Serological diagnostic techniques such as enzyme-linked immunosorbent assay (ELISA), lateral flow immunoassay, direct immune-printing, immune-filtration with magnetic nanoparticles, dot-immunobinding assay, immunocapture RT-PCR, and immunosorbent electron microscopy rely on the interaction between monoclonal and/or polyclonal antibodies and viral particles. Several companies have developed commercial detection kits for GLRaV-3 and 17 other grapevine viruses (Blouin et al., 2017). While serological diagnostic methods offer speed and simplicity, they also have limitations, including a risk of generating false-negative results. These limitations stem from factors such as the type of biomaterial, antibody quality, sensitivity, specificity, and grapevine tissue- and cultivar-specific influences. To address these limitations, recent advancements include the development of lab-on-a-chip methods designed to enhance the effectiveness of serological tests. Lab-on-a-chip methods, also known as microfluidic chips or microfluidic devices, consolidate multiple laboratory functions onto a single microchip-sized platform. This technology has recently demonstrated remarkable effectiveness in the detection of GLRaV-3, often providing results within minutes (Buja et al., 2022).

Detection of GLRaVs by Nucleic Acid Amplification-Based Methods

With the introduction and decreasing cost of PCR, many diagnostic approaches have transitioned from biological indexing and serological methods to nucleic acid amplification-based methods. These include standard PCR, RT-PCR, real-time or quantitative RT-PCR (qRT-PCR), multiplex PCR, and nested PCR (Zherdev et al., 2018). Various grapevine viruses, including GLRaV-3, GLRaV-1, and GRBV, have been successfully detected using nucleic acid amplification-based methods (Gambino, 2015; Diaz-Lara et al., 2018; DeShields and Achala, 2023). PCR-based methods offer numerous advantages, such as high sensitivity, specificity, and time efficiency. However, they do have some limitations. False results can occur due to contamination in reactions, faulty primer designs, nuclease degradation of RNA or DNA template, and occasionally, they can be affected by grapevine phenolic compounds and polysaccharides if carried over to the nucleic acid template.

Another rapid detection option that is suitable for vineyard conditions involves on-site amplification of specific DNA sequences using a set of primers through loop-mediated isothermal amplification (LAMP). By adding the reverse transcriptase enzyme to the reaction tube, the reverse transcription loop-mediated isothermal amplification assay has been used to detect GLRaV-3 (Walsh and Pietersen, 2013), as well as other grapevine viruses in vineyards. This optical detection method offers several advantages, including its high speed, high specificity, resistance to inhibitors, ease of use, and the absence of the need for a thermal cycler (Zherdev et al., 2018).

Detection of GLRaVs by DNA Microarray Methods

DNA microarray diagnostic methods are based on the principle of DNA strand hybridization. Specific DNA probes, which are based on the genomes of grapevine viruses, are attached to a solid plate. Subsequently, cDNAs from infected samples are fluorescently labeled. These labeled target sequences then bind covalently to the DNA probes, facilitating the detection of viruses. This technology allows for the simultaneous detection of multiple viral pathogens and offers sensitivity levels that fall between those of ELISA and qRT-PCR (Boonham et al., 2003). A diagnostic oligonucleotide microarray was developed for the simultaneous detection of GLRaV-3 and other grapevine viruses (Engel et al., 2010). Through two

different cDNA amplification and non-amplification methodologies, this microarray successfully identified 15 and 33 grapevine viruses, respectively. The versatility of the DNA microarray method is evident in its capacity to identify new viruses through partial attachment of target sequences to the probes. Additionally, a modified chip containing 1,578 specific viral and 19 internal probes has been developed for the detection of 38 different plant viruses, making it suitable for co-infected samples (Zherdev et al., 2018).

Detection of GLRaVs by Second Generation Sequencing (Short Read Sequencing)

High-throughput sequencing (HTS) has revolutionized the ability to swiftly and comprehensively investigate plant viromes. Unlike nucleic acid amplification-based and serological diagnostic methods, HTS offers the capability to not only detect known viruses but also identify novel ones. HTS has facilitated the detection of many known grapevine viruses and led to the identification of new ones including GRBV, grapevine Pinot gris virus, and grapevine Roditis leaf discoloration-associated virus (Zhang et al., 2011; Marais et al., 2018; Zherdev et al., 2018; Fall et al., 2020). However, despite its remarkable capabilities, HTS also has some limitations. One drawback is related to the type of nucleic acid extraction method used. For instance, viral sequences are typically present in low abundance within the total nucleic acid extract, which can limit the detection sensitivity of low-titer viruses unless target enrichment steps are included during preparation of the cDNA libraries. Furthermore, unlike bacterial and fungal metagenomics, there are no universal gene markers for amplicon sequencing of viral genomes. To overcome these challenges, various purification methods have been developed to enrich virion-associated nucleic acids, viral small interfering RNA, polyadenylated RNA, and double-stranded RNA (dsRNA). Another limiting factor is that HTS is generally more costly than other diagnostic methods as it requires high-tech and expensive sequencing equipment, as well as substantial investments in bioinformatics for data analysis. To address these challenges, various solutions have been proposed, including multiplex barcoding to minimize analytical costs, the development of user-friendly and straightforward pipelines for virus diagnosis and diversity analysis, and the exploration of single-molecule sequencing without the need for amplification.

Detection of GLRaVs by Third Generation Sequencing (Long Read Sequencing)

Third-generation sequencing technologies such as single-molecule real-time sequencing and nanopore sequencing technology may overcome some of the limitations of second-generation sequencing. For example, the MinION, a compact, portable sequencer developed by Oxford Nanopore Technologies, sequences DNA or RNA strands by passing them through a nanopore gateway protein that works by recording and base-calling electrical current fluctuations as RNA or DNA molecules traverse the nanopore protein. This technology's ability to perform parallel sequencing of multiple samples through multiplex barcoding makes it an enticing option for diagnosing grapevine viruses. This sequencing technology has demonstrated effectiveness in identifying various plant viruses across a wide range of hosts (Bronzato Badial et al., 2018; Chalupowicz et al., 2019; Fellers et al., 2019; Naito et al., 2019; Stenger et al., 2020). Recently, Javaran et al. (2023a) optimized this technology for the detection of grapevine viruses, including GLRaV-3. The study showed that this method is on par with second generation sequencing in terms of accuracy, and also highlighted its cost-effectiveness at $22 per sample. Recent improvements in chemistry and flow cell features have further reduced the cost per sample and elevated the accuracy of this technology to 99.9 percent, putting it on par with other detection methods and underscoring its potential as a cost-effective and time-effective solution for grapevine virus detection.

SPATIAL DISTRIBUTION AND TEMPORAL SPREAD OF GLD

Due to clonal propagation of grapevines to preserve trueness-to-type and varietal integrity and the obligate and intracellular nature of viruses, the primary avenue for the spread of grapevine viruses is via

infected planting stock. Consequently, it was generally believed until the 1980s that viruses such as GLRaV-3, hence GLD, spread in vineyards mainly due to the vegetative propagation and use of infected planting materials (Martelli, 2000). However, since 1989, field spread of GLD in vineyards was observed in grapevine-growing regions around the world, raising the suspicion that insect vectors might be contributing to the field spread. Several studies have recognized insects such as mealybugs (Pseudococcidae) and scale insects (Coccidae) as vectors responsible for vine-to-vine spread in vineyards (Naidu et al., 2014; Herrbach et al., 2017 and cited references). The fact that these vectors are relatively immobile means that vector-mediated spread of GLD occurs relatively slowly compared to viral diseases spread by actively mobile vectors like aphids, whiteflies, thrips, and leafhoppers. Moreover, all stages of nymphs and mature female mealybugs cannot fly due to their wingless nature and largely move by crawling (Daane et al., 2012). Female mealybugs are not capable of long-distance dispersal on their own, although it is often assumed that they can be dispersed by other means, such as human activities (e.g., via the distribution of vector infested planting stock, on machinery used for vineyard shoot thinning and harvesting, and on vineyard workers' clothing) and by wind and foraging birds. Unlike adult female mealybugs that live a mostly sedentary lifestyle, the first- and second-instar mealybug nymphs or "crawlers" are more important from the disease epidemiology perspective because of their mobility and higher transmission efficiency. The adult males are winged and can thus travel longer distances, but they are not involved in virus transmission since they have vestigial, nonfeeding mouthparts and do not feed (Daane et al., 2012).

Field studies were conducted in several grapevine-growing regions to better understand the epidemiology of GLD (Engelbrecht and Kasdorf, 1985; Habili and Nutter, 1997; Cabaleiro et al., 2008; Almeida et al., 2013; Pietersen et al., 2013; Sokolsky et al., 2013; Arnold et al., 2017; Bell et al., 2018; Donda et al., 2023). According to these studies, GLD generally spreads slowly between adjacent vines within a row and/or between neighboring rows, leading to clustering of symptomatic vines along individual rows. Monitoring GLD incidence in newly planted healthy vineyard blocks showed a gradual increase in disease incidence over successive seasons. Analyzing spatio-temporal dynamics of disease spread by a variety of methods has revealed two distinct epidemiological patterns of GLD spread. In one pattern, the primary spread into a vineyard due to planting with compromised planting stock or introduction of the virus by alighting viruliferous vectors leads to an initial random distribution of GLD-affected vines. Randomly distributed symptomatic vines during initial years then serve as primary foci of infection for vine-to-vine secondary spread by colonized mealybugs within the vineyard, leading to clustering of symptomatic vines during subsequent seasons. With continued spread over multiple seasons, these random clusters of symptomatic vines within a vineyard expand over multiple seasons due to virus spread by viruliferous vectors, ultimately coalescing to cover the entire vineyard. In another pattern of spread, called "edge effect," the virus is introduced to a newly planted healthy vineyard via immigrating viruliferous vectors from virus-infected, established vineyards, which may be nearby or some distance away. The spatial and temporal spread of GLD in subsequent seasons leads to a disease gradient in which the highest percentage of vines showing GLD symptoms occur in border rows proximal to nearby sources of infection and a gradual decline in disease incidence with increasing distance from the established (and infected) vineyard. These patterns of GLD spread appear to be similar in different wine regions across the world, irrespective of the vector species present in vineyards. The rate of vineyard spread can also be influenced by factors such as local environmental factors, cultivar and vector species composition, genetic diversity of the virus, and viticultural practices.

MANAGEMENT

Managing GLD is vital for maintaining the health and productivity of vineyards. As the most prevalent GLRaV, GLRaV-3 poses a significant threat to grapevines, impacting their quality and yield as well as nursery trade. Unfortunately, there is no known genetic resistance in grapevines to GLRaVs and

there is no single, highly effective method for controlling GLRaV-3 infection and spread. Consequently, adopting an integrated approach is paramount for GLD prevention and mitigation.

Integrated approaches to GLD mitigation have demonstrated success in vineyards across South Africa and New Zealand (Almeida et al., 2013; Bell et al., 2021; Habili et al., 2022). In South Africa, a combination of practices including applying herbicide to infected vines, using insecticide for mealybug control, and roguing of symptomatic vines and replanting resulted in a significant reduction in the GLRaV-3 infection rate. In one 41-hectare vineyard, for example, the infection rate decreased from 100 percent in 2002 to 0.027 percent in 2012 after these methods were employed (Pietersen et al., 2013). In New Zealand, the combination of vine removal and mealybug management led to a substantial reduction in infected vines over a 6-year period (Bell et al., 2018). In Napa, California, MacDonald et al. (2021) utilized five years of grower-sourced data to apply spatial and statistical models to better understand spatiotemporal trends in *Pseudococcus maritimus* populations. Results showed that when GLD incidence within a block is <1 percent, consistent monitoring and removal of diseased vines are required to contain within-block spread. As incidence increases to 1–20 percent, both insecticide applications and roguing are effective, while at levels >20 percent, roguing becomes critical for disease control.

At the regional scale, one crucial component of an integrated approach involves establishing a foundation stock, referred to as G1, which serves as the primary source of clean grapevine plants that are meticulously screened for GLRaV-3 before the establishment of G2, G3, and G4 blocks at nurseries (Golino et al., 2017). It is also a highly recommended practice to maintain an ongoing surveillance program within vineyards to monitor mealybug and soft scale vector populations and promptly remove GLRaV-3-infected vines (Pietersen et al., 2017). The success of this monitoring and testing step relies on the availability of rapid, early, cost-effective, and user-friendly detection methods (Javaran et al., 2023a), which also support certification programs within the supply chain (Javaran et al., 2023b).

Recent advances in RNAi technologies have rekindled optimism for genetic engineering focused on imparting resistance to GLRaV-3 in grapevines. RNAi, a conserved endogenous process across eukaryotes, functions through sequence-specific RNA degradation or transcriptional gene repression within the silencing pathway (Baulcombe, 2004; Csorba et al., 2009). Initially identified as post-transcriptional gene silencing in plants during the early 1990s, RNAi acts as a molecular immune system, offering a robust primary defense against viruses when triggered by the appropriate inducer molecule in infected cells (Voloudakis et al., 2022). Utilizing RNAi, it is possible to develop transgenic grapevine plants resistant to GLRaV-3 by overexpressing hairpin RNA (hpRNA) or dsRNA from specific GLRaV-3 genes. This successful strategy has been employed against viruses including zucchini yellow mosaic virus, watermelon mosaic virus, potato virus X, and legume-infecting begomoviruses (Pooggin et al., 2003; Klas et al., 2006; Kumar et al., 2017). This approach also extends to exogenous applications, including RNAi-based bioproducts for controlling GLRaV-3 titers in grapevines (Avital et al., 2021), providing a promising and sustainable approach to managing GLRaV-3. This strategy can be seamlessly incorporated into broader integrated pest management (IPM) strategies. However, despite successful laboratory studies demonstrating the efficacy of dsRNA technology in managing plant diseases, its practical field implementation faces challenges due to the high production costs of dsRNA and the limited availability of necessary adjuvants and technologies (Voloudakis et al., 2022). The following sections outline key elements of integrated approaches to effectively manage GLRaV-3 that may be employed before planting (pre-plant) and those that may be employed after planting (post-plant).

Pre-Plant Management

Certified Planting Materials

Using vines that are tested virus-free as planting materials can help prevent the incursion of GLRaV-3 into vineyards.

Quarantine Measures

Adherence to federal quarantine measures guiding the movement of planting materials can help avoid introducing GLD into a vineyard.

Genetic Resistance

Developing and deploying cultivars and rootstocks that harbor genetic resistance to viruses is a desirable strategy for the management of viral diseases (Maule et al., 2007); however, no confirmed GLD-resistant grapevine has yet become available. Multiple factors make GLD resistance breeding efforts in *Vitis* spp. especially challenging, including the perennial nature of grapevine cultivation, the non-amenability of GLRaV-3 to mechanical transmission, and the low or non-uniformity of virus inoculum in grapevines, which makes vector-mediated screening efforts difficult to accomplish (Oliver and Fuchs, 2011). To circumvent these challenges, Jiao et al. (2022) recently employed an RNA-targeting CRISPR mechanism to induce resistance against GLRaV-3 in plantlets of *V. vinifera* cv. Cabernet Sauvignon. While the performance of these engineered vines is pending field evaluation under vineyard conditions, this approach presents new GLD management opportunities using biotechnology (Fuchs, 2023).

Post-Plant Management

Monitoring and Testing

It is important to regularly monitor vineyards for signs and symptoms of GLRaV-3 infection, such as leaf discoloration, leaf rolling, reduced fruit quality, and uneven ripening. Growers can implement a testing program to periodically check for the presence of GLRaV-3 in their vines.

Roguing

Roguing and destruction of GLRaV-3-infected vines as soon as they are identified can prevent or slow down the spread of the virus within a vineyard. Spatial roguing, the removal of virus-infected vines along with their two immediate neighbors, was tested in a New York vineyard to reduce the incidence of GLRaVs (Hesler et al., 2022). Over five years, this method, combined with replacing infected vines with virus-free stock, reduced virus incidence from 5 percent to less than 1 percent (Hesler et al., 2022). The experiment demonstrated that spatial roguing, even more than insecticide use, can significantly limit the spread of GLD, making it an effective management strategy in vineyards with low disease and pest pressure.

Vector Management

IPM practices, which can include various strategies such as the use of insecticides, pheromone traps, mating disruption, and beneficial insects, can be used to control populations of GLRaV vectors such as mealybugs and soft scales. Also, controlling hemipteran-tending ants, such as the invasive Argentine ant, should be a key component of vineyard management strategies, as these ants disrupt biological control by protecting mealybugs from their natural predators (Cooper et al., 2019). Insecticide dissolved in 25 percent sugar water and toxin-laced polyacrylamide baits have proven effective in reducing ant populations, which allows natural enemies to better control mealybug infestations (Daane et al., 2006; Cooper et al., 2019;). Managing ant populations is crucial for establishing sustainable biological control, helping to maintain the balance between pests and their natural predators in vineyards. Since GLD can spread to nearby vineyards through vector dispersal, it is important to conduct vector control on a coordinated area-wide basis.

Grapevine Pruning and Canopy Management

Proper canopy management can limit contact between vines and reduce cross-vine movement of GLRaV-3-infective mealybugs and scale insect crawlers. Careful pruning is essential to prevent the spread of mealybug crawlers between vines.

Record Keeping

Maintaining records of GLRaV-3 testing and management activities can provide useful documentation to track progress and identify areas for improvement.

Holistic GLD Management

To identify points where effective management interventions could be implemented, it is essential to consider the entire grape production ecosystem (see Figure 3-6). Ultimately, combinations of different measures are more effective for GLD management than using any intervention alone (Bell et al., 2018; Chooi et al., 2024). For instance, Fuller et al. (2013) showed that losses due to GLRaV-3 are minimized when growers initially plant with certified stock and then rogue and replant with certified stock (Table 3-2), demonstrating the benefit of combining both management tactics for GLD management.

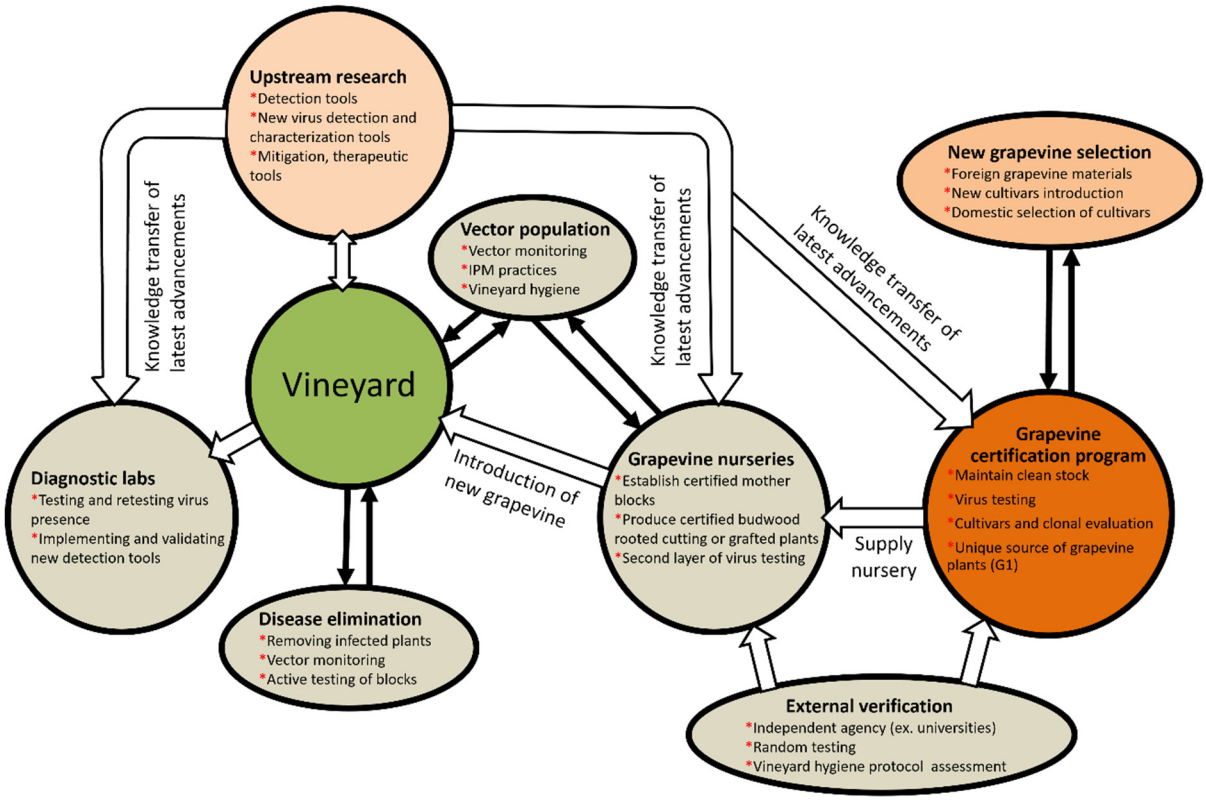

FIGURE 3-6 Opportunities for managing and mitigating GLD in the wine grape production ecosystem. SOURCE: Image from Javaran et al. (2023b).

TABLE 3-2 Estimated losses from GLD under different vineyard management scenarios in California according to Fuller et al. (2013)

			Average annual discount/25 years		Net present value/25 years	
Case	**Plantings**	**Replanting**	**Acre $/Acre/Year**	**Region $ Millions/Year**	**Acre $/Acre**	**Region $ Millions**
1	Certified	Certified	605	60.7	15,122	1,518.6
2	Certified	Non-certified	779	78.3	19,483	1,956.5
3	Certified	No replanting	790	79.3	19,745	1,982.8
4	Non-certified	Certified	914	91.8	22,847	2,294.4
5	Non-certified	Non-certified	1,138	114.3	28,449	2,857.0
6	Non-certified	No replanting	1,095	110.0	27,382	2,749.8

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4
Grapevine Leafroll and Red Blotch Diseases: Knowledge Gaps

Chapters 2 and 3 present the current state of knowledge on the grapevine red blotch virus (GRBV) and grapevine leafroll-associated virus 3 (GLRaV-3) pathosystems. This chapter highlights significant knowledge gaps that remain and discusses how additional information could be applied to inform decision making and the development of tools to manage these viruses. Where appropriate, recommendations are provided to help guide research priorities and approaches to elucidate the viruses, vectors, plant hosts, and the interactions among them, as well as to advance strategies for detection, diagnostics, and management to address the unique challenges different sectors of the industry face in responding to the spread and impacts of these grapevine viruses.

This chapter has five main sections. The first section discusses grapevine leafroll disease (GLD) knowledge gaps around causal agents, variants, virus-host interactions, and host defense mechanisms. The second section discusses grapevine red blotch disease (GRBD) knowledge gaps around causal or associated viruses, host-virus interactions, and host defense mechanisms. The third section addresses knowledge gaps common to both pathosystems, along with gaps regarding virus-virus and virus-host-environment interactions. The fourth section focuses on GRBV and GLRaV-3 diagnostics and detection, and the fifth section discusses knowledge gaps related to GRBV and GLRaV-3 vectors.

While the committee believes that all the research recommendations in this chapter are important and would generate information needed for further research /development of other control methods, tools, or strategies, the committee is also cognizant of the fact that research funds are limited and has identified the high- and medium-priority research areas. In the sections below, research recommendations of high priority are labeled HP and those of medium priority are labeled MP. Additionally, high- and medium-priority research areas are presented in a table (Table 4-1) at the end of the chapter.

GLD KNOWLEDGE GAPS

Basic Research to Generate Foundational Knowledge for Resolving the Complex Biology of GLD

Several distinct but taxonomically related closteroviruses (GLRaVs) have been reported in association with GLD, each of which may also have divergent strains or molecular variants (Martelli et al., 2002, 2012). It is generally understood that there is a stronger expression of symptoms in response to GLD in red or black-fruited than in white-fruited cultivars. Though widely reported, color-based symptomology of GLD in white-fruited varieties is often subtle and may be unrecognizable (Maree et al., 2013; Naidu et al., 2015). Symptoms, such as the patterns of interveinal chlorosis, are unreliable because they mimic those due to nutritional deficiencies. While it is true that leaf rolling in certain white fruited varieties is diagnostic for GLD, this symptomology occurs mostly in the advanced stage of GLD and often in chronically infected vines. There are also varietal differences. GLD-induced leaf rolling at harvest may be conspicuous in a cultivar like Chardonnay, but this may not be the case for cultivars like Sauvignon Blanc or Thompson Seedless (Maree et al., 2013). The reasons underlying these differences have not been well elucidated.

Because GLD is an exceptionally complex viral disease, molecular and genomic approaches are needed to better understand the many dimensions of the disease biology. Experimental systems are needed for studying viral gene functions, virus-vector-host interactions, and the transmission biology of GLRaV-3. The availability of cDNA clones for genetic variants of GLRaV-3 (Jarugula et al., 2018; Shabanian et al., 2023) can provide the critical reagents needed to apply synthetic biology approaches, the de novo synthesis of viral genomes (Wimmer et al., 2009), for understanding the role of genetically divergent variants in different aspects of GLD. These synthetic biology approaches could enable

additional studies to determine the relative pathogenicity of genetic variants of the virus(es), their relative transmission efficiencies, and disease outcomes of their co-occurrences in grapevine, among other insights.

Conclusion 4-1: Despite decades of research, knowledge on the genetic and phenotypic complexity of GLD-associated viruses remains limited.

Conclusion 4-2: Fundamental studies using synthetic biology approaches can be applied to systematically investigate how different GLRaV genotypes influence disease outcomes.

Recommendation 4-1: Support research to generate more knowledge about the impact of GLRaV-3 genetic variants on GLD development that could help guide GLD management.

Recommendation 4-2 (HP): Support foundational research to understand the intrinsic and extrinsic factors contributing to the efficient spread of GLRaV-3, including interactions with other vitiviruses.

Research questions that need to be addressed include:

- **Why is GLRaV-3 predominant among the GLRaVs? What are the biological consequences of extensive GLRaV-3 genetic diversity? What factors are driving the evolution of new GLRaV-3 genetic variants?**
- **What are possible disease outcomes of single versus mixed infections of different GLRaVs and/or distinct GLRaV-3 genetic variants?**

Virus-Host Interactions and Host Defense Mechanisms

To date, researchers have gained only limited insights into the molecular interactions between grapevine and GLRaV-3 and the defense mechanisms of grapevine, in part because grapevine's perennial and woody properties make it difficult to study. In the case of GLRaV-3, P19.7 is recognized as a putative viral suppressor of RNA silencing that plays a role in GLD symptom expression, and several up-regulated genes likely involved in RNA silencing against GLRaV-3 infection have been identified (Gouveia and Nolasco, 2012; Song et al., 2022). However, no resistance genes against GLRaV-3 or susceptibility genes in assisting GRLaV-3 infection have yet been confirmed. Further exploration of how GLRaV-3 genes may interact with grapevine RNA silencing mechanisms could yield important insights (Ding, 2010; Naidu et al., 2015; Song et al., 2022), and enhanced understanding of grapevine-virus interactions could pave the way for developing novel RNAi/CRISPR-based tools for GLRaV-3 control.

It has been reported that in greenhouse studies, some rootstock cultivars show tolerance to certain grapevine viruses, including GLRaV-1 (Zhao et al., 2024 and cited references), but the host factors involved in this defense process have not been investigated. Identifying resistance genes and genetic markers would aid in the breeding of GLRaV-3-resistant grape cultivars. Discovering the host factors required for GRLaV-3 infection in *Vitis* species would also be useful for applying genome editing to generate GLRaV-3-resistant grape cultivars. Finally, non-coding RNAs have been discovered in grapevines and the GLRaV-3 genome also contains non-coding genomic regions (Alabi et al., 2012). Non-coding RNAs are known to play significant roles in plant defense against viruses or cooperation with viruses in symptom and disease development (Wang et al., 2015; Yang et al., 2019; Ahmed et al., 2020; Shrestha and Bujarski, 2020; Javaran et al., 2021; Kumar and Chakraborty, 2021; Prasad and Prasad, 2021), but the roles of these non-coding RNAs in GLRaV-3 infection and symptom development remain largely unexplored. Understanding the role of non-coding RNAs in grapevine-virus and vector interactions could lead to important insights to inform RNAi-based biocontrol strategies for GLRaV-3.

In addition, further studies are required to understand susceptibility and symptom expression of different cultivars to GLD and why GLD symptoms are expressed only during the post-veraison stage of

the crop in red or black-fruited cultivars even though GLRaV-3 can be detected in infected vines throughout the season (Naidu et al., 2015). Elucidating the cascade of molecular events occurring during asymptomatic pre-veraison stages and symptomatic post-veraison stages could advance knowledge of the host-virus interactions that lead to symptom expression at a specific phenological stage in red or black-fruited cultivars or the lack thereof in white-fruited cultivars. The knowledge derived from these fundamental studies could also support the development of novel strategies to fight the disease, such as through the application of RNAi and CRISPR/Cas-based genome-editing technologies (see Host Plant Resistance to Viruses and Vectors section in Chapter 5).

Conclusion 4-3: Host factors required for GLRaV-3 infection and resistance in Vitis hosts have not been discovered, yet knowledge of these factors could create opportunities for developing novel control strategies.

Conclusion 4-4: The grapevine and GLRaV-3 genomes contain regions for generating non-coding RNAs whose role in infection and symptom development has not been explored.

Conclusion 4-5: Further investigations into the extent of GLRaV-3 host range within (and beyond) Vitis may generate valuable information that could be exploited for GLD management.

Recommendation 4-3 (MP): Support research to identify host factors required for GRLaV-3 infection and resistance in *Vitis* hosts and to investigate the role of non-coding regions of grapevine and GLRaV-3 genomes in infection and symptom development.

Recommendation 4-4: Support research to examine the common and unique responses of red or black- and white-fruited wine grape cultivars to GLRaV-3.

GRBD KNOWLEDGE GAPS

Basic Research to Generate Foundational Knowledge for Resolving the Complex Biology of GRBD

GRBV is the only reported causal agent of GRBD (Yepes et al., 2018). However, in light of the discovery of other grabloviruses infecting *Vitis* spp., it is important to determine whether GRBV is the only virus able to cause GRBD (Krenz et al., 2023). For example, Bayesian analyses of GRBV whole genome sequences suggested that GRBV emerged from the ancestral wild Vitis latent virus more than 9,000 years ago, prior to the domestication of *Vitis*; thus, it would make sense to assess whether wild Vitis latent virus is also a causal agent of GRBD.

GRBV isolates are generally classified as one of two genetic variants, clade 1 or clade 2, although recombinant isolates with genetic sequences from both clades have also been reported. Infectious clones based on isolates of each clade have demonstrated the ability to cause GRBD symptoms with no differences in disease expression (Yepes et al., 2018). However, there is scant evidence regarding any differences between clades in terms of symptom expression or the efficiency of transmission by the three-cornered alfalfa hopper (TCAH) *Spissistilus festinus* (Flasco et al., 2021, 2023). The question of whether there are any biologically relevant differences between clade 1 and clade 2 isolates (or whether there are any significant differences in selection pressures acting on GRBV populations in each of these clades) is still a major gap that has not been addressed (Krenz et al., 2023). The effects of co-infections of different GRBV isolates on disease are also unknown (see Complex Effects of Mixed Infections and Environmental Factors section in this chapter).

Conclusion 4-6: Knowledge of the biological differences between the major GRBV variants (clade 1 and clade 2 isolates) is incomplete.

Recommendation 4-5: Support studies to advance understanding of the epidemiological consequences of GRBV genetic diversity and interactions with other viruses.

Research questions that need to be addressed include:

- **What are the biological differences (e.g., transmission efficiencies, symptom expression, physiological responses) arising from the genetic variation of GRBV isolates?**
- **What are the consequences of co-infections of different GRBV variants?**

Virus-Host Interactions and Host Defense Mechanisms

GRBV is different from other members of the *Geminiviridae* family in some important ways (Gilbertson, 2024), and the discovery of GRBV and later ratification of this new virus species (*Grablovirus vitis*) spurred the formation of a novel genus named *Grablovirus* (Varsani et al., 2017; Fiallo-Olivé et al., 2021). Most of the putative open reading frames (ORFs) in the GRBV genome have no ascribed function to date. Transient expression of the GRBV C2 and V2 ORFs in *Nicotiana benthamiana* line 16c green fluorescent protein marker plants suggests a role for these genes in overcoming RNA silencing (Weligodage et al., 2023). Evidence for alternative splicing has been demonstrated in both the viral and complementary sense ORFs, and a novel ORF was discovered in the viral sense (V0) (Vargas-Asencio et al., 2019).

Although GRBV protein products of the V1 ORF (coat protein) and the V2 ORF (unknown function) have been physically detected in infected grapevine tissues (Buchs et al., 2018), no virions have ever been observed in GRBV-infected plants. This lack of observed virions is a gap in basic GRBV biology and an opportunity for future study since filling this gap would have practical implications for understanding transmission and improving diagnostics. Visualizing virions may be aided by discovering a suitable herbaceous model host, which has also eluded researchers thus far. Infectious GRBV clones have been inoculated into various herbaceous hosts (*Solanum lycopersicum* cv. Florida Lanai, *Nicotiana benthamiana*, and *Phaseolus vulgaris* cv. HyStyle) and GRBV replication was confirmed in inoculated leaves, but not in apical leaves (Flasco et al., 2021). The lack of systemic tractable model hosts for GRBV limits the study of virus-host interactions. Although the exact features of a pathosystem such as latency, susceptibility, and symptomatology may not be identical to what happens in grapevines, the development of an appropriate herbaceous model host can allow research to happen more quickly and in smaller spaces compared with research conducted in most grapevine varieties (Roy and Fuchs, 2024). If virus replication is higher in the herbaceous host, this would also improve the likelihood of visualizing virions. Therefore, a model herbaceous host would facilitate research on the basic biology of GRBV infection and help to identify features of interest. However, while an herbaceous host may be ideal for studying virus-host interactions, insights gained from such studies would ultimately need to be confirmed in *Vitis* spp. Due to this reason (and because an appropriate herbaceous model host has not been identified so far), it may be more practical to use Pixie grapevine, a Pinot Meunier mutant with a dwarfing and shortened internode phenotype released by the U.S. Department of Agriculture for unrestricted use, as a woody model host to study virus-host interactions. The small size and production of clusters when cultivated in a greenhouse or growth chamber can enable a broader scale or scope of research uses compared with commercial grapevine varieties, assuming Pixie becomes infected with vitiviruses similar to other *Vitis* spp.

