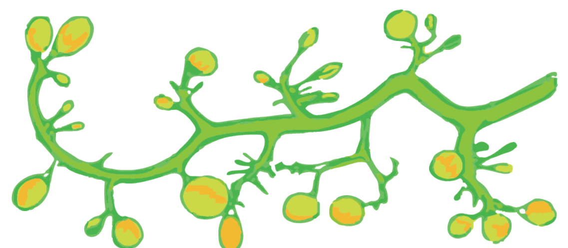
The 21st Congress of the International Council for the Study of Virus and Virus-like diseases of the Grapevine (ICVG)

Napier War Memorial Events Centre, Napier

March 23 – 27, 2026



Welcome

The 21st Congress of the International Council for the Study of Virusand Virus-like diseases of the Grapevine (ICVG)

Napier War Memorial Events Centre, Napier
March 23 – 27, 2026

Tēnā koutou katoa (Greetings everyone)

We are honoured to welcome you to the 21st ICVG conference in Napier, New Zealand. This is the first meeting held in Aotearoa New Zealand! We are excited to bring together an inspiring community of early career researchers, experienced researchers, industry practitioners, and innovators from around the world.

The ICVG congress is a unique platform to exchange ideas, share cutting-edge research, and explore new ideas and solutions that will shape our future research field and industry outcomes. The presence of both academic researchers and industry stakeholders is especially valuable and dear to our kiwi (New Zealand) hearts. We hope the conference will foster meaningful dialogue between industry and researchers, collaborations, and the translation of research into real-world impact for the industry.

Over the course of the conference, we hope you will engage deeply with the presentations, discussions, and networking opportunities. Beyond the formal activities, we also encourage you to build new relationships, friendships, and take advantage of the diverse perspectives represented at the conference. We also hope you take the time to enjoy the beautiful scenery of Napier and other scenic wonders of Aotearoa, during a particular time of uncertainty around the world.

We extend our sincere gratitude to the speakers, reviewers, and sponsors, whose mahi (efforts) made this conference possible. Most importantly, we thank you for being part of this community and for contributing your expertise and enthusiasm.

Ngā mihi nui (Warm regards)
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Meetings

1. Changins (Switzerland) 17-20 August 1964

2. Davis (California) 7-11 September 1965

3. Bernkastel Kues (W. Germany) September 1967

4. Colmar (France) 16-18 June 1970

5. Salice Terme (Italy) 16-19 September 1973

6. Cordoba-Madrid (Spain) 12-17 September 1973

7. Niagara Falls (Canada) 7-12 September 1980

8. Bari (Italy) 2-7 September 1984

9. Kiryat Anavim (Israel) 6-11 September 1987

10. Volos (Greece) 3-7 September 1990

11. Montreux (Switzerland) 5-10 September 1993

12. Lisbon (Portugal) 28 September - 2 October 1997

13. Adelaide (South Australia) 12-17 March 2000

14. Locorotondo (Italy) 12-17 September 2003

15. Stellenbosch (South Africa) 3-7 April 2006

16. Dijon (France) 31 Aug - 4 Sep 2009

17. Davis (California) 7-14 October 2012

18. Ankara (Turkey) 7-11 September 2015

19. Santiago (Chile) 9-12 April 2018

20. Thessaloniki (Greece) 25-29 September 2023

21. Napier (New Zealand) 23-27 March 2026

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ORAL ABSTRACTS

Grapevine virology highlights: 2023-2026

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INTRODUCTION

Grapevine virus research continues to thrive at the global scale. Since the last ICVG conference in Thessaloniki, Greece in 2023, a few viruses were newly identified and characterized, diagnostic tools were optimized, new spread dynamics were reported, ecological aspects of disease epidemiology were defined, novel pathogenicity traits were described, molecular underpinnings of virus-host interactions were uncovered, and innovative disease management strategies were reported. Progress in all these areas is explained by cross-disciplinary efforts that have questioned established paradigms and pushed the boundaries of knowledge. This article summarizes major accomplishments in grapevine virus research from 2023-2026.

NEWLY IDENTIFIED VIRUSES AND VIROIDS

New viruses were characterized by high-throughput sequencing (HTS) from symptomatic vines infected with multiple viruses. Grapevine leafroll-associated virus S (GVS) from the genus *Ampelovirus* in the family *Closteroviridae* was identified from table grape cultivars 'Super Sonaka' and 'Manjari Kishmish' mixed infected with seven other viruses in India (Prajapati et al. 2025). Of note, GVS clustered with subgroup I ampeloviruses such as GLRaV3 based on the coat protein gene sequence, and with subgroup II ampeloviruses such as GLRaV4 based on the RNA-dependent RNA polymerase (RdRP) and heat shock protein 70 homolog gene sequences (Prajapa0ti et al. 2025). Grapevine-associated cogu-like Idaho virus (GaCLIdV) from the family *Phenuiviridae* was identified from 'Chardonnay' vines mixed infected with at least six other viruses in Idaho, USA (Dahan et al. 2025). Similarly, grapevine cheravirus (GCheV) was identified in a 'Cabernet Sauvignon' vine displaying foliar vein yellowing symptoms in China (Fan et al. 2025), and grapevine virus P, a putative new vitivirus, was described in Japan (Ito 2024). The involvement of GVS, GaCLIdV, GCheV, and GVP in vine vigor and disease symptoms is unknown. Finally, grapevine yellow speckle viroid 3, tentatively assigned to the genus *Apscaviroid* in the family *Pospiviroidae*, was identified by mining a transcriptome dataset derived from grapevines (Prajapati et al. 2024).

The discovery of four viruses adds to the 102 viruses previously reported (Fuchs 2025), although GVP was already accounted for in this 2025 review article; therefore, the total number of viruses identified in grapevines is theoretically 105. However, grapevine was questioned as a host of Solanum nigrum ilarvirus 1 (SnIV1), a previously identified virus from the genus *Ilarvirus* in the family *Bromoviridae*, because the presence of SnIV1-infected pollen may have twisted the interpretation of HTS data obtained from grapevine tissue (Rivarez et al. 2023), as elegantly demonstrated for grapevine angular mosaic virus, another ilarvirus (Manillon et al. 2024). If SnIV1 is shown to be associated with grapevine rather than infecting it, the total number of grapevine viruses would be 104 at the present time.

PREVALENCE OF VIRUSES AND VIROIDS IN VINEYARDS AND DIVERSITY

Vineyard surveys revealed a prevalence of grapevine fanleaf virus (GFLV), grapevine virus A (GVA) and grapevine leafroll-associated virus 2 (GLRaV2) in Kazakhastan (Frolov et al. 2025), and of GFLV, GVA, grapevine leafroll-associated virus 1 (GLRaV1), GLRaV2, grapevine leafroll-associated virus 3 (GLRaV3), and grapevine leafroll-associated virus 4 (GLRaV4) in Azerbaijan

