

FINAL REPORT

Washington State Grape and Wine Research Program

Project Title: Epidemiology and management of viral diseases in Washington vineyards

Principal Investigator: Naidu Rayapati, WSU-IAREC

Summary

Managing viral diseases in vineyards is a top priority for sustainable growth of Washington's grape and wine industry. Vineyard surveys and testing samples using molecular diagnostic assays and high-throughput sequencing (HTS) technology revealed the presence of fifteen viruses in Washington vineyards. Information on the current status of viruses documented in vineyards is used in grapevine certification and quarantine programs to implement proactive measures for protecting Washington's young wine industry from 'alien' viruses reported in grapevines worldwide. Among the fifteen viruses, Grapevine leafroll-associated virus 3 (GLRaV-3) causing leafroll disease was more found to be insidious and widespread than Grapevine red blotch virus (GRBV) causing red blotch disease in the State vineyards. Since GLRaV-3 and GRBV produce fairly similar symptoms in red-fruited cultivars and mild symptoms or no obvious symptoms in white-fruited cultivars, accurate diagnosis is fundamental for managing these two distinct diseases. Data on genetic variability of GLRaVs and GRBV provided additional knowledge for improved detection and management of leafroll and red blotch diseases.

Tobacco ring spot virus (TRSV) and Grapevine fanleaf virus (GFLV) were detected in vineyards showing fanleaf degeneration/decline symptoms. However, these two viruses were found sporadically in Washington vineyards. Field studies have shown that TRSV significantly affects vineyard lifespan and fruit yield, and the dagger nematode (*Xiphinema rivesi*) present in vineyard soil can spread TRSV from infected to healthy vines. Preliminary studies indicated that *X. rivesi* is unlikely to spread GFLV. Thus, GFLV can be eliminated by using virus-tested 'clean' plants due to the absence of its nematode vector, *X. index*, whereas management of TRSV requires a combination of 'clean' plants and post-planting management of dagger nematodes.

Large-scale testing of grapevine samples at commercial diagnostic services is prohibitively costly for growers to readily embrace diagnostics for managing viral diseases in vineyards. During this project, activities were conducted to improve viral diagnostic assays for reliable detection of GLRaV-3 and GRBV. In addition, an economic study was conducted to estimate the costs associated with testing for GLRaV-3 and GRBV by molecular diagnostic methods under non-commercial settings. The results showed that employing a simplified sample preparation protocol in combination with improved molecular diagnostics assays can offer cost-effective methods for high-throughput and reliable detection of GLRaV-3 and GRBV in grapevine samples benefiting growers, nurseries and other end users.

Studies in commercial vineyards have shown that both GLRaV-3 and GRBV can cause significant impacts on fruit yield and grape quality in own-rooted and top-grafted red- and white-fruited wine grape cultivars. These impacts were found to be variable depending on the cultivar, season and vineyard location. These results provided a foundation to further elucidate grapevine-virus-environment interactions for a comprehensive understanding of impacts of viral diseases in vineyards under diverse wine grape-growing climates. Field studies have indicated absence of vine-to-vine spread of GRBV and roguing of infected vines followed by replanting with healthy

FINAL REPORT

cuttings can be used as a low-cost strategy to manage red blotch disease in vineyards. In contrast, rogueing to control GLRaV-3 was found to be successful in some vineyards, while this approach was not effective in other vineyards due to field spread of leafroll disease. This calls for additional research to study the dynamics of disease epidemics in different AVAs under varying geo-climatic conditions and vector management tactics for implementing integrated, area-wide control strategies against leafroll.

The project results were disseminated to growers, viticulturists, crop consultants and regulatory agencies at industry-sponsored meetings and workshops and field staff during vineyard visits. The project results were shared with scientific and extension communities worldwide through publications in peer-reviewed journals, industry-magazines and presentations at professional scientific meetings.

FINAL REPORT

Washington State Grape and Wine Research Program

PROJECT TITLE: EPIDEMIOLOGY AND MANAGEMENT OF VIRAL DISEASES IN WASHINGTON VINEYARDS

Project Duration: July 2017 – June 2020

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Project Objective(s):

Healthy vineyards are the foundation for sustainable and profitable grape and wine industry. Therefore, **the overall goal of this project was to conduct translational research for practical applications to mitigate negative impacts of viral diseases in Washington vineyards.** Towards this goal, the following activities were carried out synergistically with funding support from different resources and via interdisciplinary and trans-institutional collaborations to maximize research outputs and impacts. Participatory, collaborative approaches were pursued with growers to conduct the project activities in commercial vineyards. Education and outreach activities were organized to ensure timely dissemination of science-based knowledge for maximizing impacts of the project outputs.

Project activities:

Activity 1. Delineate viral spread in young vineyards: is it via planting stock and/or by vectors?
Activity 2. Early intervention strategies for management of viral diseases in new plantings.

FINAL REPORT

Activity 3. Phenomics of grapevine responses to viral infections: impacts on crop yield and fruit quality in own-rooted and top-grafted vineyards.

Activity 4. Cheaper, faster, and reliable diagnostic assays benefiting growers and nurseries.

Activity 5. Tackle minor issues right away before they become major problems: surveillance of vineyards for emerging viruses.

Note: Activity # 1 and #2 are interconnected and combined during the project period.

Summary of Major Research Accomplishments and Results by Objective:

Activity: Tackle minor issues right away before they become major problems: surveillance of vineyards for emerging viruses.

i. Current status of viruses in Washington vineyards.

Vineyard surveys were conducted during the project period to document the presence of viruses in commercial vineyards. Samples were collected from commercial vineyards in eight American Viticultural Areas (AVAs) in eastern Washington State; namely, Yakima Valley, Horse Heaven Hills, Walla Walla Valley, Rattlesnake Hills, Columbia Valley, Lake Chelan, Wahluke Slopes and Red Mountain. Leaf samples were collected from red-fruited cultivars showing typical symptoms of leafroll, red blotch and fanleaf degeneration and decline and from vines suspected for viral symptoms. Leaf samples from white grape cultivars were collected randomly, since they show either mild or no apparent symptoms of viral infections. Samples were processed using methods optimized in Rayapati's lab and tested by one step-single tube molecular diagnostic assays for the presence different viruses, with emphasis on Grapevine leafroll-associated virus 3 (GLRaV-3) and Grapevine red blotch virus (GRBV), the two most economically important viruses. Reverse transcription (RT)-polymerase chain reaction (PCR) was used for the detection of GLRaV-3 that has RNA genome and PCR was used for the detection of GRBV that has DNA genome. In the case of grapevines showing fanleaf degeneration/decline symptoms, RT-PCR-based molecular diagnostic assays were conducted to detect nematode-transmitted viruses. Where ever necessary, cloning and sequence analyses of viral genome fragments amplified in PCR assays were carried out for additional confirmation.

In addition to RT-PCR/PCR assays, next generation high-throughput sequencing (HTS) using Illumina technology was used to determine the viral composition or virome of diseased vines. This technology enabled us to not only detect commonly known viruses associated with leafroll, red blotch and fanleaf degeneration/decline symptoms, but also identify viruses not reported in Washington vineyards. A list of viruses currently documented in Washington vineyards is shown in Table 1.

PCR-based diagnostics and HTS technology will be used in our future research to estimate the relative incidence of different viruses in vineyards and use this knowledge for managing viral diseases and strengthening grapevine quarantine and certification programs to ensure 'alien' viruses are not introduced into Washington vineyards.

FINAL REPORT

*Table 1. A consolidated list of viruses currently documented in commercial vineyards**

Viruses	Disease/Disorder
Grapevine leafroll-associated virus 1 (GLRaV-1), GLRaV-2, GLRaV-3, and GLRaV-4 and two strains (GLRaV-5, GLRaV-9) of GLRaV-4.	Leafroll
Grapevine red blotch virus (GRBV)	Red blotch
Grapevine fanleaf virus (GFLV) and Tobacco ring spot virus (TRSV)	Fanleaf degeneration/decline
Grapevine Rupestris stem pitting-associated virus (GRSPaV), Grapevine virus A (GVA), GVB, GVE	Rugose wood complex
Grapevine fleck virus	Fleck
Grapevine Syrah virus 1	Syrah decline?
Grapevine rupestris vein feathering virus (GRVfV)	Unknown
Grapevine red globe virus (GRGV)	

*There are about eight five viruses reported in grapevines worldwide. Viruses other than those listed in the table may likely be present but not yet found in Washington vineyards.

ii. Relative incidence of leafroll and red blotch diseases in Washington vineyards.

Since GLRaV-3 and GRBV are economically important viruses, studies were continued during the project period to document their relative occurrence in vineyards. Samples from a total of 2,063 vines from 18 red-fruited and 7 white-fruited wine grape cultivars were collected from eight 8 AVAs during 2014 - 2016 season and tested for GLRaV-3 and GRBV by PCR-based assays optimized in Rayapati's lab. The results indicated that about 68% of total virus-positive samples were positive for GLRaV-3, whereas about 6% were positive for GRBV. Less than 10% of virus-positive samples were positive for both GLRaV-3 and GRBV, indicating low frequency of co-infections in individual vines. These results were consistent with samples tested between 2017 and 2019 seasons, where 85% of total 2,109 virus-positive samples were positive for GLRaV-3, about 12% positive for GRBV and 3% positive for both viruses. Overall results obtained during 2014 and 2019 seasons indicated that GLRaV-3 is more common than GRBV in Washington vineyards.