Conclusion 4-7: Despite some progress in determining GRBV gene function, there are still major gaps in understanding the function of the GRBV genome with regard to specific roles of GRBV proteins in plant cells.

Conclusion 4-8: To date, virions have not been observed in GRBV-infected plants using microscopy; the lack of a tractable herbaceous model host that becomes systemically infected with GRBV limits the study of virus gene functions and virus-host interactions.

Recommendation 4-6 (MP): Support research to determine optimal model hosts (e.g., Pixie grapevine and/or herbaceous hosts) to facilitate the study of molecular plant-GRBV interactions and direct research efforts to transfer this knowledge to wine grape cultivars.

Research questions that need to be addressed include the following:

- **What functionally equivalent host factors are required for GRBV infection of plants?**
- **What is the virion structure of GRBV?**
- **What or which varieties of herbaceous and/or *Vitis* hosts are the best model systems for studying virus-host interactions?**

The lack of knowledge on the length of the latency period following GRBV inoculation is another major gap that has direct implications for epidemiology and management. In particular, there is a need to refine questions regarding latency and incubation periods to focus on determining the time intervals between vector-mediated inoculation and systemic GRBV infection, between inoculation and GRBV acquisition by the vector, and between inoculation and symptom expression. It would be reasonable to expect variability in each type of latency and incubation period among different cultivars and under different environmental conditions. Insights into these factors could directly impact management recommendations by helping to inform virus testing procedures and elucidating how asymptomatic infections may contribute to virus spread.

Conclusion 4-9: Current knowledge about latency and incubation periods after GRBV inoculation is insufficient. Questions about latency and incubation, which may vary among grapevine cultivars and under different environmental conditions, need to be refined because the answers could directly impact GRBD management recommendations to growers.

Recommendation 4-7 (HP): Support research to elucidate latency periods in different cultivars and rootstock-scion combinations, including the time from virus inoculation until vector acquisition, time until symptom expression, and time until the virus is detectable in plant and/or vector tissues.

Research questions that need to be addressed include the following:

- **How much of virus load in vineyards is due to planting with infected, non-certified vines and how much is due to insect inoculation after vine establishment?**
- **How long after vector-mediated inoculation will there be a systemic GRBV infection?**
- **How long after inoculation until new vector individuals can acquire GRBV?**
- **How long after inoculation will symptoms be expressed?**
- **How do these latency periods vary among different varieties and rootstocks?**

KNOWLEDGE GAPS REGARDING EFFECTS OF MIXED INFECTIONS, ENVIRONMENTAL FACTORS, AND ROOTSTOCK-SCION INTERACTIONS

Complex Effects of Mixed Infections

Mixed infections of multiple viruses in a single plant have been reported to influence viral replication, viral evolution, disease severity, plant physiology, and vector behaviors responsible for the acquisition and transmission of viruses (Alcaide et al., 2020 and references within; Di Mattia et al., 2020; Gautam et al., 2020a,b; Moreno and López-Moya et al., 2020 and references within; Zhao and Rosa, 2020; Bello et al., 2021; Singhal et al., 2021; McLaughlin et al., 2022; Chinnaiah et al., 2023; Kwon et al., 2023). Co-infections can also influence the efficacy of host plant resistance traits (Fortes et al., 2023).

These interactions are complex and have spatial and temporal dimensions associated with the order, timing, and locations where infections occur and the outcomes of those infections.

More than 100 viruses have been reported in grapevine (Fuchs, 2023). Mixed infections of viruses and viroids are common in grapevines (Adiputra et al., 2018; Xiao et al., 2018; Yao et al., 2018; Arnold et al., 2019; Diaz-Lara et al., 2019; Jones and Nita, 2019; Soltani et al., 2020; Xiao and Meng, 2023), and, in some cases, aggregated diseases such as sudden vine collapse (Bolton, 2020) are associated with co-infection of vitiviruses and leafroll viruses (Rowhani et al., 2018). With respect to GRBV, geminiviruses in other systems have demonstrated synergism in mixed infections (Moreno and López-Moya et al., 2020); however, it is not yet clear whether any synergistic effects are associated with the co-infection of GRBV with other viruses. Synergy has been reported for co-infections of vitiviruses with GLRaVs that cause changes in symptom expression, death of the vine, or changes in virus replication (Rosa et al., 2011; Rowhani et al., 2018; Čarija et al., 2022). Four studies have examined the co-transmission of GLRaV genetic variants, or GLRaV with other virus species, and using different mealybug vectors. One study included transmission of co-infections of GLRaV-3-I and GLRaV-3-VI by *P. ficus* and *Pseudococcus viburni* (Blaisdell et al., 2015), Other studies examined transmission of GLRaV-1 + GVA, GLRaV-3 + GVA, GLRaV-1 + GLRaV-3 and GLRaV-1 + GLRav-3 + GVA by *Heliococcus bohemicus* (Bertin et al., 2016a) and by *P. ficus* and *P. citri* (Bertin et al., 2016b), and co-infections of GLRaV-3 + GVA, GVA + GRSPaV, or GVA + GVB by *P. ficus* (Blaisdell et al., 2020). The results from these studies showed the presence of multiple viruses can increase or decrease the transmission of one or more of the viruses, but changes in transmission were not observed in every study. In some studies, changes in transmission appeared to be influenced by virus-vector interactions, and in others changes in the frequency of transmission were due to virus-plant interactions after vector inoculation. Together these results highlight the complexity of virus-vector-host plant interactions that can influence transmission and host infection outcomes. The implications of the background virome (i.e., mixed infections with other viruses) for the co-transmission of GLRaVs and GRBV, expression of GLD and GRBD symptoms, fruit quality, or for GLRaV-3 and GRBV fitness, have not yet been investigated. The influence of mixed infections on the evolution and epidemiology of these viruses remains poorly understood.

Effects of Environmental Factors

There are also knowledge gaps regarding the potential influence of environmental factors such as temperature, humidity, carbon dioxide, ozone, drought, and vineyard management practices on the vector, virus, plant, and interactions among them. Laboratory or greenhouse studies can be used to investigate how these influence within-plant factors related to transmission efficiency, virus replication, and disease severity. Broader landscape-level effects also need to be understood, which would require studies at the field level or modeling studies to examine regional shifts in degree days (temperatures) that regulate insect generations, plant growth, and geographic distributions of vector and plant hosts for viruses (Trebicki, 2020; Mangang et al., 2024 and references within). Understanding the effects of changing climatic conditions and other biotic and abiotic factors that modulate the disease cycle in the field will be important for current and future research and control strategies.

Conclusion 4-10: Infection of grapevines with multiple viruses has been reported, but how mixed infections affect disease severity and evolution of GRBV and GLRaVs (or GRBD and GLD) has not been thoroughly investigated.

Conclusion 4-11: The effects of changing climatic conditions and other factors (biotic and abiotic) that modulate disease cycles, including temperature, humidity, carbon dioxide, ozone, drought, and vineyard management practices on virus-vector-host interactions have not been determined.

Recommendation 4-8: Support research on the effects of mixed infections on GRBV and GLRaV evolution and the diseases they cause, as well as research on the effects of environmental factors, grapevine management practices, and changing climatic conditions on GRBD and GLD virus-vector-host interactions and epidemiology. Industry trends and stakeholder input could be used as a guide for prioritizing scion-rootstock combinations to use in experiments.

Research questions that need to be addressed include:

- **Do co-infections of GLRaV-3 or GRBV with specific classes of grapevine viruses facilitate disease establishment or enhance its severity?**
- **What are the consequences of mixed infections of GLRaV-3 with other viruses (e.g., synergism, antagonism, neutral)?**
- **What are the consequences of mixed infections of GRBV with other viruses (e.g., synergism, antagonism, neutral)?**
- **How do abiotic factors, other stresses, and non-viral diseases influence disease caused by GLRaV-3 and GRBV?**

Identification of Rootstock-Scion Interactions Relevant to Virus Transmission

Since viruses can readily spread between scion and rootstock via successful graft union, virus-tested scion and rootstock must be used for the health and productivity of grafted vines in vineyards. Grafted vines, consisting of a scion cultivar grafted onto rootstock from a distinct genetic background, are commonly planted to mitigate impacts of soil-borne pests and diseases in vineyards. Successful grafting requires technical expertise and depends upon the compatibility between the scion and the rootstock; viral infections of scion and/or rootstock can threaten the health of the vine and lead to graft failure, resulting in death or long-term decline and economic losses. A recent study highlighted the significance of rootstock selection as a strategy to mitigate some of the negative consequences of GLRaV-3 infection (Vondras et al., 2021). Rootstock of a grafted vine is also known to influence scion traits by altering grapevine vigor and yield components, as well as performance in the face of biotic and abiotic stresses. In addition, it is now well established that soil microbial communities play an important role in supporting grapevine health and adaptation to environmental conditions. Since rootstock genotypes can influence the profile of microbiomes in the rhizosphere and the root endosphere (Lailheugue et al., 2024), long-term strategic research aimed at understanding how to exploit interactions between rootstocks and soil microbiome for improved grapevine health, including improved nutrient uptake, overall growth, fruit yield and quality, may also result in strategies to mitigate negative impacts of viral diseases in vineyards.

Recent studies have indicated differences in the sensitivity of grapevine rootstocks from different genetic backgrounds to virus infections (Vondras et al., 2021; Zhao et. al., 2024). Studies in California vineyards have also documented virus-induced graft incompatibility phenomena in grapevines grafted with specific scion and rootstock combinations (Rowhani et al., 2017b). In recent years, intensified detrimental effects were reported due to synergistic effects between leafroll viruses and vitivirus, such as grapevine virus A (Golino et al., 2015; Rowhani et al., 2016). In single infections, grapevine virus A is generally latent, but co-infection with GLRaV-3 results in synergistic interactions leading to severe symptoms and devastating pathological effects such as sudden vine collapse in wine grape cultivars grafted onto susceptible rootstocks. Similarly, co-infection of GRBV and GLRaV-3 and of these viruses with other viruses can cause severe disease symptoms depending on the scion-rootstock combinations, contributing to progressive worsening of the vineyard's performance and the shortening of its productive life span. Characterization of viral communities in vineyards by high-throughput sequencing (HTS) technologies would help set the foundation for elucidating the collective impact of multiple, co-infecting viruses on vineyard performance and longevity as well as the potential for a synergistic enhancement of disease symptoms.

It would also be informative to investigate whether there are differences in virus titer between vinifera scion cultivars and rootstocks and whether scion-rootstock combinations influence symptom expression and virus titer. Such insights would inform approaches for testing samples from the scion of grafted vines for GRBV or GLRaV-3 and could help to determine whether there is a delay in virus movement across the graft union (in contrast to own-rooted vines) leading to delayed symptom expression. Another question that is important to growers is whether delayed symptom expression in post-planting grafted vines is due to delayed expression in infected, non-certified vines or vector-mediated transmission of the virus after planting. Understanding the relative contribution of infected, non-certified vines to the spread of GLD and GRBD, compared to vector mediated spread would help guide management by identifying efforts needed for vine certification programs versus in-field management activities.

Conclusion 4-12: A variety of factors, including the scion cultivar, genetic background of rootstock, rootstock-scion interactions, virus profile in individual grafted vines, synergistic interactions between co-infecting viruses, and environmental conditions, could contribute to the presence and severity of symptoms from GRBD and GLD.

Conclusion 4-13: Resistant rootstocks along with other control strategies could help to mitigate negative effects of viral diseases in vineyards.

Recommendation 4-9 (MP): Support research on the presence and diversity of viral resistance in grapevine rootstocks with different genetic backgrounds in order to inform the incorporation of resistant rootstocks into virus control strategies.

Recommendation 4-10: Support research to determine the contribution of planting with infected, non-certified vines on virus spread.

KNOWLEDGE GAPS IN GRBV AND GLRaV-3 DIAGNOSTICS AND DETECTION

Visual scouting for GRBV- or GLRaV-3-infected vines in vineyards is unreliable due to the variability of symptoms in different types of wine grape cultivars, because symptoms may not always be expressed clearly in affected grapevines, and because typical virus symptoms are easily confused with other maladies. In particular, white-fruited grapevine cultivars often do not show discernible symptoms when infected with either virus. Moreover, red or black-fruited wine grape cultivars can show red and reddish-purple leaf symptoms in response to many factors other than viral infections, such as nutrient deficiencies, physiological disorders, mechanical injuries, infection with crown gall bacterium, or insect herbivory, making it challenging to discern the impacts of these stressors from true symptoms of GLD or GRBD. When symptoms are present, initial testing and troubleshooting must be conducted to eliminate non-viral stress factors before proceeding with using symptoms to guide site-specific management of grapevine diseases. The following section describes potential testing methods that offer diagnostics for different situations, with each offering different scales of testing across a vineyard. Investments to develop these should be made based on stakeholder input and prioritized based on which one(s) will help growers and the industry accomplish site-specific and area-wide virus management goals most effectively and economically.

Cost Effective, Field Deployable or Laboratory-Based Tools for Large Scale Detection of GRBV and GLRaV-3

Simple Plant-Based Assays for Detection

Although there have been significant strides in GRBV and GLRaV-3 diagnostics (see Diagnostics sections in Chapters 2 and 3), there is still a need for additional diagnostic tools, especially those that

could improve the early detection of GRBV and GLRaV-3 and allow for affordable, high-throughput testing of commercial vineyards. The lack of affordable diagnostic methods for on-site detection delays timely disease diagnosis and management efforts, allowing the viruses to continue to spread and lead to substantial economic losses.

To date, the feasibility of developing serological methods, such as enzyme-linked immunosorbent assay (ELISA) or squash-blot, for detecting GRBV has not been determined. Virions have not been observed in infected tissues (Buchs et al., 2018), nor have virions been purified for producing antisera. Researchers have attempted to express and produce viral proteins in experimental host plants (R. Gilbertson, personal communication, March 5, 2024) but have yet to successfully generate the quality and quantity of viral proteins necessary to produce antisera against GRBV, hindering the application of serological assays in the diagnosis of GRBV. However, despite these challenges, developing portable serological assays for on-site testing is a realistic and worthwhile goal. Using recombinant or synthetic virus proteins could lead to the production of GRBV-specific antibodies, i.e., by engineering viral proteins such as coat protein in vector plasmids to be expressed in cultured cells for making antigens. Since coat protein was detected and quantified in proteomic profiling of GRBV-infected leaf and petiole tissues (Buchs et al., 2018), coat protein is a good candidate for producing a GRBV-specific antigen for developing a serological method for detecting GRBV. This could open the door to developing affordable rapid and on-site detection assays, such as lateral flow assays, that would be accessible to growers without requiring specialized laboratory equipment.

For GLRaV-3, current diagnostic techniques often rely on either visual scouting (which is unreliable), or laboratory-based assays such as ELISA, reverse-transcription polymerase chain reaction (RT-PCR), or HTS, which are more reliable but not practical for real-time field testing due to their cost, and dependence on specialized equipment and trained personnel (Bester et al., 2012; Blouin et al., 2017; Rowhani et al., 2017a; Galvan et al., 2023). ELISA methods for laboratory-based detection remains one of the most scalable testing techniques that could be developed for large scale testing if its automation capacity can be improved. Recently, a simple crude plant extract-based reverse transcription-recombinase polymerase amplification (RT-RPA) assay was developed to detect GLRaV-3 efficiently (Kishan et al., 2024). This tool can offer a cost-effective solution that could be validated and made commercially available for on-site vineyard detection.

Conclusion 4-14: There is a need for additional affordable diagnostic tools that can detect GRBV and GLRaV-3 infections early and are suitable for extensive use in commercial vineyards.

Recommendation 4-11 (HP): Support research to develop any new, simple, and affordable high throughput tests for GRBV and GLRaV-3.

Research may include the following:

- **Producing GRBV-specific antigens that could enable development of a serological assay.**
- **Validating a simple crude plant extract-based LAMP and RPA assays for GLRaV-3 and GRBV to determine the suitability of isothermal assays for large scale and/or on-site detection.**
- **Improving the automation testing capacity for existing GLRaV-3 ELISAs to improve throughput and reduce costs.**

Volatile Organic Compound Detection

Disease detection using dogs, electronic noses (ENs), or micro-electromechanical systems could help with early detection (i.e., during latency/when visual symptoms are absent) and could also address the sampling problem/uneven distribution of pathogens in the host, which is an issue with detection methods such as PCR (Gottwald et al., 2020).

Dogs possess an impressive olfactory capability to identify distinct profiles of volatile organic compounds (VOCs) unique to specific diseases (Fuchs, 2020), as demonstrated by studies on plum pox virus (Rodoni et al., 2006), little cherry disease, citrus canker (Gottwald et al., 2020), and citrus greening (Gottwald et al., 2019). While these studies point to the potential of canine detection of virus infection and enhancing early detection efficiency (Fuchs, 2020), the most practicable and cost-effective use of dogs for field detection of GRBV and GLRaV-3 has yet to be demonstrated. Trained dogs could be effective in detecting viral infections, but due to cost, only a limited number of dogs can be trained and deployed, and dogs are not deployed for long periods of time. In a study by Gottwald et al. (2020) that used dogs to detect *Candidatus* Liberibacter asiaticus infection, a canine team was deployed in an orchard for ~30 min followed by a rest period of 30 minutes, suggesting that canine detection might be best suited for inspecting grapevine nurseries rather than surveying extensive commercial vineyards. If used in a clean stock program, the cost of canine detection (2 dogs and a handler) is estimated to be $150k to $200k for the first couple of years.[1]

Electronic noses (ENs) represent another strategy for detecting viral infections by identifying VOCs emitted by infected plants. These handheld devices, which are composed of a sensor array, a signal conditioning circuit, and pattern recognition algorithms (Cui et al., 2018), are a non-invasive, rapid, and cost-effective alternative to traditional gas chromatography-mass spectrometry techniques (Cui et al., 2018). The use of ENs to detect infection has been demonstrated in tomato plants infected with powdery mildew (Ghaffari et al., 2010); in chili plants infected with bacterial soft rot, spot, and wilt; and in papaya plants infected with bunchy top and bacterial canker (Chang et al., 2014).

Micro-electromechanical systems can also be developed to detect VOCs. Differential mobility spectrometry (DMS) is one technique capable of characterizing mixtures of gaseous compounds with detection limits in low parts per billion or high parts per trillion, depending on the chemical composition (Dodds and Baker, 2019; Cumeras et al., 2015a,b), and when used with portable gas chromatography and AI driven data analysis algorithms (Peirano et al., 2016; Anishchenko et al., 2018; Rajapakse et al., 2018; Yeap et al., 2019, 2020; Chakraborty et al., 2022; Fung et al., 2023). DMS is a type of high field asymmetric waveform ion mobility spectrometry that was developed to create mobile and portable devices for defense and security applications to screen for explosives and can be used to detect biological sources. Two DMS assays were shown to diagnose citrus trees infected with citrus greening disease with 90–99.9 percent accuracy (Aksenov et al., 2014; McCartney et al., 2024), and this approach may have applications for detection of other plant pathogens.

Conclusion 4-15: Canine olfactory capacity could be used for GRBV and GLRaV-3 field detection, but the most effective, practicable, and cost-effective way to employ dogs for monitoring and early detection has yet to be determined. Canine detection may be best suited for nurseries rather than commercial vineyards.

Conclusion 4-16: Research to profile plant responses to GRBV and GLRaV-3 (and their vectors) may reveal unique VOC profiles that could establish a basis for the development of hand-held EN or DMS devices for pathogen detection in the field.

Recommendation 4-12: Support research to identify VOCs unique to GRBV and GLRaV-3 infection or relevant vector infestations and determine the detection efficiency of VOC-based methods compared with other diagnostic tools.

Remote Sensing

Remote sensing technologies, including imaging spectroscopy, hyperspectral imaging, and RGB imaging, share a common feature of capturing detailed information about plant health status from a

[1] This estimate was provided in this article https://www.goodfruit.com/sniffing-out-diseases/

distance. These technologies can significantly aid in grapevine virus detection by enabling the identification of subtle changes in leaf color, texture, and reflectance patterns that may indicate viral infections. Remote sensing has significant potential as a tool for disease diagnosis in white grapevine cultivars, which do not exhibit conspicuous symptoms, and in infected red cultivars at the pre-veraison stage. If feasible, using imaging devices for in-field diagnosis of GRBV and GLRaV-3 would likely be attractive to growers and crop consultants because it would eliminate the need to collect plant tissue samples, and could be used to screen large areas or entire vineyards. However, due to the complexity of the symptoms associated with these viral diseases, remote sensing data collected from a vineyard would still require validation of the infection status of a large number of vines using a reliable laboratory diagnostic tool, such as PCR or ELISA. Although several research groups (MacDonald et al., 2016; Bendel et al., 2020; Nguyen et al., 2021; Galvan et al., 2023; Sawyer et al., 2023; Wang et al., 2023a,b, 2024; Lee at al., 2024; Wang and Pagay, 2024; Žibrat and Knapič, 2024) are studying the suitability of various remote sensing devices, to date this research has focused on detecting grapevine viruses based on only the leaf (or canopy) reflectance without any studies examining this method's applicability to other tissue types such as fruit berries, young twigs, branches, or trunks. Further research could help to elucidate the method's applicability to other tissue types and its feasibility for field deployment.

Conclusion 4-17: Remote sensing technology has the potential for remote or in-field diagnosis of GRBD and GLD in individual vines; however, testing the efficacy of this approach will require scalable deployment of remote sensing devices for detection of infected vines in a large-scale area.

Conclusion 4-18: Remote sensing technology can be a part of a multi-layered system to guide sampling efforts by taking advantage of different spectra and resolutions to address specific goals.

Conclusion 4-19: In addition to leaves, remote sensing devices can also potentially be used on other visible parts of the vines to detect grapevine viruses.

Recommendation 4-13 (HP): Support studies on the use of remote sensing technology to facilitate large-scale and early detection of GRBD and GLD in various tissues of commercial cultivars (including white cultivars) to increase the reliability, specificity, and sensitivity of detection with this technology.

Improved Methods for Detection of New GRBV and GLRaV-3 Variants

Nucleic Acid-Based Assays

GRBV and GLRaV-3 will continue to evolve in cultivated vines, wild vines, and other refuge plants in the vineyard ecosystem, and it is important to continually monitor for the occurrence of new GRBV and GLRaV-3 variants in vineyards and riparian habitats. Since the primers used in existing nucleic acid-based assays may not detect these newly emerging variants, nucleic acid-based detection assays need to be improved frequently by upgrading primer sequences. In addition, rolling circle amplification (RCA), which has been used to amplify the whole genome of GRBV, could be another method to detect GRBV at very low concentrations (e.g., in nursery settings). Although the feasibility of this method for diagnosing GRBV is yet to be determined, if used, sequencing the RCA products may be particularly useful for universal detection of emerging GRBV variants.

Conclusion 4-20: As GRBV and GLRaV-3 continue to evolve in vineyards and non-crop habitats, nucleic acid-based assays used for virus detection will need to be upgraded to enable reliable detection of newly emerged virus variants.

Recommendation 4-14: Support research to determine the feasibility of using RCA or other single-stranded circular DNA detection techniques to help detect GRBV at very low concentrations and for universal GRBV detection.

Recommendation 4-15 (HP): Support research aimed at improving GRBV and GLRaV-3 detection with nucleic acid-based methods that can be used in the field at large scales.

Optimal Sampling Strategies and Sample Size for Accurate Estimation of GRBV and GLRaV-3 Prevalence

Cost-effectiveness is an important consideration in the development, evaluation, and implementation of GRBD and GLD testing and diagnostic strategies. Currently, the costs associated with sample collection, preparation, and analysis restrict current testing to levels that may not be effective for diagnosing and monitoring virus infected grapevines. Collecting large numbers of samples for early diagnosis of asymptomatic vines can be labor-intensive and cost-prohibitive, which hinders nursery, certification program, and grower adoption. Many small-holder growers cannot afford testing because commercial testing is costly. The costs associated with testing also limit studies on virus spread which would ideally include multiple tests on individual vines each year, conducted over several years and locations. Current testing methods require instruments and micro-pipetting procedures that are done by specialized companies, and on-farm testing is not currently available to growers. A lack of labor and automation capabilities for sampling are also limiting factors for detecting viruses in large-scale settings. Furthermore, uneven distribution of viruses in grapevines and seasonal variations of virus titers can lead to inconsistency and false negatives of testing results in some cases, particularly for GRBV.

Strategies that require less labor could make it more feasible for growers to monitor their fields effectively, efficiently, and economically. While systematic and random sampling strategies have been investigated (Geiger and Daane, 2001; Naidu et al., 2014; Sharma et al. 2011), information is still lacking about the most effective sampling method across vineyard settings and regions. Determining the optimal sampling strategy, sample size, timing of sampling, and detection method requires consideration of various factors, including the spatial distribution of the viruses, the area of the vineyards, and the logistical feasibility of various steps in the process. For example, Meyers et al. (2011) have suggested that stratified sampling based on vineyard blocks or rows may improve accuracy by capturing spatial heterogeneity. Statistical methods such as power analysis can be employed to determine the minimum sample size needed to achieve a desired level of accuracy in prevalence estimation (McDonald, 2008; Hajian-Tilaki, 2014). However, these two approaches may not fully account for the complex dynamics of GRBV and GLRaV-3 spread within nurseries and vineyards.

Another strategy that could be considered is to detect GRBV and GLRaV-3 in insects feeding on grapevine. These phloem-restricted viruses could be a part of insects' diets when feeding on phloem of virus-infected grapevines, and phloem contents (and any microbes present) may accumulate in the insects' gut. Detecting viral markers in insects offers a unique and relatively targeted way to sample the phloem contents of a vine, and this strategy has been successfully employed to detect citrus viruses in vector and non-vector phloem-feeding insects (Saponari et al., 2008; Britt-Ugartemendia et al., 2022). Several studies have already documented the presence of GRBV in the TCAH and other insects of unknown vector status (Cieniewicz et al., 2018; LaFond et al., 2022; Wilson et al., 2022), but additional research would be needed to determine the sensitivity of this method for detecting GRBV and GLRaV-3 in insects and to define best practices for sampling insects from grapevines. Since sampling insects will generally be more time consuming than foliage sampling, it will also be important to determine the feasibility and best application of this method for virus detection.

Conclusion 4-21: Consensus is lacking on the most effective sampling technique and minimum sample size for accurately estimating GRBV and GLRaV-3 prevalence across different vineyard settings, regions, and nursery increase blocks.

Conclusion 4-22: Virus detection in vectors and other phloem feeding insects may be an alternative to testing grapevines for viruses.

Recommendation 4-16 (HP): Support research evaluating optimal sampling methods and minimum sample size for accurate estimation of GRBV and GLVaV-3 prevalence in vineyards to inform the development of best practices for adopting new technologies and for integrating multiple detection methods to improve accuracy and scale (i.e., using both molecular methods and remote sensing technology).

Standards for Diagnostic Testing in Nurseries, Commercial Vineyards, and Certification Programs

As illustrated in Figure 3-6 (Chapter 3), testing grapevines in nurseries and commercial vineyards is vital for effectively managing GRBD and GLD. However, there is currently a lack of standardized diagnostic protocols among testing laboratories. Variability in sample preparation, diagnostic methods, and data interpretation is an obstacle to the consistent and reliable detection of viruses in grapevine. To enhance the robustness and reproducibility of diagnostic protocols, it is imperative that these protocols be standardized and rigorously verified by an independent organization(s). Furthermore, all testing laboratories should obtain certification and strictly adhere to approved testing protocols with appropriate internal and external controls to ensure consistent and reliable results (see also Chapter 5).

Conclusion 4-23: Laboratory protocols for diagnostic testing of GRBV and GLRaVs have not been standardized.

Recommendation 4-17 (HP): Support efforts to develop standardized GRBV and GLRaV-3 diagnostic testing protocols that, once verified and certified, could be adopted by all laboratories that provide testing services for nurseries and commercial vineyards.

In a recent study that assessed an HTS protocol based on total RNA sequencing with RT-PCR, the HTS method demonstrated higher analytical sensitivity and inclusivity than traditional methods, detecting distant isolates and new viral species. However, the study also showed that expert judgment is essential for interpreting the results due to the potential for false positives (Rong et al., 2023) and that employing HTS in large-scale diagnostics of viruses in vineyards is not cost-effective. Conversely, long-read HTS (a DNA sequencing method that produces longer sequence reads (i.e., tens to thousands of kilobases in length) could open the door for future routine detection of GRBV and GLRaV-3 species and variants (Javaran et al., 2023). The availability of new chemistry with native barcodes will reduce the cost associated with long-read sequencing to a level comparable to PCR-based methods. Despite potential challenges in bioinformatic analysis for non-experts, the future integration of artificial intelligence algorithms and the development of user-friendly graphical interfaces for data analysis are expected to address this limitation and facilitate broader adoption of long-read sequencing for virus detection. Finally, the lack of universally accepted guidelines hinders the widespread adoption of HTS in grapevine virus diagnostics, highlighting the need for collaborative efforts to establish standardized protocols and validation frameworks in this field (Lebas et al., 2022; Massart et al., 2022).

Conclusion 4-24: HTS offers robust virus detection and discovery of new GRBV and GLRaV-3 variants, but HTS protocols need to be standardized, affordable for large-scale testing, and validated for use in diagnostic virus testing.

Recommendation 4-18: Support efforts to develop universally accepted guidelines for using HTS in GRBV and GLRaV-3 diagnostics.

Overall, there remains a clear need for new high throughput sampling, screening, and detection methods that could be used by growers, nurseries, and certification agencies to facilitate early and reliable diagnosis of viruses (and potentially their emerging variants). Future research on serological assays, remote sensing, and VOC-based detection could provide high throughput alternatives to complement available techniques. At the same time, more sensitive detection techniques such as RPA or loop-mediated isothermal amplification (LAMP), coupled with lateral flow assays, peptide nucleic acid-locked nucleic acid-mediated loop-mediated isothermal amplification (PNA-LNA mediated LAMP), and CRISPR-based novel detection techniques could further improve sensitivity and enhance disease monitoring efforts. Since these methods also have an increased ability to detect small quantities of nucleic acids, however, they carry an increased risk of false positives with even a small amount of cross-contamination of samples, making it important to consider opportunities to prevent cross contamination in these assays. Taken together, the challenges and opportunities in improving diagnostic capabilities underscore the need for collaborative efforts to drive advancements and fill the knowledge gaps in grapevine virus detection, diagnosis, and management.

KNOWLEDGE GAPS REGARDING GRBV AND GLRaV-3 VECTORS

Vector Transmission

Additional Vectors of GRBV

TCAH is the only GRBV vector that has been conclusively confirmed; however, because other geminiviruses transmitted by treehoppers (Auchenorryncha) have multiple vectors (Ammar and Nault, 2002), one question that needs to be adequately addressed is whether there are additional GRBV vectors. Numerous reports in the literature suggest additional vectors may be present but no definitive evidence for another vector (e.g., demonstration that the potential insect vector transmits the virus to grapevines) has been provided. Several reports document the presence of GRBV in other insects besides TCAH (Cieniewicz et al., 2018; LaFond et al., 2022; Wilson et al., 2022). Although this proves they acquired the virus by feeding on an infected plant, it does not prove the pathogen can move across insect membranes, enter the salivary glands, and be delivered to a recipient grapevine for successful transmission. Of the studies that have investigated transmission potential with vectors besides TCAH, not all experiments were replicated, some are missing critical details about how samples were handled for processing, and some leave open the possibility of false positive results due to the viral contamination in honeydew excreted by both vector and non-vector insects (Rosell et al., 1999). While artificial diet assays provide a way to test for transmission quickly, additional experiments are needed to confirm transmission and infection in live plants (Kahl et al., 2021).

No studies have examined sex-related differences in transmission or behavior of TCAH, even though males and females may transmit pathogens with different efficiencies or respond to environmental stimuli differently (Sakurai et al., 1998; van de Wetering et al., 1998, 1999; Beanland et al., 1999; Ghanim and Czosnek, 2000; Ning et al., 2015; Ogada and Poehling, 2015; Zhao et al., 2016; Lu et al., 2017). In the southeastern United States, TCAH males and females are present in overwintering populations, but males die soon after mating, whereas females live for an average of 38 days post-copulation (Mitchell and Newsom, 1984b). In California, both males and females have been found to be present in vineyards, but the ratios of males and females can fluctuate (Preto et al., 2019). Additional studies on TCAH population dynamics would help determine the frequency at which males and females are present in vineyards when transmission occurs and help to determine whether any sex-related differences in GRBV transmission efficiency or host feeding behaviors may be relevant to improving management approaches (see further discussion in the Virus-Vector Interactions section of this chapter).

Conclusion 4-25: While there are reports about potential additional insect vectors of GRBV, there has not been definitive evidence that other insects in addition to TCAH can transmit GRBV to grapevines.

Recommendation 4-19 (MP): Support research to identify additional vectors of GRBV using rigorous experimental approaches.