(Sultanova et al. 2023). The occurrence of grapevine rupestris stem-pitting-associated virus (GRSPaV), GLRaV2 GFLV, and grapevine fleck virus (GFkV) was reported in Argentina (Gomez Talquenca et al. 2023); GRSPaV, GLRaV1, GLRaV2, GLRaV3, grapevine Rupestris vein feathering virus (GRVFV), grapevine Red Globe virus (GRGV), grapevine Pinot gris virus (GPGV), GFkV, and grapevine red blotch virus (GRBV), GVA, grapevine virus B (GVB), GFkV, and tomato ringspot virus (ToRSV) in Canada (Xiao and Meng, 2023, Vemulapati et al. 2025); GRSPaV, GLRaV3, GFkV, tobacco ringspot virus (TRSV), GVB and GPGV in Michigan, USA (Neugebauer et al. 2025); GLRaV3 in table grape accessions in southern California, USA (Zhai et al. 2025); GFLV, GLRaV1, GFkV, GVA, and Arabis mosaic virus (ArMV) in Türkiye (Güler and Paylan 2024); GLRaV1, GLRaV2, GLRaV3, GLRaV4, TRSV, ToRSV, GFkV, GRSPaV, GVA, GVB, grapevine virus L (GVL), grapevine virus M (GVM), and GRBV in interspecific hybrids in Texas, USA (Ouro-Djobo et al. 2025); grapevine viruses E, F, I, L and T in Greece (Panailidou et al. 2025, 2024, 2026); and *Vitis* cryptic virus, GRSPaV, GPGV, GVT, GLRaV1, GVA, GRVFV, GRGV, and grapevine satellite virus in *Vitis vinifera* spp. *sylvestris* in the Black Sea region (Belkina et al. 2025). The global distribution of commonly occurring viruses is confirmed by first reports of GVB, GRSPaV, grapevine Syrah virus 1 (GSyV1), GRGV, and GPGV in Peru (Lozada et al. 2025, Sánchez-Moncada and Alvarez 2025), GLRaV4 in Hungary (Olah et al. 2024), grapevine virus F (GVF) in China (Xudong et al. 2025); grapevine geminivirus A (GGVA) and grapevine Garan Dmak virus in Lebanon (Bilen et al. 2025); and GFkV, GLRaV1, and GLRaV3 in Korea (Choi et al. 2024). Of note, GRBV was described in US accessions maintained in germplasm repositories in Australia (Kaur et al. 2025, Kinene et al. 2025). Finally, GYSVd1 was shown to be widespread in vineyards of Slovakia (Kemenczeiova et al. 2025).

The genetic diversity of GLRaV1 (Razavi et al. 2021) and GVA (Gharouni-Kardani et al. 2023) in Iran; GLRaV2 (Habili et al. 2023) and GVA (Wu et al. 2023) in Australia; grapevine Roditis leaf discoloration-associated virus in Türkiye (Serçe et al. 2024); GPGV in Serbia (Zivkovic et al. 2024) and Australia (Kaur et al. 2023); GVL in Greece (Panailidou et al. 2023); GRSPaV (Adel et al. 2024), GFLV (Laidoudi et al. 2025), and GFkV (Imene et al. 2025) in Algeria; grapevine leafroll-associated virus 13 and GLRaV4 in China (Du et al. 2025a,b) was characterized in grapevine; and GLRaV2, GLRaV3 and GVA in long-tailed mealybugs in Brazil (Fajardo et al. 2024).

ADVANCES IN DIAGNOSIS

A library preparation protocol for the detection of plant viruses and viroids in grapevine and other fruit crops by HTS was validated by three US laboratories (Abrahamian et al. 2025). Proficiency sample panels were prepared from petiole and budwood tissue, spiked with an exogeneous positive control, distributed to each laboratory and tested using a standardized set of guidelines for quality assessment by using the same nucleic acid extraction and library preparation protocols. HTS-based detection of the expected targets was achieved by all three laboratories. When normalizing all datasets, 100% virus detection rate was achieved when at least 15M sequence reads were obtained (Abrahamian et al. 2025).

A duplex diagnostic RT-qPCR was developed for GLRaV1 using primers/probe designed in the coat protein gene, as well as the phosphoenolpyruvate carboxylase as an internal control (Morán et al. 2023). For GLRaV3 diagnosis, ddPCR and LAMP were the most sensitive assays compared with RT-PCR and RT-qPCR using primers pairs designed in the RdRP gene although the number of GLRaV3 isolates and their phylogenetic affiliation were not described in this study (Sández-Salas et al. 2025). A RT-qPCR high-resolution melting curve analysis was developed for the identification of GLRaV3 and GFLV variants in Brazil (Fajardo et al. 2023). For GRBV detection, PCR and qPCR were the most accurate diagnosis methods, but LAMP and basal canopy symptom monitoring were valuable late in the growing season in Oregon, USA (DeShields and KC 2023). Optimal conditions for the identification of GRBV-infected black-berried winegrape cultivars based on visual symptom

monitoring were described (Rohrs et al. 2023). Trunk cambium tissue was used for year-round detection of GRBV by LAMP (Rohrs et al. 2024) and for discerning spatial patterns of infected vines when visual assessments are insufficient, such as in white-berried winegrape cultivars in northern California, USA (Rohrs et al. 2025). Similarly, tissue material from dormant canes was scraped with sandpaper and the corresponding nucleic acids were used in RT-qPCR for detecting GFLV, ArMV, GLRaV1, GLRaV3, GFkV, GVA and GVB in Greece (Krokida et al. 2025).

Airborne imaging spectroscopy utilizing a NASA instrument was deployed to detect GLRaV3 in vineyards of California, USA with an 85% accuracy in distinguishing noninfected, asymptomatic infected or symptomatic infected vines (Galvan et al. 2023). Hyperspectral imaging and machine learning were also applied to identify GLRaV3- and GRBV-infected grapevines in California, USA with an overall accuracy of 87% for infected leaf samples and 83% for noninfected leaf samples, but differentiation of co-infected plants was challenging (Sawyer et al. 2025). A higher accuracy (>95%) for classifying grapevine leaves with and without GLRaV3 and/or GRBV symptoms was achieved in another study (Lazcano-Garcia et al. 2025). For GRBV, hyperspectral imagery identified infected vines in a diseased vineyard with an accuracy of 76% around harvest, coinciding with the peak of disease symptom expression, and 74% prior to véraison when most vines are asymptomatic (Laroche-Pinel et al. 2025).

ADVANCES IN ECOLOGY AND TRANSMISSION

A mathematical model was developed to describe the dynamics of GLRaV3 spread and viruliferous obscure mealybugs (*Pseudococcus viburni*), as well as mealybug control through the use of natural enemies (Voigt-Geisse et al. 2025). This model could be applied to other mealybugs species and facilitate biological control of mealybug vector to reduce the spread of GLRaV3. Monitoring the incidence of GLRaV3 and GRBV in five vineyards in Mexico during 2021-2023 revealed an average increase in virus incidence of 1.7% and 9% in 2022 and 2023, respectively, with vineyard-specific spatiotemporal distributions of newly infected vines (Garcia-Reséndiz et al. 2025). Investigating the spread of GRBV and GPGV in vineyards of Ontario, Canada showed the presence of GPGV but not GRBV in sentinel vines placed in diseased vineyards. GPGV was also detected in wild grapes but not in middle-row cover crop species (Vu et al. 2023). Secondary spread of GPGV was also documented in four vineyards in Germany (Messmer et al. 2024). The spatiotemporal spread of GLRaV3 in vineyards established with virus-tested winegrape cultivars adjacent to vineyards heavily affected by leafroll disease in Washington, USA revealed a gradual increase in disease incidence over time (Donda et al. 2023). A disease gradient was observed with the highest percentage of symptomatic vines in rows proximal to infected vineyard blocks with random patterns during initial years, indicating primary spread of the virus not related to infected vines within the block. In subsequent years, clustering at the scale of neighboring vines suggested within vineyard secondary spread (Donda et al. 2023). Velvetleaf (*Abutilon theophrasti*), redroot pigweed (*Amaranthus retroflexus*), field poppy (*Papaver rhoeas*), three plant species commonly found in Croatian vineyards, were infected with GVA following transmission assays with the vine mealybug (*Pseudococcus ficus*) in the laboratory. No information is available on the natural occurrence of GVA in these plants species (Voncina et al. 2024).