The occurrence of GLRaV-3 and GRBV as single and co-infection in samples collected from individual AVAs was analyzed to determine possible differences in infection rates within and across AVAs. Overall results (Fig. 1) indicated that the infection rate of GLRaV-3 alone was significantly high in samples tested from all AVAs compared to GRBV as single and co-infection with GLRaV-3. Additionally, the occurrence GRBV as single versus co-infection with GLRaV-3 showed differences within and across AVAs. The proportion of vines infected only with GRBV was higher than co-infection with GLRaV-3 in Rattlesnake Hills, Wahluke Slope and Walla Walla Valley AVAs. In contrast, the proportion of vines infected with both GLRaV-3 and GRBV as co-infections was higher than vines infected with GRBV alone in Columbia Valley, Horse Heaven Hills and Yakima Valley AVA. Conversely, none of the samples from Red Mountain AVA tested positive for both viruses, indicating absence of co-infection of the two viruses. These results indicated that GLRaV-3 is widely distributed than GRBV in all major wine regions independent of cultivars and geographic origin.

FINAL REPORT

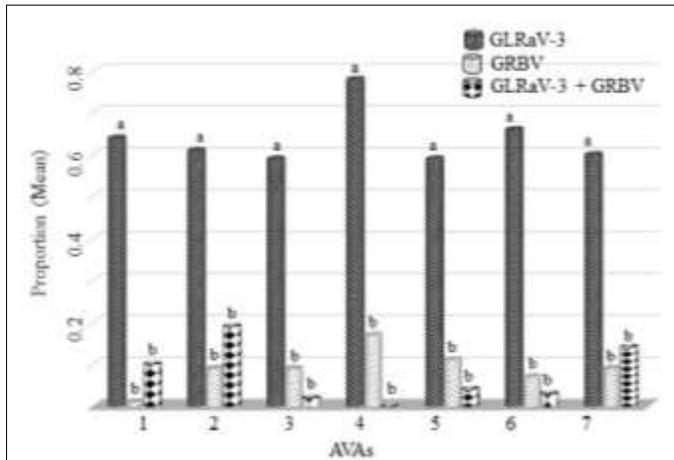


Fig. 1. Proportion of samples collected from individual vines from each AVA that were tested positive for GLRaV-3 or GRBV as single and co-infections. Samples positive for GLRaV-3 and GRBV were included in this analysis, while excluding samples negative for both viruses. Bars with different letters indicate significant difference ($P < 0.05$). AVAs shown are: 1, Columbia Valley; 2, Horse Heaven Hills; 3, Rattlesnake Hills; 4 Red Mountain; 5, Wahluke Slope; 6, Walla Walla Valley; 7, Yakima Valley.

Lake Chelan AVA was not shown due to small number of samples collected. (Reproduced from Adiputra et al., 2018. *Plant Disease* 102: 2129-2135.)

This multi-season study also indicated that symptom-based diagnosis of leafroll and red blotch diseases in vineyards is unreliable due to overlapping or similar symptoms in red-fruited cultivars. It was also clear that both diseases produce either mild or no apparent symptoms in white-fruited cultivars. Thus, reliable detection of GLRaV-3 and GRBV is essential to differentiate symptoms of between leafroll and red blotch for implementing robust measures to control these two distinct viral diseases in vineyards. Additionally, the use of diagnostic assays will help distinguishing leafroll and red blotch diseases from ‘symptoms’ induced by biotic factors and abiotic stress factors.

Results of this study were published in a peer-reviewed scientific journal indicated below:
Adiputra, J., Kesoju, S.R. and Naidu, R.A. 2018. The relative occurrence of *Grapevine leafroll-associated virus 3* and *Grapevine red blotch virus* in Washington State vineyards. *Plant Disease* 102: 2129-2135. <https://doi.org/10.1094/PDIS-12-17-1962-RE>

iii. Genetic diversity analyses of viruses associated with leafroll and red blotch diseases.

Genetic diversity is an adaptive mechanism for viruses to cope with changing circumstances and increase the potential for survival. In the context of viruses infecting grapevines, variation in viral genome can create viral populations with significantly different biological properties such as pathogenicity and spread by vectors. Thus, studying viral genetic variability helps to discriminate between pathogenic and non-pathogenic strains that are otherwise indistinguishable and elucidate spatial and temporal dynamics of genetic variants in epidemiological investigations on a local and regional scale. During this project period, a combination of molecular biology, HTS technology and bioinformatics systems were used to elucidating genome and genetic variability among GLRaVs, with emphasis on GLRaV-3, and GRBV. The baseline data generated in this study will be used for improved diagnosis and management of leafroll and red blotch diseases in vineyards.

GLRaV-3: Between 2015 and 2019, a total of 2,291 samples were collected from red- and white-fruited wine grape (*Vitis vinifera*) cultivars from fourteen commercial vineyards in four

FINAL REPORT

AVAs. Samples were tested in RT-PCR assays for the presence of GLRaV-3 using primers specific to the virus-encoded heat shock protein 70 homologue (Hsp70h) gene. The 607-base pair DNA fragments amplified from 818 samples were sequenced and compared with corresponding GLRaV-3 sequences available in public databases to examine genetic diversity. In addition, total RNA preparations from 74 grapevine samples that tested positive for GLRaV-3 were subjected to HTS to generate near-complete viral genome sequences. The sequences obtained in this study were compared with GLRaV-3 sequences reported from different grapevine-growing regions. The results showed that GLRaV-3 in Washington vineyards occurs as several genetically diverse variant groups that differ from one another by 10-20 % at the genome sequence level. Among them, GLRaV-3 isolates belonging to variant group I were found to be predominant in Washington vineyards. These results provided a foundation to optimize robust assays for detecting genetic variants in vineyards and gain insights into the epidemiology of GLRaV-3 for implementing sustainable disease management strategies in vineyards. Results of this study are being analyzed and anticipated to be published in 2021.

Since GLRaV-3 is the most insidious and widespread in vineyards, we initiated fundamental studies to elucidate the molecular biology of the virus for future applications in managing leafroll disease. Using contemporary techniques in molecular biology and recombinant DNA technology and in collaboration with Drs. William Dawson and Siddarama Gowda at the University of Florida, Lake Alfred, we have previously showed that the full-length genome (cDNA) copy of GLRaV-3 (18,498 nucleotides [nt] genome size) can faithfully replicate and form authentic virus particles in *Nicotiana benthamiana* leaves infiltrated with *Agrobacterium tumefaciens* harboring the cDNA clone the virus. This achievement was also recorded as CAHNRS Invention Disclosure “*Grapevine Leafroll-Associated Virus 3 as a Vector for Delivery and Expression of Foreign Genes and Heterologous Proteins in Plants.*” Additional experiments were conducted to map specific regions of the viral genome playing a key role in replication and virion formation. We will be using these infectious cDNA clones of GLRaV-3 to optimize methods for infecting grapevines for studying viral gene functions, grapevine-virus interactions and advancing knowledge of the transmission biology of GLRaV-3.

Results of this study were published in a peer-reviewed scientific journal indicated below: Jarugula, S., Gowda, S., Dawson, W.O. and Naidu, R.A. 2018. Development of infectious cDNA clones of *Grapevine leafroll-associated virus 3* and analyses of the 5' non-translated region for replication and virion formation. *Virology* 523: 89-99.
<https://doi.org/10.1016/j.virol.2018.07.023>

GLRaV-1: The genome of two isolates of GLRaV-1 was determined to be 18,731 and 18,946 nucleotides. The sequence data indicated that GLRaV-1 is the second largest virus among all GLRaVs. The sequence data was used to develop an RT-PCR-based restriction fragment length polymorphism assay for discrimination of genetic variants of GLRaV-1 in vineyards. Studies were completed on the expression of sub-genomic RNAs from virus-infected grapevines to advance the molecular biology of interactions between grapevines and GLRaVs.

Results of this study were published in a peer-reviewed scientific journal indicated below: Donda, B. P., Jarugula, S. and Naidu, R. A. 2017. An analysis of the complete genome sequence and subgenomic mRNAs reveals unique features of the ampelovirus, *Grapevine leafroll-*

FINAL REPORT

associated virus 1. Phytopathology 107: 1069-1079. <https://doi.org/10.1094/PHYTO-02-17-0061-R>

GLRaV-4: The complete genome of three strains of GLRaV-4 (strain 4, strain 5 and strain 9) documented in Washington vineyards was determined to be 13,824 nt, 13,820 nt and 13,850 nt, respectively. An analysis of their genome sequences revealed that the three GLRaV-4 strains from Washington vineyards have genetic features distinct from GLRaV-4 strains reported from other grapevine-growing regions. The results also indicated that exchange of genetic material could occur between strains of GLRaV-4 resulting in increased genetic diversity in the virus genome and leading to the evolution of new variants with altered biological and epidemiological properties of the virus. This knowledge is valuable in understanding the role of GLRaV-4 strains in the biology and epidemiology of leafroll disease.