Research to identify additional vectors should employ the following best practices:

- **Select vector candidates for study based on field data suggesting an association between the insect and virus spread.**
- **Replicate controlled laboratory transmission experiments, including replicating experimental units (insects and plants) each time transmission is tested under a given set of conditions and replication of experiments to draw verifiable conclusions.**
- **Allow for a minimum time of 10 days for the acquisition access period, 10 days for the latent period, and 4 days for the inoculation access period based on the minimum times reported for TCAH. Males and females should be tested separately.**
- **Because plant viruses can be excreted and detected in honeydew, it is necessary to use a cleaning procedure to remove honeydew from plant tissue prior to virus testing. Methods designed to detect a viral RNA transcript could also prevent false positives due to contaminated honeydew.**
- **Testing transmission using artificial diets represents one way to demonstrate vector competence, but transmission to grapevines is needed to confirm the epidemiological significance of vector transmission in the field.**

Additional Vectors of GLRaVs

Understanding the transmission of GLRaVs remains an ongoing area of research with several important knowledge gaps. Currently, mealybugs and scale insects are recognized as the vectors of GLRaVs, but the knowledge of the full spectrum of potential vectors and their distribution in California may be incomplete. The vine mealybug, *Planococcus ficus,* is the major vector identified in commercial vineyards. The predominant role of this vector is partially attributed to its high reproductive capacity, which increases spread over time. The grape mealybug is also a prominent vector; however, the relative contributions of individual species to GLRaV transmission in the field are unknown. The epidemiological relevance of each vector or community of vectors will be impacted by differences in abundance, distribution, life cycle, and other life history characteristics. There does not appear to be specificity or fidelity among the leafroll viruses and mealybug vectors, but most studies have focused on GLRaV-3 transmission by *Pl. ficus*. Reports suggest that GLRaVs are semi-persistently transmitted with no latent period (Cabaleiro and Segura, 1997; Tsai et al., 2008). Characteristics such as the durations of acquisition, retention, and inoculation periods have not been determined for all mealybug and scale vectors, and few transmission assays have been conducted to quantify vector acquisition and inoculation efficiencies for different species of GLRaVs or genetic variants of particular GLRaV species.

Conclusion 4-26: There are gaps in the understanding of GLRaV-3 transmission, particularly with regard to the role of different vector species and their distribution in California; the mechanisms of GLRaV-3 acquisition and transmission; the transmission efficiency of diverse GLRaV-3 isolates; the acquisition, retention, and inoculation periods of all vector species; and how environmental factors influence GLRaV-3 transmission dynamics.

Recommendation 4-20 (HP): Support research on the mechanisms and timing of acquisition, retention, and transmission of all GLRaV vector species, as well as the influence of environmental conditions and host genotype on GLRaV transmission dynamics.

Research to identify additional vectors should employ the following best practices:

- **Conduct transmission assays that individually assess acquisition, retention, and inoculation.**
- **Healthy vectors should be caged on infected plants for acquisition access periods (AAPs) that range from several hours to several days to assess acquisition efficiency.**
- **For inoculation assays, infected insects should be isolated in groups on healthy plants to assess virus transmission. Inoculation assays should utilize insects of similar developmental stage. Inoculation access periods (IAPs) can range from several hours to days, as longer IAPs yield higher transmission efficiencies.**
- **Transmission experiments in which insects feed on artificial media through a membrane can also be used to assess vector capacity, but ultimately this approach may not provide an accurate indicator of vector transmission capacity or efficiency.**
- **Transmission differences between vector species may be specific to grape cultivars and environment; therefore, comparisons of efficiency should be evaluated in controlled assays to assess the contributions of these factors to the epidemiology of vector transmission.**
- **Differences in transmission efficiency among clones or populations of vector species should be evaluated using comparable AAPs and IAPs to effectively assess the epidemiological importance of particular vector species or phenotypic variation in transmission efficiency that exists in pathogen transmission.**

Vector-Virus Interactions

Additional knowledge gaps that exist for both GRBV and GLRaVs include the mechanisms of vector-virus interactions, the effect of the environment on epidemiology, and how mixed infections with multiple viruses might impact transmission. The time required to acquire and transmit these viruses has been examined, but virus localization in the vectors has not been confirmed, and the precise viral retention sites have not been thoroughly characterized. The genetic, cellular, and physiological mechanisms underlying vector transmission remain unknown. Virus localization would confirm the mode of transmission of GLRaVs by mealybugs and scales; transmission is reported as semi-persistent, but definitive transmission studies of GLRaV-3 are generally lacking. Studies examining GRBV localization in TCAH may help explain the long latent period required for the virus to circulate through the vector and generate new hypotheses about transmission. In other pathosystems, vector or endosymbiont proteins have been reported to bind to virions circulating in insects, and endosymbionts can alter transmission of plant viruses and insect-plant interactions (Gonella et al., 2019; Wilson et al., 2019; Ghosh and Ghanim, 2021; Wu et al., 2022; Sanches et al., 2023 and references within these). These factors have not been studied for GRBV or GLRaV-3, and identification of endosymbionts, genes, proteins, and metabolites responsible for virus transmission may help generate novel control strategies designed to block these interactions (Heck, 2018; Milenovic et al., 2022; Ali and Ume-Farwa, 2024).

Conclusion 4-27: Knowledge of virus localization in the vectors and the precise role of viral retention sites in vector transmission would improve knowledge about the mode of transmission for GRBV or GLRaV-3.

Conclusion 4-28: The roles of vector endosymbionts, genes, proteins, and metabolites mediating transmission have not been studied for GRBV or GLRaVs. This information is needed to understand transmission dynamics and to develop novel tools for disrupting transmission for the management of GLD.

Recommendation 4-21: Support studies to identify interactions between GRBV and GLRaVs and their vectors that are required for transmission, as well as studies to identify

genes, proteins, and metabolites involved in virus transmission to develop control strategies based on interference of virus-vector interactions.

Vector Plant Preference and Behavior Manipulation by GRBV and GLRaVs

Impact of Known Hosts on Disease Spread and Insect Behavior Manipulation

The reported host range of GRBV and GLRaV-3 is limited to *Vitis* and non-cultivated grapevines, but the relative contributions of different species or varieties in GRBV or GLRaV-3 spread are not well understood. The variation in host utilization by vectors and the virus prevalence in host plants are also not clearly defined. Knowledge about variation in vector behavior and virus susceptibility among *Vitis* and non-cultivated grapevines may help explain patterns of spread and guide management decisions. This includes whether vector behavior changes in response to vine health or different species and varieties of *Vitis* and non-cultivated grapevines. No study to date has comprehensively compared vector preferences for plant host species. Molecular gut content analyses could help identify what plants vectors are feeding on before they move into vineyards (Cooper et al., 2016, 2019, 2022, 2023; Hepler et al., 2021, 2023; Reyes Corral et al., 2021a,b; Dorman et al., 2024; Pitt et al., 2024). Knowledge on host plant preference or suitability can also be studied by evaluating behavioral responses, such as host choice experiments, olfactometer assays, or electrophysiological studies to assess vector responses to host volatiles. Feeding assays, in the form of electrical penetration graph assays, laboratory assays, and greenhouse assays, may provide information about host preference, host suitability of different grape cultivars for specific vectors, and virus transmission which would provide information to assess the epidemiological importance of individual vector species (Fereres and Collar, 2001; Tjallingii and Prado, 2001; Fernandez-Calvino et al., 2006; Sandanayaka et al., 2013, 2014; Boquel et al., 2015; Mustafa et al., 2015; Muturi et al., 2016; Obok et al., 2018).

In addition, a growing body of work has shown that virus infection can alter insect vector behavior or fitness of host plants in ways that promote the acquisition and transmission of the viruses, a concept known as the vector manipulation hypothesis (Ingwell et al., 2012; Stafford et al., 2011; Su et al., 2015; Chen et al., 2013; Eigenbrode et al., 2018). This can occur via changes induced in the host plants that alter visual cues, volatile profiles, palatability, host defense, or nutritional quality and influence insect settling and feeding behaviors. In some pathosystems, the fitness of the vectors is improved due to metabolite profile changes in infected plants, which leads to increased reproductive or developmental rates. All of these changes may influence vector settling behaviors, feeding behaviors, and dispersal patterns related to the acquisition and spread of viruses, but changes in vector behavior (and biology) are not consistent or generalizable across or within pathosystems (Jones, 2014). This underscores the need to study how GRBV and GLRaV-3 specifically alter the behavior of the insect vector(s), which could have important epidemiological ramifications for understanding and modeling their spread.

Conclusion 4-29: GRBV and GLRaV-3 have only been reported to occur on Vitis and non-cultivated grapevines, but the relative contributions of different host species or varieties in GRBV or GLRaV-3 spread are not known.

Conclusion 4-30: Comprehensive studies to understand host plant utilization and preferences of vectors have not been completed.

Conclusion 4-31: Vector behavior might change in response to plant infection by GRBV and GLRaV-3 (i.e., changes in insect behavior mediated through the host plant), which may affect the settling, feeding, fitness, and dispersal behavior of the vectors.

Recommendation 4-22 (MP): Support research on virus-vector-host interactions to determine how the different species or varieties of *Vitis* and non-cultivated grapevines

contribute to virus spread, as well as how GRBV or GLRaV-3 infection of the host can alter vector behavior.

Recommendation 4-23 (MP): Support research to broaden the understanding of complex interactions among the virus, vector, and host to enable the development of models of disease spread and strategies to prevent disease transmission.

Possible research approaches include the following:

- **Host choice experiments, olfactometer assays, or electrophysiological studies to assess vector responses to VOCs emitted by GRBV and GLRaV-3-infected plants.**
- **Experiments with nonviruliferous (have not acquired virus) and viruliferous (have acquired virus) vectors to determine whether the presence of GRBV and GLRaV-3 alters vector behavior with respect to host plant selection, frequency of movement between plants, feeding, or reproduction.**

TCAH Host Preference and Movement Dynamics

There are major knowledge gaps in TCAH seasonal host utilization, which directly impacts epidemiology. The overwintering behavior of the TCAH has not been studied in California, and it is unknown whether TCAH spreads GRBV to grapevines in February–March when the overwintering adults have first been observed in vineyards (Preto et al., 2019). Previous studies have shown that overwintering adult TCAH actively feed, need a water source, and remain in a state of reproductive diapause except in areas with warm winter temperatures (Newsom et al., 1983; Mitchell and Newsom, 1984a). In California, the first overwintering adults have been detected February–March before bud break, with a second and larger peak detected in late June and July (Preto et al., 2019), a time when adults have tested positive for GRBV and girdling has been observed. Transmission may occur before this second peak, but this has not been tested (Cieniewicz et al., 2018). Overwintering of pathogens in adult vector populations contributed to the early season spread of Pierce's disease by the glassy-winged sharpshooter (Purcell, 1975; Almeida et al., 2005). It is unknown whether GRBV can persist in the overwintering TCAH adults that acquired GRBV before they left vineyards in the fall or whether overwintering adults acquire GRBV from cultivated or wild *Vitis* spp. during the spring. Studies have identified wild *Vitis* spp. as an alternate host for GRBV; however, surveying these hosts for TCAH and assessing their importance as a source for GRBV spread into vineyards is difficult, and even large research efforts may not be sufficient to draw meaningful conclusions.

The movement of TCAH into vineyards that is responsible for GRBV spread will be influenced by generation times, seasonal host plant availability, host plant attraction cues, host preferences, and movement behavior of TCAH. Information about TCAH movement dynamics between grapevines and alternative hosts may improve monitoring efforts and help scouts, growers, and consultants understand how to interpret observations of TCAH populations that are only transiently using vineyards while alternative hosts are largely absent during the summer (Cieniewicz et al., 2018; Preto et al., 2019). A better understanding of host preference and timing of movement by the more mobile adults of TCAH may also inform the implementation of trap cropping strategies to intercept, concentrate, and kill TCAH on alternative hosts that are more attractive than grapevines along borders of vineyards before these insects encounter grapevines.

In California, most GRBV-TCAH-grapevine studies have been conducted in the Napa Valley. However, as demonstrated by marked differences in distribution and abundance of the TCAH in other states where GRBV is spreading, and consequently differences in disease epidemiology, there are also expected differences across regions in California. This underscores the need for studies to determine the impacts of geographic factors on TCAH abundance and seasonal dynamics, which may include differences in regional viticultural practices, landscape composition, and climate patterns. At this time,

there is no optimized sampling methodology for accurate estimation of TCAH populations in vineyards. Accuracy in sampling methodology is critical for developing population models. In addition, initial temperature-based degree day population development models have been developed for TCAH (Bick et al., 2020), and additional efforts can be made to refine these models. This may include modeling vector populations as a function of local factors, including grapevine phenological stages, which may help result in more region-specific information.

Conclusion 4-32: There are major knowledge gaps regarding the TCAH overwintering behavior, seasonal GRBV spread to grapevines, and differences among distinct grapevine-growing regions in California.

Conclusion 4-33: Population models may help predict TCAH generation development associated with TCAH movement into vineyards; models may need to include information other than temperature to accurately predict population development and movement behavior.

Recommendation 4-24 (MP): Support research on the seasonal virus spread of GRBV by TCAH, focusing on year-long TCAH abundance and overwintering behavior throughout California.

Studying seasonal spread of GRBV by TCAH could involve the following:

- **Optimizing sampling methodology for the most accurate estimations of TCAH abundance.**
- **Increase sampling efforts in fall and spring when populations have been low in previous studies.**
- **Perform sampling in multiple locations across different grape production regions and in multiple years to account for inter-annual variation in population dynamics.**
- **Develop population models that may assist with the monitoring and management of TCAH.**
- **Sample for TCAH in natural vegetation and vineyard-adjacent habitat.**

Recommendation 4-25: Support research to investigate TCAH host preference and movement behavior, which could help in the development of a trap crop strategy for intercepting TCAH at vineyard borders.

Studying TCAH host preference could involve the following:

- **Greenhouse studies to determine whether TCAH readily move between grapevines and alternative hosts, or if they prefer to remain on hosts other than grapevines.**
- **Experiments with nonviruliferous (have not acquired GRBV) and viruliferous (have acquired GRBV) individuals to determine whether the presence of the virus is altering vector behavior with respect to host plant selection, frequency of movement between plants, feeding, or reproduction.**
- **If a host plant is more attractive to TCAH than grapevines such that TCAH selects and largely remains on that host, then field studies could be conducted to confirm that this behavior occurs under natural conditions.**

RESEARCH PRIORITIZATION

High and medium priority research areas (with the recommendation number) are summarized in the table below for quick reference.

TABLE 4-1 Prioritization of Research to Address Knowledge Gaps

High Priority Research
Understanding the intrinsic and extrinsic factors contributing to the efficient spread of GLRaV-3 (Rec 4-2)
Elucidation of latency periods in different cultivars and rootstock-scion combinations (Rec 4-7)
Development of any new, simple, and affordable high throughput tests for GRBV and GLRaV-3 (Rec 4-11)
Using imaging spectroscopy for large scale and early detection (Rec 4-13)
Improving GRBV and GLRaV-3 detection with nucleic acid-based methods for large scale testing (Rec 4-15)
Evaluation of optimal sampling methods and minimum sample size for accurate estimation of GRBV and GLVaV-3 prevalence in vineyards (Rec 4-16)
Development of standardized GRBV and GLRaV-3 diagnostic testing protocols (Rec 4-17)
Determining the mechanisms and timing of acquisition, retention, and transmission of all GLRaV vector species; Determining the influence of environmental conditions and host genotype on GLRaV transmission dynamics (Rec 4-20)
Medium Priority Research
Identification of host factors required for GRLaV-3 infection and resistance in *Vitis* hosts; Investigating the role of non-coding regions of grapevine and GLRaV-3 genomes in infection and symptom development (Rec 4-3)
Determining optimal model hosts to facilitate the study of molecular plant-GRBV interactions (Rec 4-6)
Determining the presence and diversity of viral resistance in grapevine rootstocks with different genetic backgrounds (Rec 4-9)
Identification of additional vectors of GRBV using rigorous experimental approaches (Rec 4-19)
Studying virus-vector-host interactions to determine contribution of different Vitis species or varieties and non-cultivated grapevines to virus spread; determining how GRBV or GLRaV-3 infection of the host can alter vector behavior (Rec 4-22)
Broadening the understanding of complex interactions among the virus, vector, and host to enable the development of models of disease spread (Rec 4-23)
Studying the seasonal virus spread of GRBV by TCAH, focusing on year-long TCAH abundance and overwintering behavior throughout California (Rec 4-24)

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5
Research and Actions that May Yield the Most Promising Management Solutions

The preceding chapters present the current state of knowledge on the grapevine red blotch virus (GRBV) and grapevine leafroll-associated virus 3 (GLRaV-3) pathosystems, as well as the significant knowledge gaps that remain in understanding these pathosystems. This chapter addresses current management approaches along with areas for future research that could enhance the management of these diseases and the sustainability of viticulture in California. Where appropriate, recommendations are provided to help guide research priorities and approaches to advance management strategies for different sectors of the industry.

Recognizing that the industry needs both short-term and long-term management solutions to these diseases, the committee sought to identify opportunities to improve "stopgap" (i.e., interim) measures to sustain the industry in the near term as research efforts make progress toward elucidating longer-term solutions. In addition, the committee considered how grower perceptions and knowledge of these diseases and their management may impact the adoption of different management practices. There is significant variation in the prevalence of grapevine red blotch disease (GRBD) and grapevine leafroll disease (GLD) among wine grape production regions of California. Likewise, current management practices vary among regions, as well as among growers within regions. For regions where both diseases are established, growers may or may not fully appreciate the differences in each pathosystem in terms of vector ecology and behavior, and although the pathosystems are substantially different, current management practices often share certain general similarities. Because GRBV is a more recent emerging pathogen, less research has been conducted on its management compared with GLRaV-3, and according to Hobbs et al. (2022), the lack of information and education regarding GRBD has likely impeded the adoption of appropriate management practices. All of this underscores the urgent need to generate and effectively disseminate knowledge about each of these viral diseases and strategies for managing them.

This chapter also addresses the importance of integrating management programs for GRBD, GLD, and other pest issues and vector-borne diseases in California vineyards so that new approaches for the management of one pest do not disrupt the management of others. In this vein, it is encouraging that the California wine grape industry currently employs an array of tactics to mitigate disease spread, especially for GLD (Farrar et al., 2016). Given that all management practices have their own unique pros and cons with regard to development and implementation, the committee attempted to identify the strengths as well as the potential weaknesses or downsides of each strategy so that the industry can make informed decisions about pathways forward.

While the committee believes that all the recommendations in this chapter are important, it is also cognizant of the fact that research funds are limited and has identified the high- and medium-priority research areas and actions. In the sections below, research recommendations and actions of high priority are labeled HP and those of medium priority are labeled MP. Additionally, high- and medium- priority research areas and actions are presented in a table (Table 5-1) at the end of the chapter.

CLEAN PLANTS

Since viruses can spread via clonal propagation of grapevines (both scion cultivars and rootstocks), using "clean" planting material is the first line of defense in establishing healthy vineyards.

The National Clean Plant Network (NCPN)[1] supports grape clean plant centers in California, Washington State, Missouri, New York, North Carolina, and Florida that maintain foundation collections (i.e., Generation 1 or G1 planting stock) utilizing standard operating diagnostic and pathogen elimination protocols to ensure that plants are free of economically important viruses. These clean plant centers maintain the G1 grapevines on a long-term basis under conditions that mitigate the risk of infection. As an example of the high level of emphasis placed on ensuring the quality and safety of this G1 material, Foundation Plant Services at the University of California, Davis, recently began the process of moving its core foundation collection from an open-field vineyard into a greenhouse environment to further protect it from potential sources of infection.

Commercial nurseries use G1 stock to propagate mother blocks of G2 grapevines, which can be certified and registered under state grape certification programs. Grapevines propagated directly from registered G2 blocks are then amplified as G3 and G4 vines and supplied as certified stock to growers for planting new vineyards (see Figure 5-1).

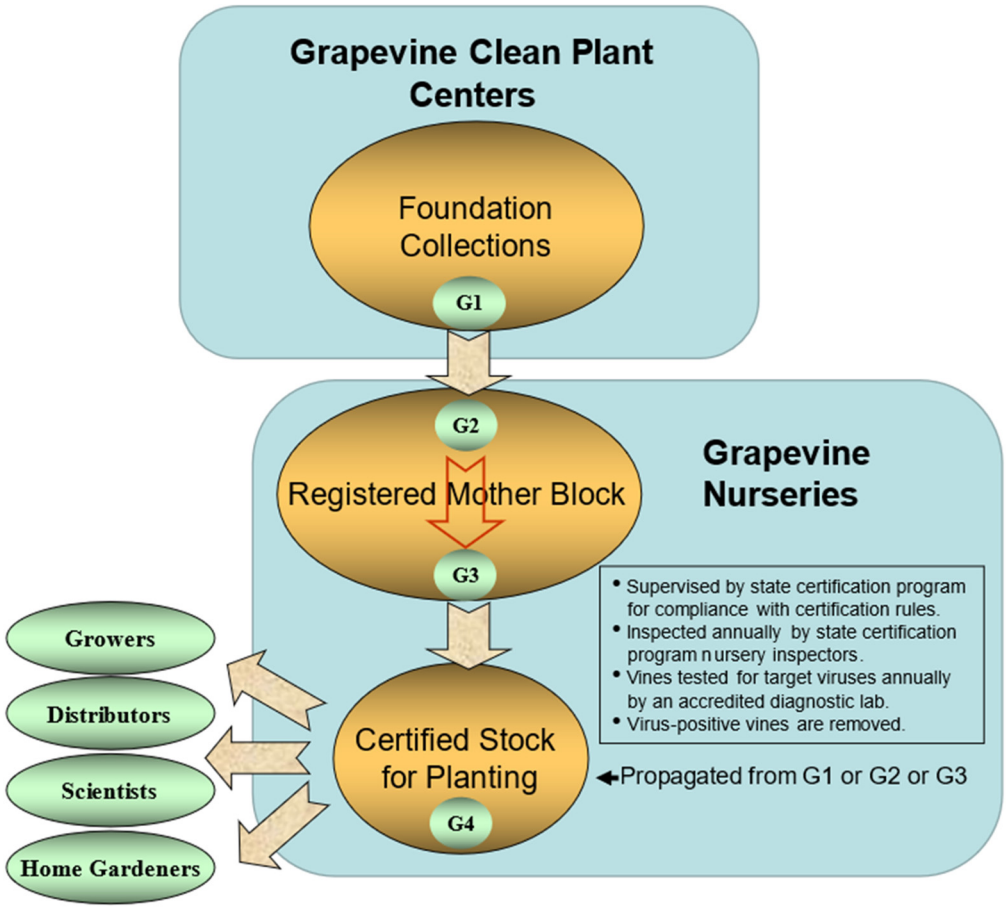

FIGURE 5-1 An illustration of the supply chain for clean grapevine planting material.
SOURCE: Naidu Rayapati, WSU-IAREC.

At each stage along the supply chain for grapevine planting materials, grapevines (both scions and rootstocks) may be inspected and tested for economically important viruses. As mother blocks represent the transition point between foundation collections and the broader distribution of grapevine

[1] See https://www.nationalcleanplantnetwork.org/.

stock, ensuring that registered mother blocks remain free of diseases and harmful viruses is especially critical to strengthening clean planting material supply chains, meeting state certification and quarantine criteria, and maintaining growers' confidence in the value of using clean stock for planting new vineyards. Toward this goal, it is vital to employ robust sampling strategies and state-of-the-art, sensitive, and reliable diagnostic methods (see Box 5-1) to test grapevines in registered mother blocks for harmful viruses. It is not sufficient to rely on symptom-based scouting of grapevine nursery materials, since symptoms can be similar for GLD and GRBD and symptoms for both diseases can vary among different cultivars. Instead, reliable diagnostic methods should be employed and samples from a few wine grape cultivars and rootstocks should periodically be subjected to high-throughput sequencing (HTS) to validate diagnostic results.

BOX 5-1
State-of-the-Art Practices: Key Elements for Reliable Grapevine Virus Detection

Accurate detection of GLRaV-3 and GRBV relies on robust protocols that minimize the risk of false positives and negatives. Both polymerase chain reaction (PCR)-based and high-throughput sequencing (HTS)-based detection methods have specific requirements to ensure diagnostic reliability. Recent advancements, including the development of assays to detect genetically diverse GLRaV-3 variants and the refinement of HTS protocols, have significantly enhanced the accuracy of grapevine virus detection. Below are key elements to consider in developing testing protocols; for more detailed information, refer to the cited publications and the American Phytopathological Society's Diagnostic Assay Validation Network (DAVN).[a] DAVN offers tools, a community of practice, and knowledge resources to support the development, implementation, and understanding of validated diagnostic assays for plant pathogens. Adhering to these practices can optimize PCR and HTS methods to deliver reliable and accurate detection of grapevine viruses and reduce the likelihood of diagnostic errors.

PCR-Based Detection Protocols

PCR-based methods, such as reverse-transcription PCR (RT-PCR) and quantitative PCR (qPCR), are well-established for detecting specific viruses but require careful attention to several factors to avoid inaccuracies.

Specificity and sensitivity: Design virus-specific primers that target conserved regions to ensure both specificity and sensitivity for the virus of interest and its associated variants. For instance, an available RT-qPCR assay has been shown to detect all known GLRaV-3 variants, including highly divergent ones, by targeting a conserved region in the 3' untranslated terminal region of the virus genome (Diaz-Lara et al., 2018). As new GLRaV-3 variants emerge, it is important to reevaluate the assay periodically to encompass current knowledge on the virus genetic diversity.

***Sample quality*:** Ensure high-quality RNA/DNA extraction to avoid degradation, which could lead to false negatives. The success of PCR-based detection is highly dependent on the quality of the extracted nucleic acids and the sampling time (Chooi et al., 2013; Setiono et al., 2018).

***Control use*:** Incorporate positive and negative controls in each run to validate the results and identify potential contamination or errors (Chooi et al., 2013).

Reaction conditions: Optimize PCR conditions (e.g., annealing temperature, cycle number) to prevent non-specific amplification, which could result in false positives (Ruiz-Villalba et al., 2017). The use of internal and external references in qPCR assays further enhances assay robustness (Setiono et al., 2018).

***Confirmation*:** Validate positive results with sequencing or additional independent tests to confirm the presence or absence of the virus.

continued

BOX 5-1 *continued*

HTS-Based Detection Protocols

HTS-based protocols provide a comprehensive approach to virus detection but require meticulous validation, appropriate controls for each step, and analysis to prevent false results.

***Sample preparation and integrity*:** Maintain high-quality sample processing to ensure that RNA integrity is preserved, which is critical for reliable sequencing outcomes (Hamim et al., 2022).

***Bioinformatics analysis and sensitivity*:** Use rigorous bioinformatics pipelines to accurately assemble and align sequences, and implement contamination monitoring tools (e.g., alien controls) to avoid misinterpretation of data (Massart et al., 2022; Rong et al., 2023). The introduction of new protocols, such as combining petiole and cane sampling across seasons, has been shown to increase the sensitivity of HTS to 100 percent (Soltani et al., 2021).

***Controls and thresholds*:** Apply external controls and set appropriate detection thresholds to distinguish true viral sequences from noise or contaminants (EPPO Standard on Diagnostics PM 7/151 (1), 2022). The false virus discovery rate should be minimized to reduce the likelihood of false positives, as shown in recent validation studies (Massart et al., 2022).

Expert evaluation: Rely on expert judgment in interpreting results, especially in cases where new or unexpected viruses are detected (Rong et al., 2023). HTS data often requires careful interpretation due to the complexity of viral populations within a sample (Javaran et al., 2023).

***Validation*:** Cross-verify HTS findings with traditional methods such as RT-PCR and Sanger sequencing to confirm results and rule out false positives or negatives (Rong et al., 2023). Consistent performance metrics, such as high sensitivity and reproducibility, are essential for HTS to be adopted in routine diagnostics (Massart et al., 2022).

[a] See https://www.apsnet.org/DAVN/Pages/default.aspx.

Productive collaborations between grape clean plant centers, state certification programs and departments of agriculture, certified nurseries, growers, and the broader wine grape industry are vital for nurturing the long-term economic prosperity of the grape and wine industry, while gaps can lead to missed opportunities and undermine the health of the industry. One potential gap is that testing laboratories may not always be providing reliable results to growers and nurseries. In particular, a major concern is whether laboratories testing for GLRaVs are using the most up-to-date testing protocols to keep pace with the rapid evolution of the viruses (Maree et al., 2015; Li et al., 2022). The University of California, Davis Foundation Plant Services (FPS) Center devotes considerable effort to optimizing detection and identification protocols; encouraging commercial testing labs to adopt standard protocols could decrease the risk of false negatives going forward. To ensure that appropriate protocols are used, industry members could take the position of only using labs that employ "FPS-validated" protocols for testing (e.g., Protocol 2010).[2] In the context of large-scale testing, it is ideal to employ affordable diagnostic tests that accurately detect the widest range of genetic variants; for example, the California Department of Food and Agriculture uses enzyme-linked immunosorbent assay (ELISA), an approach that is also aligned with Protocol 2010 recommendations, for testing in the California Registration and Certification Program for Grapevines (Li et al., 2022).

A second concern is that commercial testing laboratories in California are largely unregulated in their technical standards, and there have been reports of inconsistencies in diagnostic results across

[2] See https://fps.ucdavis.edu/fgr2010.cfm.

laboratories.[3] This variation in laboratory results could arise from not using standard assays, as discussed above, or from a lack of proper training, technique, or equipment. A certification scheme for testing laboratories would help ensure that different laboratories are using best practices and up-to-date diagnostics for virus detection. Such certification programs for laboratory standards already exist in other agricultural arenas. For example, the U.S. Department of Agriculture's Animal and Plant Health Inspection Service oversees accreditation programs for detection of sudden oak death (caused by *Phytophthora ramorum*) and citrus greening/Huanglongbing (HLB) (caused by *Candidatu*s Liberibacter asiaticus).[4] An alternative to government oversight is industry-driven accreditation, as exemplified by an industry group[5] that provides certification for plant and soil nutrient analyses. The industry may consider developing an industry-driven ring-test process with the assistance of FPS to help assure that laboratories are providing valid results with the most up to date assay protocols (Cardwell et al., 2018). Whatever model is used, providing greater assurance in the reliability and accuracy of testing would benefit efforts to detect and manage these grapevine diseases, both for ensuring that new planting materials are disease-free and for informing management strategies once viruses are present in a vineyard. Since the cost of commercial testing may also be an impediment for growers, a more standardized approach could also potentially help bring costs down or at least offer reassurance of the quality of diagnostic results and thereby encourage greater use of testing to inform management tactics such as rouging virus-positive tested vines (Speirs et al., 2013).

Conclusion 5-1: Using clean planting material is the first line of defense in establishing healthy vineyards because viruses can spread via clonal propagation of grapevines.

Conclusion 5-2: There are concerns regarding the reliability of results from testing laboratories; these stem from questions about whether testing for GLRaVs and GRBV is being done using the most up-to-date protocols to detect all variants, and from the fact that commercial testing laboratories are largely unregulated in their technical standards, potentially resulting in inconsistencies in diagnostic results across laboratories.

Recommendation 5-1 (HP): Encourage the adoption and implementation of higher sanitary standards in registered mother blocks using robust, state-of-the-art, sensitive, and reliable diagnostic methods; and roguing of infected vines to maintain disease-free stock and provide clean planting materials for growers.

This could include engaging FPS in exploring the potential of developing a ring-test process or similar validation scheme to better assure the validity and reliability of diagnostics from laboratories working with the industry.

ROGUING INFECTED VINES

Grape growers may employ roguing to reduce the spread of viruses within vineyards. Roguing during the first years after establishment of a block of vines has proven effective in reducing and even eliminating GLD in individual vineyards (Almeida et al., 2013; Pietersen et al., 2013; Ricketts et al., 2015). In South Africa, roguing when the incidence of GLRaV-3 is below 20 percent is recommended as part of the GLD integrated management program, which also includes planting clean vines and management of the vector (mealybug) using systemic insecticides and biological control. This strategy has nearly eliminated GLRaV-3 from South African vineyards that produce high-end wines (Pietersen,

[3] This concern was mentioned by a grower association representative at the open meeting in UC Davis on March 5, 2024.
[4] See https://www.aphis.usda.gov/plant-pests-diseases/citrus-diseases/citrus-greening.
[5] See https://alta.ag/about.

2024). However, the threshold decision point of when roguing still remains cost-effective rests on assumptions that may not be valid (Pietersen et al., 2013). Consequently, it is essential to evaluate this threshold to improve the adoption rate of roguing as a management strategy.

Optimal roguing schemes may be different for different production regions in California, which vary widely with regard to their market economics and the environmental conditions that affect vector and virus dynamics (Ricketts et al., 2017). Cunniffe et al. (2022) present a framework for modeling complex interactions among viruses, vectors, and plants. This framework aims to better characterize disease spread and identify key points in the process for targeted management. Implementing such a framework could enhance decision making in viral disease management. However, refining roguing schemes requires addressing some important knowledge gaps. For example, mealybugs, which transmit GLRaVs, are known to move on a local "plant to plant" scale as well as by long-range passive dispersal, while movement patterns of the three-cornered alfalfa hopper (TCAH) and the spread of GRBV are not as well characterized (see TCAH Host Preference and Movement Dynamics section in Chapter 4). Knowledge gaps regarding TCAH flight behavior and the impact and behavior of other potential GRBD vectors substantially impair the development of roguing schemes and other management practices in the context of GRBD. Limited information from Oregon indicates that roguing can mitigate the spread of GRBD (Achala et al., 2022), but the abundance of TCAH in that area is unknown. The relative importance of the primary versus secondary spread of GRBV is also unclear. In addition to determining when roguing is the best management choice and which vines should be rogued, there is also a need to define methods for vine removal that minimize the risk of re-infection. In the case of GLD, leaving root systems of rogued vines in the vineyard has been shown to create a reservoir for GLRaV-3 and mealybugs that can develop within the remnant root systems and continue to spread the virus (Bell et al., 2009).

Implementing roguing and replanting can be difficult for growers to justify because infected grapevines can still be productive whereas replanted vines are not immediately productive. Also, the effectiveness of roguing and replanting may be impacted by abundance of virus inoculum and vectors within the surrounding landscape. This points to a critical need for economic analysis on the cost-effectiveness of roguing and replanting schemes (Sisterson and Stenger, 2012). Modeling efforts have shown that as vector density and dispersal increase, roguing individual vines for GLD becomes less effective in suppressing disease spread, and economically may be less cost-effective than replanting entire vineyard blocks (Mannini and Digiaro, 2017; Bell et al., 2021). Less information is available for developing roguing schemes for GRBD. Aside from the expense, roguing and replanting can also complicate viticultural practices, as having vines at different stages (such as when younger vines are replanted into older blocks) or having gaps where vines are simply rogued and not replaced can require adjustments to management practices within a vineyard (Ricketts et al., 2015; Mannini and Digiaro 2017).