For GRBV, the three-cornered alfalfa hopper (*Spissistilus festinus*) was documented as a vector of epidemiological relevance in vineyards of northern California, USA (Flasco et al. 2023a). Characterizing its dietary history by amplicon high-throughput sequencing revealed a total of 171 genera from 60 plant families with a preeminence of Asteraceae, Fabaceae, and Vitaceae over two growing seasons, illustrating dietary profiles composed primarily of natural vegetation rather than winegrapes (Hoyle et al. 2025a). Distinct seasonal feeding trends related to weather patterns and host repertoires unique to some vineyard sites were observed with estimated vector travel distances of up to 2 km. An ecological relatedness between free-living vines in riparian corridors and vineyards for

GRBV transmission was documented (Hoyle et al. 2025a). GRBV acquisition and transmission is influenced by the treehopper genotype and sex, and by the virus isolate (Flasco and Fuchs, 2023, Hoyle et al. 2024a, Hoyle et al. 2025b). Transmission is not transovarial (Hoyle et al. 2024a), but treehoppers retain GRBV for at least 60 days without access to an infected plant (Hoyle et al. 2025b). Dispersal of the treehopper vector by walking and jumping contribute to short distance spread of GRBV (Hoyle et al. 2024a) but movement by flying contributes to long distance spread, particularly for male insects that exhibit significantly longer and farther flights (570m in average) compared to females (240m in average) using a flight mill apparatus under laboratory conditions. Flight propensity, however, was not influenced by insect sex or age (Antolinez et al. 2023). Symptomless GRBV-infected grapevines in spring display a relatively high virus titer that may favor treehopper-mediated GRBV transmission (Flasco et al. 2023b). Another treehopper species (*Tortistilus wickhami*) is more abundant than the three-cornered alfalfa hopper in northern California vineyards, but it is an unsubstantiated vector of GRBV (Hoyle et al. 2024b). Annual surveys in a vineyard planted in 2015 in California revealed red blotch disease onset in 2018 and an aggregation of vines infected with GRBV isolates of phylogenetic clade 1, the least prevalent clade, suggesting the rootstock as the virus inoculum source. GRBV clade 1 isolates were predominant in 2018-2019 but displaced by clade 2 isolates in 2021-2022, revealing an influx of the latter isolates from outside sources. An adjacent vineyard exhibited disease symptoms one-year post-planting, likely due to infected scion material (Flasco et al. 2023c). In a third vineyard in California, disease incidence increased from 3.9% in 2014 to 36.4% in 2023 with rapid GRBV spread proximal to a transmission hotspot (Flasco et al. 2025). Remarkably, precipitation (3-4 years later) and air temperature (the same or 1 year later) significantly influenced epidemic parameters, and asymptomatic infections contributed to spatial aggregations at increasing lags (Flasco et al. 2025).

ADVANCES IN VIRUS-HOST INTERACTIONS

The effect of GLRaVs on ‘Crimson Seedless’ infected with GLRaV-3 or GLRaV-4 strain 9 and GVA showed that all fruit quality indices significantly affected by viral infection over two years, while titratable acidity varied annually (Salot et al. 2024). The anthocyanin and phenol content of infected vines were significantly reduced compared with noninfected vines, while no quantitative relationship between viral load and berry composition was found (Salo et al. 2024). The p23, HSP70 and CP of GLRaV4 were found to interact in a yeast two-hybrid system and a bimolecular fluorescence complementation assay (Du et al. 2025). Grapevine berry inner necrosis virus (GINV) and GYSVd1 decreased soluble sugar and increased organic acids in berries and wine in ‘Welschriesling’ (Wu et al. (2025). GINV decreased glucose content and increased malic acid content by regulating acid invertase, isocitrate dehydrogenase, phosphoenolpyruvate carboxylase, and malate dehydrogenase activity, while GYSVd1 decreased glucose content and increased malic acid content by regulating acid invertase and citrate synthase activity. However, in mixed infection with GINV and GYSVd1, the negative effects on soluble sugars were not observed (Wu et al. (2025). Early symptoms of GLRaV3 are good predictors of post-véraison severity, yield and sugar content in fruit juice. Some GLRaV3 isolates always caused mild symptoms or remained asymptomatic 10 years after grafting but remained a source of infection for vectors (Cabaleiro et al. 2023).

Grapevine genes associated with systemic acquired resistance were upregulated in vines infected by GLRaV4 strain 9 and GVA that exhibited Shiraz disease symptoms, while genes related to vine immunity were downregulated. The nature of upregulated genes suggested that callose deposition and the blocking of phloem sieve elements are likely employed as a defense strategy to limit the spread of viruses (Onetto et al. 2025). Inoculating 'Red Globe' and 'Summer Black' plants with an infectious clone of GGVA resulted in upward leaf curling, chlorosis, malformation, and necrosis, documenting that this virus causes severe foliar symptoms (Liu et al. 2025). A cDNA clone of GPGV with a large movement protein (MP) was engineered and its pathogenicity was tested via

Agrobacterium tumefaciens-mediated delivery (Karki et al. 2025). The new infectious clone unexpectedly resulted in a faster GPGV replication and caused more severe disease symptoms than other infectious GPGV cDNA clones with a large or a short MP. A single C to T mutation at the polymorphic MP gene site resulted in a more severe disease symptoms and a two-fold higher RNA accumulation compared to the wildtype infectious clone (Karki et al. 2025). These authors proposed that the GPGV titer rather than its genetic makeup trigger the onset of disease symptoms. Studies on protein-protein interactions between GLRaV3 and *Vitis vinifera* using a yeast two-hybrid library screened against viral open reading frames encoding structural proteins and those potentially involved in systemic spread and silencing of host defense mechanisms revealed five interacting protein pairs, three of which were validated *in planta* (Moster et al. 2023). The minor coat protein of GLRaV3 was shown to interact with 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase 02, a protein involved in primary carbohydrate metabolism and the biosynthesis of aromatic amino acids. Interactions were also identified between GLRaV3 p20A and an 18.1-kDa class I small heat shock protein, as well as MAP3K epsilon protein kinase 1. Both proteins are involved in the response of plants to various stressors, including pathogen infections (Moster et al. 2023)
A differential expression of miRNAs in 'Merlot' infected with GRBV at the pre- and post-véraison stages was found with GRBV-derived siRNAs (vsiRNAs) predominantly ranging from 19 to 24 nt, with the 21nt size being the most abundant (Ault et al. 2024). Mapping vsiRNAs across the GRBV genome revealed hotspots predominantly located in the V3 open reading frame (Ault et al. 2024). Proteins C2 and V2 of GRBV function as viral silencing suppressors (VSRs) with synergistic or additive effects observed at the mRNA level when these two proteins are expressed together transiently (De Silva Weligodage et al. 2023). Similarly, GFLV proteins 1A, 1B, and 1AB, which is predicted as an intermediary product of RNA1 polyprotein proteolytic processing, were identified as VSRs. These VSRs differentially altered the expression of plant host genes involved in RNA silencing (Choi et al. 2023). The three GFLV VSRs carry conserved WG/GW motifs. Mutating tryptophan (W) to alanine (A) in the WG motif of proteins 1A and 1AB abolished their ability to induce systemic RNA silencing suppression, limit siRNA accumulation, downregulate *NbAGO2* expression, and resulted in a non-infectious virus. Mutating W to A in the GW motif of proteins 1B and 1AB reduced their ability to suppress systemic RNA silencing, abolished the downregulation of *NbDCL2*, *NbDCL4,*, and *NbRDR6* expression, delayed infection at the local level, and inhibited systemic infection *in planta*. Double mutations of W to A in protein 1AB abolished its ability to induce RNA silencing suppression, limit siRNA accumulation, and downregulate *NbDCL2* and *NbRDR6* expression. Thus, the conserved WG/GW motifs are critical not only for the VSR function but also for the virus viability. *In silico* protein structure prediction indicated that a W to A substitution modifies the structure and physicochemical properties of the three GFLV VSRs (Choi et al. 2024).