Results of this study were published in a peer-reviewed scientific journal indicated below: Adiputra, J., Jarugula, S. and Naidu, R.A. 2019. Intra-species recombination among strains of the ampelovirus *Grapevine leafroll-associated virus 4*. Virology Journal 16: 139. <https://doi.org/10.1186/s12985-019-1243-4>

GRBV: The genome sequence of 42 isolates of GRBV was completed and their phylogenetic analysis revealed the presence of two distinct variant groups in Washington vineyards. This knowledge provided a better understanding of the genetic diversity of GRBV in Washington vineyards relative to virus variants present in other grapevine-growing regions in North America. These results are helping to improve our understanding of the epidemiology of red blotch and for the design of effective control strategies. (This data was part of the publication [Adiputra et al., 2018. Plant Disease 102: 2129-2135] mentioned above).

iv. Status of nematode-transmitted viruses in vineyards.

During the project period, grapevine samples suspected for fanleaf degeneration/decline symptoms were collected and tested for nematode-transmitted viruses known to infect grapevines by molecular diagnostic assays and HTS technology. The results indicated the presence of TRSV in three red-fruited cultivars (Grenache, Tempranillo and Syrah) in one commercial vineyard in Yakima Valley AVA and GFLV in two red-fruited cultivars (Cabernet franc and Merlot) planted in two commercial vineyards located in Yakima Valley and Walla Walla AVAs. Overall results indicated sporadic distribution of TRSV and GFLV in Washington vineyards.

During the previous project (July 1, 2014 to June 30, 2017), we reported the spread of TRSV from infected grapevines to healthy plants using cucumbers as transmission baiting plants. Further, the dagger nematode (*Xiphinema rivesi*) was identified as the likely vector based on nematode morphological features and genome sequence analysis. To address the spread of TRSV between grapevines, virus-tested Cabernet franc (CF) cuttings were planted in close proximity to virus-infected Grenache vines in spring 2014 (Fig. 2A). Samples were collected from individual CF vines annually between 2015 and 2019 seasons and tested by RT-PCR for the presence of TRSV. As shown in Fig. 2B, samples from all 24 CF vines tested negative in 2015 indicating no infection of healthy vines. However, samples from 5 CF vines tested positive in 2016, an

FINAL REPORT

additional 4 vines in 2017, 1 vine in 2018 and 3 vines in 2019 tested positive for the virus. Thus, 54% of vines (13/24) tested positive during 2016 and 2019 seasons after planting healthy cuttings in 2014 season. Overall, these results indicate that TRSV can spread from infected Grenache vines to healthy CF vines by *X. rivesi* present in vineyard soil.

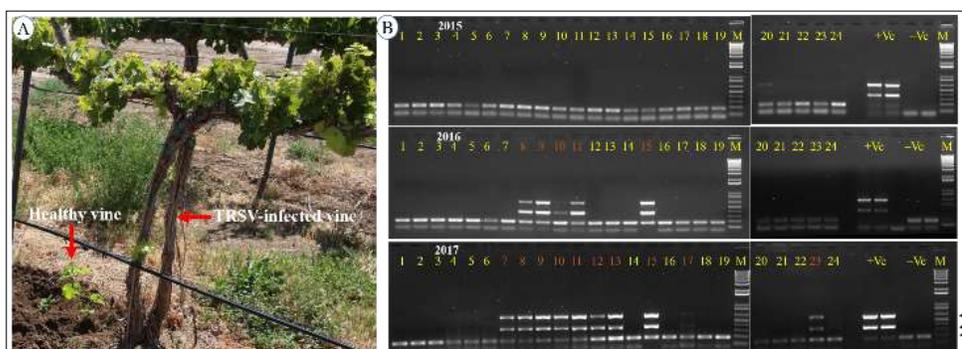


Fig. 2. (A). Healthy Cabernet franc (CF) vines ($n = 24$) were planted next to TRSV-infected Grenache vines in 2014. (B). Samples from CF vines tested by RT-PCR for TRSV in 2015, 2016 and 2017 seasons. Lanes 1-24= samples from individual CF vines, with virus-positive vines marked in red color. Two arrows on right show DNA bands specific to RNA 1 and RNA 2 genome segments of TRSV. Arrow head on right denotes DNA band specific to a 'house-keeping gene' of host origin used as an internal control to verify functionality of each RT-PCR assay.

In a different commercial vineyard, cucumber baiting assay was conducted during 2017 season to examine the spread of GFLV from infected Merlot vines to healthy cucumber plants. Cucumber plants planted in June 2017 were tested by RT-PCR for the presence of TRSV in October 2017. The results were negative, indicating that GFLV was unlikely to be transmitted by nematodes present in the vineyard soil. Although two dagger nematodes (*X. rivesi* and *X. pachtaicum*) were observed in the soil, the absence of GFLV in cucumber samples suggested that these two dagger nematode species are not capable of spreading the virus in the Merlot vineyard block. It should be noted that the results are based on one season and the cucumber baiting assay need to be repeated to confirm these observations.

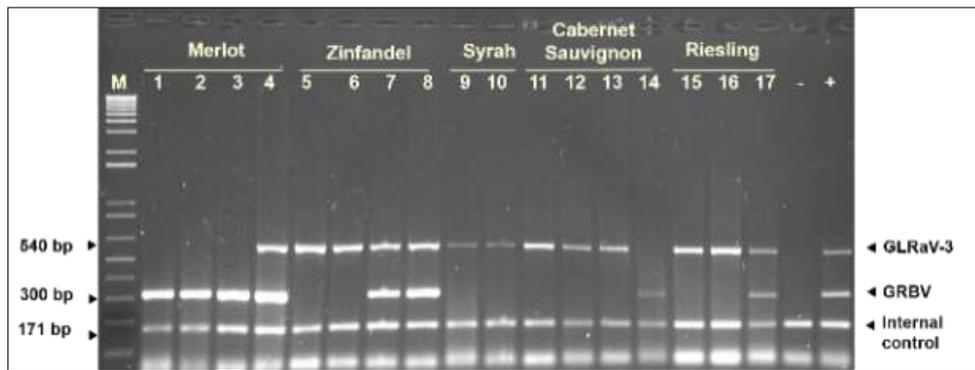
Based on these results, it can be inferred that the dagger nematode, *X. rivesi*, present in vineyard soils is capable of spreading TRSV but not GFLV. Thus, a two-pronged approach consisting of planting virus-tested cuttings and nematode control measures is necessary for the management of TRSV. Conversely, removing infected vines and replanting with virus-tested cuttings would help preventing the spread of GFLV in vineyards due to the absence of its vector *X. index*.

Activity: Cheaper, faster, and reliable diagnostic assays benefiting growers and nurseries.

Effective management of viral diseases in vineyards relies on accurate identification of virus(es) infecting grapevines. Since red-fruited cultivars express red leaf symptoms due to viral infection as well as other biotic (crown gall infection) and abiotic (mechanical damage, cold damage and insect feeding damage) stress factors, we conducted research to optimize methods for the detection of GLRaV-3 and GRBV to differentiate viral symptoms from non-viral symptoms and provide affordable diagnostic methods benefiting growers. Towards this objective, we developed a multiplex PCR protocol for simultaneous

FINAL REPORT

detection of GLRaV-3 and GRBV in individual samples and offer high throughput diagnostic services at an affordable price (Fig. 3). The multiplex PCR was validated by testing 535 samples (467 samples from 9 red-fruited cultivars and 68 samples from 3 white-fruited cultivars) for the presence of GLRaV-3 and GRBV. The results showed single infection of GLRaV-3 and GRBV, respectively, in 347 (65 %) and 33 (6 %) samples and co-infection of both viruses in 63 samples (12 %). A total of 82 samples (15 %) tested negative for both viruses. For validation of these results, the same set of sample extracts were tested in separate PCR assays (called uniplex PCR) for GLRaV-3 and GRBV. In linear regression statistical analysis, multiplex PCR results showed 98.69% correlation with uniplex PCR results, with a correlation coefficient of 99.9%. In all assays, amplification of DNA specific to grapevine genome sequence facilitated proper interpretation of negative results while testing for GLRaV-3 and GRBV in grapevines. Together with simplified sample extraction method previously established in Rayapati's lab, this multiplex PCR assay will help processing large number of samples in a relatively short period of time. This multiplex-PCR assay is currently being used in epidemiological studies to test grapevine samples collected from commercial vineyards and help growers and nurseries to test cuttings prior to planting new vineyards in a cost-effective manner.



*Fig. 3. Detection of GLRaV-3 and GRBV by multiplex PCR. The size of 540 and 300 base pair (bp) DNA band specific to GLRaV-3 and GRBV, respectively, are shown by arrow head on left. Name of viruses and internal control gene are shown by arrow head on right. A 171 bp DNA band specific to the internal control gene, a 300 bp DNA band specific to GRBV and a 540 bp DNA band specific to GLRaV-3 are shown by an arrow head on left. Lanes '+' and '-' = positive and negative controls, respectively. Lane M = DNA size marker. (reproduced from Adiputra et al., 2018. *Plant Disease* 102: 2129-2135 mentioned above).*

Because high costs levied by commercial diagnostic services are not affordable to many growers, we examined the cost-effectiveness of molecular assays for detecting GLRaV-3 and GRBV in a non-commercial setting. The prevailing costs of various components used in sample processing and virus testing, which includes variable costs (personnel salaries, research supplies, molecular reagents, other consumable goods) and fixed costs (depreciation of equipment costs used in diagnostic analyses), were collected. Subsequently, costs of testing petiole and cane samples for GLRaV-3 by RT-PCR and GRBV by PCR were estimated. Four scenarios, namely processing and testing one sample, 10 samples, 50 samples, or 100 samples per day, were considered to estimate overall cost of testing samples for the presence of GLRaV-3 and GRBV.