Conclusion 5-3: Roguing has been shown to be effective in GLD management and in mitigating GRBD spread, but it can be difficult for growers to justify removing infected but still productive vines and replacing them with new vines that will not immediately bear fruits. Both roguing and roguing followed by replanting also complicate viticultural practices in vineyards.

Conclusion 5-4: There is insufficient information available for developing effective roguing schemes for GLD and GRBD. Specifically, more data is needed on the determination of threshold decision points, the cost-effectiveness of roguing under various conditions, and the influence of movement patterns and flight behavior of TCAH and other potential GRBV vectors on the spread of GRBD.

Conclusion 5-5: Roguing schemes need to be optimized for California production regions in light of differences in market economics and in the environmental conditions that affect vector and virus dynamics. Additional epidemiological research may reveal the optimum roguing and

replanting schemes for both GLD and GRBD in different production regions and for vineyards with differing business models.

Recommendation 5-2 (HP): Support research to develop optimal roguing and replanting schemes and techniques to manage GLD and GRBD, and to facilitate their implementation by growers.

This could include studies to determine:

- **The cost-effectiveness of roguing and/or replanting based on disease incidence and rate of spread.**
- **How long it typically takes for newly-planted clean grapevines to become infected and become sources of inoculum.**
- **Best practices for removal of remnant root systems of rogued vines to prevent them from serving as reservoirs for the vector and virus.**
- **Roguing schemes suited to the different grape production regions in California.**

VECTOR MANAGEMENT

Because of the inherent complexities of the GLD and GRBD pathosystems, no single tactic is likely to provide a complete and sustainable management solution. To maximize the benefits of clean plant programs, the use of clean plants and roguing schemes needs to be complemented by effective strategies for managing the vectors that carry viruses into and within vineyards (Daane and Haviland, 2024). Vector management will have greater importance as climate warming is likely to exacerbate mealybug populations. Increasing temperatures will allow additional mealybug generations to develop, and there may be disproportionate population increases as increasing temperatures may increase protective behaviors in mealybug tending ants (Zhou et al., 2017). Overall, increasing temperatures have been predicted to lead to increased mealybug populations and decreasing efficacy of natural enemies because of greater asynchrony in their temporal and spatial distributions (Gutierrez et al., 2008). Increasing temperatures will also affect the phenology of TCAH hosts in and around vineyards and the development of the treehopper, which could lead to earlier dispersal into vineyards and greater populations (Jordan Jr., 1952; Preto et al., 2019; Bick et al., 2020).

Monitoring vector populations is fundamental to successful vector management. Information from monitoring programs not only allows growers to determine when to employ pest management measures but also to evaluate their effectiveness. The University of California guidelines for grape pest management[6] includes well-developed resources for implementing monitoring programs for mealybugs and scales. Sex pheromones that can be employed in monitoring efforts are commercially available for certain mealybugs, including the vine mealybug (Millar et al., 2002). For monitoring TCAH in vineyards, both sweep net sampling and yellow sticky traps have been used effectively (Wilson et al., n.d.), although sweep net sampling has been found to provide a more accurate estimate of adult populations and sex ratios than sticky card trapping (Johnson and Mueller, 1990).

Any consideration of the use of insecticides for vector management must include careful attention to optimizing the insecticide type and application strategy used. It is also important to consider the policy context for insecticide use, which may affect the types of products or practices that will be allowable in the future. In 2023 the California Department of Pesticide Regulation published a "Sustainable Pest Management Road Map" for the state, which outlines a goal of identifying and eliminating the use of certain "Priority Pesticides" (defined based on hazards to human and ecosystem health) by 2050 (CDPR, 2023). Awareness of pesticides that may eventually be phased out under this initiative can help to inform where research investments focused on insecticides for GLD and GRBV vectors are likely to be most impactful in the long run.

[6] See https://ipm.ucanr.edu/agriculture/grape/#gsc.tab=0.

Insecticides for Mealybug Management

Insecticides can be effective for mealybug management; however, insecticides alone will not stop the spread of GLD and should be considered a complementary approach to be combined with other tactics. In addition, only certain types of insecticides are likely to be effective. The tendency for mealybugs to aggregate in concealed areas can reduce their exposure to contact insecticides. Systemic insecticides such as neonicotinoids and spirotetramat, which move in the plant phloem and xylem, offer better management options. Because systemic insecticides can move to enclosed areas of plants, they can reach mealybugs in concealed areas; they also tend to have relatively long residual activity in plants (Van Timmeren et al., 2012). However, systemic insecticides require insects to feed in order to ingest the toxins. Given that mealybugs can transmit GLRaVs in a short period of time (about 1 hour) (Tsai et al., 2008), systemic insecticides would not likely disrupt feeding quickly enough to stop virus transmission. Therefore, the value of these insecticides lies in overall population suppression (O'Hearn and Walsh, 2020).

Increasing the transport of the active ingredients of systemic insecticides throughout vine tissues and increasing their longevity in the plant could further improve the effectiveness of these insecticides for mealybug management. For example, spirotetramat must be metabolized in the plant to spirotetramat-enol, which is the toxin that kills immature mealybugs and can reduce adult fecundity. Since the conversion efficiency to the enol metabolite depends on environmental factors and physiological conditions of the plant (Martin, 2021), variation in these conditions can result in differences in the amount of toxin present in leaf samples after an application. Within-plant distribution of the toxin also may not be uniform, allowing mealybugs in areas such as the trunks to be more likely to escape exposure or to be exposed to lower doses. Environmental conditions can also influence the efficacy of systemic insecticides. For example, soil type affects neonicotinoid activity; imidacloprid has greater efficacy in lighter, sandier soils whereas thiamethoxam has greater efficacy in heavier loam and clay soils (Kurwadkar et al., 2013). This suggests that the local environmental conditions are an important consideration to guide insecticide selection.

The strategy and timing of delivery for insecticides also influences their effectiveness. Delivering therapeutics at the right time and in the right amount to woody plant vasculature is a challenge for controlling insect vectors in many pathosystems. One way to overcome this is to consider the use of unconventional pesticide delivery methods such as trunk injection (see Trunk Injection of Systemic Pesticides section in Chapter 6). In addition, because of the broad use of insecticides such as neonicotinoids in grapevines for multiple pests, determining the optimal timing for insecticide sprays is critical to maximize efficacy and avoid ineffective and unnecessary applications (Hamby et al., 2015; Mermer et al., 2021). For example, applications of contact insecticides targeting mealybug crawlers would be ineffective if they are made when crawlers are not active.

Finally, the reliance on a limited number of insecticides with similar mode of action predisposes the grape industry to the development of insecticide resistance (Venkatesan et al., 2016). Spirotetramat has been widely used over the past decade with growers anecdotally reporting decreasing efficacy. Likewise, the widespread use of imidacloprid for glassy-winged sharpshooter management could contribute to the evolution of resistance to this pesticide in mealybugs and TCAH. As such, there is a critical need for implementing insecticide resistance management programs and for the development of new active ingredients for vector management. Venkatesan et al. (2016) provide recommendations for resistance management. There has been progress in identifying more selective insecticides for mealybug management and more effective products for organic production (Poliakon et al., 2017; Tacoli et al., 2018; Deza-Borau et al., 2020). Other natural products, such as plant essential oils, have potential to cause significant mortality (Tacoli et al., 2018).

Conclusion 5-6: Contact insecticides are not effective in controlling mealybugs due to the cryptic nature of mealybug behavior. Systemic insecticides will not likely disrupt feeding quickly enough to stop transmission of GLRaVs, but they could be effective in reducing mealybug populations. In

addition to their crypsis, the sessile nature of mealybugs suggests that systemic insecticides, even if slow acting, could reduce secondary spread of GLRaV-3. Primary spread from mealybugs entering vineyards would require a more rapid kill time.

Conclusion 5-7: Knowledge of factors that affect the efficacy of insecticides (such as physiology of the plant, environmental conditions, soil type, insect behavior, insecticide application methods) is important in developing improved guidelines for their application.

Conclusion 5-8: Reliance on a small set of insecticides for mealybug control increases the likelihood that mealybugs will develop resistance to them.

Recommendation 5-3 (HP): Support research to determine the optimal conditions for the application of systemic insecticides to achieve better mealybug control.

Recommendation 5-4 (HP): Develop and implement insecticide resistance management programs and support research to develop new active ingredients for mealybug management, including by evaluating the efficacy of natural products such as plant essential oils, that could provide additional options for both organic and conventional vineyards.

Insecticides for TCAH Management

Insecticide application could play an important role in TCAH management, but more information is needed to assess the pros and cons and ensure the effectiveness of this tactic. In particular, it will be important to have a better understanding of virus transmission dynamics. Although secondary spread of GRBV (between vines within an already-infected vineyard) has been documented, the relative importance of primary spread (introduction into a previously uninfected vineyard from outside the vineyard) versus secondary spread is unknown (Cieniewicz et al., 2019). Insecticide control is rarely successful in preventing primary spread, whereas insecticides may be effective in limiting secondary spread (Perring et al., 1999), so clarifying the relative importance of primary versus secondary spread in the context of GRBV could help to guide decisions about insecticide use.

A better understanding of vector-virus dynamics is also needed. TCAH appears to be transient in grapevines, and insecticides are not effective in controlling primary disease spread from transient vectors with a short inoculation time of nonpersistent pathogens (Perring et al., 1999). Systemic insecticides, such as neonicotinoids, require the insect to feed on treated plants to acquire a lethal dose of the insecticide, which generally makes them ineffective for managing pathogens requiring brief inoculation times (Almeida et al., 2013). However, certain systemic insecticides may interfere with feeding by hemipteran vectors, which can reduce the transmission of plant viruses by individual vectors (Garzo et al., 2020). Beyer et al. (2017) reviewed the insecticide recommendations for TCAH in annual crops (peanut, soybean) and perennial forages (alfalfa) and found that most of the products listed are broad spectrum insecticides, such as pyrethroids, carbamates, and neonicotinoids. In their review of insecticide treatments against TCAH on soybeans, alfalfa, and peanuts, Bradley and Kuhar (2023) noted that flupyradifurone (Sivanto), a Group 4D butanolide, was highly efficacious. The circulative transmission mode and lengthy inoculation access period of GRBV by the TCAH suggests that insecticide applications have the potential to be effective, as has been demonstrated for other circulatively-transmitted pathogens (Garzo et al., 2020). In addition, because transmission of GRBV appears to require a long feeding time for acquisition, there is potential to develop a decision support system based on diagnosing the abundance of viruliferous TCAH in a region to guide the timing of insecticide applications, if warranted (Stillson et al., 2020).

Should insecticide use become a broadly implemented technique for GRBV management, insecticide resistance monitoring would become important and regional testing for insecticide susceptibility among populations of TCAH would provide critical baseline data and help to minimize the

risks of insecticide failures (Roush and Miller, 1986; Prabhaker et al., 2006). In addition, since wine grapes are subject to damage from a range of pests, any insecticide use must take into account the effects of insecticides on both target pests and non-target insects. For example, some of the insecticides identified as candidates for managing TCAH may also be used against mealybugs and glassy-winged sharpshooter, suggesting that applying them to control TCAH in vineyards could have implications for resistance management where multiple pests occur on one crop. It is also important to recognize that insecticide use can inadvertently exacerbate pest problems. Spinosyn-based insecticides may trigger outbreaks of secondary pests, including planthoppers, because of the elimination of natural enemies of those secondary pests (Duso et al., 2022), while pyrethroids that can be used to target planthoppers may trigger outbreaks of spider mites and other types of planthoppers. These outbreaks can result from hormesis, the phenomenon in which sublethal doses of insecticides promote insect reproduction, as well as the elimination of natural enemies of secondary pests (Trichilo and Wilson, 1993).

Finally, economic or action thresholds for insecticide application to manage the vectors of GLRaVs and GRBV are still lacking. Although there is essentially a zero-mealybug tolerance for wine grapes, it is not known if this standard is appropriate (Daane et al., 2013). The establishment of economic thresholds for management of any vector should be based on a thorough understanding of the epidemiology of the diseases involved (Perring et al., 1999).

Conclusion 5-9: A better understanding of GRBV acquisition and transmission dynamics is needed to improve the effectiveness of insecticide application as a control tactic against TCAH, and appropriate economic or action thresholds are needed to guide insecticide application programs.

Recommendation 5-5 (HP): Support research to determine the optimum conditions for the application of insecticides to achieve better TCAH control and to establish economic or action thresholds to guide insecticide application programs.

Mating Disruption

Mating disruption, a technique that uses artificial stimuli (e.g., synthetic sex pheromone) that confuse individuals and disrupt mate location or courtship behaviors to block the reproductive cycle, has been used for mealybug management in California vineyards for two decades. Currently, sex pheromone for mating disruption is commercially available for the vine mealybug (*Planococcus ficus*) only. Sex pheromones have been identified for grape mealybugs (*Pseudococcus maritimus*) (Figadère et al., 2007), obscure mealybugs (*Pseudococcus viburni*) (Millar et al., 2005), and longtailed mealybugs (*Pseudococcus longispinus*) (Millar et al., 2009); experiments with the use of sex pheromones for mating disruption are underway for grape mealybugs (Millar et al., 2005; Bahder et al., 2013).

Mating disruption programs have shown clear decreases in vine mealybug populations and damage. To maximize the effectiveness of mating disruption for mealybug control, research findings suggest that it is important to deploy pheromones throughout the growing season and especially during the late season (September–October) when male vine mealybug flights peak (Daane et al., 2020). Research also shows that mating disruption tends to be most effective when it is employed over longer timescales and on larger spatial scales, indicating the benefit of using areawide programs with consistent deployment of pheromones during critical population periods (Sharon et al., 2016; Cocco et al., 2018; Hogg et al., 2021). However, no studies have been undertaken to determine the impact of mealybug mating disruption on GLRaV-3 spread, likely due to barriers from lack of rapid virus detection methods and funding for long-term studies.

Additional information is needed to improve the efficacy of mating disruption, in particular regarding the appropriate number and type of pheromone dispensers to use to ensure optimal coverage in time and space. Pheromones can be released into the environment through various dispenser types or by direct application of the chemical to an area (Benelli et al., 2019). Researchers have evaluated the efficacy

of using passive dispensers (Cocco et al., 2014; Sharon et al., 2016; Mansour et al., 2017; Lucchi et al., 2019; Daane et al., 2021; Hogg et al., 2021), aerosolized canisters (Benelli et al., 2019; Daane et al., 2021), and flowable microencapsulated formulations (Daane et al., 2021) to release sex pheromones targeting the vine mealybug. Microencapsulated formulations are distinct from other dispersion methods because they are applied in the same manner as other flowable agrochemicals, thereby eliminating some of the logistical and technical constraints of using dispensers to disperse pheromones in vineyards (Daane et al., 2021). All pheromone application methods have been shown to lower densities and/or damage of the vine mealybug, and some show reductions in the first year and following seasons (Cocco et al., 2014; Lucchi et al., 2019; Daane et al., 2020), but lasting results appear to be influenced by location and year (Daane et al., 2021). To further improve efficiency and reduce costs, researchers are examining ways to lower the densities of dispensers and use programmable dispensers to align pheromone dispersion with flight times (Daane et al., 2021).

A better understanding of basic information about mealybug mating behavior, seasonal adult male flight behavior, seasonal sex ratios, and regional differences in the timing of male flights and generation numbers would help to elucidate how and where mating disruption programs will be the most effective. Mealybug females display diel periodicity in the release of pheromones, which affects male activity and timing of mating (Levi-Zada et al., 2014) Characterization of how environmental and endogenous (e.g., female age) factors may affect pheromone release could be used to improve mating disruption programs (e.g., timing of pheromone release from puffer devices (Daane et al., 2020). In addition, having a better understanding of the mechanism by which pheromone releases disrupt mating behavior through either noncompetitive disruption (in which female pheromones are masked by the inundation of synthetic pheromones) or competitive disruption (in which dispensers create false pheromone plumes that males follow, instead of following real plumes from females) would also help inform the optimal placement of pheromone dispensers in the field. Competitive and noncompetitive disruption have been studied for lepidopteran pests (Miller et al., 2006; McGhee, 2014; Miller and Gut, 2015); however, the biology and behavior of moths and butterflies is markedly different from mealybugs with regard to location, lifespan, flight capabilities, and other factors, making it difficult to translate these research insights to inform mealybug management. Finally, additional information is needed to guide the timing of pheromone dispersion, including information about generation development, which influences when male flights occur; the establishment of effective in-field or predictive population models of mealybug generation could help guide the timing of mating disruption activities.

Even with improvements in the techniques used, mating disruption is unlikely to completely eliminate vine mealybug populations, suggesting that it should be complemented by additional management tactics such as insecticide application and/or biological control. Long-term studies are needed to assess the persistence of mealybug population suppression using mating disruption, the short- and long-term importance and economics of continued insecticide applications compared with mating disruption, and the optimal timing for applications of insecticides and pheromones when these tactics are used in combination. Since mating disruption works better when mealybug densities are low, the use of insecticides at the start of a mating disruption program may help to reduce populations early and increase the efficacy of mating disruption (Walton et al., 2006; Cocco et al., 2018; Daane et al., 2020; Hogg et al., 2021). Insecticides may also be needed at different time points after the initiation of mating disruption programs in areas where mealybug density is high or rebounds. It would be helpful to further elucidate the efficacy of different flowable pheromone products, to refine thresholds for spraying insecticides in combination with mating disruption, and to understand the dynamics of mealybug suppression beyond a two-year period, especially where low population densities are achieved (Hogg et al., 2021). Results of one study showed that two and three applications of a flowable pheromone formulation reduced vine mealybug populations to the same and a greater extent, respectively, compared with a grower-standard insecticide treatment from June through August in a California wine grape vineyard (Daane et al., 2021). Since the densities of mealybugs on trunks do not always decrease concurrent with trap captures, further studies could also help to refine the frequency of continued management after pheromone trap captures of male mealybugs decrease. In addition, more information is needed about potential synergies between

mating disruption programs and biological control (Shapira et al., 2018). Because biological control agents are also more effective at reducing mealybug populations when the pest populations are low, it would be useful to examine whether growers can reap additional pest suppression benefits by complementing mating disruption with biological control (Daane et al., 2012).

Mating disruption via sex pheromones is unlikely to be effective in reducing the spread of GRBV because sex pheromones are unknown among the family Membracidae and do not appear to be part of mating behavior in TCAH (Wood, 1993). However, since hemipteran insects such as TCAH use acoustic signals and substrate-borne vibrations to locate mates (Hunt, 1993), they may be susceptible to acoustic or vibrational disruptions that interfere with mating behavior (Mankin, 2012). Initial research on disruption of substrate borne mating vibrations for a leafhopper (*Scaphoideus titanus*) that vectors the grape phytoplasma, Flavescence dorée, has been carried out in Italy. Research has demonstrated that mating in the open field can be disrupted with vibrations generated by a specialized shaker device (Polajnar et al., 2016). However, such a mating disruption system may not be practical for TCAH because mating likely occurs off of grapevines and outside of vineyards (Mitchell and Newson, 1984; Sisterson et al., 2022).

Conclusion 5-10: Mating disruption tends to be most effective in reducing mealybug populations when used over longer timescales and on larger spatial scales. More information is needed to determine the optimum number and type of pheromone dispensers to use to ensure coverage in time and space while reducing the cost of employing this technique.

Conclusion 5-11: Mating disruption has been shown to decrease vine mealybug populations and damage, but no studies have been done to determine the impact of mating disruption on GLRaV-3 spread.

Conclusion 5-12: Knowledge about the mating disruption mechanism in mealybugs (i.e., competitive or noncompetitive) and about mealybug biology, behavior, and generation development could help identify optimal times for dispersing pheromones to disrupt mating. In-field or predictive population models of mealybug generation may also help guide timing of mating disruption activities.

Conclusion 5-13: Studies are needed to determine how long mating disruption can suppress mealybug populations and guide the use, frequency, and timing of insecticide applications to keep mealybug populations low.

Conclusion 5-14: Studies are needed to determine and compare the short- and long-term efficacy and economics of various techniques for applying pheromones in mating disruption programs.

Conclusion 5-15: Studies are needed to inform integrated pest management (IPM) decision making by elucidating the potential impacts of biological control tactics such as leveraging natural enemies alongside mating disruption programs.

Conclusion 5-16: Mating disruption is not likely to be a practical management tactic for TCAH as leafhoppers do not appear to use long-range sex pheromones to locate mates but instead use substrate-borne vibrational signals that occur off of grapevines.

Recommendation 5-6 (HP): Support research to generate information needed for improving the efficacy of mating disruption for mealybug control and to determine the benefits (economic and otherwise) of employing this technique as part of an integrated approach to manage insect vectors in grapevines.

This could include studies to determine:

- **The optimum number and type of pheromone dispensers for ensuring coverage over an extended period over a large area.**
- **Mealybug mating behavior, seasonal adult male flight behavior, seasonal sex ratios, regional differences in the timing of male flights, generation development, and the mechanism of mating disruption in mealybugs.**
- **How long mating disruption can suppress mealybug populations and how insecticides and natural enemies can be used to complement mating disruption to keep mealybug populations low.**
- **The impact of mating disruption on GLRaV-3 spread.**

Ultraviolet Light for Mealybug Management

In recent years, the effect of ultraviolet (UV-C) light has been explored as a non-chemical strategy to manage insect populations that cause damage to crops as pests and disease vectors. The use of UV-C has shown promising results as a control measure against common insect pests, such as two-spotted spider mites (*Tetranychus urticae* Koch), chili thrips (*Scirtothrips dorsalis* Hood), and western flower thrips (*Frankliniella occidentalis* Pergande) in strawberries (Montemayor et al., 2023). UV-C applications have also been shown to help combat powdery mildew on strawberries (Onofre et al., 2021) and grapevines (McDaniel et al., 2024b) with no adverse effects on fruit yield and quality. A recent study by McDaniel et al. (2024a) reported potential impacts of UV-C light treatment on grape mealybug nymph mortality, suggesting that UV-C could represent a valuable IPM approach to suppress mealybug populations in vineyards. UV-C applications in vineyards may not be practical for TCAH likely because they do not reside primarily within vineyards.

Conclusion 5-17: Emerging research suggests the use of UV-C light could help to suppress pest populations without negatively impacting crop yield. However, further refinement of this method is needed to make it an effective tool for vine mealybug management in vineyards.

Recommendation 5-7: Support research to further refine UV-C treatment of grapevines to complement other IPM strategies to suppress field populations of mealybug vectors in vineyards.

CULTURAL CONTROL

Although they are only known to spread GRBV to *Vitis* species, TCAH spend significant amounts of time on other plants. Cultural control practices such as removal of reproductive hosts or the use of trap crops could offer opportunities for reducing populations of viruliferous TCAH on grapevines.

TCAH appear to favor leguminous plants as reproductive hosts (Kron and Sisterson, 2020). Recent research shows that removal of vegetation between rows of grapevines in the spring may reduce populations of TCAH within vineyards by reducing the availability of such reproductive hosts (Bick et al., 2020; Billings et al., 2021). The complete removal of vegetation by discing at times specified by degree-day modeling has proven to be more effective than mowing (Bick et al., 2020), and Billings et al. (2021) found that discing ground covers in the early spring could reduce the abundance of TCAH in vineyards. All cover crops in this study were mixtures that contained legumes; specific mixtures of ground covers, especially limited to non-leguminous hosts, designed to reduce TCAH were not evaluated, no comparison of cover crop termination methods to clean cultivation were made, and no measures were included to assess changes in the rates of disease spread. Billings et al. (2021) also do not fully address the costs and benefits of ground cover removal. Vegetation removal can increase soil erosion, especially in steep terrains (Xu et al., 2013), and also raises concerns regarding the potential effects on natural

enemies of all vineyard pests (Sáenz-Romo et al., 2019), which could adversely affect biological control of both mealybugs and TCAH. Legumes can comprise a large component of weedy vegetation inside and outside vineyards and have certain features that enhance vineyard health; legume cover crops have been used in vineyards as a sustainable means to provide nitrogen to vines (Ovalle et al., 2010), and they may provide floral resources for natural enemies of TCAH and other grape pests. Therefore, large-scale vegetation removal could have important downsides that would need to be weighed against the potential benefits for TCAH management.

Trap crops are defined as plants "that serve to attract, divert, intercept, and/or retain targeted insects or the pathogens they vector in order to reduce damage to the main crop" (Shelton and Badenes-Perez, 2006). While trap cropping has proven beneficial in numerous cropping systems, their utility for the management of TCAH and GRBV is unknown. Trap crops can be employed in different ways to reduce pest populations (Sarkar et al., 2018); they have been used, but with limited success, with intercropping and border plantings to limit pathogen spread. One of the most notable examples of trap crop use is in reducing infection of potato with potato virus Y, a non-persistently transmitted aphid-borne virus (Dupuis et al., 2017). Trap crops reduce the spread of non-persistently transmitted viruses when the vector encounters them before moving to the main crop. The vector feeds on and transmits the virus to the non-host trap crop, which significantly depletes or eliminates the virus from the vector mouthparts so there is little or no virus transmitted by the time the vector moves into and feeds on the main crop. In the case of GRBV, which persists in the TCAH, this strategy would only be effective if the trap crop is attractive enough to concentrate TCAH and prevent the vector from moving into vineyards before an insecticide can be applied to the trap crop. This strategy has been demonstrated in tarnished plant bugs (*Lygus lineolaris*) treated with an insecticide while concentrated on a mullein trap crop (Dumont and Provost, 2022). Alfalfa has also been used as a trap crop to reduce colonization of strawberry by *L. lineolaris*. Since TCAH also has an affinity for alfalfa (Wistrom et al., 2010), alfalfa may have potential as a trap crop for TCAH. More research is needed to better understand TCAH-plant host interactions and assess whether this type of habitat manipulation is a viable strategy for reducing the spread of GRBV. Because the ecology and biology of mealybugs and GLRaVs differ from those of TCAH, the use of trap crops for GLD management would likely be ineffective.

Conclusion 5-18: Removal of vegetation (such as legumes, which serve as reproductive hosts) between rows of grapevines in the spring may reduce populations of TCAH within vineyards, but information about the cost and benefits of this practice is lacking.

Conclusion 5-19: Trap crops have been shown to reduce the spread of non-persistently transmitted viruses, but the feasibility of using trap crops to control GRBV, which is persistently transmitted by TCAH, has not been determined.

Recommendation 5-8 (MP): Support research to determine the costs and benefits of removing vegetation that harbors TCAH in and around vineyards and the use of trap crops to inform grower decision-making regarding the employment of these methods for managing TCAH in vineyards.

BIOLOGICAL CONTROL

Biological control strategies can be used to help reduce the population of an insect pest or vector by creating conditions under which that insect will be more vulnerable to the effects of predators, parasites, or other biological agents that threaten its survival or reproduction. Some progress has been made in developing biocontrol strategies for mealybugs; less is known about potential biocontrol strategies for TCAH.

Several parasitoids and predators of the vine mealybug have been identified that may contribute to mealybug control in California. Most IPM programs have emphasized conservation biological control

(i.e., minimizing disruptions to naturally occurring populations of natural enemies). However, deliberate releases can also be used; for example, several insectaries produce the parasitoid *Anagyrus pseudococci* for inundative releases in the spring.[7] Releasing this parasitoid before naturally occurring populations typically become active may help to overcome the lag between mealybug population increases and the effective control by parasitoids (Malakar-Kuenen et al., 2001). In addition to parasitoids, several predators have been documented preying on mealybugs in vineyards. They include lady beetles (Coleoptera: Coccinellidae), especially the mealybug destroyer (*Cryptolaemus montrouzieri*), brown and green lacewings (Neuroptera: Hemerobiidae, Chrysopidae), predatory gall midges (Diptera: Cecidomyiidae), and spiders. However, most of the mealybug predators are generalists (i.e., they prey on a wide range of small, soft-bodies insects) and information is lacking on their effectiveness as biocontrol agents in vineyards (Daane et al., 2012). In Italy, combined inundative releases of the parasitoid *Anagyrus sp. near pseudococci* and *C. montrouzieri* have proven effective in managing *P. ficus* when insecticides have not been applied against it or other pests (Lucchi and Benelli, 2018).

Entomopathogenic fungi (EPF) offer an additional biocontrol tactic. Numerous species of EPF have been identified that impact vine mealybug, grape mealybug, and other vectors of GLRaVs (Sharma et al., 2018). The successful use of EPFs in biological control is dependent on identifying the most effective strains or isolates to use for a particular pest in a particular crop. Commercial formulations of certain EPFs are available and registered for use in grapes, where they can be used as microbial insecticides for inundative biological control. However, the available formulations require repeated applications to contribute to pest suppression (Fuxa, 1987; Jaronski, 2010), and EPFs in general tend to lose infectivity and virulence under harsh environmental conditions. High temperatures and ultraviolet light, typical of California vineyards during the growing season, tend to degrade EPFs. Identification and mitigation of abiotic and biotic factors that degrade EPFs could make it possible to establish self-perpetuating populations of EPFs to provide ongoing suppression of vector populations within vineyards. In addition, previously unidentified EPF strains may be more efficacious than the currently commercially available strains, although such novel strains would need to demonstrate appropriate safety with regard to human health when applied to a food crop, as well as environmental safety with regard to impacts on non-target organisms, in order to be approved for use.[8] The use of locally obtained strains may facilitate the registration process (Lima, 1992).

The efficacy of biocontrol strategies can be compromised by ants, which tend mealybugs to access the honeydew that the mealybugs excrete. Ants disrupt the natural enemies of mealybugs and also promote mealybug survival and development through other mechanisms (Daane et al., 2007). Parasitism levels of mealybugs are significantly higher in the absence of ants than when ants are present (Daane et al., 2007). In addition to disrupting biological control, certain species of ants may to move mealybugs to new locations within or between vines (Daane et al., 2007; Grasswitz and James, 2008). Although this behavior has been observed, the extent to which it may facilitate the dispersal of mealybugs, and the development of new mealybug colonies has not been studied and remains unknown at this time.

Bait stations, similar to those used in residences, have been developed to help manage ants in vineyards (Daane et al., 2008; Cooper and Varela, 2015). However, they are too costly for deployment over large areas for extended periods of time. There are also concerns regarding the environmental sustainability of various bait technologies (Mercer et al., 2024). The development of biodegradable bait stations may offer a more environmentally appropriate delivery system, and one that would be suitable for organic producers (Le et al., 2024). In addition to the carrier and species-appropriate bait formulations (e.g., sugar, protein, sugar+protein), further development and registration of effective active ingredients is needed. California also requires adjuvants be registered as pesticides. A greater emphasis on ant management in vineyards could provide a means to help suppress mealybug populations and increase the impact of other biological control strategies.

[7] See https://www.countyofnapa.org/DocumentCenter/View/32581.

[8] See https://www.aphis.usda.gov/tradeimportsorganism-and-soil-imports/biological-control-organism-permits/microbial-organisms-used.

Little research has been conducted on the biocontrol of TCAH. Most natural enemies that have been identified are generalist arthropods and avian predators (Jordan Jr., 1952); assassin and nabid bugs can also prey on less mobile nymphal TCAH instars, but adults may escape predation because of their mobility and hard exoskeleton (UC IPM, n.d.). Nickerson et al. (1977) note that, similar to mealybugs, TCAH nymphs can be tended by ants, which may interfere with natural enemies attacking them. If TCAH is reproducing on non-crop plants within vineyards, ant management targeting mealybugs could have a benefit in TCAH management.

Conclusion 5-20: Parasitoids, predators, and EPF have been identified that could be further studied for development as biocontrol agents for use in IPM programs targeting mealybugs.

Conclusion 5-21: EPF strains currently available for use on grapevines require repeated applications to be effective and may lose virulence when exposed to high temperatures and UV light; identification and mitigation of factors that degrade EPFs could help improve their utility in IPM programs or in situations where the use of chemical insecticides is not an option.

Conclusion 5-22: Because ants support mealybug survival in vineyards, more emphasis on ant management is needed to help suppress mealybug populations and increase the impact of other biocontrol strategies.

Conclusion 5-23: There is a dearth of research on biocontrol of TCAH; if research is pursued, it will be important to address the impacts of ants, which tend TCAH nymphs, on potential biocontrol agent(s).

Recommendation 5-9: Support research to find, evaluate, and develop more efficacious biocontrol agents and their integration with other management tactics within IPM programs or in situations, such as organic production systems, where chemical insecticides are not an option for vector management in grapevines.

SANITATION

Sanitation practices can help to prevent the spread of GLRaV-3 and GRBV by suppressing the spread of vector populations. Research is needed to identify the most effective and practical procedures to support sanitation and minimize the spread of vectors throughout a vineyard.

It is known that mealybug crawlers stick to workers' clothing and vineyard tools and disperse among grapevines (Walton et al., 2009; Roda et al., 2013). The sanitation of workers' protective covers, tools, and farming equipment was reported to be effective in limiting the dispersal of mealybugs in citrus orchards (Middleton and Diepenbrock, 2022). Spraying workers' protective covers, small tools, and containers with commercially available isopropanol or hot water can be done to reduce the chances of dispersing crawlers like mealybugs in vineyards. For farming equipment, the application of hot steam can kill most crawlers, although there is a need for further research to identify the optimum temperature and duration of steam applications to ensure efficacy (Hansen et al., 2011; Middleton and Diepenbrock, 2022).

In vineyards that employ mechanical strategies to harvest fruits or to thin and prune vines, insects can be disturbed by the equipment, causing them to spread more actively, and accelerate dispersal of crawlers along the track of the moving machines (Charles et al., 2009). This can significantly increase the chances for dispersed viruliferous mealybugs to spread GLRaV-3. To reduce insect dispersal, mechanical equipment can be high-pressure washed with soapy water to remove plant stem, cane, and leaf debris that could carry crawlers; research is needed to determine the optimal frequency of washing to effectively reduce insect dispersal without negatively impacting vineyard operations and productivity. In addition, there may be opportunities to reduce the spread of vectors and virus by adjusting the order of operations

in vineyards, such as by starting with blocks that are free of vectors or have lower densities before moving on to more extensively infested blocks. Dispersal across vine rows may be reduced by reorienting discharge chutes of machinery.