Predictive modeling of GFLV proteins confirmed previously validated functions or previously identified putative functions and predicted novel functions with varying levels of confidence (Roy et al. 2024). Infection of *Nicotiana benthamiana* plants with wildtype GFLV strain GHu and mutant GFLV strain F13 $1E_{G802K}$, both carrying a lysine in position 802 of protein 1E (RdRP), resulted in a significantly lower number of root tips (-30%), and a significantly increased average root diameter (+ 20%) in comparison with roots of mock inoculated plants (Roy and Fuchs 2024). In contrast, the root system architecture (RSA) of plants infected with wildtype GFLV strain F13 and mutant GFLV strain GHu $1E_{K802G}$, both carrying a glycine in position 802 of protein 1E, resembled that of mock inoculated plants. Root tissue transcriptome analysis indicated dysregulation of pattern recognition receptors, plant hormones, RNA silencing, and genes related to the production of reactive oxygen species (Roy and Fuchs 2024). Changes in RSA resulted in differential dagger nematode (*Xiphinema index*)-mediated GFLV transmission rates in relation to the viral strain and the identity of the amino acid in position 802 of protein 1E, with a glycine favoring transmission and a lysine reducing transmission (Roy et al. 2025).

Extensive reprogramming of the *N. benthamiana* transcriptome was observed upon infection with ToRSV in symptomatic leaves, including upregulation of genes characteristic of biotic stress responses such as cysteine-rich antimicrobial peptides, and downregulation of genes associated with the function and translation of chloroplasts (Paudel et al. 2025). The majority of plant genes that were differentially regulated during the symptomatic stage returned to their basal levels after plant recovery. Genes that remained upregulated after the symptomatic stage or were specifically induced at the symptom recovery stage were also identified. Several plant miRNAs were differentially expressed in ToRSV-infected plants, and their predicted targets were differentially regulated. Many of the miRNA predicted targets were related to plant defense responses and may contribute to symptom induction and/or symptom recovery (Paudel et al. 2025).

MITIGATION AND MANAGEMENT

Epidemiological findings on GRBV suggest zonal roguing, the removal of diseased and surrounding vines, as a disease management option (Flasco et al. 2025). Cultural approaches were investigated in diseased vineyards to mitigate the effects of GRBV, but mixed results were obtained (Girardello et al. 2024, Kurtural et al. 2023, Roberts et al. 2025a, b). Editing technologies such as CRISPR were explored to engineer resistance against grapevine viruses (Spencer et al. 2025). By investigating the early activation of RNA interference (RNAi) in GRBV-infected grapevines, small RNA sequencing identified nine genomic virus-derived small interfering RNA (vsiRNA)-producing regions involved in yielding 24-nt vsiRNA species associated with transcriptional gene silencing. Administring double-stranded RNA molecules derived from these vsiRNA-producing regions to GRBV-infected plants via root soaking of *Agrobacterium tumefaciens* carrying infectious viral clones, significantly reduced viral gene expression in leaves and petioles (Mandelli and Deluc 2025). Remarkably, the exogenous application of HSVd-specific double-stranded RNA significantly reduced HSVd levels in infected grapevine plantlets through RNAi. Notably, the inhibitory effect persisted through three successive generations without additional treatment (Kang and Jeong 2025).

REVIEW ARTICLES

A review article reported more than 100 viruses in grapevines (Fuchs 2025). Other reviews focused on GRBV (Cieniewicz and Fuchs 2025) and detailed transmission protocols of GRBV by the three-cornered alfalfa hopper (Hoyle et al. 2025c). Flasco et al. (2023d) published an opinion piece on GRBV transmission, given a flurry of misleading reports on this topic, and made recommendations on best practices for transmission assays with insect vector candidates. Krenz et al. (2023) offered a series of open research questions on GRBV to guide more investigations on this virus. Other reviews centered on leafroll disease (Buciumeanu et al. 2025) and GLRaV3 (Fust et al. 2025), or provided an historical account of GLRaV3 in New Zealand (Chooi et al. 2024) and of GLRaV2 in Australia (Habili et al. 2023). The occurrence of viruses and their management in Mexico were discussed (Diaz-Lara et al. 2023). Furthermore, the applications of HTS were evaluated for the detection of known and novel viruses in grapevine with an emphasis on the performance of different bioinformatics approaches. Best practices were recommended for the deployment of HTS based on sensitivity, specificity and analytical sensitivity (Stevens and Al Rwahnih 2024). Investigating the winegrape industry in the western USA resulting in the identification of ways to improve the dissemination of knowledge for increasing the adoption of viral disease management strategies (Hobbs et al. 2023). Finally, Tzanetakis et al. (2024) identified “phantom agents”, i.e., putative pathogenic agents that are cited in the literature but lack supporting evidence of their existence, in specialty crops and recommended their removal from regulatory lists to streamline germplasm exchange. Fuchs et al. (2025) complemented the list of phantom agents in grapevines by considering nine additional viruses, 14 virus-like diseases and nine viroids to be excluded from regulatory oversight or should not be regulated. This list is anticipated to assist policy makers adopt regulations

that expedite the safe exchange of *Vitis* germplasm across regulatory boundaries while reducing incentives for illicit introductions.

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A large-scale, multi-year virome survey in Belgium reveals potential phytosanitary risks through the first detection of five regulated non-quarantine pests (RNQPs)

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INTRODUCTION

Over the past decade, Belgian viticulture has undergone a remarkable transformation, expanding from only a handful of hectares to ~1,000 ha by 2024 (SPF Economie, 2025). This rapid rise, fueled by climate change and consumer enthusiasm, has resulted in a brand-new winemaking territory in Europe.-Yet, with this growth comes uncertainty: which virus or viroid-based diseases may have been introduced with planting material or have already established and are causing disease? And what phytosanitary risks do they pose to Belgian vineyards in the long term?

The VITIBEL project, launched in May 2023, was designed to address these questions. Built on a unique partnership between scientists and growers, the project now delivers the first comprehensive overview of grapevine viruses and viroids in Belgium. By combining large-scale virome surveys based on high-throughput sequencing with targeted prevalence studies and continuous engagement with growers, VITIBEL goes beyond establishing an inventory: it fills a critical knowledge-gap and provides the foundation for understanding national and local epidemiological risks.

MATERIALS AND METHODS

In early summers 2023 and 2024, representative surveys were conducted, combining analysis on both asymptomatic pooled samples and individual symptomatic plants to establish the baseline virome of Belgian vineyards. It was accompanied by a grower's interview to collect metadata (cultivar, origin, year of planting and some other cultural and pest management practices). For all samples, leaf tissue was used as the biological matrix. Symptomatic samples underwent total RNA extraction, while asymptomatic pools were processed using an antibody-based dsRNA enrichment protocol (Blouin et al., 2023). Extracts were subjected to Illumina high-throughput sequencing (HTS), and viral detections were validated through RT-PCR and Sanger sequencing.

In 2025, prevalence surveys were conducted within selected parcels where regulated non-quarantine pests (RNQPs) for the European Union had been detected during the initial survey. Targeted viruses were *Nepovirus foliumflabelli* (GFLV), *Ampelovirus univitis* and *Ampelovirus trivitis* (GLRaV-1, -3), *Nepovirus arabis* (ArMV), and *Nepovirus rubi* (RpRSV). Sample size per vineyard was calculated using a binomial proportion approach, corrected for finite population size and design effect.

Sampling followed a systematic grid across vines and rows, with individual leaves collected and pooled in groups of five. DAS-ELISA analyses are currently ongoing, and positive pools will be resolved by retesting individual vines. For GFLV, the final round of sampling is still in progress, and in addition neighboring plants of symptomatic vines are specifically targeted for testing. Soil samples will be collected and analysed for nematode detection and identification. Confirmed viral isolates will subsequently undergo Sanger sequencing for genetic diversity analysis.