FINAL REPORT

Results of this study indicated that it is economical to test more samples (50-100 samples) than a few samples (less than 10 samples) at a given time. Overall, our data indicated that the cost per sample (if 100 samples are tested at a given time) for GLRaV-3 detection by RT-PCR is about \$5.38 for testing petiole samples and \$6.63 for testing cane samples. In contrast, the cost per sample (if 100 samples are tested at a given time) for GRBV detection by PCR is about \$4.66 for testing petiole samples and \$5.91 for testing cane samples. It should be noted that the cost is slightly higher for GLRaV-3, since additional reagents are needed for RT-PCR detection of the viral RNA genome, compared to the detection of DNA genome of GRBV by PCR. Similarly, the cost is slightly higher for testing cane samples compared to petiole samples for both viruses due to extra time involved in sample preparation from dormant canes. Nevertheless, our estimated costs under non-commercial settings is significantly lower compared to costs levied by commercial testing labs (Fig. 4).

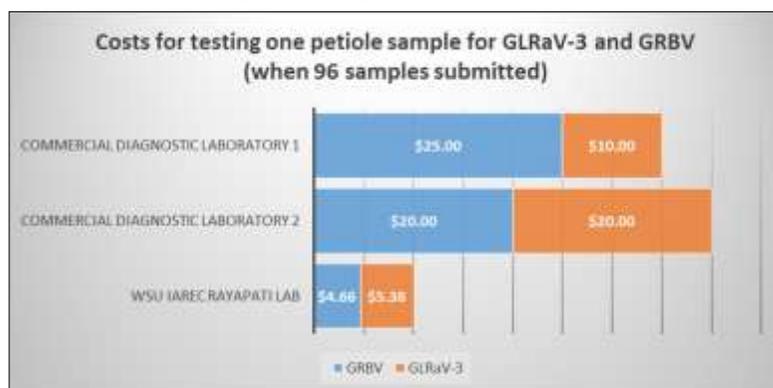


Fig. 4. A comparison of costs for testing petiole samples for GLRaV-3 and GRBV using commercial diagnostic laboratories versus the cost estimations under non-commercial settings. Costs are for testing one virus per sample. Blue color: GRBV, Orange color: GLRaV-3. (Hadaway et al. Unpublished data).

It should be noted that estimated costs of viral diagnostics is dependent on list price of chemicals, enzymes and other reagents, lab consumables and local personnel salaries and associated overhead costs. These costs are variable under different settings and locations and might change in future. Choice of reagents used in diagnostic assays, cost reductions associated with large volume of samples tested or discount pricing offered for virus testing by commercial service are unlikely to have a significant effect on outcomes of our cost estimations. In this context, it should be noted that the cost analysis of diagnostics carried out in this study is pertinent to non-commercial settings and do not undermine the services currently provided by commercial diagnostic services.

Activity: Delineate viral spread in young vineyards: is it via planting stock and/or by vectors?
Activity: Early intervention strategies for management of viral diseases in new plantings.

Many growers are removing old vineyard blocks that have become less productive and uneconomical due to virus-infections and replanting with virus-tested 'clean' planting stock obtained from certified nurseries. However, some growers are using self-propagated and compromised planting stock for new plantings due to the shortage of certified planting stock. One of the key issues in replanting vineyards is the risk of virus spread via planting stock and from external sources. Rogueing or removing symptomatic vines is one of the cultural practices used during post-planting for preventing viral spread in new plantings. To examine rogueing as a low-cost control strategy, vineyards planted with red-fruited cultivars in two AVAs were monitored for leafroll and red blotch diseases. In each vineyard, the location of symptomatic

FINAL REPORT

vines in each season was recorded in an XY matrix with the X-axis being the row number and the Y-axis being the within-row location of individual plants. Samples from a few symptomatic vines were tested during each season by PCR and RT-PCR, respectively, for the presence of GRBV and GLRaV-3. The data collected from individual vineyard blocks during successive seasons was used to prepare maps showing spatial patterns of the distribution of symptomatic vines in each vineyard block and to deduce the effectiveness of roguing in reducing virus incidence in young vineyards. The results of this study are described below:

i. Roguing for controlling red blotch.

During the past five years, possible spread of GRBV was monitored in vineyard blocks planted with cvs. Merlot, Cabernet franc, and Sangiovese in Horse Heaven Hills, Red Mountain, Yakima Valley, Walla Walla AVAs. Our observations indicated no spread of GRBV in these blocks, suggesting the absence of vine-to-vine spread by insect vectors. Instead, GRBV spread was observed to occur primarily via contaminated planting materials. In view of the lack of vector-mediated spread, roguing was pursued as a practical approach to control red blotch disease in commercial vineyards. This study was conducted in a 10-acre vineyard block planted in 2013 with cv. Merlot (clone 15). As shown in Fig. 5, 15.27% vines showed red blotch symptoms and spatial mapping of these vines during 2017 season showed random distribution across the entire vineyard block. In this block, samples from symptomatic vines tested positive for GRBV and negative for GLRaV-3. In collaboration with the grower, replacing symptomatic vines with virus-tested vines was implemented during three seasons. In each season, roguing of symptomatic vines during winter and replanting with virus-tested cuttings in the following spring resulted in reduced incidence of the disease from 15.27% in 2017 to 1.96% in 2018 to 0.42% in 2019 (Fig.5). These encouraging results suggest that replacing infected vines with healthy plants can be effectively used for controlling red blotch disease in vineyards. Discussions with the grower indicated that roguing of infected vines enabled to harvest high quality grapes and wine maker was able to produce high-end wine from these grapes. This block will be monitored during 2020 season to further confirm effectiveness of roguing in controlling red blotch disease.

Similar approaches involving roguing of symptomatic vines and replanting with virus-tested cuttings were pursued in vineyard blocks mentioned above to reduce red blotch incidence. Similar to the Merlot block shown in Fig.5, roguing was found effective in these blocks in controlling red blotch disease. Based on these results, it can be concluded that field spread of red blotch is less likely to occur in Washington vineyards and roguing can be implemented as an effective strategy for controlling red blotch disease.

FINAL REPORT

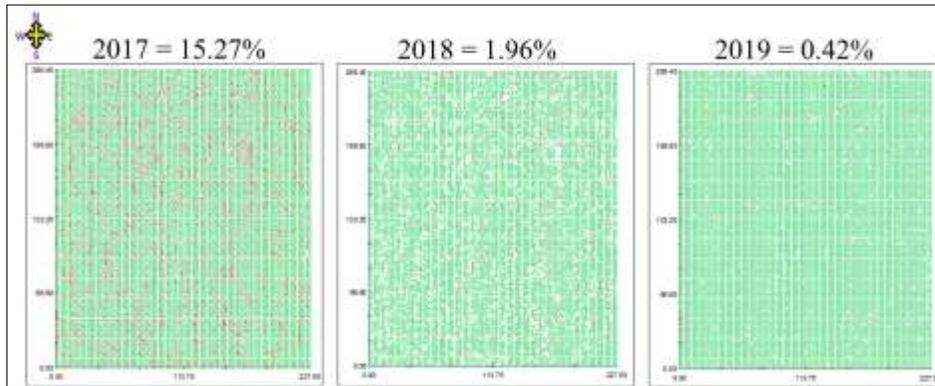


Fig. 5. Spatial distribution of vines showing red blotch symptoms in a 10-acre Merlot block (total vines = 13,708). Roguing of symptomatic vines followed by replanting with healthy vines reduced disease incidence from 15.27% in 2017 (left) down to 1.96% in 2018 (middle) and further to 0.42% in 2019 (right).

ii. Roguing for controlling leafroll disease.

Since GLRaV-3 can be disseminated via planting materials and spread by vectors, newly planted vineyard blocks were monitored each season to better understand leafroll disease spread and for implementing roguing as a control tactic to prevent the disease spread in young vineyards. For this purpose, three vineyard blocks in two geographic locations in Yakima Valley and Horse Heaven Hills AVAs were selected to test roguing as a non-pesticidal control strategy. The results are presented below:

iii. Roguing in a vineyard block planted with compromised cuttings obtained from a grower vineyard.

In spring 2015, grower planted a 5.5-acre block with Cabernet Sauvignon cuttings obtained from another grower's vineyard compromised with leafroll disease (Fig. 6). This block is located in Yakima Valley AVA. In fall 2015, 11.88% vines showed leafroll symptoms that tested positive for GLRaV-3 and negative for GRBV. In collaboration with the grower, we implemented roguing symptomatic vines followed by replanting with virus-tested cuttings during 2015 and 2019 seasons. The results showed reduced number of symptomatic vines from 11.88% in 2015 to 0.29% in 2017. However, a few vines showed new infections in 2018 and 2019, leading to a slight increase in the number of symptomatic vines to 0.46% in 2018 and 0.8% in 2019 seasons. Nevertheless, the disease incidence remained below 1% in this block, underscoring the beneficial effect of roguing as a practical approach for reducing leafroll disease incidence in young vineyards. This block will be monitored during 2020 season to assess whether the disease incidence remains at a low level or an increase in number of symptomatic vines would occur compared to 2019 season.