There is a general lack of information about best practices for sanitation in vineyard settings and the degree to which sanitation measures are employed is unknown. Research in other cropping systems indicates that the efficacy of cleaning programs varies with mealybug species and life stage (Middleton and Diepenbrock, 2022). In addition, since the biology and behavior of TCAH differ from those of mealybugs, sanitation of workers' protective equipment, tools, and farming equipment may not be effective in reducing TCAH populations.

Conclusion 5-24: Cleaning harvesting and pruning equipment, tools, and workers' protective equipment has been shown to limit the dispersal of mealybugs; however, there is a general lack of publicly available information about best practices for sanitation in vineyard settings and the degree to which sanitation measures are employed is unknown.

Recommendation 5-10 (HP): Support research to determine the most effective and practical farm and worker equipment sanitation measures and harvesting and pruning strategies that can help minimize the spread of insect vectors.

PHYSICAL BARRIERS

Physical barriers can be used to prevent or discourage pests or disease vectors from accessing a crop. Three examples that may be relevant in the context of TCAH include fencing, kaolin clay, and reflective mulches.

Passive devices such as fencing can intercept pests as they fly toward a vineyard. The success and practicality of screening fences depend on the flight behavior of the target pest. Although data are limited, there may be opportunities use barrier screens to limit the movement of TCAH into vineyards from riparian areas. One study found that the vast majority of TCAH around soybean fields were captured near ground level (less than 33 centimeters above the surface) (Johnson and Mueller, 1989). Recent flight mill studies indicate that both male and female TCAH can travel hundreds of meters per day, but males fly substantially farther in individual flight sessions than do females (Antolínez et al., 2023).

In Florida, the tactic of installing protective screens over citrus trees has proven effective for growing trees in an enclosed environment and keeping them disease-free (Vashisth et al., 2021). However, this approach is expensive and likely to be most applicable for smaller acreages of specialty crops, such as fresh fruit varieties with a high return on investment. The use of individual protective covers (IPCs), protective mesh bags applied to individual trees, can be economically more feasible for varieties grown on large acreages (Gaire et al., 2022). IPCs provide an alternative to soil drenches and foliar insecticides, which cannot always prevent infection by the HLB pathogen (*Candidatus* Liberibacter asiaticus), especially in light of the increasing levels of psyllid resistance to neonicotinoid insecticides, which have been used extensively for almost a decade to protect young trees from the Asian citrus psyllid (*Diaphorina citri*). Psyllid exclusion by using IPCs is, therefore, a promising tool that has sparked interest in recent years, with many growers adopting this technology in citrus orchards in Florida (Alferez et al., 2021; Gaire et al., 2022, 2024).

The use of nets is considered a highly effective tactic for reducing bird damage to agricultural crops such as grapevines (Fuller-Perrine and Tobin, 1991; Taber and Martin, 1998). This tactic, which is becoming increasingly common in vineyards worldwide in response to changing bird migratory patterns, has been shown to have no detrimental effects on Cabernet franc yield or on the quality of the fruit and wine, especially when netting is installed early in the growing season (Pagay et al., 2013). The use of a net cover on grapevine, either on a vine row or individual vine, could be explored to exclude TCAH and other potential insect vectors of GRBV in vineyards in California. This tactic is more effective against

flying insects; hence, it may not be effective against resident mealybugs within vineyards. Whether it can limit the risk of vine infestation by immigrant (wind-aided) mealybug crawlers is unknown.

Kaolin clay applications leave a white non-toxic residue on plant surfaces. This residue alters the physical appearance of plants and may also disrupt feeding by small insects, leading to reduced pathogen transmission (Reitz et al., 2008). Kaolin has been shown to interfere with the host plant settling and probing behavior of *Diaphorina citri* in citrus (Miranda et al., 2018); however, impacts on the behavior of TCAH have not been studied. Kaolin can also have diverse ancillary effects on the physiology of treated plants (Rosati, 2007); for grapes grown in Mediterranean climates, for example, kaolin applications mitigate physiological stresses from excess heat and drought conditions (Dinis et al., 2018). But in a multilocation study, kaolin treatments of vineyards in New Zealand and Italy were ineffective in controlling populations of *Pseudococcus calceolariae*, *Pseudococcus longispinus*, and *Planococcus ficus* (Tacoli et al., 2018).

Reflective mulches (i.e., aluminum or silver polyethylene mulches that reflect sunlight upwards) can reduce the dispersal of insect pests into crops by disrupting the visual cues that insects need to locate potential host plants (Greer and Dole, 2003). In studies, the application of reflective mulches between crop rows reduced the abundance of leafhoppers in Cabernet franc grapes grown in Niagara, Canada (Coventry et al., 2005), Asian citrus psyllid in citrus (Croxton and Stansly, 2014), and vectors of *Candidatus* Phytoplasma pruni, the causal agent of X disease in cherry (Marshall et al., 2024). Marshall et al. (2024) noted that the reflective ground cover used in their study limits insect access to alternative host plants, which may further reduce vector populations within orchards. Coventry et al. (2005) also assessed effects on vine physiology and berry quality and found that the use of reflective mulches brought benefits for vine photosynthesis, advanced the timing of veraison, and increased levels of total soluble solids and total phenolics in berries. However, one downside is that these mulches deteriorate over time; Croxton and Stansly (2014) discuss potential opportunities to improve the longevity and durability of the materials.

Conclusion 5-25: Information about TCAH flight behavior and movement could be used to devise and evaluate possible physical barriers such as screening fences and kaolin clay to impede TCAH movement from riparian areas to vineyards.

Conclusion 5-26: Installing protective screens over citrus trees is effective for keeping them disease-free; however, this tactic is costly and may be most applicable for smaller acreages of crops with a high return on investment.

Conclusion 5-27: A less costly tactic for vector exclusion is covering individual trees with mesh bags (i.e., individual protective covers); this tactic has been widely adopted by citrus growers in Florida as an IPM tool to control HLB.

Conclusion 5-28: Reflective mulches have the potential to reduce leafhopper populations in grapes without any detrimental effects on vine physiology and berry quality; however, these mulches degrade over time.

Recommendation 5-11 (MP): Support research to evaluate the efficacy of physical barriers in deterring TCAH movement from natural or vineyard-adjacent habitats to vineyards.

Recommendation 5-12 (MP): Support research to evaluate the efficacy of reflective mulches in reducing the abundance of insect vectors in vineyards and research on improving the longevity and durability of reflective mulches.

AREAWIDE PEST MANAGEMENT

Areawide pest management (AWM), an approach for reducing pests by uniformly applying pest mitigation measures across large geographical areas instead of using a field-by-field approach, has the potential to facilitate management of both GLD and GRBD. This approach is particularly well suited for highly mobile pests or disease vectors (Hendrichs et al., 2007), and can overcome limitations farmers face when the activity of pests is on a larger spatial scale than that of the individual farms affected. For the bacterial disease Huanglongbing (citrus greening), citrus yields were positively correlated with the number of growers within a region participating in a coordinated AWM program (Singerman et al., 2017). However, despite the benefits, growers can be reluctant to participate in AWM programs, either from a preference to work independently or a lack of confidence that their neighbors will carry out the program mandates (Singerman et al., 2017), underscoring the need to build trust and educate stakeholders on the value of large-scale programs (Hendrichs et al., 2007).

In the context of GRBD and GLD, growers' willingness to cooperate in AWM programs may be limited without outreach to ensure that they understand the severity of the disease threats and the long-term sustainability benefits of participating in areawide programs. Growers may be particularly hesitant to invest in programs in which they perceive their investment disproportionately benefits neighboring competitors (Perring et al., 1999). The California grape industry has experience with AWM programs to support management of Pierce's disease (Haviland et al., 2021), and lessons from those programs can be used to develop and implement AWM programs for GRBD and GLD that attract broad participation among growers. Although they comprise a smaller scale than the California wine industry, production regions in New Zealand and South Africa have launched concerted, coordinated programs to manage GLD on an areawide basis (Pietersen et al., 2013; Chooi et al., 2024). Central to these programs are increased efforts to use virus free material for planting, improved efforts to monitor and remove infected vines, and cooperative efforts to manage mealybug vectors across vineyards. These case studies can also provide valuable information for GLD and GRBD management in California.

Conclusion 5-29: Areawide pest management, which is well suited for pests that move beyond the boundaries of individual farms, can help in managing insect-vectored viruses in vineyards across larger areas.

Recommendation 5-13 (HP): Support efforts to develop areawide GLD and GRBD vector management programs for regions of California with different threat levels from these diseases, along with activities to encourage grower participation in these programs.

COORDINATING MANAGEMENT OF MULTIPLE VECTORS

Given the significance of Pierce's disease as well as GLD and GRBD in California's wine grape industry, it is imperative to coordinate management tactics for these different pathosystems to ensure that they are complementary, cost-effective, and do not disrupt overall pest management. Since the vectors for all three diseases are hemipterans, insecticides that are effective against one species are likely to have activity against the other vectors. As a result, the timing of insecticide applications requires careful consideration to facilitate management of the entire vector complex, and it is important to be aware that applications can lead to insecticide resistance in species other than the one being targeted. In addition, if a pest is successfully managed through biological control, insecticide applications targeting other pests should minimize disruptions to biological control. Finally, the costs and benefits of tactics such as habitat manipulation should be weighed in the context of overall pest management.

Conclusion 5-30: Pierce's disease, GLD, and GRBD are all spread by hemipterans and insecticides used to control one vector species may also affect the other vectors; hence, it is important to coordinate vector management tactics for vectors of all three diseases.

HOST PLANT RESISTANCE TO VIRUSES AND VECTORS

Host plant resistance to plant viruses and insect vectors is an effective and sustainable approach for the control of vector-borne diseases. This approach relies on the plant defense response, which is driven by innate genetic traits in the plant. The availability of grapevine cultivars resistant to GLRaVs and GRBV (and/or their vectors) would have a long-term impact on the economics of wine grape production by increasing yields and quality, and could potentially reduce the inputs and costs associated with vector control. However, resistance traits must be carefully managed and would need to be part of a sustained IPM strategy for these vector-borne viruses.

Potential paths that can be explored for developing plants that are resistant to GLRaVs and GRBV include traditional breeding and bioengineering. Due to the complexity of the disease pathosystems, a multidisciplinary approach is essential and any pathway would require the involvement of experts in plant breeding, plant biotechnology, plant virology, and vector biology. It is also important to screen for virus-resistant germplasm assessing resistance (Djennane et al., 2021; Cousins, 2024), since the vector not only introduces biologically relevant amounts of the virus but may also deliver effector molecules that modulate plant defenses and create a favorable environment for virus replication (Ray and Casteel, 2022).

For traditional strategies using grape breeding and large-scale screens for genetic resistance, it is important to study both existing cultivars (*Vitis vinifera*) and wild grapes (other *Vitis* species), as resistance genes may be found in wild varieties or non-traditional varieties. This approach led to the successful identification of resistance genes for Pierce's disease and grapevine fanleaf virus (Djennane et al., 2021; Huerta-Acosta et al., 2022). However, using traditional breeding to screen for resistance genes and then incorporating them into commercially viable cultivars is time-consuming and labor-intensive, but can be rewarding. Identifying traits conferring resistance to *Xylella fastidiosa*, the causal agent of Pierce's disease, was a research priority for over 20 years before the first wine grape cultivars incorporating the PdR1b resistance gene from *V. arizonica* were commercially released (Walker and Tenscher, 2019). Efforts to identify and incorporate additional *X. fastidiosa* resistance traits into *V. vinifera* cultivars are still in progress (Rapicavoli et al. 2018; Huerta-Acosta et al., 2022).

Once an effective resistance gene is identified, it can be transferred into popular wine cultivars using traditional breeding strategies or various bioengineering approaches. Bioengineering approaches such as RNA interference (RNAi)-based or transgenic resistance and genome editing can also be used to design and implement new genetic modifications that may yield effective, long-lasting resistance. Resistance to virtually any virus can be created using knowledge of virus diversity and sequence conservation. RNAi-based resistance for plant viruses has been shown to be highly effective and durable for annual and perennial plants including plum (Scorza et al., 2013), papaya (Tripathi et al., 2007), squash (Tricoll et al., 1995), and bean (Aragão et al., 2013). Resistance to a geminivirus, bean golden mosaic virus, has been developed and deployed in beans for human consumption (Aragão et al., 2013). Such approaches could facilitate the development of a transgene that targets multiple genomic sequences in GLRaV-3 and GRBV. This would provide California wine grape growers with a single trait that provides protection from both virus threats, potentially with a lower regulatory burden than would be involved in obtaining approvals for multiple traits separately. Transgenic resistance mediated by RNAi may be one of the fastest and most effective approaches to get virus resistance into the field; several publications outlining best practices for RNA-based plant virus resistance provide useful guidance in this endeavor (Zhao et al., 2019; Kumar et al., 2022), and the PD/GWSS Board has already supported research to develop RNAi-based resistance to GLRaV-3 and mealybug vectors.

Genome editing of susceptibility genes (genes that are required for the virus replication cycle in the plant) is another bioengineering pathway with increasing utility for non-model plants such as grapevines (Zhao et al., 2019; Khan et al., 2022). Filling the knowledge gaps of virus-host interactions may identify conserved host gene targets for directed mutation of downregulation or resistance genes for activation. It may be possible to target multiple GLRaVs and provide broad-spectrum resistance by modifying a conserved host factor. For example, there may be conserved pathways for GLRaV replication

complex assembly that could be modified by CRISPR/Cas genome editing to inhibit virus replication factory formation and confer resistance to viruses without compromising host growth under normal conditions. This approach would require detailed knowledge of the molecular virus-grapevine host interactions that occur during virus infection. At this time, there is limited knowledge of such host factors, although significant progress has been made toward genome editing in grapevines (Tricoli, 2024). A comprehensive analysis of GLRaV-3 and GRBV virus-host interactions in important wine grape cultivars and/or in the model grapevine cv. Pixie could facilitate further progress; the complex dynamics of GLD and GRBD in red or black- and white-fruited cultivars warrant fundamental studies employing contemporary tools in molecular biology, multi-omics, and plant biology to elucidate host-virus interactions using a systems biology approach to bridge the gap between genomics and phenomics of these diseases (Naidu et al., 2015). If susceptibility genes can be identified and modified with single-base changes, this may represent a path toward disease resistance with minimal regulatory burden. Progress in genome editing approaches may also enable cisgenics (the modification of plants using a natural gene from a sexually compatible plant) to allow knock-in (i.e., insertion at a particular locus) incorporation of viral resistance genes identified using traditional breeding approaches into important wine grape cultivars.

In addition to conferring resistance to viral infections, similar bioengineering approaches can also be used to confer resistance to insect vectors. Researchers have made some progress toward identifying germplasm with resistance to mealybugs (Naegele et al., 2020). Genome editing of susceptibility genes in host plants is an additional avenue that could be explored for vector control.

At present, genome editing techniques are not subject to the same regulatory framework as genetically modified organisms in the United States because an edited genome also could potentially have been eventually produced through traditional breeding (Hundleby and Harwood 2022; Genetic Literacy Project, 2023). The lower regulatory standards could lead to faster commercialization of resistant cultivars developed through gene editing compared with RNAi-based transgenic approaches. However, detailed knowledge of virus-host interactions necessary for determining appropriate targets for edits has yet to be generated, and it is likely that separate gene targets for GLRaV-3 and GRBV will need to be identified.

For all host plant resistance approaches, a plan for moving the research from the lab to the field should be part of the research vision and is imperative for real-world application. These activities can include assessing potential off-target effects on grapevines and the quality of juice from new cultivars, full exploration of the regulatory hurdles required for approval, and consideration of consumer concerns and the acceptability of crops involving different types of bioengineering techniques.

Conclusion 5-31: Host plant resistance is an effective and sustainable tactic for controlling vector-borne virus diseases, especially when used as a component of an IPM strategy.

Conclusion 5-32: The choice of approach (traditional breeding or bioengineering strategies such as transgenic approaches or gene editing) for achieving host resistance has implications for the length of time required to create a resistant grapevine cultivar, the expediency of obtaining regulatory approval, and consumer acceptance.

Conclusion 5-33: RNAi-based resistance to plant viruses has been shown to be highly effective and durable for annual and perennial crops; this approach could produce a resistant grape cultivar within a relatively short period of time.

Conclusion 5-34: Genome editing for developing host resistance to GLRaVs, GRBV, and their vectors requires knowledge of virus-host and vector-host interactions and the collaborative efforts of researchers from multiple disciplines.

Conclusion 5-35: Gene-edited crops are not subject to the same regulatory processes as genetically modified organisms in the United States and could therefore lead to faster

commercialization of a resistant grapevine cultivar; however, information on virus-host and vector-host interactions necessary for determining appropriate edits is not yet available.

Recommendation 5-14 (HP): Support research using traditional and bioengineering approaches for developing GLD and GRBD resistance; when conducting resistance screening assays, the biological vector should be used as much as possible.

Recommendation 5-15: Support research on the use of transgenic RNAi for developing plants with virus and/or insect resistance; creating a trangene(s) combining resistance to GLRaV-3 and GRBV could provide effective resistance to both viruses and help reduce the burden of regulatory approval.

Recommendation 5-16: Develop grapevine as a model system to advance fundamental understanding of the entire network of virus-host interactions across cultivars.

Recommendation 5-17 (HP): Establish multidisciplinary and trans-institutional collaborations to enhance synergies in pursuing bioengineering approaches, such as RNAi-mediated resistance and CRISPR/Cas-based genome-editing technologies, as an alternative to traditional breeding for resistance against GLD and GRBD.

CROSS-PROTECTION STRATEGIES

Cross protection (also referred to as mild strain cross protection) is the use of a mild virus strain to infect a plant in order to protect it from subsequent infection by a more aggressive strain of the same virus that causes severe symptoms and damage. Cross protection has been applied to several important plant viruses, including in citrus against citrus tristeza virus (CTV; Folimonova, 2013; Folimonova et al., 2020), in cacao against cacao swollen shoot virus (Ameyaw et al., 2016), in zucchini against zucchini yellow mosaic virus, and in tomato against pepino mosaic virus (Hernando and Aranda, 2024). One of the challenges in implementing cross protection is identifying suitable mild strains that induce protective effects across all phylogenetic groups of the same virus without causing damage to the plant or having a negative impact on crop yield, as occurred in efforts to develop cross protection for fanleaf degeneration (Komar et al., 2008; Vigne et al., 2009; Kubina et al., 2022).

Researchers have made some initial progress in laying the groundwork for developing cross-protection strategies for GLD. In 2013, Poojari et al. identified an asymptomatic strain of GLRaV-2 (designated as GLRaV-2-SG) that was found to have no significant effect on fruit yield, total soluble solids, juice pH, or total anthocyanins of berry skin in cv. Sangiovese. In 2019, Thompson et al. found a novel genetic variant of GLRaV-3 (designated as ID45) in Idaho and reported that the ID45 variant caused no foliar symptoms in Cabernet Sauvignon in the fall (Thompson et al., 2019); its effect on fruit yield or fruit quality has not been determined. However, the discovery of mild strains is only the first step in developing a cross-protection strategy for GLD management; the success of cross protection across grapevine cultivars under varying environmental conditions requires careful consideration of host-virus interactions and the impact of climate change events that can diminish grapevine responses to viral infections (Perrone et al., 2017; Velásquez et al., 2018). Moreover, despite efforts to understand the protection conferred using mild strains in initially infected plants, the molecular mechanism(s) behind cross protection remain(s) largely unclear (Zhang et al., 2018; Pechinger et al., 2019). Having a better understanding of the pathogenicity factors across all GLRaV-3 phylogenetic groups will be essential in developing a cross-protection strategy for GLD. Research into cross protection for GLD management could be guided by lessons learned from cross-protection efforts for other viruses, such as CTV (Folimonova, 2013), cacao swollen shoot virus (Ameyaw et al., 2016), sugarcane mosaic virus (Xu et al., 2021), and pepper mild mottle virus (Yoon et al., 2006).

Conclusion 5-36: The identification of a mild and asymptomatic strain of GLRaV-2 (GLRaV-2-SG) that does not cause any significant damage to grapevine, and a mild strain of GLRaV-3 (ID45) points to the potential to apply cross protection in GLD management.

Recommendation 5-18: Support research to explore cross protection as a possible tactic for managing GLD.

RISK ASSESSMENT MODELS TO GUIDE DECISION MAKING

GLRaV-3 and GRBV are two of the most economically damaging viruses that infect grapevines. Developing risk assessment models for GLRaV-3 and GRBV could enhance decision making and improve GLD and GRBD management. An example of such a model is the Bayesian Belief Network (BBN) model, which has been found to have application in forecasting crop diseases (Bi and Chen, 2011; Yang et al., 2019) and IPM decision making (Singh and Gupta, 2017). One advantage of the BBN model is that it provides a causally correct method to explore scenarios using both quantitative and qualitative inputs, distinguishing between statistical correlation and causal effects (Pearl, 2009, 2014; Topuz et al., 2023). It could be used to identify the risk factors associated with the highest likelihood of a GLRaV-3 or GRBV outbreak and to assess vineyard vulnerability to such a threat based on these identified risk factors. This model can also be used to inform the timing of insecticide applications to improve the effectiveness of GLRaV-3 and GRBV vector control. This type of model can provide a comprehensive framework for identifying the risk factors that increase the likelihood of a GLRaV-3 or GRBV outbreak and for assessing the relative risk of these factors either individually or in combination.

Conclusion 5-37: The Bayesian Belief Network model, which can be used to assess the probability of GLRaV-3 and GRBV outbreaks, could be helpful in informing GLD and GRBD management decision making.

Recommendation 5-19: Support research to evaluate the potential utility of the Bayesian Belief Network model in informing growers' decisions related to GLRaV-3 and GRBV management.

RESEARCH PRIORITIZATION

High and medium priority research areas and actions (with the recommendation number) are summarized in Table 5-1 below for quick reference. The recommended research and actions related to the use of clean plants, roguing, vector management, sanitation, physical barriers, and areawide pest management would contribute to GRBD and GLD management in the short term. Recommendations and actions related to host plant resistance would contribute to GRBD and GLD management in the long term.

TABLE 5-1 Prioritization of Research that May Yield Most Promising Short- and Long-Term Management Solutions

High Priority Research and Actions
Encouraging the adoption and implementation of higher sanitary standards in registered mother blocks using robust, state-of-the-art, sensitive, and reliable diagnostic methods; Roguing of infected vines to maintain disease-free stock (Recommendation 5-1)
Developing optimal roguing and replanting schemes and techniques to manage GLD and GRBD, and facilitating their implementation by growers (Rec 5-2)

continued

TABLE 5-1 *continued*

High Priority Research and Actions
Determining the optimal conditions for application of systemic insecticides to achieve better mealybug control (Rec 5-3)
Development and implementation of insecticide resistance management programs and development of new active ingredients for mealybug management (Rec 5-4)
Determining the optimum conditions for the application of insecticides to achieve better TCAH control and to establish economic or action thresholds to guide insecticide application programs (Rec 5-5)
Generating information needed for improving efficacy of mating disruption for mealybug control; Determining the benefits (economic and otherwise) of mating disruption as part of an integrated approach to manage insect vectors in grapevines (Rec 5-6)
Determining the most effective and practical farm/worker equipment sanitation measures and harvesting/pruning strategies that can help minimize spread of insect vectors (Rec 5-10)
Developing areawide GLD and GRBD vector management programs for regions of California with different GLD and GRBD threat levels; Developing activities to encourage grower participation in areawide programs (Rec 5-13)
Developing GLD and GRBD resistance using traditional and bioengineering approaches (Rec 5-14)
Establishing multidisciplinary and trans-institutional collaborations to enhance synergies in pursuing bioengineering approaches to develop GLD and GRBD resistance (Rec 5-17)
Medium Priority Research
Determining the costs and benefits of removing vegetation that harbors TCAH in and around vineyards and the use of trap crops to inform grower decision-making (Rec 5-8)
Evaluating the efficacy of physical barriers in deterring TCAH movement from riparian areas to vineyards (Rec 5-11)
Supporting research to evaluate the efficacy of reflective mulches in reducing the abundance of insect vectors in vineyards and research on improving the longevity and durability of reflective mulches (Rec 5-12)

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6
Considerations for Future Research on Grapevine Viruses and Diseases

This final chapter discusses future considerations for the California Department of Food and Agriculture (CDFA) Pierce's Disease/Glassy-Winged Sharpshooter (PD/GWSS) Board as it continues to support research to develop viable solutions to virus diseases that threaten vineyard health and the sustainability of the California wine grape industry. The following sections include discussions of genetic pest management strategies; relevant insights and additional research directions from other pathosystems; and approaches to engage a wider range of investigators in grapevine virus research, address immediate research needs, and facilitate knowledge sharing and collaboration among researchers, extension agents, growers, and other constituents of the wine grape industry.

While the committee believes that all the recommendations in this chapter are important, it is also cognizant of the fact that research funds are limited and has identified the high and medium priority research areas and actions. In the sections below, research recommendations and actions of high priority are labeled hp and those of medium priority are labeled MP. Additionally, high- and medium-priority research areas and actions are presented in a table (Table 6-1) at the end of the chapter.

GENETIC PEST MANAGEMENT STRATEGIES

Genetic pest management is an approach to pest control that involves "releasing modified versions of a pest species to mate with wild pests in the target area" (Leftwich et al., 2021). The two main strategies for genetic pest management are suppression and replacement. For suppression, sterile males are released into the environment where they compete with wild type males for mating, reducing each female's chance of successful reproduction and ultimately reducing the size of the population (Abraham et al., 2007). A population suppression approach using genetically-modified male *Aedes aegypti* mosquitoes has proven to be successful in reducing vector populations and the prevalence of the mosquito-borne dengue virus (Carvalho et al., 2015; de Castro Poncio et al., 2023; Oxitec, 2024). A variation of the suppression strategy is precision guided Sterile Insect Technique (pgSIT). With this technique, genetically-modified, laboratory-reared males are released into the environment to mate with wild females and propagate genes that result in sterility among male offspring and death among female offspring. This method ensures that only sterile males survive, and genetically-modified males can be introduced into the population at any life stage to effectively suppress insect populations (Kandul et al., 2019; Li et al., 2021). The success of this strategy requires a sex that does not cause harm (for example, male mosquitoes feed on nectar only and therefore do not transmit dengue virus), the ability to rear genetically-modified individuals successfully in controlled conditions, and genetic modifications that do not reduce mating fitness. For replacement, individuals that have been genetically modified to be incapable of transmitting pathogens are released into the environment where they reproduce freely, propagating the genetic modification and reducing the population's overall ability to carry disease (Shaw and Catteruccia, 2019). CRISPR-based genome editing has transformed the ability to perform precise genome manipulations that spread target genes rapidly through a population.

The importance of vine mealybug as a vector of grapevine leafroll-associated virus 3 (GLRaV-3) and the invasive nature of this insect (Daane et al., 2018) make this species a good candidate for consideration for genetic pest management and feasibility studies for genome editing. Male mealybugs may be good targets for the pgSIT approach because they do not feed on grapevines or transmit viruses, but additional knowledge of their genomes, reproductive biology, seasonal dynamics, laboratory rearing procedures, and molecular transmission mechanisms are needed to begin investigating this approach for

vector control. Some factors that may impact the success of a genetic pest management approach that would need to be included in models are the short lifespan of male mealybugs, and their dispersal capability and sensitivity to environmental conditions. Preliminary modeling studies may prove useful in predicting whether genetic pest management methods would be effective in controlling these pests as a component of integrated pest management (IPM) programs (Barclay, 2021). One benefit of this approach is that it relies on modification of the insect rather than the plant (and thus does not involve modification of products intended for human consumption).

To implement potential genetic pest management, additional research is needed to further characterize the primary vectors of GLRaV-3, the vine mealybug and grape mealybug. This includes addressing the knowledge gaps identified in Chapters 4 and 5, as well as research to gain insights into the vector's genome and bioengineering projects. Interdisciplinary research teams that include molecular biologists, entomologists, modelers, field biologists, and extension specialists are key for developing strategies and predicting real-world implications. It is also important to study sociological aspects and consumer acceptance to understand how biotechnology-based strategies may be perceived and adopted and anticipate potential downsides or barriers to adoption.

The replacement strategy, which targets the molecular determinants of vector competence or expression of genes to reduce vector competence (Buchman et al., 2020; Dong et al., 2020; Carballar-Lejarazú et al., 2023), also represents a potentially viable option for grapevine leafroll disease (GLD) and/or grapevine red blotch disease (GRBD) management. For GLRaV-3, which is transmitted in a semi-persistent manner, this would likely involve targeting molecules on the surface of the vector foregut, the proposed site of virus retention. In contrast, grapevine red blotch virus (GRBV) is transmitted in a persistent manner and likely is transcytosed (i.e., transported across a biological barrier) through the treehopper gut cells to the hemolymph after initial interactions with specific molecules in the gut. For both pathosystems, virus receptors in the insects have not been identified; a multidisciplinary approach to defining receptors could enable research to develop incompetent vectors. However, due to the importance of mealybugs as direct pests in addition to disease vectors, a population suppression approach may be more desirable and might eventually be part of an IPM approach alongside the use of pheromones, biological controls, and insecticides.

Conclusion 6-1: Genetic pest management strategies, in which the insect vector is modified rather than the plant, offer opportunities to curb the spread of disease by reducing vector populations or their ability to transmit viruses. The biology of mealybug vectors makes them good targets for genetic pest management.

Conclusion 6-2: Multidisciplinary research teams composed of molecular biologists, entomologists, modelers, and field biologists or extension researchers are needed to develop genetic pest management strategies and to predict their real-world implications.

Conclusion 6-3: Sociological aspects and consumer acceptance are important considerations when developing genetic pest management strategies.

Recommendation 6-1: Support basic research to enable genetic pest management strategies for GLD and GRBD vectors and support modeling and sociological research to predict whether these strategies will be effective in the field and be accepted by the public.

INSIGHTS AND ADDITIONAL RESEARCH DIRECTIONS FROM OTHER PATHOSYSTEMS

Tactics for Controlling Insect Vectors

This section discusses approaches or tools used to control vectors of pathogens that infect other economically important crops, which may be applicable to insect vector management in grapevines.

RNA Interference (RNAi)

RNAi is a conserved cellular defense mechanism in eukaryotes targeting double-stranded RNA (dsRNA) that can be harnessed for insect control. To use this approach, dsRNA molecules with a sequence complementary to that of the single-stranded messenger RNA (mRNA) of an essential insect gene are introduced into the insects via feeding, injection, or other methods. The insect's cells perceive the dsRNA as a pathogen-associated molecular pattern, a sign of invasion by a foreign entity or an unnatural phenomenon. This in turn triggers the RNAi response, resulting in degradation of the target mRNA. The functionality of the essential (i.e., target) gene in the insect is thereby reduced by this knockdown of mRNA abundance, resulting in developmentally challenged individuals or even the death of the insect.

Several research groups have explored the application of RNAi for insect control (Gordon and Waterhouse, 2007; Vogel et al., 2019; Christiaens et al., 2020; Zhu and Palli, 2020). For example, this approach is gaining considerable interest in Huanglongbing (HLB) research as insecticides have become less effective in controlling the vector of this disease, Asian citrus psyllid (ACP, *Diaphorina citri*). Studies of RNAi knockdown in the ACP across all life stages have been reviewed by Yu and Killiny (2020); an RNAi approach developed for controlling ACP and for disrupting essential genes of the glassy-winged sharpshooter have been shown to be effective and species-specific (Hunter et al., 2012, 2019, 2020; El-Shesheny et al., 2013; Killiny et al., 2014; Andrade and Hunter, 2016, 2017; Taning et al., 2016; Ghosh et al., 2018; Kishk et al., 2017; Hunter and Sinisterra-Hunter, 2018; Tian et al., 2018; Yu and Killiny, 2018; Liu et al., 2020). As further illustrated in studies using exogenous RNAi to significantly reduce insect vectors, including reports of gene target suppression after ingestion of dsRNA by plant-feeding hemipterans (examples and references in Li et al., 2013, 2015; Christiaens and Smagghe, 2014; Adeyinka et al., 2020; Fletcher et al., 2020; Jain et al., 2020), the strong response to dsRNA triggers shows that RNAi has the potential for managing insect vectors and pests. However, caution is needed when designing an RNAi for a targeted insect in order to avoid harming beneficial insects or other non-target species.

Another promising aspect of this approach is that two RNAi-based mode of action products were approved by the U.S. Environmental Protection Agency and commercially released in 2024 (Yan et al., 2024); one is a sprayable formulation that targets the Colorado potato beetle (CPB, *Leptinotarsa decemlineata*) (Zhu et al., 2011; San Miguel and Scott, 2016; Máximo et al., 2020; Mehlhorn et al., 2020; Petek et al., 2020; Doğan et al., 2021), and one is a transgenic crop that expresses dsRNA to target the western corn rootworm (WCR, *Diabrotica virgifera virgifera* LeConte) (Bolognesi et al., 2012; Ramaseshadri et al., 2013; Bachman et al., 2016; Head et al., 2017). The first sprayable dsRNA biopesticide, Calantha™ (active ingredient ledprona), triggers RNAi to silence expression of an enzyme, which leads to death of CPB (Rodrigues et al., 2021). To manage WCR, RNAi is triggered after root-feeding larvae ingest dsRNAs from corn plants genetically engineered to express them. These new products provide novel modes of action for pest management and it is worth investigating the potential use of similar approaches in vector management for GLD and GRBD.

Conclusion 6-4: RNAi has the potential for use in managing viruses, their insect vectors, and potential other grapevine pests. Applied RNAi biopesticides should have narrow activity based on target-specific dsRNA that will trigger RNAi suppression only in the targeted organism, and no activity in other beneficial insects. Genetically engineered plants expressing dsRNA may more effectively manage mealybugs and other insects that reside under bark where it is hard to contact them with insecticide sprays.

Recommendation 6-2 (MP): Consider supporting interdisciplinary research teams to advance RNAi research for the suppression of vectors in vineyards.