RESULTS AND DISCUSSION

The combined 2023-2024 HTS campaigns provided the first comprehensive overview of grapevine viruses and viroids in Belgium. Across both years, 86 vineyards and three genetic collections were surveyed, covering all production areas in Belgium. Approximately 4,400 vines samples from more than 50 cultivars, different planting years and nursery origins were sampled and deep-sequenced. Illumina data revealed over 35 distinct viral and viroid species, spanning at least 8 families and 12 genera, which corresponds to a substantial proportion of the currently known global grapevine virus diversity. Twenty-one species were confirmed by RT-PCR and Sanger sequencing, while the remaining detections are under validation. Most correspond to first records of grapevine viruses and viroids in Belgium, highlighting both the diversity of the virome and the detection of five regulated non-quarantine viruses (GFLV, GLRaV-1, -3, ArMV, and RpRSV) that may threaten production.Most correspond to first records of grapevine viruses and viroids in Belgium.
Building on these findings, the 2025 prevalence survey was launched to estimate field prevalence of the five RNQPs. In total, 5,318 vines from 25 parcels were sampled across 16 vineyards and 3 genetic collections. Laboratory analyses are still in progress. Preliminary results confirmed independently the detection of the five RNQP viruses and enabled initial estimation of their prevalence and distribution in the different vineyards. These prevalence data, combined with the HTS-based virus inventory, will strengthen the phytosanitary baseline for Belgian viticulture and inform both growers and regulators on the magnitude of viral threats and open a question about future impact of new viral diseases for this emerging sector. In parallel, correlations between virus occurrence and vineyard metadata (cultivar, nursery origin, planting year, and management practices) are being explored to identify potential sources and pathways of virus introduction and spread within the country.

ACKNOWLEDGEMENTS

We warmly thank all participating winegrowers for their invaluable collaboration. This work was supported by the Federal Public Service (FPS) Health, Food Chain Safety and Environment of Belgium, under the VITIBEL project (RF 23/22).

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The Grapevine Certification Program in New York State, USA

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INTRODUCTION

The United States is the third fresh grape producer globally after China and Italy (OIV 2025). Its grape industry is one of the most valuable specialty crop industries in the country, contributing over $323 billion to the economy in 2024. New York is the third grape producer in the United States for both production volume and value. The New York grape industry supports winemaking, juice production, and the fresh market with an economic value of $6.65 billion in 2024 (NYWGF 2024).

More than 100 viruses have been identified in grapevines, of which some are causing or are associated with economically concerning diseases (Fuchs 2025). The management of these viruses primarily relies on preventive measures to limit their presence in the propagation and planting material. Clean, virus-tested vines are the backbone of sustainable viticulture and are primarily maintained in foundation vineyards referred to as G1 blocks with usually 2-5 individuals kept for each accession (i.e., cultivar, clone, genotype, selection) (Fuchs 2020). Propagative material from G1 vines is then transferred to nurseries and established in increase vineyards, referred to as G2 blocks or eventually in G3 and G4 blocks, to bulk up the number of propagative units needed for the production of planting stocks to be sold to growers (Fuchs 2020). The cleanliness of G1 vines in the United States is determined by high-throughput sequencing and bioinformatic tools at institutions involved with the National Clean Plant Network (NCPN).

The health status of clean G1-G4 vines that are maintained outdoors can be compromised due to vector-mediated virus infections or unintentional human errors. Testing regularly these vines for viruses is essential to quickly identify and eliminate virus-infected individuals and avoid secondary spread. This can be achieved via certification, a system of monitoring and testing vines for the presence of undesired viruses in combination with site isolation and plant maintenance criteria. In the United States, certification it managed by individual states rather than by the federal government. In New York, a grapevine certification program was initiated in the early 1960's. This program ceased to exist in the mid 1990's due to a lack of funding and limited availability of clean stocks during an expansion phase of the industry. However, a widespread occurrence of leafroll viruses (Fuchs et al. 2009) and their recognized economic impact (Atallah et al. 2012) in the state triggered a strong interest to revitalize a certification program. Starting in 2017, the New York grapevine nurseries and representatives of the grape and wine industries built a partnership with the New York State Department of Agriculture and Markets and Cornell University to imagine a realistic and meaningful program for the 21st century. Below is a summary of some of the major accomplishments of the New York certification program in 2024-2025.

MATERIALS AND METHODS

New York nurseries maintain clean G2 vines in isolated vineyard sites with minimal dagger nematode (*Xiphinema americanum* sensu lato) populations after introducing clean, virus-tested G1 material from Cornell University, Foundation Plant Services at the University of California in Davis, the Clean Plant Center Northwest at the University of Washington or the Midwest Center at Missouri State University. These four institutions are part of NCPN that supports the production, maintenance

and distribution of G1 propagative material free of undesired pathogens and pests. However, only the centers in California, Washington, and Missouri carry foundation G1 vineyards.
Staff from the New York State Department of Agriculture and Markets work with the New York nurseries to collect leaf samples from 25% of all vines in G2 blocks for virus testing each year. Samples are submitted for virus testing to Cornell University. Blind testing is performed with leaf samples delivered to the lab with just a number so that their origin is concealed to prevent biases. To facilitate high throughput testing, composites of 5-25 leaf samples are analyzed by PCR or DAS-ELISA in the lab to lower the number of assays without affecting the accuracy of the tests. If a composite unit tests positive for a virus, the number of samples is split for re-testing until the infected sample(s) is identified. Viruses of concern are tomato ringspot virus (ToRSV), tobacco ringspot virus (TRSV), grapevine fanleaf virus (GFLV) in the spring, and grapevine leafroll-associated virus 1 (GLRaV1), grapevine leafroll-associated virus 2 (GLRaV2), grapevine leafroll-associated virus 3 (GLRaV3), grapevine leafroll-associated virus 4 (GLRaV4), and grapevine red blotch virus (GRBV) in the fall. The quality of the virus test results is thoroughly evaluated by the lab prior to being communicated to the Department of Agriculture and Markets. In turn, the Department of Agriculture and Markets discusses the cleaniness of the G2 vines with the nurseries and the removal of virus-infected G2 vines.

RESULTS AND DISCUSSION

New York reinstated a certification program in 2022. It is managed by the Department of Agriculture and Markets that acts as a third party to monitor the health status of approximately 53 hectares of G2 vines maintained by three nurseries in New York. In 2024-2025, approximately 0.1% of the G2 vines tested were positive for viruses, including ToRSV (0.1%, 32 of 30,807), TRSV (0.01%, 3 of 30,807), GLRaV1 (0.02%, 6 of 30,807) and GLRaV3 (0.01%, 2 of 30,807). None of the leaf samples tested in both years were positive for GFLV, GLRaV2, GLRaV4 or GRBV. Testing 25% of vines in G2 blocks each year is unprecedented for a certification program, but this level of testing is essential to maintain the cleanliness of G2 vines no tolerance for economically damaging viruses. This scope of testing guarantees that every vine in G2 blocks is tested in the course of four years. It is also critical to build confidence in the certification program for grape growers interested in high-quality planting stocks (Li et al. 2025). Certified vines are sold by nurseries to the satisfaction of the growers at a $0.50 premium for their superior quality. Options to revise the program requirements for overall improvement are discussed annually by all the partners.

ACKNOWLEDGEMENTS

Support for the New York certification program is provided by the New York State Environmental Protection Fund, USDA-APHIS, New York nurseries, and the New York Wine and Grape Foundation.