FINAL REPORT

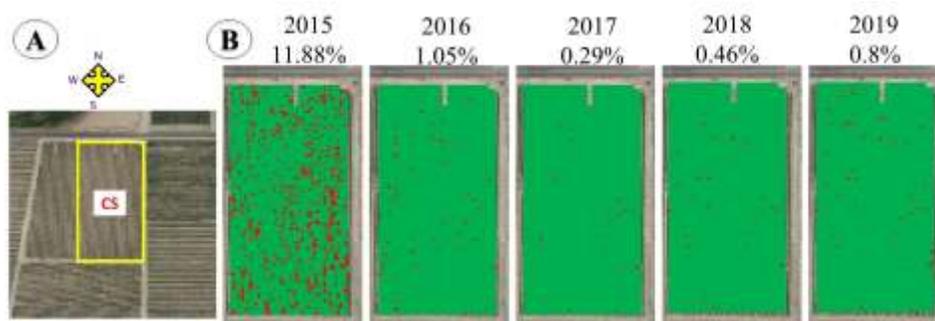


Fig. 6. Roguing in a Cabernet Sauvignon block planted with compromised planting stock. (A). Google map showing the Cabernet Sauvignon block (yellow rectangle border). Note that this block was surrounded with GLD-infected vineyard blocks on all sides except North side. (B). Distribution of symptomatic vines and incidence of GLD in successive seasons after roguing of symptomatic vines on an annual basis.

ii. Roguing in vineyard blocks planted with cuttings brought from a certified nursery.

Two Cabernet Sauvignon blocks planted, respectively, in 2013 and 2014 with cuttings purchased from a certified nursery were monitored to examine the effectiveness of roguing in controlling GLD. These two blocks are spatially separated in a commercial vineyard in Horse Heaven Hills AVA. Samples from a select number of symptomatic vines in both blocks were tested annually and found to be positive for GLRaV-3, but not for GRBV. The information was provided to the grower at the end of each season to remove symptomatic vines and replant with virus-tested cuttings. The data collected from these two blocks during 2015, 2016, 2017, 2018 and 2019 seasons are presented in Fig.7 and Fig. 8. In the 19-acre block planted in 2014 (Fig. 7), roguing symptomatic vines annually helped to keep leafroll disease at sufficiently low levels (<2%) during the first three seasons (2015-2017) post-planting. However, the number of vines showing symptoms increased during 2018 and 2019 seasons resulting in 2.44% and 7.4% disease incidence, respectively. In the 13-acre block (Fig.8), roguing and replanting with healthy cuttings resulted in nearly 50% reduction in disease incidence from 2.26% in 2015 to 1.25% in 2018 season. However, a dramatic increase in disease incidence to 4.46% was observed in 2019 season. These results indicated that roguing symptomatic vines was less effective due to new infections from external sources. The two blocks will be monitored during 2020 season to examine the spread of GLD.

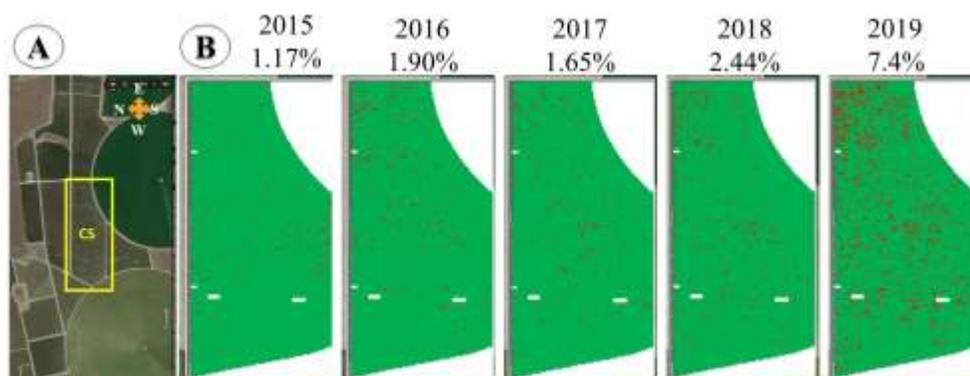


Fig. 7. A 19-acre block of Cabernet Sauvignon (clone #2) planted in 2014 (total vines = 13,833). (A). Google map showing the newly planted Cabernet Sauvignon block (yellow rectangle

FINAL REPORT

border). Note that this block is surrounded with GLD-infected vineyard blocks on the North side and partly on East, South and West sides. (B) The spatial and temporal distribution of symptomatic vines during 2015 and 2019 seasons. The red dots represent symptomatic vines.



Fig. 8. A 13-acre block of Cabernet Sauvignon (clone #8) planted in 2013 (total vines = 10,467). (A). Google map showing the Cabernet Sauvignon block (yellow rectangle border). Note that GLD-infected vineyard blocks present on the North side and no vineyards on other sides of this newly planted vineyard block. (B) The spatial and temporal distribution of symptomatic vines during 2015 and 2019 seasons. The red dots represent symptomatic vines.

The results presented in Fig. 7 and 8 would suggest that symptoms expressed by apparently healthy vines during each season could represent new infections. Spatial and temporal distribution of symptomatic vines in these two blocks indicate an ‘edge effect’ suggesting that the disease spread occurred from external sources and clustering of symptomatic vines with time would indicate vine-to-vine spread within newly planted blocks. Overall data presented in Figs 6, 7 and 8 indicate variation in virus spread and disease incidence in vineyards. Therefore, studies on comprehensive analyses of spatiotemporal spread of GLRaV-3 in different AVAs and the influence of environmental and geographical factors on disease dynamics are necessary to facilitate epidemiological models that inform decision-making for area-wide management of GLD. The efficacy of a rogueing strategy is constrained due to variability in symptom expression displayed by different cultivars. Therefore, future studies should also involve examining the correlation between symptoms and presence of viruses to improve visual diagnostics as a reliable identifier of viral infections to support rogueing as a practical and cost-effective approach in red-fruited cultivars. The lack of foliar symptoms makes visual diagnosis of infected vines unreliable in white-fruited cultivars and, consequently, diagnostic methods are critical to support rogueing for controlling viral diseases in white-fruited cultivars.

Activity: Phenomics of grapevine responses to viral infections: impacts on crop yield and fruit quality in own-rooted and top-grafted vineyards.

i. Impacts of leafroll and red blotch in red-fruited cultivars.

During this project, studies were conducted to examine cultivar-specific responses to viral infections across vintages in different AVAs. These studies were conducted in commercial vineyards using own-rooted and top-grafted wine grape cultivars listed below to advance our understanding of site- and cultivar-specific responses to viral infections.

FINAL REPORT

Self-rooted cultivars:

- i. Merlot, Petite Sirah, Malbec, Zinfandel, and Cabernet Sauvignon infected with leafroll.
- ii. Merlot, Cabernet Sauvignon, Syrah, and Sangiovese infected with red blotch.
- iii. Tempranillo and Grenache infected with fanleaf.

Top-grafted cultivars:

- i. Cabernet Sauvignon (top-grafted on Riesling) infected with leafroll.
- ii. Syrah (top-grafted on Riesling and Gewürztraminer) infected with leafroll and red blotch.

Twenty to thirty symptomatic vines positive for a candidate virus (either GLRaV-3 or GRBV) and an equal number of non-symptomatic vines that tested negative for these viruses were selected in each block to measure fruit yield and examine grape quality attributes. At the time of commercial harvest in September/October, clusters in each vine were harvested manually and counted the number of clusters per vine and measured weight of all clusters to estimate total fruit yield per vine. In addition, 50 grapes/vine were collected randomly from symptomatic and healthy vines. Extracts from grapes were used to measure total soluble solids or sugars (measured as °Brix), titratable acidity (TA), pH, and total anthocyanins using protocols established in Rayapati's lab. The data was analyzed statistically for significant differences between vines with and without viral infections. Overall results obtained during the past few seasons are summarized in Tables 2 to 4.

Table 2: Impacts of grapevine leafroll disease (GLRaV-3) on own-rooted wine grape cultivars

Cultivar (AVA)¹	Fruit Yield²	°Brix²	TA²	pH²	Anthocyanins²
Cabernet Sauvignon (YV) (3 seasons: 13, 14, 15)	-12.13	-8.34	+6.09	-0.62	-15.24
Syrah (YV) (3 seasons: 13, 15, 17)	-13.28	-6.06	+5.54	+0.13	-1.05
Zinfandel (HHH) (5 seasons: 14, 15, 16, 17, 18)	-0.65	+4.40	+7.89	-0.09	-6.60
Petite Sirah (HHH) (5 seasons: 14, 15, 16, 17, 18)	-0.65	-5.48	+1.12	-0.77	-15.84
Cabernet Sauvignon (HHH) (5 seasons: 14, 15, 16, 17, 18)	-19.06	-0.80	-3.24	-0.20	+6.49

¹YV = Yakima Valley AVA, HHH= Horse Heaven Hills AVA

²Negative and positive values indicate, respectively, percent decrease and increase in virus-infected vines compared to healthy vines.