Nanobodies

Nanobodies, or single-domain antibodies, have emerged as promising tools for managing grapevine fanleaf virus (GFLV) (Hemmer et al., 2018). These small antibody fragments, derived from camelid species, have high affinity and specificity for their target antigens, including GFLV proteins (Hemmer et al., 2018). Although GFLV can overcome resistance conferred by the Nb23 nanobody, researchers have developed Nb75, which provides dual resistance to GFLV and arabis mosaic virus, and are exploring the combination of different nanobodies for more durable resistance (Hemmer et al., 2018; Orlov et al., 2020). Other studies have shown nanobodies can effectively inhibit viral infection, replication, and disease symptoms in plants (Ghannam et al., 2015; Ingram et al., 2018). Transgenic expression of nanobodies in plants also offers a promising approach to control viral infections like GLRaV-3 and GRBV, potentially neutralizing viruses and preventing their spread. However, challenges such as the scalability and cost-effectiveness of nanobody production need to be addressed for practical field application. A U.S. Department of Agriculture (USDA) team in collaboration with AgroSource, Inc. recently demonstrated that nanobodies can be produced in a plant system and could have agricultural and public health applications. As a proof-of-concept, this team demonstrated the production of functional nanobodies targeting SARS-CoV-2, showcasing their potential for various uses beyond agriculture (USDA-ARS, 2022). Their recent efforts are focused on testing the plant-based delivery system, which they are calling Symbiont technology, to prevent and treat citrus greening disease using nanobodies (USDA-ARS, 2024). Field trials and risk assessments are needed to evaluate the long-term stability and effectiveness of nanobodies under field conditions.

Conclusion 6-5: Nanobodies present a promising strategy for managing grapevine viruses like GLRaV-3 and GRBV, given their high specificity and efficacy in targeting viral proteins. However, successful application in vineyards depends on overcoming challenges related to scalable production, cost-effectiveness, and long-term stability under field conditions.

Recommendation 6-3: Consider supporting research to advance the development of nanobodies for the control of GLRaV-3 and GRBV through transgenic or exogenous approaches. This could include monitoring and funding multidisciplinary, collaborative efforts to refine nanobody production methods to improve scalability and affordability, as well as supporting field trials to rigorously assess the performance and durability of nanobodies in diverse vineyard environments to ensure they are a practical and sustainable solution for virus management.

Trunk Injection of Systemic Pesticides

Trunk injection of systemic pesticides is often used to treat vascular bacterial, fungal, or nematode diseases of forest trees and has been applied to various tree crops. For example, trunk injection of oxytetracycline (OTC) has been shown to manage HLB in citrus, almond leaf scorch (*Xylella fastidiosa*) in almonds, mycoplasma infections in apricots, and phytoplasma infections causing lethal bronzing of palms (Brooks et al., 1994; Takai et al., 2000; Koch et al., 2010; Archer et al., 2023). Studies investigating the efficacy of antibiotics, brassinosteroids, plant growth regulators, RNAi, insecticides, systemic acquired resistance inducers, endophytes, and other agents by trunk injection show positive results in citrus (Shwarz et al., 1972; Boina and Bloomquist, 2015; Hu and Wang, 2016; Hu et al., 2017; Archer et al., 2022a,b, 2023). Much of this work has been focused around the management of HLB, which has brought Florida's iconic citrus industry to the brink of collapse and is spreading in other major citrus production areas in Texas and California (Halbert and Manjunath, 2004; Bové, 2006; Gottwald, 2010; Grafton-Cardwell et al., 2013; Hall et al., 2013; McCollum and Baldwin, 2016; Graham et al., 2020). While the vascular nature of HLB renders therapies that are applied via foliar sprays, such as bactericides, ineffective (Killiny et al., 2020), trunk injection methods can overcome these obstacles. In

Florida, trunk injections of OTC have been found to reduce *Candida*tus Liberibacter asiaticus titer levels, improve fruit and juice quality, and prevent HLB-induced decline (Archer and Albrecht, 2023; Archer et al., 2023).

The effectiveness of trunk injection methods in controlling vasculature diseases and insect pests in other tree crops suggests the approach could be applicable to the grapevine industry in California. Studies conducted in European vineyards have demonstrated the potential to control esca disease complex by injecting fungicides and chemicals into the grapevine trunk (Di Marco et al., 2000; Calzarano et al., 2004; Dula et al., 2007; Del Frari et al., 2018). Using trunk injection as a way of managing woody plant pathogens and pests offers several distinct advantages relative to foliar sprays. First, they can eliminate chemical loss due to spray drift (Berger and Laurent, 2019). Second, they offer precise delivery and allow for a higher concentration in the plant tissue, thus requiring fewer applications (Vincent et al., 2022). Additionally, they can reduce risks for non-target organisms and worker contact with materials, thus causing less concern for human health and the environment. Finally, therapeutics administered directly into plant tissue are less likely to be removed by rain or degraded by sunlight, resulting in greater stability and extended residual activity of the therapeutics (reviewed in Batuman et al., 2024).

Conclusion 6-6: Trunk injection has been used for delivering pesticides directly to the plant vasculature to control diseases in citrus, almond, apricot, and palm trees. This delivery method, which is more precise and has a lower risk of non-target effects, may be applicable in controlling phloem-limited pathogens as well as phloem-feeding insects in vineyards.

Recommendation 6-4 (MP): Consider supporting research to investigate the potential utility of trunk injection to control vectors and viruses with various pesticides (including new approaches such as RNAi and nanobodies) in grapevines.

Prediction Models and Risk Indexes as Management Tools

Various models and risk indexes have been developed to help predict timing of insect infestations into crops, insect development, and the risk of disease spread into crops. Incorporating elements such as degree day models of insects and/or crops, crop phenological models, models of pathogen spread, and information about factors that promote or suppress crop injury or disease spread, these tools help stakeholders identify critical periods and geographic areas for management by shedding light on site-specific risk based on seasonality, location, and production practices. Further development of these tools may help growers better predict insect population dynamics to improve scouting and timing of management activities. To advance this work and improve prediction accuracy requires multi-year and multi-location data to accurately identify factors driving insect and pathogen dynamics, validation after development, and ongoing evaluation to ensure models are kept current based on changing production practices and improved knowledge. The University of California Statewide IPM program currently hosts a variety of insect degree day and crop phenology models;[1] if developed, models specific to GLRaV-3 and GRBV could be housed there for public use.

Tomato Spotted Wilt Virus Field Risk Index and Thrips Projections

Several region-specific tools have been developed to help manage infestations of tobacco thrips (*Frankliniella fusca* [Hinds]) and the spread of tomato spotted wilt virus (TSWV). In California, a dedicated webpage[2] has been established that provides predictions of western flower thrips (*Frankliniella occidentalis* [Pergande]) population development and alerts growers and pest control advisers when they may consider implementing thrips control measures in tomato fields (Batuman et al., 2020). The website

[1] See https://ipm.ucanr.edu/WEATHER/index.html#PESTPLANTMODELS.
[2] See https://ucanr.edu/sites/TSWVfieldriskindex/.

also provides a TSWV field risk index that growers can use to predict the potential for disease outbreaks in a given field. These resources are updated regularly to provide grower updates and alerts before, during, and after the growing season (Batuman et al., 2015).

Thrips Infestation Predictor for Cotton

The Thrips Infestation Predictor (Chappell et al., 2020a) provides an online tool[3] that cotton growers in the Southeast and Mid-South can use to learn when adult thrips will be present to infest seedling crops and how the risk of cotton injury is likely to vary depending on planting date. As its data source, the tool uses a degree day model that captures landscape-level dynamics and can be used to determine when infestations will occur for any crop that is infested with thrips. The Thrips Infestation Predictor is specific to cotton and models how seedling cotton growth interacts with periods of insect flights to identify windows of susceptibility when pesticide applications are needed. To use the online tool, growers select their location on a map and enter their planting date. Based on these inputs, the model uses publicly available weather data to provide forecasts of insect dispersal into fields, crop injury risk, and provides graphical outputs and explanations. Growers can use this tool to adjust planting dates or identify priority fields for management based on the planting date used. Because cotton growth and thrips population dynamics are seasonally predictable, information in this tool does not need to be updated annually unless cotton growth parameters or responses of insect population dynamics to the environment change.

Peanut Rx

Peanut Rx is a disease index developed to help U.S. peanut growers identify the risk of diseases including TSWV, leaf spot, white mold, and root-knot nematode in peanut fields (Kemerait et al., 2004; Chappell et al., 2020b). Risk points are assigned based on factors known to increase or decrease incidence of specific diseases in the crop. Factors included in the model include peanut variety, planting date, plant population, at-plant insecticide, row pattern, tillage, herbicide, crop rotation, field history, and irrigation. The tool helps growers identify production practices that lower their risk of specific pathogens to prevent yield loss. Peanut Rx is available online[4] and is updated during the annual meeting of the Land Grant University peanut breeders, agronomists, entomologists, and plant pathologists in the Southeastern United States who discuss any changes occurring in ongoing disease pressure, efficacy of management tools, production practices, and varieties.

Conclusion 6-7: Models have been valuable tools for stakeholders to understand pest risk, apply practices that mitigate risk, and know critical windows of time for scouting and management activities.

Recommendation 6-5 (HP): Fund research that will lead to the development of publicly available, regionally relevant insect population models and disease risk models that can be used to guide local and areawide management activities for GLD and GRBD.

ENGAGING A WIDER RANGE OF RESEARCHERS IN ADDRESSING RESEARCH NEEDS

The CDFA PD/GWSS Board and its Requests for Proposals (RFPs)[5] focus on Pierce's disease and its vector, the glassy-winged sharpshooter, which continues to pose an important economic threat to California wine grape production. However, researchers who work on other pathosystems and are not

[3] See https://products.climate.ncsu.edu/ag/cottontip/.
[4] See https://peanutrx.org/.
[5] See https://www.cdfa.ca.gov/pdcp/grants/.

familiar with the PD/GWSS Board research and outreach grant program may not realize that the program also supports research on other grapevine viruses and pests, such as GLRaVs and GRBV and their vectors. It is important for the broader research community (domestic and international) to be aware of the scope of research that is supported by the PD/GWSS Board.

One way to increase the pool of researchers who engage with this grant program is to allocate funding specifically for early and mid-career researchers. This approach can also help to build a new network of scientists to address long-term questions.

Conclusion 6-8: Researchers who are not familiar with the PD/GWSS Board research and outreach grant program may not be aware that this program also funds research on other grapevine viruses and pests, such as GLRaVs and GRBV and their vectors. Allocating funding specifically for early and mid-career scientists may help expand the pool of researchers working on grapevine virus diseases.

Recommendation 6-6 (HP): To draw in diverse researchers, consider changing the name of the PD/GWSS Board research and outreach grants to accurately reflect the scope of its RFPs, which include multiple grapevine virus diseases and their insect vectors.

Recommendation 6-7: To increase awareness of the work of the PD/GWSS Board and bring in new scientists to address grapevine vector-borne diseases of national and global significance, expand efforts to promote the funding portfolio and RFPs to more diverse research communities via social media, professional societies, and other mechanisms.

Recommendation 6-8 (MP): Consider offering specific funding for early and mid-career researchers to encourage engagement in grapevine virus diseases research and build a network of scientists to address long-term questions.

Inviting specific researchers or research groups to address particular knowledge gaps or research needs may also increase the pool of interested researchers. For example, the Citrus Research and Development Foundation occasionally issues invitations for a "Directed Research" proposal to conduct immediate studies on specific topics outside of the RFP process. The foundation also accepts off-cycle proposals to work on projects that have potential to generate results that can significantly improve the health or yield of citrus trees infected with citrus greening;[6] success in these projects can lead to longer-term projects in subsequent funding cycles. As another example, Bayer Crop Science develops RFPs directed at specific research needs (e.g., for developing innovative solutions for real-time, remote monitoring of greenhouse spaces and for developing next-generation genomic tools in agriculture).[7,8]

Conclusion 6-9: In addition to traditional RFP cycles, research may be funded through other mechanisms such as inviting researchers to address specific topics.

Recommendation 6-9 (HP): Consider developing additional funding mechanisms to address particular needs for GLD or GRBD research, such as through inviting specific researchers to address particular knowledge gaps or accepting off-cycle proposals for projects that have potential to generate information for dramatically improving GLD and GRBD management.

[6] See https://citrusrdf.org/apply-for-funding/.
[7] See https://www.halo.science/research/agriculture/greenhouse-level-image-monitoring?utm_campaign=n-2784899&utm_source=notification-campaigns&utm_medium=email&_luid=2453&_nid=2784899).
[8] See https://www.bayer.com/media/en-us/bayers-grants4ag-program-awards-21-crop-science-research-grants-for-2023/.

ADDRESSING THE NEED FOR LONGER-TERM STUDIES AND REPLICABILITY

The study of vector-borne diseases requires extensive coordination and organization among diverse scientists and constituents, and the added complexity of the perennial cropping system necessitates long-term studies to accurately describe disease biology and make recommendations for disease or vector management. As a result, some research projects would benefit from longer funding periods than the current three-year maximum.

For some studies, a five-year funding period may enable researchers to make significant new discoveries on the biology and ecology of GLD and GRBD. To provide checkpoints along the way and ensure continued progress, projects with this extended period of funding would need to demonstrate significant progress each year in order to justify receiving the next year of funding. This extended period of funding may be particularly valuable for multidisciplinary studies that advance control recommendations, translational research, and projects that integrate economic and societal impacts.

To support replicability (which is defined as obtaining consistent results across studies that address the same scientific questions and have generated their own data; NASEM, 2019) and confirm emerging findings, the PD/GWSS Board may also consider supporting studies to replicate experiments that address the same research questions in different locations and/or grape growing regions. This may be particularly important for advancing knowledge about GRBV because many questions remain unanswered about basic virus biology and transmission by vectors. For example, the PD/GWSS Board could choose to support research projects in more locations, encourage collaboration among researchers in different locations, and design new funding mechanisms for collaborative proposals to support these larger efforts. One approach could be to employ the National Science Foundation's strategy for funding collaborative proposals, in which projects are submitted "as a single proposal, in which a single award is being requested (with subawards administered by the lead organization); or by simultaneous submission of proposals from different organizations, with each organization requesting a separate award" (NSF, n.d.). In some cases, these collaborations may also benefit from an international perspective where new disease control strategies have been developed or implemented.

Conclusion 6-10: The study of complex systems such as vector-borne diseases in perennial crops may take longer than three years and require more funding to accurately describe disease biology and make recommendations for disease or vector management.

Conclusion 6-11: Replicability of results is an important issue, especially with GRBV because of knowledge gaps in virus biology and vector transmission.

Conclusion 6-12: Collaborative research proposals provide a mechanism to support multiple research teams addressing the same research questions.

Recommendation 6-10 (HP): Consider funding longer-term projects (lasting more than three years) such as studies that advance control recommendations, translational research, and projects that integrate economic and societal impacts.

Recommendation 6-11 (HP): Consider funding research to replicate experimental results in more than one location and with different research teams to obtain more robust and reliable insights.

Recommendation 6-12: Consider new ways to leverage available funds using different proposal and award structures to encourage collaboration.

KNOWLEDGE SHARING AND COLLABORATIVE RESEARCH

Interdisciplinary Approach to Vector-Borne Disease Research

The study of plant diseases requires a multi-faceted, interdisciplinary approach to understand the complex interactions within a given pathosystem (Jeger et al., 2021). This approach has been employed and works well in the study of soil-borne diseases involving interactions of multiple biotic agents (e.g., fungi and nematodes) (Zhang et al., 2020) and in the study of vector-borne plant diseases in which vector control is utilized for disease management (Jeger et al., 2021). While the PD/GWSS Board encourages multidisciplinary teams in its RFPs, more effort is needed to improve collaboration and communication among researchers from various disciplines, institutions, and wine grape producing regions in order to gain a more holistic understanding of GLD and GRBD and inform the development of more effective control strategies.

Moving from a framework of multidisciplinary to interdisciplinary research could facilitate this goal. In multidisciplinary research, a common topic is addressed by experts using different disciplinary perspectives, but the findings are not integrated in the end (Van den Besselaar and Heimeriks, 2001). In contrast, interdisciplinary research entails the integration of knowledge generated by experts from various disciplines. Interdisciplinary research has been defined as "a mode of research by teams or individuals that integrates information, data, techniques, tools, perspectives, concepts, and/or theories from two or more disciplines or bodies of specialized knowledge to advance fundamental understanding or to solve problems whose solutions are beyond the scope of a single discipline or area of research practice" (IOM, 2005). The integration of knowledge is crucial to the "systems thinking" approach to solving a complex problem, such as vector-borne diseases. Effective management of vector-borne diseases requires research to understand the virus(es), vector(s), hosts, and their interactions, as well as the influence of environmental factors on the pathosystem. Knowledge of the pathosystem is needed for developing detection and diagnostic tools and methods for use in clean plant programs and in monitoring and early detection of GLRaVs and GRBV in vineyards. In addition, knowledge of socioeconomic factors that may prevent growers from adopting disease management strategies or participating in areawide pest management is also crucial, as is finding and implementing the most effective educational and outreach strategies (see Figure 6-1).

> *Conclusion 6-13: GLD and GRBD research would benefit from an interdisciplinary approach, wherein findings and perspectives of experts from various disciplines and growers are integrated to gain a holistic understanding of a complex problem.*
>
> **Recommendation 6-13: Consider allocating funding specifically for research projects that employ an interdisciplinary approach.**

Fostering Information Sharing, Interactions, and Collaboration

In the past, the PD/GWSS Board sponsored an annual symposium that facilitated information sharing and interactions among funded researchers. With the COVID-19 pandemic, this symposium was moved online and then canceled in most recent years. Although holding an annual in-person symposium can be costly, it is important for researchers studying GLD and GRBD (in California or elsewhere in the United States and the world) to interact and share information in a timely manner, and to have some mechanism for integrating information generated by research teams and forming synergistic collaborations. PD/GWSS project progress reports are available online[9] and shared among researchers and stakeholders; however, additional efforts are required to integrate the information on GLD and GRBD generated to date. Greater sharing and integration of research findings could be facilitated by the

[9] See https://www.cdfa.ca.gov/pdcp/research.html.

establishment of a dedicated working group that includes all or most researchers who study GLD and GRBD, and/or through expanded opportunities for U.S. and international researchers to interact and share ideas at in-person meetings.

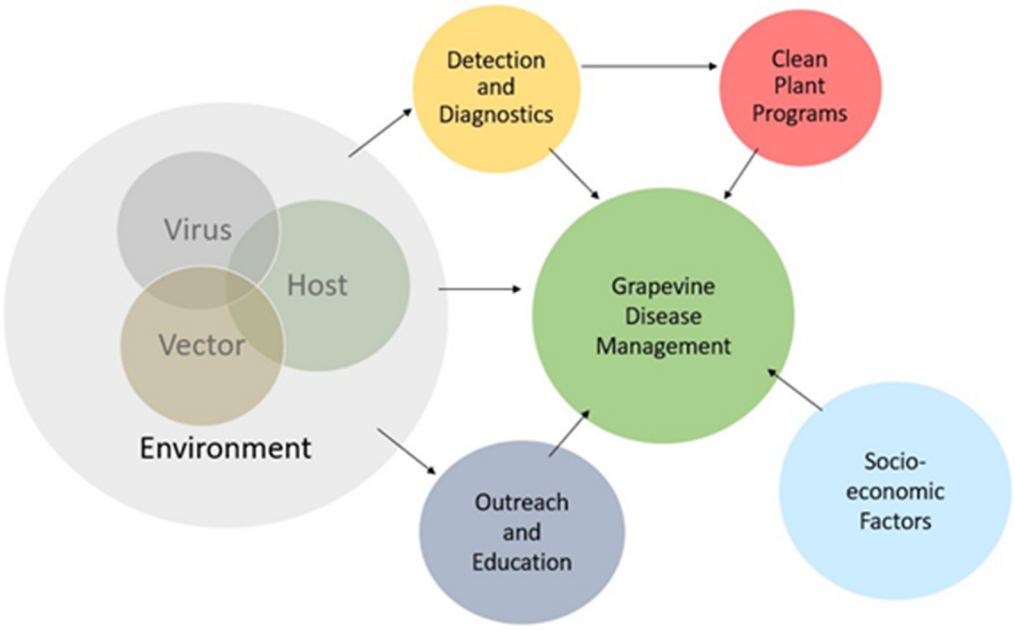

FIGURE 6-1 Diagram representing research areas that provide the knowledge necessary for developing, improving, and implementing strategies for effective GLD and GRBD management. The figure illustrates the connections between research areas and the need for multidisciplinary and interdisciplinary research and systems thinking to tackle the complex diseases caused by vector-borne viruses of grapevine.

Engagement with a USDA-sponsored Multistate Research Coordinating Committee and Information Exchange Group represents one model. One such exchange group is the WERA20,[10] which meets annually and facilitates the exchange of information and ideas related to fruit crops, including grapevines. WERA20 focuses on a wide range of diseases caused by graft-transmissible pathogens, such as viruses, viroids, phytoplasmas, and systemic bacterial pathogens (Fuchs et al., 2021). It includes official representatives from various states, although multiple representatives from the same state attend annual meetings, which are held in states with significant fruit crop industries, such as California, Washington, New York, and Michigan.[11] Annual meetings typically consist of two days of state reports and research presentations followed by a half-day tour of the local fruit crop industries. WERA20 already includes strong representation by researchers involved in grapevine virus research, including researchers funded by the PD/GWSS Board. Increasing the formal presence of the PD/GWSS Board at this annual meeting by sponsoring a mini workshop on topics of interest to the CDFA could represent a relatively low-cost substitute for a yearly research symposium. The opportunity for researchers studying GLD and GRBD to interact with researchers studying other fruit crops such as stone fruits, citrus, and berries would offer the additional advantage of facilitating a broader exchange of ideas and research breakthroughs.

Coordinating with other existing organizations and events could provide additional venues for GLD and GRBD researchers to share research and exchange ideas. For example, it may be possible for the PD/GWSS Board to organize sessions within the annual conference of the American Society for

[10] See https://nimss.org/projects/view/mrp/outline/18910.
[11] See https://nimss.org/seas/52334.

Enology and Viticulture or the annual Unified Wine and Grape Symposium.[12] To facilitate further collaboration among researchers across states, California's wine grape industry could also advocate for the creation of a multistate research or exchange project under the Hatch Multistate Research Fund, which is administered by the USDA National Institute of Food and Agriculture and supports agricultural research to address problems across multiple states.[13]

The Emerging Viruses in Cucurbits Working Group (EVCWG) offers another potential model for facilitating collaboration on GLD and GRBD issues. The EVCWG was established in 2022 through the initiative of cucurbit researchers with support from the U.S. cucurbit industry and funding from the Southern IPM Center (see Box 6-1).[14] It is composed of members from all sectors (research, production, extension/outreach, and regulation) of the U.S. cucurbit industry. Establishing a working group under a similar model for grapevine viruses could facilitate communication and dissemination of resources and findings among researchers, extension agents, growers, and other members of the wine grape industry. Additionally, such a group can be useful for facilitating sharing of prepublication data related to pathogen sequences and biology, which has enabled faster responses to emerging diseases (Hadfield et al., 2018; Dhami et al., 2022; Open Wheat Blast).[15] Greater collaboration and data sharing can also enable rapid response to changes in virus populations (for example, by modifying molecular detection methods to better detect viruses in the field; Thompson et al., 2019) and build a network of scientists ready to respond as virus diseases of grapevine evolve and emerge.

Conclusion 6-14: Sharing of information and collaboration among researchers are essential to interdisciplinary research and to facilitating a "systems thinking" approach for solving complex problems.

Conclusion 6-15: Groups such as WERA 20 and EVCWG have effectively facilitated the dissemination of information and the exchange of ideas about virus diseases in crops among researchers, extension agents, growers, and other stakeholders.

Recommendation 6-14 (MP): As an alternative to the annual Pierce's disease symposium, consider coordinating with other organizations to hold sessions on GLD and GRBD at events such as the annual conference of the American Society for Enology and Viticulture and the Unified Wine and Grape Symposium. These sessions could also serve as a platform to facilitate new collaborations involving scientists working on other grape diseases or working in other wine grape producing regions.

Recommendation 6-15: Consider enhancing PD/GWSS Board participation in WERA20 annual meetings through sponsorship of workshops to build synergies and facilitate cross-pollination of strategies and technologies across specialty crops.

Recommendation 6-16 (HP): Explore the feasibility of creating a working group, supported by the wine grape industry and funded by another entity, that can facilitate information sharing and foster collaboration among GLD and GRBD researchers.

[12] See https://www.unifiedsymposium.org/.
[13] See https://www.nifa.usda.gov/grants/programs/capacity-grants/hatch-act-1887-multistate-research-fund.
[14] The Southern IPM Center is funded by the USDA National Institute of Food and Agriculture to promote IPM. It is the hub of a multi-state partnership and communication network for connecting researchers, growers, extension educators, commodity organizations, environmental groups, pest control professionals, government agencies, and others. The Western Region IPM Center, headquartered at the University of California, Davis (https://westernipm.org/), is the western counterpart to the Southern IPM Center.
[15] See http://openwheatblast.net/.

BOX 6-1
The Emerging Viruses in Cucurbits Working Group

The creation, mission, and accomplishments of the Emerging Viruses in Cucurbits Working Group (EVCWG) provide an illustrative example of academic-industry collaboration that could help to guide similar efforts to facilitate information exchange on grapevine virus diseases.

During the 2021 annual Plant Health meeting of the American Phytopathological Society, Bill Wintermantel of the USDA Agricultural Research Service and Rebecca Melanson of Mississippi State University Extension led a discussion of cucurbit-infecting viruses among interested researchers. A follow-up discussion with participants from the cucurbit industry was held two months later. During these discussions, participants identified critical needs for the cucurbit industry to better address the challenges of cucurbit-infecting viruses, including increased knowledge of current and potential virus threats and improved educational resources on virus threats for a variety of industry stakeholders. To address these challenges and facilitate coordination and knowledge sharing among scientists and industry members across multiple sectors of the cucurbit industry, the EVCWG was established in 2022 through the initiative of academic researchers with support from the U.S. cucurbit industry and funding from the Southern IPM Center.

The EVCWG is composed of 27 members representing various sectors (research, production, extension/outreach, and regulation) of the U.S. cucurbit industry and a few members from other countries. It is led by two co-chairs and has nine steering committee members (two from industry, three from government research organizations, and four from university research or extension organizations).

The mission of the EVCWG is to improve knowledge of and communication about viruses and their spread across the industry, promote strategies to identify and mitigate virus threats to cucurbit production, and educate stakeholders on emerging viruses of cucurbits and the mission and initiatives of the EVCWG both in the U.S. and internationally. To achieve its mission, the EVCWG holds quarterly meetings; maintains a website[16] to share and communicate EVCWG initiatives, activities, and educational resources; develops educational webcasts, videos, recorded presentations, and factsheets on virus threats to cucurbit production; and delivers educational presentations to stakeholders on emerging viruses and their management. Workgroup members have given talks and hosted outreach events on cucurbit virus threats and the EVCWG at industry, trade, and scientific meetings across several states. Together, these activities have fostered closer cooperation among scientists in academia, government, and industry to address a common interest of tackling viruses in cucurbit production systems.

EDUCATION AND OUTREACH

Information Dissemination

As understanding of GLD and GRBD continues to advance, it is critical to make the expanding body of knowledge available to wine industry stakeholders, including grape growers, crop consultants, and wineries, to help with disease management decision making. Many solutions for insect-vectored virus diseases in grapevines that are relatively simple to implement have not been widely adopted, a gap that has been attributed to a lack of effective communication with and knowledge dissemination to decision makers (Fuchs, 2020). Hobbs et al. (2022) identified growers' lack of knowledge regarding the cause of GLD as an important barrier to the adoption of control tactics, in addition to the economic costs of implementation. Similarly, the adoption of current GRBD management practices has likely been hampered by a lack of information and education (Hobbs et al., 2022). Innovative strategies in education and outreach that utilize dynamic information technologies would help provide greater connectivity between those with scientific knowledge and those who can use it to maintain the health and productivity of their vineyards. Having open communication with growers across the California wine grape production areas could also help with aligning what growers consider as priority issues and what researchers perceive as priority research needs. Efforts in regional education and outreach like the Lodi Winegrape

[16] See https://ecucurbitviruses.org.

Commission[17] and the Napa County Vine Mealybug Management Program[18] provide models for other winegrape regions in California.

Conclusion 6-16: Gaps in communication and knowledge dissemination contribute to the underutilization of GLD and GRBD management practices, underscoring the importance of having more effective educational and outreach strategies as knowledge of GLD and GRBD advances.

Recommendation 6-17: Consider allocating funds for projects to advance innovative educational and outreach strategies to help improve grower and extension educator knowledge of GLD and GRBD and strategies for their control.

Recommendation 6-18 (HP): Provide opportunities for funded researchers to share findings and recommendations regarding grapevine viruses via a dedicated website or a virtual town hall that facilitates interactive discussions about GLD and GRBD among researchers, extension agents, and growers.

Grower Adoption of Disease Control Strategies

Science and technology alone do not solve problems—people do, and very little about their behavior is predictable or even rational (Kahneman, 2011). If it is challenging for people to adopt changes with proven benefits such as boiling water to make it safe for drinking (Rogers, 1962) or putting wheels on suitcases (Marçal, 2021), it is understandable that agricultural systems will face challenges in grappling with complex decisions around infectious disease. Fortunately, the social sciences have advanced significantly since Ryan and Gross (1943) investigated why some farmers in Iowa adopted hybrid seed corn and why some did not and identified the stages in the innovation adoption process (Rogers, 1995). That early work gave rise to the diffusion of innovations paradigm, later expanded on in "*Diffusion of Innovations"* (Rogers, 1962), which provides insights about how an idea or product (i.e., innovation) spreads through a social system (LaMorte, 2022). In the context of agriculture, there is growing awareness of the effects of human actors in addition to the host, vectors, and pathogens in the epidemiology of plant diseases. Garcia-Figuera et al. (2024) point out "a need to better characterize how attributes of epidemics determine the usefulness of collective management, what influences actors' decisions to participate, what governance systems fit different plant health threats, and how these subsystems interact to lead to plant health outcomes." These are researchable topics of equal significance to other dimensions of the effort to mitigate the damage of GRBV and GLRaVs. For example, the careful use of well-designed surveys and appropriate statistical analyses can reveal distinct producer "archetypes" and their unique priorities and help map the network of information flow through stakeholders. These studies are especially significant when collective action is required for success of a disease intervention (Lowder et al., 2024a,b).

Regardless of the science that supports any pest management recommendation, there will still be a high degree of uncertainty with regard to adoption of control tactics by growers. Adoption is most critical and apparent with respect to practices that depend on areawide implementation for success. Because an infectious disease knows no boundaries, a few growers who do not opt into a best practice for vector control could undermine the effectiveness of control across a larger region. This is known as a "weakest link public good problem." It is, therefore, of utmost importance that growers and researchers become collaborators in the search for practical solutions and that information flows freely and frequently in both directions. It is also important to convey information via growers' social networks, which is linked to practice adoption (Lubell, 2024).

[17] See https://lodigrowers.com/growereducation/viruses/.
[18] See https://www.countyofnapa.org/1499/Vine-Mealybug.

Conclusion 6-17: Successful control of vector-borne diseases relies not only on understanding the pathosystem and devising strategies to control the pathogen or its vector, but also on what growers decide to do, known as "willingness to adopt" (e.g., to participate in areawide pest management programs or not).

Conclusion 6-18: Social science research has shown that social networks play an important role in social learning (learning by observing others) and subsequently, in the adoption of innovations (e.g., pest management practices).

Recommendation 6-19 (HP): Support research to better understand the sociological aspects of managing vector-borne diseases through collective action (i.e., areawide pest management) and find ways to increase grower participation in areawide pest management programs.

Recommendation 6-20 (HP): Support research on understanding and improving the flow of information across grower social networks and on outreach efforts to understand the drivers and barriers to successful adoption of GLD and GBRD management practices.

RESEARCH PRIORITIZATION

High and medium priority research areas and actions (with the recommendation number) are summarized in the table below for quick reference.

TABLE 6-1 Future Considerations Prioritization

High Priority Research
Epidemiology research that will lead to the development of publicly available, regionally relevant insect population models and disease risk models that can be used to guide local and areawide management activities for GLD and GRBD (Rec 6-5)
Research to better understand the sociological aspects of managing vector-borne diseases through collective action (i.e., areawide pest management) and find ways to increase grower participation in areawide pest management programs (Rec 6-19)
Research on understanding and improving the flow of information across grower social networks and on outreach efforts to understand the drivers and barriers to successful adoption of GLD and GBRD management practices (Rec 6-20)
Medium Priority Research
Research to investigate the potential utility of trunk injection to control vectors and viruses with various pesticides (including new approaches such as RNAi and nanobodies) in grapevines (Rec 6-4)
High Priority Actions
To draw in diverse researchers, changing the name of the PD/GWSS Board research and outreach grants to accurately reflect the scope of its RFPs, which include multiple grapevine virus diseases and their insect vectors (Rec 6-6)
Developing additional funding mechanisms to address particular needs for GLD or GRBD research, such as through inviting specific researchers to address particular knowledge gaps or accepting off-cycle proposals (Rec 6-9)
Funding longer-term projects such as studies that advance control recommendations, translational research, and projects that integrate economic and societal impacts (Rec 6-10)
Funding research to replicate experimental results in more than one location and with different research teams to obtain more robust and reliable insights (Rec 6-11)

Creating a working group, supported by the wine grape industry and funded by another entity, that can facilitate information sharing and foster collaboration among GLD and GRBD researchers (Rec 6-16)

Providing opportunities for funded researchers to share findings and recommendations regarding grapevine viruses via a dedicated website or a virtual town hall (Rec 6-18)

Medium Priority Actions

Supporting interdisciplinary research teams to advance RNAi research for the suppression of vectors in vineyards (Rec 6-2)

Offering specific funding for early and mid-career researchers to encourage engagement in grapevine virus diseases (Rec 6-8)

As an alternative to the annual Pierce's disease symposium, coordinating with other organizations to hold sessions on GLD and GRBD at events such as the annual conference of the American Society for Enology and Viticulture and the Unified Wine and Grape Symposium (Rec 6-14)

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Appendix A
Committee Member Biographical Sketches

Anna E. Whitfield (*Chair*) is William Neal Reynolds Distinguished Professor of entomology and plant pathology at North Carolina State University, which she joined in 2017 as a Chancellor's Faculty Excellence Program cluster hire in Emerging Plant Diseases and Global Food Security. Previously, she was a professor of plant pathology at Kansas State University (KSU). She is known internationally for her work on plant-virus-vector interactions. The long-term goal of her research is to develop biologically based strategies for controlling viruses and arthropod vectors. Whitfield's research scholarship around virus-vector relationships is enabling development of innovative strategies that disrupt the cycle of disease in the field. Her awards include a National Science Foundation (NSF) Faculty Early Career Development Award for her work addressing the molecular mechanisms of virus-vector interactions, the KSU College of Agriculture Excellence in Graduate Teaching Award (2014), the 2016 Diversity Award from the Kansas State University College of Agriculture, the Sigma Xi Kansas State University 2016 Outstanding Scientist Award, and the Syngenta (2017) and Ruth Allen (2023) awards from the American Phytopathological Society for her research and teaching accomplishments. Whitfield received her master of science degree from the University of California, Davis and her doctoral degree from the University of Wisconsin.