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An ongoing grower-led holistic grapevine virus outreach and extension project in California, U.S.A

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INTRODUCTION

The Lodi Winegrape Commission is a formal organization created in 1991 by winegrowers in Lodi, California, U.S.A for the purposes of research, education and promotion. The winegrowers tax themselves on the sale of their winegrapes to fund activities which are directed by an elected Board and carried out by full-time staff. The Lodi Winegrape Commission currently represents approximately 750 growers farming over 33,100 hectares of vineyards.
The sheer size and fast growth of the California viticulture industry have led to a massive oversight in terms of grapevine viruses and their vectors, which continue to spread throughout the state. No single state body oversees the holistic management of virus and vector prevention, outreach, and testing. The state's grapevine certification program provides a false sense of security due to severely inadequate supply chain testing, commercial virus testing is unmonitored, and the average winegrower is uninformed (or even worse – misinformed) about viruses and vectors.
Since the Lodi winegrowers were losing millions of dollars and watching vine mealybugs and leafroll 3 virus spread like wildfire, they organized Research Focus Groups to find both short- and long-term solutions to this crisis.

MATERIALS AND METHODS

In early 2017, the Lodi Winegrape Commission established the Mealybug Biocontrol Research Focus Group. This group was led by Dr. Stephanie Bolton and included growers, pest control advisors, a university Farm Advisor, and industry members who shared decades of real-world experiences. Dr. Kent Daane (University of California) served as the "on-call" expert advisor, and the group met monthly at a local diner for breakfast for over a year, discussing how to best implement mealybug biocontrol practices in Lodi.
Later in 2017, the Lodi Winegrape Commission established the Lodi Grapevine Virus Research Focus Group, building on the success and knowledge gained from the Mealybug Research Focus Group. The Virus Research Focus Group was also a collaborative team of growers, pest control advisors, scientists, nurseries, viticulturists, and extension personnel with Prof. Gerhard Pietersen (South Africa) and Prof. Marc Fuchs (Cornell University) as advising "on-call" experts. Dr. Maher Al Rwahnih (Foundation Plant Services) also proved invaluable as a consultant.
Like the Mealybug Research Focus Group, the Virus Research Focus Group met monthly at a local diner for breakfast and had clear objectives. The overall objective was to learn how to best manage and prevent grapevine virus disease in Lodi, California and beyond. The following sub-objectives were established:

1. To conduct a grapevine virus literature search, including collecting case studies about grapevine viruses both locally, state-wide, and internationally.
2. To learn how to best test and rogue infected grapevines for virus management, developing and incorporating economic thresholds into outreach materials.

3. To learn best practices for replacement of an existing infected vineyard.
4. To formulate a long-term management plan for economically feasible and impactful virus control strategies in Lodi.
5. To develop and deliver timely, relevant educational materials and workshops for best virus management practices for Lodi growers.
6. To establish priorities for further grapevine virus research projects.

The Virus Focus Group conducted in-depth studies, mostly via reading and conversations with each other and numerous others, on the topics of: viruses and their vectors, the California grapevine certification program, virus testing, virus transmission, vector and virus management, nursery management, foundation block management, winegrower perceptions, industry myths, and more.

As new information was gained, it was continuously shared to various audiences within Lodi, across California, nationally, and internationally via numerous formats including written blog posts, newsletter articles, in-person meetings indoors and in the field, hands-on trainings, videos, booklets, a book (Bolton 2020), a website, emails, graphics, presentations, and one-on-one conversations.

We hosted "Grapevine Virus Influencer Dinners" to bring together thought leaders in the field and "Virus Testing Days" to encourage testing. We held a Family Field Day with a beneficial insect drone demonstration and a female *Anagyrus* wasp superhero. We created physical outreach materials such as laminated mealybug scouting cards in English and Spanish, custom vine mealybug flagging tape, and custom vine roguing flagging tape.

RESULTS AND DISCUSSION

When we all learn together, we can better work together and find solutions faster. Teamwork and neighbor-to-neighbor education are critical to reduce devastating virus infections in California. The first step in the outreach project was to help growers understand why they need to care enough to learn about viruses and their management. Many growers are in a state of denial where they do not want to face the virus issue, despite their vineyards being severely infected.

A key to success was that Research Focus Group members had a financially vested interest in finding mealybug and virus management solutions, and that they were willing to work towards a greater good for everyone. California growers are learning more about viruses and taking intentional action towards their management and prevention, and our statewide industry is working more cooperatively and communicating better. Nonetheless, despite tangible progress, there is still a lot of work to do.

ACKNOWLEDGEMENTS

A very special thank you to the many, many growers, industry members, and scientists who have shared their expertise and continue to learn along with us. We are also grateful for funding from the Lodi Winegrape Commission, the American Vineyard Foundation, the PD/GWSS Board, and USDA Western SARE.

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Communicate early and often – Responding to virus detections in foundation grapevine collection

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INTRODUCTION

Foundation Plant Services (FPS) is the primary source for certified, virus-tested, and true-to-variety grapevine plant material distributed to nurseries under the California Department of Agriculture's Grapevine Registration and Certification (R&C) Program, through which participating nurseries produce and distribute most grapevines planted in the United States. FPS has successfully maintained collections of grapes in open foundation vineyards on the UC Davis campus for more than 70 years. However, the Russell Ranch Foundation Vineyard (RRV) became infected with grapevine red blotch virus (GRBV), an economically significant insect-vectored grapevine virus that was introduced into RRV from outside sources. To ensure no spread of GRBV in planting stock, FPS stopped distributing material from RRV. Fortunately, the Classic Foundation Vineyard has remained relatively unaffected by GRBV and is rigorously tested to continue supplying the grape industry with clean planting stock. Annual vineyard testing results are posted in the news feed of the FPS website and shared at stakeholder meetings. FPS relies on frequent, transparent communication with the industry, to maintain trust in the collection and testing protocols (Figure 1).

Due to the importance of this grape collection and the potential threat of insect-vectored viruses, FPS and the grape industry determined the best course of action is to protect the core collection of grapevine selections indoors, in a greenhouse and screenhouse designed to exclude both known and unknown vectors.

Foundation Plant Services

HOME | GRAPE | PRUNUS | PISTACHIO | UC STRAWBERRY | ROSE | SWEET POTATO | BERRIES | VARIETY ID | VIRUS DIAGNOSTICS

Foundation Plant Services News

Additional Testing of Classic Foundation Vineyard Finds No GLRaV-3 or GRBV

17-Mar-25

Foundation Plant Services (FPS) operates under a "Test-to-Order" policy for grapevine material, implemented in 2018 in response to the detection of grapevine red blotch virus (GRBV) at the Russell Ranch and Classic Foundation vineyards. In addition to the annual late-summer screening of each individual Classic Foundation Vineyard vine, every vine that is used for dormant material distribution is tested again in the winter. To minimize the risk of false negatives, a total of twelve canes are sampled from each vine; six from each side. Cuttings from each Classic Foundation Vineyard vine that was the source of dormant material distributed in 2025 were re-tested for grapevine leafroll-associated virus 3 (GLRaV-3) and GRBV, with NO virus detected.

In November 2024 FPS shared the news that GLRaV-3 had been detected in one vine during the annual all-vine testing of the Classic Foundation Vineyard. The vine was removed, and the nearby vines intensively sampled for follow-up testing. In addition to screening dormant cuttings from all ordered vines this winter, the vines around the GLRaV-3 positive vine were tested again, with no GLRaV-3 detected. Monitoring of the surrounding vines will continue through the summer.

Figure 1: News story posted to FPS website to announce results of dormant vine testing at Classic Foundation Vineyard.

Efforts to secure grape material for the indoor collection began in earnest in 2019, and continue today. The goal of FPS is to propagate, test, and if necessary, treat, priority grapevine selections that can be maintained in the Foundation Greenhouse as a source of high-quality healthy grapevine stock for nurseries in CDFA's Grapevine R&C Program.

MATERIALS AND METHODS

The indoor facilities do not have capacity for all selections found in the outdoor vineyard, so priorities were determined by surveying FPS customers and reviewing distribution records. We prioritized industry ranked and high-demand selections, and new introductions after they complete the quarantine process. Once priority was determined, dormant budwood was collected from grapevines in the Classic Foundation Vineyard, tested to determine virus status and propagated in a controlled facility (Figure 2).