FINAL REPORT

Table 3: Impacts of grapevine red blotch disease (GRBV) on own-rooted wine grape cultivars

Cultivar (AVA)¹	Fruit Yield²	°Brix²	TA²	pH²	Anthocyanins²
Cabernet Sauvignon (HHH) (5 seasons: 14, 15, 16, 17, 18)	-21.67	-6.90	+10.80	-0.96	+9.23
Merlot (HHH) (7 seasons: 13, 14, 15, 16, 17, 18, 19)	-17.15	-10.36	+11.12	+0.35	-11.63
Syrah (YV) (6 seasons: 13, 14, 15, 16, 17, 18)	-42.26	+0.62	+11.54	+6.48	-18.07
Merlot (YV) (2 seasons: 17, 18)	-9.25	-7.02	+9.76	-5.54	-9.91

¹HHH=Horse Heaven Hills, YV=Yakima Valley; ²Negative and positive values indicate, respectively, percent decrease and increase in virus-infected vines compared to healthy vines.

Table 4: Impacts of grapevine leafroll (GLRaV-3) and red blotch (GRBV) diseases on top-grafted wine grape cultivars

Cultivar (AVA)¹	Fruit Yield²	°Brix²	TA²	pH²	Anthocyanins²
Syrah (3 seasons: 17, 18, 19) ³	-12.11	-14.60	+18.99	-2.61	-19.07
Syrah (1 season: 17) ³	-10.89	-8.45	+30.73	+1.06	-24.23
Merlot (3 seasons: 17, 18, 19) ³	-11.95	-4.30	+13.80	-0.88	+0.41
Cabernet Sauvignon (3 seasons: 17, 18, 19) ³	-4.72	-8.30	+8.81	+0.80	-13.32
Merlot (3 seasons: 17, 18, 19) ⁴	-9.24	-1.69	+5.26	-1.70	+5.19
Merlot (3 seasons: 17, 18, 19) ⁵	-12.98	-4.76	+11.12	-2.43	+0.93
Syrah (1 season: 17) ⁶	-31.29	-2.07	-0.92	+5.58	-8.48
Sangiovese (3 seasons: 15, 16, 18) ⁶	-17.43	-8.94	+1.58	+2.51	-17.93
Syrah (1 season: 17) ⁷	-25.31	-5.00	+1.83	+3.07	-29.09

¹All blocks were in Yakima Valley, except Sangiovese block located in Walla Walla AVA.

²Negative and positive values indicate, respectively, percent decrease and increase in virus-infected vines compared to healthy vines; ³GLRaV-3; ⁴GLRaV-4; ⁵GLRaV-3+GLRaV-4; ⁶GRBV;

⁷GLRaV-3+ GRBV.

Based on the results presented in Tables 2 to 4, it can be concluded that impacts of GLRaV-3 and GRBV on fruit yield and quality attributes are highly variable across the locations and among the cultivars. In general, fruit yield and quality, especially sugars and berry anthocyanins that are hallmarks of grape quality, are negatively affected due to leafroll and red blotch infections in both own-rooted and top-grafted vineyards. In contrast, impacts of leafroll and red blotch on

FINAL REPORT

grape juice pH are not as pronounced as impacts on fruit yield and berry sugars. However, higher levels of TA were observed in grapes from vines infected with leafroll and red blotch. These results provided a foundation to pursue phenomics approaches (study of the influence of genetic and environmental interactions on physical traits such as grapevine health and fruit quality) to elucidate cultivar- and site-specific responses to viral infections across vintages and in different AVAs for a comprehensive understanding of impacts of virus diseases in vineyards under diverse wine grape-growing climates.

ii. Impacts of leafroll in white-fruited cultivars.

Although white grape cultivars account for nearly 42% of total grape production in Washington State, no studies were conducted on impacts of viral diseases on vine health and fruit quality in white-fruited cultivars. Therefore, we have conducted studies to generate research-based knowledge on impacts of viral diseases in two white-fruited cultivars relative to viral disease impacts in red-fruited cultivars. This study was conducted in two commercial vineyards planted with Chardonnay and Sauvignon Blanc in Yakima Valley AVA. Vines showing mild symptoms or suspected for symptoms and tested positive for GLRaV-3 and non-symptomatic vines that tested negative for the virus were selected for this study. Methods described above for red-fruited cultivars were used for measuring impacts on fruit yield and quality. The results (Table 5) showed significant impacts on fruit yield and berry sugars or total soluble solids in both Chardonnay and Sauvignon Blanc. Similar to red-fruited cultivars, juice pH was not affected significantly, whereas significantly higher levels TA was observed in virus-infected vines of both cultivars compared to healthy vines.

Table 4. Impacts of leafroll disease (GLRaV-3) on fruit yield and quality in cvs. Chardonnay and Sauvignon Blanc

Cultivar (AVA)¹	Fruit Yield²	°Brix²	TA²	pH²	Anthocyanins²
Chardonnay (YV) (2 seasons: 18, 19)	- 69.72	- 16.38	+ 23.48	- 4.13	N/A
Sauvignon Blanc (YV) (1 season: 17)	-42.39	-20.15	+14.28	-4.82	N/A

¹*Both blocks were in Yakima Valley (YV).*

²*Negative and positive values indicate, respectively, percent decrease and increase in virus-infected vines compared to healthy vines. N/A = Not applicable.*

In summary, studies in commercial vineyards have shown that both GLRaV-3 and GRBV can cause significant impacts on fruit yield and grape quality in own-rooted and top-grafted red- and white-fruited wine grape cultivars. It can be concluded from these studies that viral infection negatively affects fruit yield and sugars (and anthocyanins in red-fruited cultivars) in both red- and white-fruited wine grape cultivars. Although juice pH is not affected, higher levels of TA was observed in both red and white grapes due to viral infections. In general, impacts of viruses on fruit yield and berry quality were found to be variable depending on the cultivar, season and vineyard location. The results provided a foundation to further elucidate grapevine-virus-

FINAL REPORT

environment interactions for a comprehensive understanding of impacts of viral diseases in vineyards under diverse wine grape-growing climates.

Outreach and Education Efforts - Presentations of Research:

Research-based knowledge generated from this project was disseminated to various stakeholders by a variety of dissemination pathways. A list of publications, industry presentations, posters, and other educational and outreach activities are listed below.

Note: R.A. Naidu and Naidu Rayapati are the same person.

i. Peer-reviewed scientific publications

1. Adiputra, J., Jarugula, S. and **Naidu, R.A.** 2019. Intra-species recombination among strains of the ampelovirus *Grapevine leafroll-associated virus 4*. *Virology Journal* 16: 139.
2. Sinha, R., Khot, L.R., Rathnayake, A.P., Gao, Z. and **Naidu, R.A.** 2019. Visible-near infrared spectroradiometry-based detection of grapevine leafroll-associated virus 3 in a red-fruited wine grape cultivar. *Computers and Electronics in Agriculture* 162: 165-173.
3. Jarugula, S., Gowda, S., Dawson, W.O. and **Naidu, R.A.** 2018. Development of infectious cDNA clones of *Grapevine leafroll-associated virus 3* and analyses of the 5' non-translated region for replication and virion formation. *Virology* 523: 89-99.
4. Adiputra, J., Kesoju, S.R. and **Naidu, R.A.** 2018. The relative occurrence of *Grapevine leafroll-associated virus 3* and *Grapevine red blotch virus* in Washington State vineyards. *Plant Disease* 102: 2129-2135.
5. Pandey, B., **Naidu, R.A.** and Grove, G.G. 2018. Detection and analysis of mycovirus-related RNA viruses from grape powdery mildew fungus *Erysiphe necator*. *Archives of Virology* 163: 1019-1030.
6. Donda, B. P., Jarugula, S., and **Naidu, R. A.** 2017. An analysis of the complete genome sequence and subgenomic mRNAs reveals unique features of the ampelovirus, *Grapevine leafroll-associated virus 1*. *Phytopathology* 107:1069-1079.

ii. Peer-reviewed Book Chapters

1. **Naidu, R.A.** 2017. *Grapevine leafroll-associated virus 1*. In: Meng B., Martelli G., Golino, D., Fuchs M (eds.), *Grapevine Viruses: Molecular Biology, Diagnostics and Management*. Springer, Cham, Switzerland, pages 127-139.
2. Burger, J., Maree, H.J., Gouveia, P., and **Naidu, R.A.** 2017. *Grapevine leafroll-associated virus 3*. In: Meng B., Martelli G., Golino, D., Fuchs M (eds.), *Grapevine Viruses: Molecular Biology, Diagnostics and Management*. Springer, Cham, Switzerland, pages 167-195.

iii. Extension publications

Hoheisel, G., Moyer, M.M., Daniels, C.H., Miller, T.W., Walsh, D., Zasada, I., **Naidu, R. A.**, and Davenport, J.R. 2017. *Pest Management Guide for Grapes in Washington*. EB0762, 56 pp.

iv. Industry trade journals

Naidu, R.A. Naidu, R.A. 2017. Grafting wine grapes. *Good Fruit Grower* June 2017. Vol. 68, pages 38-39.