Olufemi J. Alabi is a plant virologist and a professor and extension specialist in the Department of Plant Pathology and Microbiology, Texas A&M University System. His applied research and extension program addresses economically important diseases of fruit (such as citrus and grape) and vegetable (such as cucurbits) crops in South Texas via translational research into disease causation and management, along with education and outreach to growers, industry stakeholders, and the public. He is a member of the American Phytopathological Society (APS), past chair of the APS Virology Committee, member of the International Council for the Study of Viruses and Virus-like Diseases of the Grapevine, and member National Clean Plant Network Education and Outreach Committee. He also serves on the Technical Advisory Committee of the Texas Citrus Pest and Disease Management Corporation and the Emerging Viruses in Cucurbits Working Group Steering Committee. Alabi holds a master of science degree in crop protection and environmental biology from University of Ibadan, Ibadan, Nigeria (2003). He received his doctorate degree in plant pathology from Washington State University (2009), where he worked on grapevine leafroll disease and other grapevine viruses. His research program in Texas also focusses on grapevine red blotch and leafroll diseases and their associated viruses.

Ozgur Batuman is an associate professor in the Department of Plant Pathology at the Southwest Florida Research and Education Center, University of Florida. His current research focuses on pathogen identification and disease management in citrus and tomato production systems. To develop integrated pest management (IPM) in these crops, he studies plant disease etiology, pathogen biology, and epidemiology and develops novel disease management strategies. Batuman's current research and extension activities cover fundamental and applied aspects of citrus diseases and emerging resistant-breaking viral diseases of tomato, including orthotospoviruses and tobamoviruses. He also studies plant-pathogen-vector interactions and

characterizes insect-specific viruses of the vectors in these pathosystems. Previously, he was a postdoctoral researcher and project scientist at the Department of Plant Pathology at the University of California, Davis, where he worked on thrips population dynamics and tomato spotted wilt virus incidence in processing tomato, pepper, and lettuce and developed effective IPM strategies. He also identified and characterized several viruses and virus-like diseases (i.e., viroid and phytoplasma) of other vegetable crops in various countries, including the Dominican Republic, Guatemala, Mali, and Ghana. Batuman holds an M.Sc. degree in plant pathology from the University of Cukurova, Turkey, and a Ph.D. from the Hebrew University of Jerusalem, Israel.

Elizabeth J. Cieniewicz has been an assistant professor of plant virology at Clemson University since 2019. Her research is focused primarily on the ecology of virus diseases of fruit crops including stone fruits and various small fruits such as blackberry, strawberry, and grapevine, and on virus-vector interactions. Her professional background is in vector-borne grapevine viruses, especially grapevine red blotch virus. In addition to teaching responsibilities at Clemson, she also directs the Clemson Clean Plant Center, which is affiliated with the National Clean Plant Network to ensure the supply of virus-negative propagation material for the fruit tree industry. She is also a member of the American Phytopathological Society and Entomological Society of America. She currently serves as senior editor for Plant Disease journal.

Mamadou L. Fall is a research scientist at Agriculture and Agri-Food Canada (AAFC) and associate professor at Université de Sherbrooke. He holds a B.S. in biology from Université de Moncton, NB, Canada, and a DEUG in biology from Université Cheikh Anta Diop, Dakar, Senegal. He earned his Ph.D. in plant pathology from Université de Sherbrooke and completed postdoctoral research at Michigan State University. He leads the virus epidemiology laboratory at AAFC's Science and Technology Branch and teaches a grapevine viruses course at Université de Québec en Outaouais as part of the certification program in Cold Climate Viticulture and Oenology. Fall's research spans from host-virus interaction studies to applied field research on disease management in horticultural agroecosystems, such as grapevines and small fruits. His team has developed virus detection tools and patented a dsRNA binding protein-based extraction method for virus detection and characterization. They also discovered that hybrid grapevine cultivars, like Vidal, show no symptoms despite the presence of grapevine leafroll viruses (GLD), indicating that removing symptomatic grapevines, an effective strategy elsewhere, is not suitable for vineyards in Québec. Fall is a senior editor of the Plant Disease journal and an associate editor of the Canadian Journal of Plant Pathology and the British Plant Pathology journal. He also serves as a guest editor for the Frontiers in Fundamental Virology and Frontiers in Evolutionary and Genomic Microbiology journals.

Alana L. Jacobson is an associate professor at Auburn University. She holds an M.S. degree from Purdue University and Ph.D. from North Carolina State University. In 2014, Jacobson joined the faculty in Auburn University's Department of Entomology and Plant Pathology. Research on insect vectors of plant viruses has been a primary focus of her work, including understanding the biological, ecological, and genetic factors that influence vector-virus interactions underlying the transmission, spread, evolution, and management of plant viruses. As part of this work, she has conducted studies on thrips-transmitted tomato spotted wilt orthotospovirus; aphid-transmitted cotton leafroll dwarf virus; and whitefly transmitted

begomoviruses including tomato yellow leaf curl virus, tomato mottle virus, and viruses that cause cassava mosaic disease. She also has ongoing projects evaluating tools and strategies for management of insect pests of row crops, as well as projects aimed at understanding factors driving the evolution of resistance to management strategies. She is a member of the American Association for the Advancement of Science, the Entomological Society of America, and the American Phytopathological Society.

Alexander V. Karasev is a professor of plant virology at the University of Idaho. He held faculty positions at Thomas Jefferson University and the University of Florida prior to coming to Idaho in 2006. He has been studying plant viruses and plant virus diseases for over 30 years, pioneering research in the molecular biology of closteroviruses, and in particular citrus tristeza virus at the University of California, Riverside and the University of Florida. At the University of Idaho, Karasev's research has focused on understanding interactions between plant viruses and their hosts, and on how resistance genes drive virus evolution. A major emphasis of his current research is on the genetic determinants of pathogenicity of plant viruses affecting potato, common beans, grapevines, and sugar beet. In 2019, Karasev was elected a Fellow of the American Phytopathological Society (APS), and in 2022 he was promoted to the rank of University Distinguished Professor at the University of Idaho. From 2019-2021 he served as an Editor-in-Chief of Plant Disease and as a member of the APS Publication Board. Karasev received his Ph.D. in virology from Moscow State University in Russia and continued his training as a postdoctoral research fellow at the University of California, Riverside.

Kirsten Pelz-Stelinski is the director of the University of Florida/Institute of Food and Agricultural Sciences Mid-Florida Research & Education Center and a professor in the University of Florida's Department of Entomology and Nematology. Her research program focuses on the biology and microbial ecology of insect vectors of plant diseases, with an emphasis on developing microbial-based management strategies for insect pests. Currently, she is investigating transmission of the Huanglongbing (HLB) pathogen *Candidatus* Liberibacter asiaticus by the Asian citrus psyllid (ACP; *Diaphorina citri*) to further the development of successful ACP management programs. Aspects of this research include evaluating the effects of antimicrobials on ACP fitness and pathogen transmission and investigating the function of ACP endosymbionts. She is a member of the American Association for the Advancement of Science, the Entomological Society of America, and the American Society for Microbiology. Pelz-Stelinski received her Ph.D. (2008) and M.Sc. (2004) degrees in entomology from Michigan State University.

Wenping Qiu is a research professor in the W. H. Darr College of Agriculture, Missouri State University and directs the Midwest Center of the National Clean Plant Network-Grapevine, which provides virus-tested clean grapevines and virus testing services. His research group focuses on understanding the molecular and genetic basis of disease resistance in grapevines and finding effective strategies for preventing and managing diseases that cause significant losses to the grape industry. Among his research teams' achievements is the discovery of the first DNA virus of grapevines, grapevine vein clearing virus (GVCV). Qiu was awarded the Clif & Gail Smart Professorship from 2012-2019 and the Missouri State University Foundation Award in Research in 2020. He serves on the National Clean Plant Network-Grapes Tier II Committee and is a member of American Phytopathological Society. He received his Ph.D. in plant

pathology/biotechnology from North Carolina State University in 1997 and his M.S. degree in plant virology from Wuhan Institute of Virology, Chinese Academy of Science in 1988.

Naidu A. Rayapati has served as director of the Irrigated Agriculture Research and Extension Center at Washington State University since May 2018. As a faculty member since 2004 in the Department of Plant Pathology, College of Agricultural, Human, and Natural Resource Sciences (CAHNRS), he leads an integrated program of research, teaching, and extension and outreach in plant virology with a strong focus on grapevine viruses and viral diseases in Washington vineyards. He has made significant advances in basic and applied research on economically significant grapevine viruses, leading to a better understanding of their molecular biology and epidemiology and improved management in vineyards. Previously, Rayapati worked as a senior scientist at the International Crops Research Institute for the Semi-Arid Tropics contributing to crop improvement against viral diseases in subsistence agriculture in Asia and Africa. Rayapati received the International Service Award in 2007 from the American Phytopathological Society, the IPM Team Excellence Award at the 6th IPM International Symposium in 2009 for his superior contributions in plant pathology, and the Land Grant Mission Award in 2020 from CAHNRS for his outstanding contributions to research, teaching, and extension. He is a member of several professional organizations, including the American Phytopathological Society. He received his doctoral degree in plant virology from Sri Venkateswara University, Tirupati, India.

Stuart R. Reitz is the director of Oregon State University's Malheur Experiment Station and a professor of cropping systems. His research addresses the management of arthropod pests, especially vectors of plant pathogens, and interactions between pest management and cultural management in cropping systems in the inland Pacific Northwest. Prior to joining Oregon State University, Reitz served as a Research Entomologist for the U.S. Department of Agriculture's Agricultural Research Service (USDA-ARS), where his research focused on the ecology and management of insect vectors of plant pathogens, in particular thrips and tospoviruses. He was a member of the W-2008 research team that was recognized in 2018 with the Western Region Excellence in Multistate Research Award for their efforts in managing onion pests and diseases. During his tenure with USDA-ARS, he received the Florida Entomological Society Research Award and was a member of the team that received the Southern Region IPM "Pulling Together" Team Award. He holds a Ph.D. in entomology from Clemson University. Reitz served as chair of the National Research Council Insect Control Panel and member of the National Research Council Committee on Review of Research Proposals on Citrus Greening from 2008-2009.

Thomas H. Turpen serves as president and CEO of Sensit Ventures, Inc. He is also an advisor with the Food System 6 organization, a member of the Sustainability Council of the 2Blades Foundation, and a principal consultant with Technology Innovation Group. Turpen is a serial entrepreneur, a registered patent agent, and a founder of both for-profit and non-profit organizations including Eliance Biotechnology (acquired by MacroGenics) and the Citrus Research Development Foundation. His synthetic biology research contributed to the understanding and design of disease resistance traits in agriculture and helped pioneer the use of plant biomass for industrial biotechnology applications including self-assembling nanoparticles, vaccines, and pharmaceuticals at Zoecon Research Institute (Sandoz Crop Protection), Biosource, and Large Scale Biology. He has a passion for connecting innovation to societal needs and has served as a director of early-stage life science companies and as an appointed

volunteer in several institutional and civic advisory committees. He was elected a Fellow of the American Association for the Advancement of Science in 2017. He received his Ph.D. in plant pathology from the University of California, Riverside.

Appendix B
Public Meeting Agendas

OPEN SESSION WITH STUDY SPONSOR
JUNE 30, 2023
VIRTUAL

3:00–3:15	Welcome and Introductions; Meeting Goal(s) **Anna Whitfield,** *Committee Chair*; Committee Members Brief Overview of the National Academies and the Study Process **Camilla Ables,** *Study Director*
3:15–3:25	Overview of PD/GWSS Board and Grapevine Viruses/Diseases Research **Matt Kaiser,** California Department of Food and Agriculture
3:25–3:55	Context for and Expectations from the Study **Matt Kaiser,** California Department of Food and Agriculture **Kristin Lowe,** *President*, Vine Balance Consulting (PD/GWSS Board Consultant)
3:55–4:00	Q&A **CDFA Reps and Committee Members**
4:00	Adjourn Open Session

WEBINAR #1
GRAPEVINE LEAFROLL DISEASE: VECTOR BIOLOGY AND MANAGEMENT
DECEMBER 18, 2023

3:00	Welcome, Introductions, and Overview of the Open Session/Webinar Agenda **Anna Whitfield,** *Committee Chair*
3:10	Presentation: Grapevine Leafroll Disease: Vector Biology and Management **Kent Daane,** University of California, Berkeley The committee asked the speaker to address the following topics/questions during this webinar: ▪ Provide your perspective on knowledge advancements and critical gaps in leaf roll disease management. ▪ Describe the relative importance of leafroll vectors in California and their biology and ecology, and current vector management strategies and effectiveness. ▪ Provide perspectives about red blotch virus in California: incidence, spread, and its economic importance relative to leafroll on a state-wide basis; what are gaps in knowledge and areas where additional research would be most impactful? ▪ Describe the potential for grapevine-virus vector management: mating disruption (pheromones); biocontrol; and other strategies. ▪ Identify areas where significant progress could be made toward understanding virus-vector biology and developing effective control strategies. What barriers need to be overcome? Q&A

4:30 Adjourn Open Session

WEBINAR #2
GRAPEVINE RED BLOTCH AND LEAFROLL VIRUSES: BIOLOGY, ECOLOGY, AND MANAGEMENT
FEBRUARY 16, 2024

3:00 Welcome, Introductions, and Overview of the Open Session/Webinar Agenda
Anna Whitfield, *Committee Chair*

3:10 Presentation: Grapevine Red Blotch and Leafroll Viruses: Biology, Ecology, and Management
Marc Fuchs, Cornell University
The committee asked the speaker to address the following topics/questions during this webinar:
What is the status of virus-vector interaction research for GRBD (and GLD if relevant), and what are the major questions that need to be addressed?

- Describe GRBV transmission biology by *Spissistilus festinus.*
- Provide your perspective on GRBV transmission ecology by *S. festinus* in different vineyard ecosystems.
- Are there regional differences or even site differences in the epidemiology of red blotch and leafroll in California?
- Provide an update on your research aimed at controlling vector-borne viruses impacting wine grapes. Provide your perspective on the potential and acceptance of biotechnological approaches for virus and vector control.
- Identify areas where significant progress could be made toward understanding vector biology/ecology and developing effective control strategies. What barriers need to be overcome?
- Discuss the role of the NCPN in grapevine certification program in New York and the challenges that the program has encountered.
- Share your perspective on grower adoption of management strategies and similarities and differences across virus-vector systems.

Q&A

4:30 Adjourn Open Session

COMMITTEE MEETING IN DAVIS, CALIFORNIA
SESSION 1—OPEN
MARCH 4, 2024

8:30 Welcome and Introductions; Overview of Open Session
Anna Whitfield, *Committee Chair*

8:45 Epidemiology of Grapevine Virus Diseases and Plant Disease Management
Neil McRoberts, *Professor of Plant Pathology*, University of California, Davis; *Director*, Western Plant Diagnostic Network
Topics/questions to be addressed:

- Describe the spatial and temporal spread of red blotch and leafroll in California; Are there regional patterns within California and beyond the borders of California?
- How do biotic and abiotic factors, such as climate variations and viral mixed infections, contribute to the epidemiology of grapevine leafroll and red blotch, and

how can they be integrated into modeling efforts for a more comprehensive understanding?
- Considering the dynamic nature of plant diseases, especially in vineyards, what challenges and considerations are involved in modeling the long-term effects of grapevine leafroll and red blotch, and how reliable are current models in making predictions for future disease prevalence and spread?
- Provide your insight into growers' perspective/understanding of red blotch and leafroll disease, including economic and social perspectives relevant to these diseases, willingness to adopt new technologies, and areawide management (barriers to adoption).
- Identify knowledge gaps in the biology and epidemiology of grapevine leafroll and red blotch that need to be addressed to provide understanding of the spread and control of these diseases.
- What are the key challenges with regard to extension? How is information translated, and what research areas have the greatest potential for payoff in disease and vector control?
- What are the barriers to the adoption of management tactics by growers?

9:30 Grapevine Red Blotch and Leafroll Disease Management
Monica Cooper, *Director & ANR Advisor (Viticulture)*, UC Cooperative Extension, Napa County
Topics/questions to be addressed:
- Describe the significance of red blotch and leafroll diseases to wine grape and table grape production in California.
- Are there regional differences or even site differences in importance and severity of red blotch and leafroll in California?
- Describe challenges to areawide pest management programs and considerations to keep in mind for future efforts.
- What areas of red blotch and leafroll research and possible intervention points show promise for providing solutions for these vector-borne viruses? Describe challenges and considerations to keep in mind for future efforts (e.g., wine quality, social perception of technologies, grower concerns, etc.).
- What are the barriers to the adoption of management tactics by growers?
- Discuss any other aspects of red blotch and leafroll that you feel are important for the committee to take into consideration.

10:45 Coffee Break

11:00 Grapevine Breeding for Disease/Vector Resistance
Peter Cousins, *Grape Breeder*, Winegrowing Research, E. & J. Gallo Winery
Topics/questions to be addressed:

- Significance of red blotch and leafroll diseases to wine grape production in California.
- Status of grapevine breeding for virus and vector resistance: best models and progress with red blotch and leafroll disease.
- What are the major technical barriers to progress in grape breeding for virus and/or vector resistance, and how could we overcome these barriers? How important is the inoculation method when screening for resistance to viruses?

- Describe challenges to grapevine breeding for virus resistance and considerations to keep in mind for future efforts (e.g., wine characteristics, social perception of technologies, grower concerns, etc.).
- Discuss any other aspects of red blotch and leafroll that you feel are important for the committee to take into consideration.

11:45 Adjourn Open Session

COMMITTEE MEETING IN DAVIS, CALIFORNIA
SESSION 2—OPEN
MARCH 4, 2024

12:20 Welcome and Introductions; Overview of Open Session
Anna Whitfield, *Committee Chair*

12:30 Grapevine Red Blotch Virus and Vectors
Frank Zalom, *Distinguished Professor of Entomology*, Agricultural Experiment Station Entomologist and Extension Specialist, University of California, Davis
Mysore Sudarshana, *Research Biologist*, USDA-ARS Crops Pathology and Genetics Research, Davis, California
Topics/questions to be addressed:

- Status of virus-vector interaction research and what are the major questions that need to be addressed?
- Discuss GRBV transmission biology by *Spissistilus festinus*.
- Discuss GRBV transmission ecology by *S. festinus* in different vineyard ecosystems.
- Chemigation: What is the grapevine uptake efficiency? When is the optimal time for chemical application? What does the future of red blotch and leafroll vector chemical control look like?
- Progress toward identification of additional (or all possible?) red blotch vectors and key challenges.
- Identify areas where significant progress could be made toward understanding virus-vector biology and developing effective control strategies. What barriers need to be overcome?
- What are the most promising new technologies for vector control and challenges to be overcome for their application?
- Discuss any other aspects of red blotch and leafroll that you feel are important for the committee to take into consideration.

1:30 Impacts of GRBV on Grapes and Wine Composition
Anita Oberholster, *Cooperative Extension Specialist in Enology*, University of California, Davis
Topics/questions to be addressed:

- Provide an update on your research studying the effect of red blotch virus on grape and wine composition.
- Describe progress toward mitigating grapevine red blotch virus impact on final wine composition. How does the industry mitigate red blotch impact?
- Discuss the important biotic and abiotic factors that may further complicate the effect of red blotch on grapes and wine and cultivar responses to virus.
- Discuss any other aspects of red blotch and leafroll that you feel are important for the committee to take into consideration.

2:00 New Technologies for Virus Control

Protoplast-Mediated Gene Editing for Disease Resistance

David Tricoli, *Manager*, Plant Transformation Facility, University of California, Davis

Topics/questions to be addressed:

- Provide an update on your research studying the effect of red blotch virus on grape and wine composition.
- Describe progress toward identification of targets for gene editing for virus resistance.
- Describe progress toward efficient gene editing in grapevines.
- Identify areas where significant progress could be made toward developing effective control strategies. What knowledge gaps barriers need to be overcome?
- Provide your perspective on the potential and acceptance of biotechnological approaches for virus and vector control.
- Discuss any other aspects of red blotch and leafroll that you feel are important for the committee to take into consideration.

Grapevine Virus-Based RNA Interference (RNAi) Approaches to Target Grapevine Leafroll-Associated Viruses

Yen-Wen Kuo, *Assistant Professor of Plant Pathology*, University of California, Davis

Topics/questions to be addressed:

- Describe your progress toward new virus and/or vector control using grapevine virus-based RNA interference (RNAi) approaches to target grapevine leafroll-associated viruses.
- Provide an update on your research aimed at controlling vector-borne viruses impacting wine grapes.
- Discuss the progress and potential for vector insect-specific viruses to control vectors and/or viruses.
- Identify areas where significant progress could be made toward developing effective control strategies. What knowledge gaps and barriers need to be overcome?
- Provide your perspective on the potential and acceptance of biotechnological approaches for virus and vector control.
- Discuss any other aspects of red blotch and leafroll that you feel are important for the committee to take into consideration.

3:00 Grapevine Phytosanitary Regulations and the California Grapevine Registration and Certification Program

Maher Al Rwahnih, *Director*, University of California, Davis Foundation Plant Services

Topics/questions to be addressed:

- Provide your perspective on the significance of red blotch and leafroll diseases to wine grape and table grape production and relative importance to the industry.
- Describe the status of grapevine phytosanitary regulations (in general), the status of the California certification program, challenges, and strategies that are working well and areas for improvement. Describe certification, protocols, and quarantine strategies implemented at the state level and policies and protocols for bringing in planting materials from outside of California.
- How is the planting material supply chain (from Foundation Plant Services to Nurseries to Growers) working?
- Describe the major barriers to progress in the implementation of phytosanitary measures to address virus issues in wine grapes; provide your opinion on strategies to overcome these barriers.
- How do international collaborations and agreements impact the implementation and enforcement of grapevine phytosanitary regulations?

- How do economic considerations and the financial impact of implementing phytosanitary measures influence the decision making of grape growers?
- Are there specific regions or grape varieties that are more vulnerable to the economic and qualitative impacts of red blotch and leafroll diseases?
- What are the major gaps in diagnostics for these viruses?
- What additional tool(s) and knowledge would be helpful for Foundation Plant Services with their efforts on clean plant production and to help growers implement phytosanitary standards?
- Are there specific knowledge gaps that, if addressed, could significantly improve the efficiency and accuracy of clean plant production programs?

3:45 Adjourn Open Session

OPEN SESSION
HYPERSPECTRAL IMAGING Q&A SESSION
APRIL 19, 2024
VIRTUAL

3:00 Welcome, Introductions, and Overview of the Open Session
Anna Whitfield, Committee Chair
The committee asked the speaker to address the following topics/questions in their recorded presentations, which were submitted beforehand for the committee to view (available at the study website):

- What is the current state of research on hyperspectral imaging for detecting grapevine viruses such as leafroll and red blotch?
- What are the major roadblocks in applying hyperspectral imaging to virus detection?
- How early in the infection process can hyperspectral imaging detect viral infections in grapevines? Is it effective for detecting asymptomatic infections?
- What specific spectral signatures or patterns are indicative of grapevine viruses, and how reliable are they for accurate diagnosis?
- Are the challenges different for asymptomatic and symptomatic plants? Do you expect that co-infection also complicates this?
- What do you prefer as a ground truthing method (i.e., ELISA/PCR?) and is ground truthing data a challenge?
- Are there any ongoing research projects or future directions in this field that you find particularly promising or exciting?
- Are there are any specific camera instruments/vendors that you would recommend for hyperspectral imaging given that the performance continues to improve and cost to fall (Cubert Hyperspectral, for example)?

3:10 Q&A on Hyperspectral Imaging for Grapevine Virus Disease Detection
Empowering Autonomous Virus Detection in Vineyards: Hyperspectral Vision Systems Bridging Science and Industrial Application
Luca Brilliante, Fresno State University
Combination of Spectroscopy and Data Analytics for the Early Detection of Red Blotch Infection in Grapevines
Nitin Nitin, University of California, Davis

4:00 Adjourn Open Session

COMMITTEE MEETING IN WASHINGTON, DC
SESSION 1—OPEN
MAY 8, 2024

9:30 Welcome and Introductions; Overview of Open Session
Anna Whitfield, *Committee Chair*

9:40 Q&A on Grapevine Leafroll Disease (GLD) and its Management in South Africa
Gerhard Pietersen, *Senior Researcher*, Patho Solutions; Committee
The committee asked the speaker to address the following topics/questions in their pre-recorded presentation, which was submitted beforehand for the committee to view (available at the study website):

- Can you provide an overview of the status of grapevine leafroll disease (GLD) in South Africa, including its prevalence and impact on vineyards?
- What are the primary methods used in South Africa for diagnosing GLD?
- What are the key strategies employed in South Africa for managing GLD, both in terms of prevention and control?
- Can you discuss any specific cultural practices or vineyard management techniques that have been effective in reducing the spread and impact of GLD?
- Are there any challenges or unique considerations in managing GLD in the South African context, such as climate, grapevine varieties, or regulatory issues?
- What are the best management practices for implementing rouging and replanting strategy in South Africa, including their approach to early identification of infected vines, rouging protocols, thresholds for replanting, and the economics of implementation?
  - What is the industry-wide adoption rate of the rouging and replanting strategy?
  - What are some of the barriers to adopting the rouging and replanting strategy?
  - How do South African growers deal with the management of multiple generations grapevines as a consequence of the rouging and replanting strategy?
- Can you speak to the issue of the so-called ‘bad neighbors’ with respect to GLD management and how this is dealt with in South Africa?

10:30 Coffee Break

11:00 Q&A on Why Growers Adopt Best Management Practices
Mark Lubell, *Professor*, University of California, Davis; Committee
The committee asked the speaker to address the following topics/questions in their pre-recorded presentation, which was submitted beforehand for the committee to view (available at the study website):

- What are the barriers to grower adoption of disease/pest management practices? How can grower demographics (age, income, primary language, etc.) and communication strategies influence grower adoption?
- Can you share any case studies or examples of successful collaborations between the social science research community and growers in addressing disease and pest management challenges?
- In your experience, what communication methods or platforms have been most effective in disseminating information about disease and pest management practices to growers?
- How can we increase research utilization and ensure that the best available knowledge is used to inform grower practices?

- What are the key considerations for designing outreach and education programs aimed at improving grower adoption of disease and pest management practices? What are the best practices for fostering communal (area-wide) management of diseases and pests?
- How can we address potential socioeconomic disparities in access to resources and information related to disease and pest management in farms (vineyards)?

12:00 Break

SESSION 2—OPEN

1:30 Q&A on Airborne and Spaceborne Imaging Spectroscopy for Early Grapevine Viral Disease Detection

Katie Gold, *Assistant Professor of Grape Pathology*, Cornell University; Committee

The committee asked the speaker to address the following topics/questions in their pre-recorded presentation, which was submitted beforehand for the committee to view (available at the study website):

- What is the current state of research on hyperspectral imaging for detecting grapevine viruses such as leafroll and red blotch?
- What are the major roadblocks in applying hyperspectral imaging to virus detection?
- How early in the infection process can hyperspectral imaging detect viral infections in grapevines? Is it effective for detecting asymptomatic infections?
- What specific spectral signatures or patterns are indicative of grapevine viruses, and how reliable are they for accurate diagnosis?
- Are the challenges different for asymptomatic and symptomatic plants? Do you expect that co-infection also complicates this?
- What do you prefer as a ground truthing method (i.e., ELISA/PCR?) and is ground truthing data a challenge?
- Are there any ongoing research projects or future directions in this field that you find particularly promising or exciting?
- Are there are any specific camera instruments/vendors that you would recommend for hyperspectral imaging given that the performance continues to improve and cost to fall (Cubert Hyperspectral, for example).

2:15 Adjourn Open Session

Appendix C
Conclusions and Recommendations

High- and medium-priority research or actions are identified in this table (HP = high priority and MP= medium priority).

GLD KNOWLEDGE GAPS TO ADDRESS TO HELP WITH DEVELOPING PROMISING SHORT- AND LONG-TERM SOLUTIONS
GLD Biology, Interactions Between GLRaVs and their Hosts, and Host Defense Mechanisms
Conclusion 4-1: Despite decades of research, knowledge on the genetic and phenotypic complexity of GLD-associated viruses remains limited. **Conclusion 4-2:** Fundamental studies using synthetic biology approaches can be applied to systematically investigate how different GLRaV genotypes influence disease outcomes.
Recommendation 4-1: Support research to generate more information about GLRaV-3 genetic variants that could help guide GLD management. **Recommendation 4-2 (HP):** Support foundational research to understand the intrinsic and extrinsic factors contributing to the efficient spread of GLRaV-3, including interactions with other vitiviruses. Research questions that need to be addressed include: • Why is GLRaV-3 predominant among the GLRaVs? What are the biological consequences of extensive GLRaV-3 genetic diversity? What factors are driving the evolution of new GLRaV-3 genetic variants? • • What are possible disease outcomes of single versus mixed infections of different GLRaVs and/or distinct GLRaV-3 genetic variants?
Conclusion 4-3: Host factors required for GLRaV-3 infection and resistance in *Vitis* hosts have not been discovered, yet knowledge of these factors could create opportunities for developing novel control strategies. **Conclusion 4-4:** The grapevine and GLRaV-3 genomes contain regions for generating non-coding RNAs whose role in infection and symptom development has not been explored. **Conclusion 4-5:** Further investigations into the extent of GLRaV-3 host range within (and beyond) *Vitis* may generate valuable information that could be exploited for GLD management.
Recommendation 4-3 (MP): Support research to identify host factors required for GRLaV-3 infection and resistance in *Vitis* hosts and to investigate the role of non-coding regions of grapevine and GLRaV-3 genomes in infection and symptom development. **Recommendation 4-4:** Support research to examine the common and unique responses of red or black- and white-fruited wine grape cultivars to GLRaV-3.
GRBD KNOWLEDGE GAPS
GRBD Biology, Interactions Between GRBV and its Hosts, and Host Defense Mechanisms
Conclusion 4-6: Knowledge of the biological differences between the major GRBV variants (clade 1 and clade 2 isolates) is incomplete.
Recommendation 4-5: Support studies to advance understanding of the epidemiological consequences of GRBV genetic diversity and interactions with other viruses. Research questions that need to be addressed include: • What are the biological differences (e.g., transmission efficiencies, symptom expression, physiological responses) arising from the genetic variation of GRBV isolates? • What are the consequences of co-infections of different GRBV variants?

Conclusion 4-7: Despite some progress in determining GRBV gene function, there are still major gaps in understanding the function of the GRBV genome with regard to specific roles of GRBV proteins in plant cells.

Conclusion 4-8: To date, virions have not been observed in GRBV-infected plants using microscopy; the lack of a tractable herbaceous model host that becomes systemically infected with GRBV limits the study of virus gene functions and virus-host interactions.

Recommendation 4-6 (MP): Support research to determine optimal model hosts (e.g., Pixie grapevine and/or herbaceous hosts) to facilitate the study of molecular plant-GRBV interactions and direct research efforts to transfer this knowledge to wine grape cultivars. Research questions that need to be addressed include the following:

- What functionally equivalent conserved host factors are required for GRBV infection of plants?
- What is the virion structure of GRBV?
- What or which varieties of herbaceous and/or Vitis hosts are the best model systems for studying virus-host interactions?

Conclusion 4-9: Current knowledge about latency and incubation periods after GRBV inoculation is insufficient. Questions about latency and incubation, which may vary among grapevine cultivars and under different environmental conditions, need to be refined because the answers could directly impact GRBD management recommendations to growers.

Recommendation 4-7 (HP): Support research to elucidate latency periods in different cultivars and rootstock-scion combinations, including the time from virus inoculation until vector acquisition, time until symptom expression, and time until the virus is detectable in plant and/or vector tissues. Research questions that need to be addressed include the following:

- How much of virus load in vineyards is due to planting with infected, non-certified vines and how much is due to insect inoculation after vine establishment?
- How long after vector-mediated inoculation will there be a systemic GRBV infection?
- How long after inoculation until new vector individuals can acquire GRBV?
- How long after inoculation will symptoms be expressed?
- • How do these latency periods vary among different varieties and rootstocks?

KNOWLEDGE GAPS REGARDING EFFECTS OF MIXED INFECTIONS, ENVIRONMENTAL FACTORS, AND ROOTSTOCK-SCION INTERACTIONS

Complex Effects of Mixed Infections and Effects of Environmental Factors

Conclusion 4-10: Infection of grapevines with multiple viruses has been reported, but how mixed infections affect disease severity and evolution of GRBV and GLRaVs (or GRBD and GLD) has not been thoroughly investigated.