Priority selections were considered for microshoot tip culture therapy if: 1) viruses or virus-like agents are detected in the testing phase above and/or 2) the source vine (mother plant) has not received microshoot tip culture. Microshoot-tip tissue culture therapy involves the excision of a very

small (less than 0.5 mm) piece of the shoot tip of the grape, growing it in media into a new vine and testing the new grapevine for pathogens. Performing microshoot-tip culture can take 6 to 24 months, and once a plantlet is transferred from tissue culture media to soil, it must experience two winter dormancies before we are confident enough in the virus titer to complete testing.
To recertify priority selections that have completed microshoot tip therapy, plants are subject to PCR/HTS testing to ensure pathogens have been eliminated. Propagates derived directly from cuttings collected in the Classic Foundation Vineyard were also tested to confirm their virus status in relation to CDFA R&C standards. In addition, the regulations for the R&C program require that the certified grapevines in the program are true to variety. Therefore, all plants entering the FPS Foundation Greenhouse Collection will be tested using DNA microsatellite technology to ensure correct variety identification of each selection.

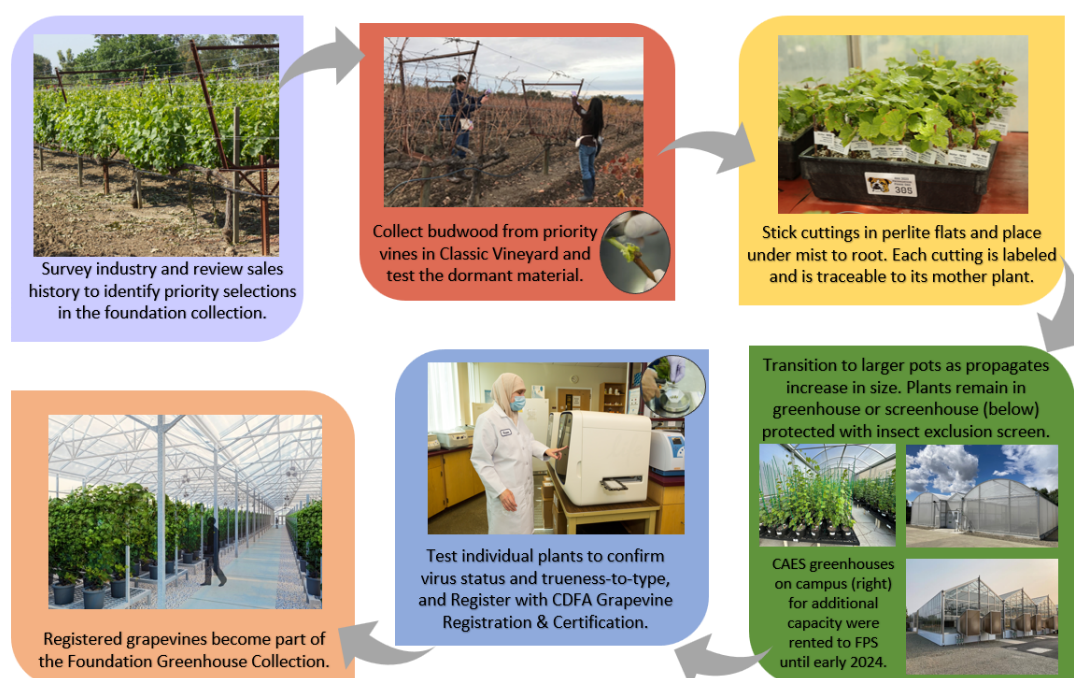

Figure 2: Journey of grapevine material from outdoor vineyard, to indoor greenhouse and screenhouse.

RESULTS AND DISCUSSION

The Phase 1 Greenhouse, completed in March 2024, has been filled and currently contains over 1300 clones. Construction of the Phase 2 Screenhouse to increase collection capacity began in September 2025 and is expected to be completed in Spring 2026. California's nursery and stakeholder groups continue to support FPS, both through purchase of tested material from the collection, and by funding greenhouse construction and propagation work to secure the collection. We strongly believe that being transparent when viruses are detected in the grapevine foundation and communicating clearly and regularly with stakeholders is a key to this success.

ACKNOWLEDGEMENTS

With the tremendous support of the California Fruit Tree, Nut Tree, and Grapevine Improvement Advisory Board (IAB), the California Grape Rootstock Research Foundation, and California Grape Rootstock Commission, FPS secured funding for construction of the Phase 1 Greenhouse in 2023-2024. CDFA Pierce's Disease/Glassy-Winged Sharpshooter Board, National Clean Plant Network, and IAB provided funding for propagation of the grapevine collection. Phase 2 screenhouse construction is made possible with generous loan of funds from UC Davis College of Agricultural and Environmental Sciences and donations from industry individuals and companies.

Network-based extension supports regional coordination for leafroll and red blotch disease management

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INTRODUCTION

Agricultural producers face increasing pest and disease challenges resulting from factors such as globalization and changing climatic conditions. Globally, $220 billion in plant production losses are attributable to plant diseases and $70 billion to invasive insects (UN FAO, 2022). Thus, the development, through research and experimentation, and implementation of plant protection practices are essential activities that support global agricultural production.

Adoption of agricultural practices hinges upon factors that can be broadly classified into three categories (Hobbs et al., 2023). First, the producer must gain knowledge of the pathosystem, and the practice or suite of practices needed to achieve successful management outcomes. Knowledge may be acquired through a variety of pathways, including seminars, workshops, or other experiential learning opportunities, as well as through print or video resources. Knowledge may be shared through agricultural networks that include producers, consultants, industry experts, and outreach professionals. Second, the producer must have the resources and capacity to implement the practice. This includes the financial means, as well as technical expertise, personnel, and equipment. Lastly, social conditions within the organization and industry should be favorable to adaptation. Agricultural organizational structure should support transparent communication, participatory decision-making, and effective team processes, including collaboration and training. At the industry level, regional communication and collaboration are key aspects. More broadly, public policy instruments should provision resources and support the development and implementation of evidence-based practices.

Management of grapevine leafroll and red blotch diseases presents a unique opportunity for producers to enact a collective response. This is ascribed to the need for inoculum removal, whose benefits can be augmented when conducted at the regional scale. Numerous studies have demonstrated that inoculum removal is a critical aspect of leafroll disease management (Bell et al. 2018; MacDonald et al., 2021). Emerging evidence suggests that similar attention to inoculum removal will be required to reduce the economic consequences of red blotch disease (Dalton et al., 2019; Flasco et al., 2025). In the leafroll disease system, coordinated, regional mating disruption programs may reduce vector population pressure (Hogg et al., 2021).

Network-based extension recognizes the resource limitations of traditional outreach programs. It leverages community relationships and social networks to augment communication channels and disseminate information more broadly, through well-connected and respected members of the agricultural community. Despite their benefits, the mechanics of agricultural social networks and their functions are poorly described in the literature. The goals of this work were therefore to document the impacts of existing peer networking groups on knowledge dissemination for viral disease management, explore their structure and function, describe the experiences of group members and leaders, and provide guidance for future efforts to harness the potential of agricultural networks to respond to emerging challenges.

MATERIALS AND METHODS

We studied peer networking groups, principally in the wine grape production region of Napa, California, USA, but also in nearby Lodi, California. We interviewed group coordinators (n=12) and surveyed group members (n=46). We summarized descriptive survey data, including participant characteristics, meeting attendance, impacts and ratings of success. We conducted a theme analysis

of the interview data, identifying six top-level themes, each with numerous sub-themes. We conducted a social network analysis of 249 individuals who communicated about pest and disease management in Napa, CA.