FINAL REPORT

v. Professional Scientific Meetings

1. Mitra, A., Jarugula, S., Donda, B., Jordan, E. and **Naidu, R.A.** 2019. Elucidating the genetic diversity of *Grapevine leafroll-associated virus 3* for managing grapevine leafroll disease in vineyards. Proceedings of the Northwest Center for Small Fruits Research 2019. December 2-4, 2019, Ferndale, WA.
2. Hadaway, K., Kogan, C., Jarugula, S. and **Naidu, R.A.** 2019. Elucidating differences in red leaf symptoms produced by biotic and abiotic stresses in grapevines. IEEE Women in Engineering Leadership Summit, July 30, 2019, Richland, WA (**Received best poster award**)
3. Mitra, A., Jarugula, S., Donda, B., Jordan, E. and **Naidu, R.A.** 2019. Genetic diversity of *Grapevine leafroll-associated virus 3* in Washington State vineyards. 2019 American Phytopathological Society Annual Meeting, August 3-7, 2019, Cleveland, OH. (**Received “Phytobiomes Student Poster Award” from the scientific journal ‘Phytobiomes’ at the American Phytopathological Society**).
4. Chingandu, N., Jarugula, S., Movva, A. and **Naidu, R.A.** 2019. The occurrence of economically detrimental viruses in certified nurseries and commercial vineyards in Washington State. 70th American Society for Enology and Viticulture (ASEV) National Conference, June 17-20, 2019, Napa Valley, CA.
5. Jarugula, S., Mitra, A., Adegbola, R., Swamy, P. and **Naidu, R.A.** 2019. Impacts of viral diseases in field-grafted vineyards in Washington State. 70th American Society for Enology and Viticulture (ASEV) National Conference, June 17-20, 2019, Napa Valley, CA.
6. Mitra, A., Jarugula, S., Donda, B., Jordan, E. and **Naidu, R.A.** 2018. *Grapevine leafroll-associated virus 3* is present as genetically distinct strains in Washington State vineyards. The American Phytopathological Society- Pacific Division. Portland, OR, June 25-27, 2018.
7. Adiputra, J., Jarugula, S. and **Naidu, R.A.** 2018. Genetic diversity of *Grapevine leafroll-associated virus 4* strains in Washington State vineyards. The American Phytopathological Society- Pacific Division. Portland, OR, June 25-27, 2018.
8. Olden, T., Funk, C., Nilson, N., Natra, N., and **Naidu, R.A.** 2018. Impact of *Tobacco ringspot virus* on vine growth and grape quality. Gathering of Scholars Symposium – Advancing Academic Research and Creativity in the Arts”, Heritage University, April 3, 2018, Toppenish, WA. (**Received 2nd best poster award**)
9. Funk, C., Nilson, N., Olden, T., Natra, N., and **Naidu, R.A.** 2018. Impact of *Tobacco ringspot virus* on vine growth and grape quality. 7th Annual Showcase for Undergraduate Research and Creative Activities (SURCA), Washington State University, Pullman, WA, April 2, 2018.
10. Natra, N.T., Akinbade, S., Schultz, A., and **Naidu, R.A.** 2018. The tale of two nepoviruses in Washington state vineyards. The 9th International IPM Symposium, Improving Health, Environment and Global Sustainability, Baltimore, MD, March 19-22, 2018.
11. Adiputra, J., and **Naidu, R.A.** 2017. State-wide surveys indicated widespread distribution of grapevine leafroll than red blotch in Washington vineyards. 2017 American Phytopathological Society Annual Meeting, August 5-9, 2017, San Antonio, TX.
12. Natra, N., Akinbade, S., Schultz, A., and **Naidu, R.A.** 2017. Epidemiology of Tobacco ringspot virus causing fanleaf degeneration and decline symptoms in wine grape (*Vitis vinifera*) cultivars. 2017 American Phytopathological Society Annual Meeting, August 5-9, 2017, San Antonio, TX.
13. Sinha, R., Gao, Z., Rathnayake, A.P., Khot, L.R., and **Naidu, R.A.** 2017. Visible–near infrared spectroscopy-based Grapevine leafroll–associated virus–3 detection from

FINAL REPORT

- undetached leaves under field condition. Paper No. 1700499, ASABE 2017 Annual International Meeting, Spokane, WA, July 16-19, 2017.
14. Bagewadi, B., Movva, A., Natra, N., and **Naidu, R.A.** 2017. Sanitary Status of Grapevine Nurseries in Washington State. 68th American Society for Enology and Viticulture (ASEV) National Conference, June 26-29, 2017, Bellevue, WA.
 15. Swamy, P., Jarugula, S., and **Naidu, R.A.** 2017. Cultivar- and vintage-specific impacts of grapevine leafroll and red blotch diseases in commercial vineyards. 68th American Society for Enology and Viticulture (ASEV) National Conference, June 26-29, 2017, Bellevue, WA.
 16. Donda, B., Jarugula, S., and **Naidu, R.A.** 2017. The complete genome sequence of *Grapevine leafroll-associated virus 1* isolates from Washington vineyards. 68th American Society for Enology and Viticulture (ASEV) National Conference, June 26-29, 2017, Bellevue, WA.

vi. Industry Presentations (Oral)

1. Naidu, R.A. 2019. Emerging seed-borne viruses in vegetable crops. Pacific Northwest Vegetable Association 33rd Annual Conference. November 20-21, 2019. Kennewick, WA.
2. Naidu, R.A. 2019. An update on grapevine viruses in Washington vineyards. G.S. Long Co., Inc. 2014 Grower Meeting, January 16, 2019, Yakima, WA.
3. Naidu, R.A. 2019. Current and on-going research on roguing in vineyards. Washington Winegrowers Convention and Trade Show, February 12-14, 2019, Kennewick, WA, USA.
4. Naidu, R.A. 2018. *Viruses: What They Are, Are Not, Their Damage, And More.* Regionalizing Grape Quarantine and Certification Programs in the Pacific Northwest. May 1, 2018, Yakima, WA.
5. Naidu, R.A. 2018. *Risk of Viruses in field-grafted vineyards: Case studies.* In: Spanish Language Session-When viticulture matters to winemaking. Washington Winegrowers Association Annual Meeting, Convention & Trade Show, February 6, 2018, Kennewick, WA.
6. Naidu, R.A. 2018. *Top-grafting and Virus diseases: Case studies.* In: Panting and grafting – How to? Washington Winegrowers Association Annual Meeting, Convention & Trade Show, February 8, 2018, Kennewick, WA.
7. Naidu, R.A. 2017. The booming wine grape industry in the Pacific Northwest and threats from viral diseases. The 44th Annual Hermiston Farm Fair Seminars & Tradeshow, Hermiston, OR, December 1, 2017.
8. Naidu, R.A. 2017. Grapevine leafroll disease and current sampling and testing methods. Western Horticultural Inspection Society, Benton City, October 12, 2017.
9. Naidu, R.A. 2017. Scouting for virus disease symptoms in vineyards - what, when, and how? Cold Creek vineyards, Mattawa, WA. September 8, 2017.
10. Naidu, R.A. 2017. Grapevine leafroll disease: a ‘unique’ pathosystem with many intrigues. WERA 1007 Multistate Research Coordinating Committee and Information Exchange Group meeting, IAREC, Prosser, WA. July 24, 2017.
11. Naidu, R.A. 2017. Current research on leafroll and red blotch diseases at Washington State University. Grapevine Redleaf Disease Research Review Meeting. Foundation Plant Services, UC-Davis, CA. May 9-10, 2017.
12. Naidu, R.A. 2017. Grapevine virus diseases and their impacts. Walla Walla Community College, May 5, 2017.

FINAL REPORT

13. Naidu, R.A. 2017. On-going research projects on grapevine viruses at Washington State University. Ste. Michelle Wine Estates (SMWE) Viticulture Team Meeting. 14 Hands Winery, Prosser. May 4, 2017.
14. Naidu, R.A. 2017. Best management practices for managing virus diseases in vineyards. SMWE Viticulture Staff Annual Retreat. Wine Science Center, Richland. March 31, 2017.

vii. Industry Presentations (Poster)

1. Jarugula, S., Adegbola, R., Mitra, A., Chingandu, N., Thammina, C., Swamy, P., Bagewadi, B. and Naidu, R.A. 2020. Rogueing symptomatic vines for controlling viral diseases in vineyards. Washington Winegrowers Convention and Trade Show, March 2-5, 2020, Kennewick, WA, USA. **(1st place under People Choice category and 3rd prize under Professional Category)**.
2. Mitra, A., Jarugula, S., Donda, B., Jordan, E., and Naidu, R.A. 2020. Elucidating the genetic makeup of *Grapevine leafroll-associated virus 3* for managing leafroll disease in Washington State vineyards. Washington Winegrowers Convention and Trade Show, March 2-5, 2020, Kennewick, WA, USA.
3. Chingandu, N., Jarugula, S., Movva, A. and Naidu, R.A. 2020. The absence of Grapevine red blotch virus in Washington's certified grapevine nurseries. Washington Winegrowers Convention and Trade Show, March 2-5, 2020, Kennewick, WA, USA.
4. Mitra, A., Jarugula, S., Donda, B., Jordan, E., and Naidu, R.A. 2019. Elucidating the genetic makeup of *Grapevine leafroll-associated virus 3* for managing leafroll disease in Washington State vineyards. Washington Winegrowers Convention and Trade Show, February 12-14, Kennewick, WA, USA. **(Graduate Student Category: 1st Prize under Oral presentation and 3rd prize under poster presentation)**
5. Hadaway, K.M. Ball, T., Folwell, R., Jarugula, S. and Naidu, R.A. 2019. To test or not to test? Providing affordable diagnostic assays for the benefit of grapevine nurseries and growers. Washington Winegrowers Convention and Trade Show, February 12-14, Kennewick, WA, USA.
6. Chingandu, N., Jarugula, S., Movva, A. and Naidu, R.A. 2019. The occurrence of economically detrimental viruses in certified nurseries and commercial vineyards in Washington State. Washington Winegrowers Convention and Trade Show, February 12-14, Kennewick, WA, USA. **(3rd prize for poster presentation under Professional Category)**.
7. Naidu, R.A., Jarugula, S., Swamy, P., Natra, N., Mitra, A. and Movva, A. 2018. The mantra of 'Start Clean, Stay Clean' for healthy vineyards. Washington Winegrowers Association Annual Meeting, Convention & Trade Show, February 6-8, 2018, Kennewick, WA. **(1st Prize under Professional Category)**.
8. Jarugula, S., Swamy, P. and Naidu, R.A. 2018. Impacts of two distinct virus diseases in Washington State vineyards. Washington Winegrowers Association Annual Meeting, Convention & Trade Show, February 6-8, 2018, Kennewick, WA. **(2nd Prize under Professional Category)**.
9. Funk, C., Nilson, N., Olden, T., Natra, N. and Naidu, R.A. 2018. Impact of *Tobacco ringspot virus* on vine growth and grape quality. Washington Winegrowers Association Annual Meeting, Convention & Trade Show, February 6-8, 2018, Kennewick, WA. **(1st Prize under Undergraduate Student Category)**.
10. Mitra, A., Jarugula, S., Donda, B., Jordan, E. and Naidu, R.A. 2018. Understanding the genetic diversity landscape of *Grapevine leafroll-associated virus 3* for implementing leafroll