Conclusion 4-11: The effects of changing climatic conditions and other factors (biotic and abiotic) that modulate disease cycles, including temperature, humidity, carbon dioxide, ozone, drought, and vineyard management practices on virus-vector-host interactions have not been determined.

Recommendation 4-8: Support research on the effects of mixed infections on GRBV and GLRaV evolution and the diseases they cause, as well as research on the effects of environmental factors, grapevine management practices, and changing climatic conditions on GRBD and GLD virus-vector-host interactions and epidemiology. Industry trends and stakeholder input could be used as a guide for prioritizing scion-rootstock combinations to use in experiments.
Research questions that need to be addressed include:

- Do co-infections of GLRaV-3 or GRBV with specific classes of grapevine viruses facilitate disease establishment or enhance its severity?
- What are the consequences of mixed infections of GLRaV-3 with other viruses (e.g., synergism, antagonism, neutral)?
- What are the consequences of mixed infections of GRBV with other viruses (e.g., synergism, antagonism, neutral)?
- How do abiotic factors, other stresses, and non-viral diseases influence disease caused by GLRaV-3 and GRBV?

Identification of Rootstock-Scion Interactions Relevant to Virus Transmission
Conclusion 4-12: A variety of factors, including the scion cultivar, genetic background of rootstock, rootstock-scion interactions, virus profile in individual grafted vines, synergistic interactions between co-infecting viruses, and environmental conditions, could contribute to the presence and severity of symptoms from GRBD and GLD. **Conclusion 4-13:** Resistant rootstocks along with other control strategies could help to mitigate negative effects of viral diseases in vineyards.
Recommendation 4-9 (MP): Support research on the presence and diversity of viral resistance in grapevine rootstocks with different genetic backgrounds in order to inform the incorporation of resistant rootstocks into virus control strategies. **Recommendation 4-10:** Support research to determine the contribution of planting with infected, non-certified vines on virus spread.
KNOWLEDGE GAPS IN GRBV AND GLRaV-3 DIAGNOSTICS AND DETECTION
Cost Effective, Field Deployable Tools to Detect GRBV and GLRaV-3
Conclusion 4-14: There is a need for additional affordable diagnostic tools that can detect GRBV and GLRaV-3 infections early and are suitable for extensive use in commercial vineyards.
Recommendation 4-11 (HP): Support research to develop any new, simple, and affordable high throughput tests for GRBV and GLRaV-3. Research may include the following: • Producing GRBV-specific antigens that could enable development of a serological assay. • Validating a simple crude plant extract-based LAMP and RPA assays for GLRaV-3 and GRBV to determine the suitability of isothermal assays for large scale and/or on-site detection. • Improving the automation testing capacity for existing GLRaV-3 ELISAs to improve throughput and reduce costs.
Conclusion 4-15: Canine olfactory capacity could be used for GRBV and GLRaV-3 field detection, but the most effective, practicable, and cost-effective way to employ dogs for monitoring and early detection has yet to be determined. Canine detection may be best suited for nurseries rather than commercial vineyards. **Conclusion 4-16:** Research to profile plant responses to GRBV and GLRaV-3 (and their vectors) may reveal unique VOC profiles that could establish a basis for the development of hand-held EN or DMS devices for pathogen detection in the field.
Recommendation 4-12: Support research to identify VOCs unique to GRBV and GLRaV-3 infection or relevant vector infestations and determine the detection efficiency of VOC-based methods compared with other diagnostic tools.
Conclusion 4-17: Remote sensing technology has the potential for remote or in-field diagnosis of GRBD and GLD in individual vines; however, testing the efficacy of this approach will require scalable deployment of remote sensing devices for detection of infected vines in a large-scale area. **Conclusion 4-18:** Remote sensing technology can be a part of a multi-layered system to guide sampling efforts by taking advantage of different spectra and resolutions to address specific goals. **Conclusion 4-19:** In addition to leaves, remote sensing devices can also potentially be used on other visible parts of the vines to detect grapevine viruses.
Recommendation 4-13 (HP): Support studies on the use of remote sensing technology to facilitate large-scale and early detection of GRBD and GLD in various tissues of commercial cultivars (including white cultivars) to increase the reliability, specificity, and sensitivity of detection with this technology.
Improved Methods for Detection of New GRBV and GLRaV-3 Variants
Conclusion 4-20: As GRBV and GLRaV-3 continue to evolve in vineyards and non-crop habitats, nucleic acid-based assays used for virus detection will need to be upgraded to enable reliable detection of newly emerged virus variants.

Recommendation 4-14: Support research to determine the feasibility of using RCA or other single-stranded circular DNA detection techniques to help detect GRBV at very low concentrations and for universal GRBV detection.

Recommendation 4-15 (HP): Support research aimed at improving GRBV and GLRaV-3 detection with nucleic acid-based methods that can be used in the field at large scales.

Optimal Sampling Strategies and Sample Size for Accurate Estimation of GRBV and GLRaV-3 Prevalence

Conclusion 4-21: Consensus is lacking on the most effective sampling technique and minimum sample size for accurately estimating GRBV and GLRaV-3 prevalence across different vineyard settings, regions, and nursery increase blocks.

Conclusion 4-22: Virus detection in vectors and other phloem feeding insects may be an alternative to testing grapevines for viruses.

Recommendation 4-16 (HP): Support research evaluating optimal sampling methods and minimum sample size for accurate estimation of GRBV and GLVaV-3 prevalence in vineyards to inform the development of best practices for adopting new technologies and for integrating multiple detection methods to improve accuracy and scale (i.e., using both molecular methods and remote sensing technology).

Standards for Diagnostic Testing in Nurseries, Commercial Vineyards, and Certification Programs

Conclusion 4-23: Laboratory protocols for diagnostic testing of GRBV and GLRaVs have not been standardized.

Recommendation 4-17 (HP): Support efforts to develop standardized GRBV and GLRaV-3 diagnostic testing protocols that, once verified and certified, could be adopted by all laboratories that provide testing services for nurseries and commercial vineyards.

Conclusion 4-24: HTS offers robust virus detection and discovery of new GRBV and GLRaV-3 variants, but HTS protocols need to be standardized, affordable for large-scale testing, and validated for use in diagnostic virus testing.

Recommendation 4-18: Support efforts to develop universally accepted guidelines for using HTS in GRBV and GLRaV-3 diagnostics.

KNOWLEDGE GAPS REGARDING GRBV AND GLRaV-3 VECTORS

Vector Transmission

Conclusion 4-25: While there are reports about potential additional insect vectors of GRBV, there has not been definitive evidence that other insects in addition to TCAH can transmit GRBV to grapevines.

Recommendation 4-19 (MP): Support research to identify additional vectors of GRBV using rigorous experimental approaches. Research to identify additional vectors should employ the following best practices:

- Select vector candidates for study based on field data suggesting an association between the insect and virus spread.
- Replicate controlled laboratory transmission experiments, including replicating experimental units (insects and plants) each time transmission is tested under a given set of conditions and replication of experiments to draw verifiable conclusions.
- Allow for a minimum time of 10 days for the acquisition access period, 10 days for the latent period, and 4 days for the inoculation access period based on the minimum times reported for TCAH. Males and females should be tested separately.
- Because plant viruses can be excreted and detected in honeydew, it is necessary to use a cleaning procedure to remove honeydew from plant tissue prior to virus testing. Methods designed to detect a viral RNA transcript could also prevent false positives due to contaminated honeydew.
- Testing transmission using artificial diets represents one way to demonstrate vector competence, but transmission to grapevines is needed to confirm the epidemiological significance of vector transmission in the field.

Conclusion 4-26: There are gaps in the understanding of GLRaV-3 transmission, particularly with regard to the role of different vector species and their distribution in California; the mechanisms of GLRaV-3 acquisition and transmission; the transmission efficiency of diverse GLRaV-3 isolates; the acquisition, retention, and inoculation periods of all vector species; and how environmental factors influence GLRaV-3 transmission dynamics.

Recommendation 4-20 (HP): Support research on the mechanisms and timing of acquisition, retention, and transmission of all GLRaV vector species, as well as the influence of environmental conditions and host genotype on GLRaV transmission dynamics. Research to identify additional vectors should employ the following best practices:

- Conduct transmission assays that individually assess acquisition, retention, and inoculation.
- Healthy vectors should be caged on infected plants for acquisition access periods (AAPs) that range from several hours to several days to assess acquisition efficiency.
- For inoculation assays, infected insects should be isolated in groups on healthy plants to assess virus transmission. Inoculation assays should utilize insects of similar developmental stage. Inoculation access periods (IAPs) can range from several hours to days, as longer IAPs yield higher transmission efficiencies.
- Transmission experiments in which insects feed on artificial media through a membrane can also be used to assess vector capacity, but ultimately this approach may not provide an accurate indicator of vector transmission capacity or efficiency.
- Transmission differences between vector species may be specific to grape cultivars and environment; therefore, comparisons of efficiency should be evaluated in controlled assays to assess the contributions of these factors to the epidemiology of vector transmission.
- • Differences in transmission efficiency among clones or populations of vector species should be evaluated using comparable AAPs and IAPs to effectively assess the epidemiological importance of particular vector species or phenotypic variation in transmission efficiency that exists in pathogen transmission.

Vector-Virus Interactions

Conclusion 4-27: Knowledge of virus localization in the vectors and the precise role of viral retention sites in vector transmission would improve knowledge about the mode of transmission for GRBV or GLRaV-3.

Conclusion 4-28: The roles of vector endosymbionts, genes, proteins, and metabolites mediating transmission have not been studied for GRBV or GLRaVs. This information is needed to understand transmission dynamics and to develop novel tools for disrupting transmission for the management of GLD.

Recommendation 4-21: Support studies to identify interactions between GRBV and GLRaVs and their vectors that are required for transmission, as well as studies to identify genes, proteins, and metabolites involved in virus transmission to develop control strategies based on interference of virus-vector interactions.

Vector Plant Preference and Behavior Manipulation by GRBV and GLRaVs

Conclusion 4-29: GRBV and GLRaV-3 have only been reported to occur on Vitis and non-cultivated grapevines, but the relative contributions of different host species or varieties in GRBV or GLRaV-3 spread are not known.

Conclusion 4-30: Comprehensive studies to understand host plant utilization and preferences of vectors have not been completed.

Conclusion 4-31: Vector behavior might change in response to plant infection by GRBV and GLRaV-3 (i.e., changes in insect behavior mediated through the host plant), which may affect the settling, feeding, fitness, and dispersal behavior of the vectors.

Recommendation 4-22 (MP): Support research on virus-vector-host interactions to determine how the different species or varieties of *Vitis* and non-cultivated grapevines contribute to virus spread, as well as how GRBV or GLRaV-3 infection of the host can alter vector behavior.

Recommendation 4-23 (MP): Support research to broaden the understanding of complex interactions among the virus, vector, and host to enable the development of models of disease spread and strategies to prevent disease transmission. Possible research approaches include the following:

- Host choice experiments, olfactometer assays, or electrophysiological studies to assess vector responses to VOCs emitted by GRBV and GLRaV-3-infected plants.
- Experiments with nonviruliferous (have not acquired virus) and viruliferous (have acquired virus) vectors to determine whether the presence of GRBV and GLRaV-3 alters vector behavior with respect to host plant selection, frequency of movement between plants, feeding, or reproduction.

Conclusion 4-32: There are major knowledge gaps regarding the TCAH overwintering behavior, seasonal GRBV spread to grapevines, and differences among distinct grapevine-growing regions in California.

Conclusion 4-33: Population models may help predict TCAH generation development associated with TCAH movement into vineyards; models may need to include information other than temperature to accurately predict population development and movement behavior.

Recommendation 4-24 (MP): Support research on the seasonal virus spread of GRBV by TCAH, focusing on year-long TCAH abundance and overwintering behavior throughout California. Studying seasonal spread of GRBV by TCAH could involve the following:

- Optimizing sampling methodology for the most accurate estimations of TCAH abundance.
- Increase sampling efforts in fall and spring when populations have been low in previous studies.
- Perform sampling in multiple locations across different grape production regions and in multiple years to account for inter-annual variation in population dynamics.
- Develop population models that may assist with the monitoring and management of TCAH.
- Sample for TCAH in natural vegetation and vineyard-adjacent habitat.

Recommendation 4-25: Support research to investigate TCAH host preference and movement behavior, which could help in the development of a trap crop strategy for intercepting TCAH at vineyard borders. Studying TCAH host preference could involve the following:

- Greenhouse studies to determine whether TCAH readily move between grapevines and alternative hosts, or if they prefer to remain on hosts other than grapevines.
- Experiments with nonviruliferous (have not acquired GRBV) and viruliferous (have acquired GRBV) individuals to determine whether the presence of the virus is altering vector behavior with respect to host plant selection, frequency of movement between plants, feeding, or reproduction.
- If a host plant is more attractive to TCAH than grapevines such that TCAH selects and largely remains on that host, then field studies could be conducted to confirm that this behavior occurs under natural conditions.

RESEARCH AND ACTIONS THAT MAY YIELD THE MOST PROMISING MANAGEMENT SOLUTIONS

Clean Plants

Conclusion 5-1: Using clean planting material is the first line of defense in establishing healthy vineyards because viruses can spread via clonal propagation of grapevines.

Conclusion 5-2: There are concerns regarding the reliability of results from testing laboratories; these stem from questions about whether testing for GLRaVs and GRBV is being done using the most up-to-date protocols to detect all variants, and from the fact that commercial testing laboratories are largely unregulated in their technical standards, potentially resulting in inconsistencies in diagnostic results across laboratories.

Recommendation 5-1 (HP): Encourage the adoption and implementation of higher sanitary standards in registered mother blocks using robust, evidence-based sampling strategies; state-of-the-art, sensitive, and reliable diagnostic methods; and roguing of infected vines to maintain disease-free stock and provide clean planting materials for growers.
This could include engaging FPS in exploring the potential of developing a ring-test process or similar validation scheme to better assure the validity and reliability of diagnostics from laboratories working with the industry.

Roguing Infected Vines

Conclusion 5-3: Roguing has been shown to be effective in GLD management and in mitigating GRBD spread, but it can be difficult for growers to justify removing infected but still productive vines and replacing them with new vines that will not immediately bear fruits. Both roguing and roguing followed by replanting also complicates viticultural practices in vineyards.

Conclusion 5-4: There is insufficient information available for developing effective roguing schemes for GLD and GRBD. Specifically, more data is needed on the determination of threshold decision points, the cost-effectiveness of roguing under various conditions, and the influence of movement patterns and flight behavior of TCAH and other potential GRBV vectors on the spread of GRBD.

Conclusion 5-5: Roguing schemes need to be optimized for California production regions in light of differences in market economics and in the environmental conditions that affect vector and virus dynamics. Additional epidemiological research may reveal the optimum roguing and replanting schemes for both GLD and GRBD in different production regions and for vineyards with differing business models.

Recommendation 5-2 (HP): Support research to develop optimal roguing and replanting schemes and techniques to manage GLD and GRBD, and to facilitate their implementation by growers.
This could include studies to determine:

- The cost-effectiveness of roguing and/or replanting based on disease incidence and rate of spread.
- How long it typically takes for newly-planted clean grapevines to become infected and become sources of inoculum.
- Best practices for removal of remnant root systems of rogued vines to prevent them from serving as reservoirs for the vector and virus.
- Roguing schemes suited to the different grape production regions in California.

Vector Management

Conclusion 5-6: Contact insecticides are not effective in controlling mealybugs due to the cryptic nature of mealybug behavior. Systemic insecticides will not likely disrupt feeding quickly enough to stop transmission of GLRaVs, but they could be effective in reducing mealybug populations. In addition to their crypsis, the sessile nature of mealybugs suggests that systemic insecticides, even if slow acting, could reduce secondary spread of GLRaV-3. Primary spread from mealybugs entering vineyards would require a more rapid kill time.

Conclusion 5-7: Knowledge of factors that affect the efficacy of insecticides (such as physiology of the plant, environmental conditions, soil type, insect behavior, insecticide application methods) is important in developing improved guidelines for their application.

Conclusion 5-8: Reliance on a small set of insecticides for mealybug control increases the likelihood that mealybugs will develop resistance to them.

Conclusion 5-9: A better understanding of GRBV acquisition and transmission dynamics is needed to improve the effectiveness of insecticide application as a control tactic against TCAH, and appropriate economic or action thresholds are needed to guide insecticide application programs.

Recommendation 5-3 (HP): Support research to determine the optimal conditions for the application of systemic insecticides to achieve better mealybug control.

Recommendation 5-4 (HP): Develop and implement insecticide resistance management programs and support research to develop new active ingredients for mealybug management, including by evaluating the efficacy of natural products such as plant essential oils, that could provide additional options for both organic and conventional vineyards.

Recommendation 5-5 (HP): Support research to determine the optimum conditions for the application of insecticides to achieve better TCAH control and to establish economic or action thresholds to guide insecticide application programs.

Conclusion 5-10: Mating disruption tends to be most effective in reducing mealybug populations when used over longer timescales and on larger spatial scales. More information is needed to determine the optimum number and type of pheromone dispensers to use to ensure coverage in time and space while reducing the cost of employing this technique.

Conclusion 5-11: Mating disruption has been shown to decrease vine mealybug populations and damage, but no studies have been done to determine the impact of mating disruption on GLRaV-3 spread.

Conclusion 5-12: Knowledge about the mating disruption mechanism in mealybugs (i.e., competitive or noncompetitive) and about mealybug biology, behavior, and generation development could help identify optimal times for dispersing pheromones to disrupt mating. In-field or predictive population models of mealybug generation may also help guide timing of mating disruption activities.

Conclusion 5-13: Studies are needed to determine how long mating disruption can suppress mealybug populations and guide the use, frequency, and timing of insecticide applications to keep mealybug populations low.

Conclusion 5-14: Studies are needed to determine and compare the short- and long-term efficacy and economics of various techniques for applying pheromones in mating disruption programs.

Conclusion 5-15: Studies are needed to inform integrated pest management (IPM) decision making by elucidating the potential impacts of biological control tactics such as leveraging natural enemies alongside mating disruption programs.

Conclusion 5-16: Mating disruption is not likely to be a practical management tactic for TCAH as leafhoppers do not appear to use long-range sex pheromones to locate mates but instead use substrate-borne vibrational signals that occur off of grapevines.

Recommendation 5-6 (HP): Support research to generate information needed for improving the efficacy of mating disruption for mealybug control and to determine the benefits (economic and otherwise) of employing this technique as part of an integrated approach to manage insect vectors in grapevines.
This could include studies to determine:

- The optimum number and type of pheromone dispensers for ensuring coverage over an extended period over a large area.
- Mealybug mating behavior, seasonal adult male flight behavior, seasonal sex ratios, regional differences in the timing of male flights, generation development, and the mechanism of mating disruption in mealybugs.
- How long mating disruption can suppress mealybug populations and how insecticides and natural enemies can be used to complement mating disruption to keep mealybug populations low.
- • The impact of mating disruption on GLRaV-3 spread.

Conclusion 5-17: Emerging research suggests the use of UV-C light could help to suppress pest populations without negatively impacting crop yield. However, further refinement of this method is needed to make it an effective tool for vine mealybug management in vineyards.

Recommendation 5-7: Support research to further refine UV-C treatment of grapevines to complement other IPM strategies to suppress field populations of mealybug vectors in vineyards.

Cultural Control

Conclusion 5-18: Removal of vegetation (such as legumes, which serve as reproductive hosts) between rows of grapevines in the spring may reduce populations of TCAH within vineyards, but information about the cost and benefits of this practice is lacking.

Conclusion 5-19: Trap crops have been shown to reduce the spread of non-persistently transmitted viruses, but the feasibility of using trap crops to control GRBV, which is persistently transmitted by TCAH, has not been determined.

Recommendation 5-8 (MP): Support research to determine the costs and benefits of removing vegetation that harbors TCAH in and around vineyards and the use of trap crops to inform grower decision-making regarding the employment of these methods for managing TCAH in vineyards.

Biological Control

Conclusion 5-20: Parasitoids, predators, and EPF have been identified that could be further studied for development as biocontrol agents for use in IPM programs targeting mealybugs.

Conclusion 5-21: EPF strains currently available for use on grapevines require repeated applications to be effective and may lose virulence when exposed to high temperatures and UV light; identification and mitigation of factors that degrade EPFs could help improve their utility in IPM programs or in situations where the use of chemical insecticides is not an option.

Conclusion 5-22: Because ants support mealybug survival in vineyards, more emphasis on ant management is needed to help suppress mealybug populations and increase the impact of other biocontrol strategies.

Conclusion 5-23: There is a dearth of research on biocontrol of TCAH; if research is pursued, it will be important to address the impacts of ants, which tend TCAH nymphs, on potential biocontrol agent(s).
Recommendation 5-9: Support research to find, evaluate, and develop more efficacious biocontrol agents and their integration with other management tactics within IPM programs or in situations, such as organic production systems, where chemical insecticides are not an option for vector management in grapevines.
Sanitation
Conclusion 5-24: Cleaning harvesting and pruning equipment, tools, and workers' protective equipment has been shown to limit the dispersal of mealybugs; however, there is a general lack of publicly available information about best practices for sanitation in vineyard settings and the degree to which sanitation measures are employed is unknown.
Recommendation 5-10 (HP): Support research to determine the most effective and practical farm and worker equipment sanitation measures and harvesting and pruning strategies that can help minimize the spread of insect vectors.
Physical Barriers
Conclusion 5-25: Information about TCAH flight behavior and movement could be used to devise and evaluate possible barriers such as screening fences and kaolin clay to impede TCAH movement from riparian areas to vineyards. **Conclusion 5-26:** Installing protective screens over citrus trees is effective for keeping them disease-free; however, this tactic is costly and may be most applicable for smaller acreages of crops with a high return on investment. **Conclusion 5-27:** Covering individual vines with mesh bags may be a less costly tactic for vector exclusion; this approach has been widely adopted by citrus growers in Florida as an IPM tool to control HLB. **Conclusion 5-28:** Reflective mulches have the potential to reduce leafhopper populations in grapes without any detrimental effects on vine physiology and berry quality; however, these mulches degrade over time.
Recommendation 5-11 (MP): Support research to evaluate the efficacy of physical barriers in deterring TCAH movement from natural or vineyard-adjacent habitats to vineyards. **Recommendation 5-12 (MP):** Support research to evaluate the efficacy of reflective mulches in reducing the abundance of insect vectors in vineyards and research on improving the longevity and durability of reflective mulches.
Areawide Pest Management
Conclusion 5-29: Areawide pest management, which is well suited for pests that move beyond the boundaries of individual farms, can help in managing insect-vectored viruses in vineyards across larger areas.
Recommendation 5-13 (HP): Support efforts to develop areawide GLD and GRBD vector management programs for regions of California with different threat levels from these diseases, along with activities to encourage grower participation in these programs.
Coordinating Management of Multiple Vectors
Conclusion 5-30: Pierce's disease, GLD, and GRBD are all spread by hemipterans and insecticides used to control one vector species may also affect the other vectors; hence, it is important to coordinate vector management tactics for vectors of all three diseases.
Host Plant Resistance to Viruses and Vectors
Conclusion 5-31: Host plant resistance is an effective and sustainable tactic for controlling vector-borne virus diseases, especially when used as a component of an IPM strategy. **Conclusion 5-32:** The choice of approach (traditional breeding or bioengineering strategies such as transgenic approaches or gene editing) for achieving host resistance has implications for the length of time required to create a resistant grapevine cultivar, the expediency of obtaining regulatory approval, and consumer acceptance. **Conclusion 5-33:** RNAi-based resistance to plant viruses has been shown to be highly effective and durable for annual and perennial crops; this approach could produce a resistant grape cultivar within a relatively short period of time.

Conclusion 5-34: Genome editing for developing host resistance to GLRaVs, GRBV, and their vectors requires knowledge of virus-host and vector-host interactions and the collaborative efforts of researchers from multiple disciplines. **Conclusion 5-35:** Gene-edited crops are not subject to the same regulatory processes as genetically modified organisms in the United States and could therefore lead to faster commercialization of a resistant grapevine cultivar; however, information on virus-host and vector-host interactions necessary for determining appropriate edits is not yet available.
Recommendation 5-14 (HP): Support research using traditional and bioengineering approaches for developing GLD and GRBD resistance; when conducting resistance screening assays, the biological vector should be used as much as possible. **Recommendation 5-15:** Support research on the use of transgenic RNAi for developing plants with virus and/or insect resistance; creating a trangene(s) combining resistance to GLRaV-3 and GRBV could provide effective resistance to both viruses and help reduce the burden of regulatory approval. **Recommendation 5-16:** Develop grapevine as a model system to advance fundamental understanding of the entire network of virus-host interactions across cultivars. **Recommendation 5-17 (HP):** Establish multidisciplinary and trans-institutional collaborations to enhance synergies in pursuing bioengineering approaches, such as RNAi-mediated resistance and CRISPR/Cas-based genome-editing technologies, as an alternative to traditional breeding for resistance against GLD and GRBD.
Cross Protection Strategies
Conclusion 5-36: The identification of a mild and asymptomatic strain of GLRaV-2 (GLRaV-2-SG) that does not cause any significant damage to grapevine, and a mild strain of GLRaV-3 (ID45) points to the potential to apply cross protection in GLD management.
Recommendation 5-18: Support research to explore cross protection as a possible tactic for managing GLD.
Risk Assessment Models to Guide Decision Making
Conclusion 5-37: The Bayesian Belief Network model, which can be used to assess the probability of GLRaV-3 and GRBV outbreaks, could be helpful in informing GLD and GRBD management decision making.
Recommendation 5-19: Support research to evaluate the potential utility of the Bayesian Belief Network model in informing growers' decisions related to GLRaV-3 and GRBV management.
CONSIDERATIONS FOR FUTURE RESEARCH ON GRAPEVINE VIRUSES AND DISEASES
Genetic Pest Management
Conclusion 6-1: Genetic pest management strategies, in which the insect vector is modified rather than the plant, offer opportunities to curb the spread of disease by reducing vector populations or their ability to transmit viruses. The biology of mealybug vectors makes them good targets for genetic pest management. **Conclusion 6-2:** Multidisciplinary research teams composed of molecular biologists, entomologists, modelers, and field biologists or extension researchers are needed to develop genetic pest management strategies and to predict their real-world implications. **Conclusion 6-3:** Sociological aspects and consumer acceptance are important considerations when developing genetic pest management strategies.
Recommendation 6-1: Support basic research to enable genetic pest management strategies for GLD and GRBD vectors and support modeling and sociological research to predict whether these strategies will be effective in the field and be accepted by the public.
INSIGHTS AND ADDITIONAL RESEARCH DIRECTIONS FROM OTHER PATHOSYSTEMS
Tactics for Controlling Insect Vectors
Conclusion 6-4: RNAi has the potential for use in managing viruses, their insect vectors, and potential other grapevine pests. Applied RNAi biopesticides should have narrow activity based on target-specific dsRNA that will trigger RNAi suppression only in the targeted organism, and no activity in other beneficial insects. Genetically

engineered plants expressing dsRNA may more effectively manage mealybugs and other insects that reside under bark where it is hard to contact them with insecticide sprays.
Recommendation 6-2 (MP): Consider supporting interdisciplinary research teams to advance RNAi research for the suppression of vectors in vineyards.
Conclusion 6-5: Nanobodies present a promising strategy for managing grapevine viruses like GLRaV-3 and GRBV, given their high specificity and efficacy in targeting viral proteins. However, successful application in vineyards depends on overcoming challenges related to scalable production, cost-effectiveness, and long-term stability under field conditions.
Recommendation 6-3: Consider supporting research to advance the development of nanobodies for the control of GLRaV-3 and GRBV through transgenic or exogenous approaches. This could include monitoring and funding multidisciplinary, collaborative efforts to refine nanobody production methods to improve scalability and affordability, as well as supporting field trials to rigorously assess the performance and durability of nanobodies in diverse vineyard environments to ensure they are a practical and sustainable solution for virus management.
Conclusion 6-6: Trunk injection has been used for delivering pesticides directly to the plant vasculature to control diseases in citrus, almond, apricot, and palm trees. This delivery method, which is more precise and has a lower risk of non-target effects, may be applicable in controlling phloem-limited pathogens as well as phloem-feeding insects in vineyards.
Recommendation 6-4 (MP): Consider supporting research to investigate the potential utility of trunk injection to control vectors and viruses with various pesticides (including new approaches such as RNAi and nanobodies) in grapevines.
Prediction Models and Risk Indexes as Management Tools
Conclusion 6-7: Models have been valuable tools for stakeholders to understand pest risk, apply practices that mitigate risk, and know critical windows of time for scouting and management activities.
Recommendation 6-5 (HP): Fund epidemiology research that will lead to the development of publicly available, regionally relevant insect population models and disease risk models that can be used to guide local and areawide management activities for GLD and GRBD.
ENGAGING A WIDER RANGE OF RESEARCHERS IN ADDRESSING RESEARCH NEEDS
Conclusion 6-8: Researchers who are not familiar with the PD/GWSS Board research and outreach grant program may not be aware that this program also funds research on other grapevine viruses and pests, such as GLRaVs and GRBV and their vectors. Allocating funding specifically for early and mid-career scientists may help expand the pool of researchers working on grapevine virus diseases.
Recommendation 6-6 (HP): To draw in diverse researchers, consider changing the name of the PD/GWSS Board research and outreach grants to accurately reflect the scope of its RFPs, which include multiple grapevine virus diseases and their insect vectors. **Recommendation 6-7:** To increase awareness of the work of the PD/GWSS Board and bring in new scientists to address grapevine vector-borne diseases of national and global significance, expand efforts to promote the funding portfolio and RFPs to more diverse research communities via social media, professional societies, and other mechanisms. **Recommendation 6-8 (MP):** Consider offering specific funding for early and mid-career researchers to encourage engagement in grapevine virus diseases research and build a network of scientists to address long-term questions.
Conclusion 6-9: In addition to traditional RFP cycles, research may be funded through other mechanisms such as inviting researchers to address specific topics.
Recommendation 6-9 (HP): Consider developing additional funding mechanisms to address particular needs for GLD or GRBD research, such as through inviting specific researchers to address particular knowledge gaps or accepting off-cycle proposals for projects that have potential to generate information for dramatically improving GLD and GRBD management.
ADDRESSING THE NEED FOR LONGER-TERM STUDIES AND REPLICABILITY
Conclusion 6-10: The study of complex systems, such as vector-borne diseases in perennial crops may take longer than three years and require more funding to accurately describe disease biology and make recommendations for disease or vector management.

Conclusion 6-11: Replicability of results is an important issue, especially with GRBV because of knowledge gaps in virus biology and vector transmission.

Conclusion 6-12: Collaborative research proposals provide a mechanism to support multiple research teams addressing the same research questions.

Recommendation 6-10 (HP): Consider funding longer-term projects (lasting more than three years) such as studies that advance control recommendations, translational research, and projects that integrate economic and societal impacts.

Recommendation 6-11 (HP): Consider funding research to replicate experimental results in more than one location and with different research teams to obtain more robust and reliable insights.

Recommendation 6-12: Consider new ways to leverage available funds using different proposal and award structures to encourage collaboration.

KNOWLEDGE SHARING AND COLLABORATIVE RESEARCH

Interdisciplinary Approach to Vector-Borne Disease Research

Conclusion 6-13: GLD and GRBD research would benefit from an interdisciplinary approach, wherein findings and perspectives of experts from various disciplines and growers are integrated to gain a holistic understanding of a complex problem.

Recommendation 6-13: Consider allocating funding specifically for research projects that employ an interdisciplinary approach.

Fostering Information Sharing, Interactions, and Collaboration

Conclusion 6-14: Sharing of information and collaboration among researchers are essential to interdisciplinary research and to facilitating a "systems thinking" approach for solving complex problems.

Conclusion 6-15: Groups such as WERA 20 and EVCWG have effectively facilitated the dissemination of information and the exchange of ideas about virus diseases in crops among researchers, extension agents, growers, and other stakeholders.

Recommendation 6-14 (MP): As an alternative to the annual Pierce's disease symposium, consider coordinating with other organizations to hold sessions on GLD and GRBD at events such as the annual conference of the American Society for Enology and Viticulture and the Unified Wine and Grape Symposium. These sessions could also serve as a platform to facilitate new collaborations involving scientists working on other grape diseases or working in other wine grape producing regions.

Recommendation 6-15: Consider enhancing PD/GWSS Board participation in WERA20 annual meetings through sponsorship of workshops to build synergies and facilitate cross-pollination of strategies and technologies across specialty crops.

Recommendation 6-16 (HP): Explore the feasibility of creating a working group, supported by the wine grape industry and funded by another entity, that can facilitate information sharing and foster collaboration among GLD and GRBD researchers.

EDUCATION AND OUTREACH

Information Dissemination

Conclusion 6-16: Gaps in communication and knowledge dissemination contribute to the underutilization of GLD and GRBD management practices, underscoring the importance of having more effective educational and outreach strategies as knowledge of GLD and GRBD advances.

Recommendation 6-17: Consider allocating funds for projects to advance innovative educational and outreach strategies to help improve grower and extension educator knowledge of GLD and GRBD and strategies for their control.

Recommendation 6-18 (HP): Provide opportunities for funded researchers to share findings and recommendations regarding grapevine viruses via a dedicated website or a virtual town hall that facilitates interactive discussions about GLD and GRBD among researchers, extension agents, and growers.

Grower Adoption of Disease Control Strategies
Conclusion 6-17: Successful control of vector-borne diseases relies not only on understanding the pathosystem and devising strategies to control the pathogen or its vector, but also on what growers decide to do, known as "willingness to adopt" (e.g., to participate in areawide pest management programs or not). **Conclusion 6-18:** Social science research has shown that social networks play an important role in social learning (learning by observing others) and subsequently, in the adoption of innovations (e.g., pest management practices).
Recommendation 6-19 (HP): Support research to better understand the sociological aspects of managing vector-borne diseases through collective action (i.e., areawide pest management) and find ways to increase grower participation in areawide pest management programs. **Recommendation 6-20 (HP):** Support research on understanding and improving the flow of information across grower social networks and on outreach efforts to understand the drivers and barriers to successful adoption of GLD and GBRD management practices.