FINAL REPORT

disease management strategies in Washington State vineyards. Washington Winegrowers Association Annual Meeting, Convention & Trade Show, February 6-8, 2018, Kennewick, WA.

11. Natra, N., Jarugula, S., Mitra, A., Movva, A. and Naidu, R.A. 2018. Strengthening the grapevine supply chain for healthy vineyards. Washington Winegrowers Association Annual Meeting, Convention & Trade Show, February 6-8, 2018, Kennewick, WA.
12. Natra, N. and Naidu, R.A. 2018. Current state of knowledge about nematode-transmitted viruses in Washington vineyards. Washington Winegrowers Association Annual Meeting, Convention & Trade Show, February 6-8, 2018, Kennewick, WA.
13. Gao, Z., Naidu, R.A., Zhang, Q. and Khot, L. 2018. Feasibility of hyperspectral imaging towards early detection of grapevine leafroll disease. Washington Winegrowers Association Annual Meeting, Convention & Trade Show, February 6-8, 2018, Kennewick, WA.

viii. WAVEx

1. Grape Virus Analytics and Testing on February 19, 2020 at WSU-IAREC, Prosser.
2. Managing Grapevine Viral Diseases for Healthy Vineyards on March 15, 2018 at WSU-Wine Science Center, Richland.
3. Slowing the spread of grapevine viruses on April 19, 2017 at Walter Clore Center, Prosser.

ix. Outreach through University News outlets (CAHNRS and WSU Extension Marketing and News Services)

1. What's gone viral? Washington State Magazine Spring 2019. (<https://magazine.wsu.edu/2019/02/02/whats-gone-viral/>).
2. WSU scientists clone virus to help stop overwhelming grape disease. WSU Insider. August 29, 2018. (<https://news.wsu.edu/2018/08/29/wsu-scientists-clone-grape-virus-help-stop-devastating-disease/>).
3. Vineyard, WSU scientists team up to battle orchard virus threat. WSU News. June 14, 2017. (<https://news.wsu.edu/2017/06/14/plant-virus-major-threat-to-growers/>).

x. Outreach through industry magazines and other news outlets.

1. WSU scientists clone grapevine leafroll virus. Path to stopping overwhelming grape disease. American Vineyard Vol. 28, No.3, 2019, pages 16-18.
2. Virus cloning may lead to leafroll control. Good Fruit Grower December 27, 2018. (<https://www.goodfruit.com/virus-cloning-may-lead-to-leafroll-control/>).
3. Leafroll-associated virus 3 cloned to help stop grape disease. International Society for Plant Pathology ISPP Newsletter 48 (10), October 2018. (http://www.isppweb.org/newsletters/pdf/48_10.pdf)
4. Virus cloned to help stop overwhelming grape disease. Euresia Review September 3, 2018. (<http://www.eurasiareview.com/03092018-virus-cloned-to-help-stop-overwhelming-grape-disease/>).
5. Breakthrough to combat devastating grape disease. Technologynetworks August 31, 2018. (<https://www.technologynetworks.com/analysis/news/breakthrough-to-combat-devastating-grape-disease-308363>).
6. Scientists clone virus to help stop overwhelming grape disease. Cell & Microbiology August 30, 2018. (<https://phys.org/news/2018-08-scientists-clone-virus-overwhelming-grape.html>).

FINAL REPORT

7. Scientists clone virus to help stop overwhelming grape disease. ScienceDaily August 30, 2018. (<https://www.sciencedaily.com/releases/2018/08/180830143053.htm>).
8. On a mission to vanquish viruses in vineyards. Good Fruit Grower February 26, 2018. (<https://www.goodfruit.com/on-a-mission-to-vanquish-viruses-in-vineyards/>).
9. Soil-borne virus stunts crop growth; causes concern for farmers. KNDO/KNDU Right Now (NBC affiliate based in Yakima and Tri-Cities, WA). July 12, 2017. (<http://www.nbcrightnow.com/story/35872486/soil-borne-virus-stunts-crop-growth-causes-concern-for-farmers>).
10. Tobacco ring spot virus found in Washington wine grapes. Capital Press, June 23, 2017. (<http://www.capitalpress.com/Washington/20170623/tobacco-virus-found-in-washington-wine-grapes>).
11. WSU study finds virus threaten grape crops. KING 5 (NBC affiliate based in Seattle, WA) June 15, 2017. (<http://www.king5.com/tech/science/environment/ws-researchers-find-harmful-worms-in-wine-growing-soil/449413104>).

Research Success Statement:

A mix of basic and applied research activities conducted during the project period helped to improve our understanding of leafroll and red blotch, the two economically important viral diseases affecting Washington vineyards. Information on the current status of viruses in vineyards is used in grapevine certification and quarantine programs to implement proactive measures for protecting Washington's young wine industry from 'alien' viruses reported in grapevines worldwide. Due to practical challenges associated with symptom-based diagnosis of leafroll and red blotch diseases in vineyards, accurate detection and diagnosis is a fundamental for coming up with management options for leafroll and red blotch diseases. Improvements in sample processing and diagnostic protocols provided cost-effective methods for high-throughput and reliable detection of GLRaV-3 and GRBV in grapevine samples benefiting growers, nurseries and other end users. Research on nematode-transmitted viruses indicated that GFLV can be eliminated by using virus-tested 'clean' plants due to the absence of dagger nematode vector, *X. index*. In contrast, management of TRSV involves a combination of 'clean' plants and post-management of dagger nematode vector, *X. index*. Field studies have shown that rogueing infected vines and replanting with healthy cuttings can be used as a low-cost tool to manage red blotch disease in vineyard blocks. In contrast, leaf roll management requires a more integrated approach involving planting vineyard blocks with virus-tested planting stock, rogueing symptomatic vines and vector control. Thus, future studies should focus on spatiotemporal spread of GLRaV-3 in different AVAs and the influence of environmental and geographical factors on disease dynamics to facilitate the development of epidemiological models that inform decision-making for area-wide management of GLD.

Funds Status:

The following table shows funding provided for three years of the project.

FINAL REPORT

	Year 1 FY	Year 2 FY	Year 3 FY
	Jul 2017 - Jun 2018	Jul 2018 - Jun 2019	Jul 2019 - Jun 2020
Item			
Salaries	66,842	69,515	72,296
Benefits	30,199	31,802	0
Wages	11,613	12,077	12,561
Benefits	1,185	1,233	0
Supplies	28,075	28,075	28,075
Travel	4,818	4,818	4,818
Miscellaneous	0	0	0
Total	142,732	147,520	115,367

YEAR 1: \$142,732

13B-3361-7620: \$38,575 Wine Tax Funds

13Z-3361-7621: \$104,157 WA Wine Auction

YEAR 2: \$147,520

13B-3661-7620: \$77,343 Wine Tax Funds

13Z-3361-7621: \$70,177 WA Wine Auction

YEAR 3: \$115,367

10A-3361-8000: \$66,982 State Funds

13B-3361-7620: \$48,385 Wine Tax Funds

An amount of \$67,202 available from year 3 of the project will be used to support 50% FTE of a post-doctoral research associate during the new project (Jul 20- Jun 23). This information was provided in the new project approved by the Washington State Grape and Wine Research Program.

OTHER FUNDING SOURCES/SUPPORT

During July 2017 and June 2020, funding was obtained from the following resources to conduct research on virus diseases of grapevines. It should be noted that these projects involve multiple institutions and some activities of these projects are distinct and others complement activities of the project funded by the WSGWRP. Collectively, the research-based information generated from these projects is advancing our understanding of virus diseases benefiting grape growers and nursery certification and improvement programs in Washington State.

1. Washington State Commission on Pesticide Registration:
Jan-Dec 2018: \$24,908, Jan-Dec 2019: \$25,310.
2. Northwest Center for Small Fruits Research:
Oct 2016-Sept 2017: \$33,406
3. WSDA-Specialty Crop Block Grant Program:
 - i. September 30, 2015 to April 30, 2018: \$247,878
 - ii. September 30, 2016 to September 29, 2019: \$248,587