RESULTS AND DISCUSSION

The peer networking groups had eight to 25 core members who participated in most meetings, and an additional 12 to 70 members who received email updates and attended meetings sporadically. Most survey respondents worked in vineyards, with viticulturist being the most commonly represented job title (39%). Group coordinators described multiple positive impacts of group participation. These included sharing knowledge and data, improved networking and connectedness, along with reduced neighborhood conflict and reduced vector population pressure and spread of leafroll disease. Group activities were perceived to increase the adoption of practices, effect regulatory change, and provide resilience through the establishment of a collective network of participants who continually engaged with each other to share ideas and address challenges. Established groups also served as models and inspiration for the formation of new groups or alliances.

Commitment of coordinators is critical for group success. Coordinators must have the time, resources, and support to lead the group and facilitate interactions among members. Certain groups employed dual coordinators to reduce the time burden and ensure continuity. Coordinators guided the group members on purpose and activities. They proposed scheduling, organized meetings and facilitated discussions. Coordinators voiced challenges with recruitment. They struggled to identify potential members to target for recruitment, and determine who should be included or excluded. Overall, participants viewed their groups as successful (67%). Membership in the group provided networking opportunities (87% of respondents), which led to collaborations outside the group including sharing staff expertise (63%), data (61%) or coordinating straightforward practices (52%). Although shared financial responsibilities are often mentioned anecdotally as potential outcomes, in actuality few respondents (11%) had shared materials or financial resources. Group members who were more involved in group activities were more likely to learn about and adopt new practices (57%), suggesting that continued participation can have positive outcomes for collective pest and disease management, which is a strong motivator for recruitment and engagement.

ACKNOWLEDGEMENTS

This work received financial support from the American Vineyard Foundation. We are extremely grateful to the peer networking group coordinators and members who dedicate their time to building and nurturing the relationships and collaborations that achieve collective gains for the industry.

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Ecological network management of leafroll virus

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INTRODUCTION

Grapevine leafroll-associated virus 3 (GLRaV-3) is the most damaging virus in New Zealand (NZ) vineyards. Being graft transmissible, GLRaV-3 is also transmitted between grapevines by hemipteran insects (mealybugs, soft scale insects). An integrated response to GLRaV-3 was adapted for NZ from 2009. Despite advances, key knowledge gaps persist, for which we propose an ecosystem-based biological response. Via a case study, we highlight research on the mealybugs *Pseudococcus calceolariae* and *P. longispinus*, the main insect vectors of GLRaV-3 in NZ. The practice of removing a virus-infected grapevine frequently identified *P. calceolariae* feeding on remnant roots, a GLRaV-3 reservoir. This association is likely a virus transmission pathway increasing the susceptibility of young replacement grapevines to GLRaV-3. Reports suggest 30–70% of replacement grapevines show foliar symptoms of GLRaV-3 within 2-years of planting. Developed 15 years ago, the protocol to manage this risk no longer works for growers. Hence, understanding the interface between mealybugs and grapevine remnant roots is needed to determine how we might alter this association to reduce the risk of GLRaV-3 transmission. Working with industry, we seek to combine existing tactics with the principles of an ecosystem-based biological response to reduce the influence of GLRaV-3.

MATERIALS AND METHODS

Presently, the industry standard for GLRaV-3 recommends using certified material when planting grapevines. While growers can access laboratory-based technology to identify GLRaV-3, the more efficient and cost-effective option for many is visual symptom identification (VSI), a process limited to symptomatic red berry varieties (Bell et al. 2009). Undertaken by trained assessors, VSI results guide the removal (roguing) of GLRaV-3-infected grapevines. There is emphasis on removing as much of the root system as practicable to a depth of about 25 cm, which correlates to the maximum depth for most *P. calceolariae*. Finally, monitoring aids understanding of mealybugs and helps guide decisions on where and when to apply insecticides compatible with biological control (Bragato Research Institute 2024).

Data from commercial vineyards collected over multiple years shows adopting the integrated response is sometimes failing to meet grower expectations. A research project in the planning phase but not yet funded, will evaluate various strategies to reduce the influence of the remnant root/*P. calceolariae* association. It includes assessing if ‘barriers’ like non-*Vitis* herbaceous plants reduce the frequency of viruliferous mealybugs resident on remnant roots and thus severing vine/virus/vector interactions.

Nationally, we are working with growers who are recording non-*Vitis* plant biodiversity in the vineyard environment. Focusing on broad-leafed flowering plants, they record plant species growing naturally as well as those they have introduced. In listing plant species growing in and around the vineyard ecosystem, the goal is to develop a planting ‘recipe’ guiding regional planting programmes.

RESULTS AND DISCUSSION

In reiterating we are proposing research in advance of funding, we do so in recognition of a problem for which science cannot yet offer a viable solution to affected growers. An early first step – NZ's integrated response to GLRaV-3 – delivered many tangible benefits across the NZ wine sector (Bell et al. 2018). Notwithstanding this advance, some wineries indicate they cannot implement some protocols because of practical constraints. Physically extracting rogued grapevine remnant roots is an example. These roots are a GLRaV-3 reservoir, and they appear to support feeding mealybugs, some of which tested virus-positive (Bell et al. 2009). The prospect of the remnant root/subterranean mealybug association being another virus transmission pathway has prompted some wineries to defer replanting indefinitely knowing that to do so will result in a significant proportion of young grapevines testing virus-positive soon after. This inaction is an obstacle to production goals: one winery estimated the cumulative effect of lost production was NZ$1 million annually (Chooi et al. 2024).

The grapevine monoculture typifies many NZ vineyards today: broad-acre grapevine plantings, where the under-vine zone is managed by herbicides; the inter-row, dominated by grass and some naturally occurring broadleaf flowering plants, is mown several times during a growing season. Under these circumstances pests like mealybugs have few host choices beyond the grapevine. However, some vineyards have started integrating broadleaf flowering plants into the wider vineyard ecosystem. This encouraging development not only helps increase beneficial species biodiversity (and mealybug biocontrol), but it may also help sever the mealybug/grapevine association. Of special interest are groundcover plants known to support mealybugs, but which do not host GLRaV-3. One example is Grasslands Huia white clover (GHWC, Gough et al. 2024). This, and possibly other species, represents an opportunity to test the 'barrier' concept, whereby residual populations of subterranean mealybugs resident on remnant roots are 'attracted' to a non-*Vitis* alternative host. Movement by viruliferous mealybugs onto a GHWC host results in virus loss and apparently reduced motivation to recolonize the grapevine (Gough et al. 2024). By identifying plants compatible with vineyards in different regions of NZ, we expect to assess their capacity to enhance network connections to parasitoids and predators. Such knowledge offers potential to develop tools helping growers select plants by region and which attract and sustain beneficial species (Bragato Research Institute 2025).

ACKNOWLEDGEMENTS

We thank New Zealand Winegrowers, Bragato Research Institute, and the many cooperating growers. We remember the late Dr Rod Bonfiglioli and acknowledge his virology research.

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The prevalence of viruses in Australian viticulture from 2016-2025

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INTRODUCTION

There are 19 viruses known to occur in Australian dried, table and wine grape and rootstock varieties. Routine diagnostic testing using RT-PCR is only done for grapevine leafroll associated viruses (GLRaV) -1, -2, -3 and -4, grapevine viruses A (GVA) and B (GVB), grapevine fleck virus (GFkV), grapevine Pinot gris virus (GPGV), grapevine rupestris stem pitting associated virus (GRSPaV) and grapevine red blotch virus (GRBV). During the last ten years most of the virus testing was done by the Australian Wine Research Institute and Waite Diagnostics (The University of Adelaide), Crop Health Services (CHS) and DPIRD Diagnostics and Laboratory Services. This testing supports provision of high health planting material via certification programs across the country, and it assists growers in making decisions about vineyard renovation, including topworking to new varieties, grapevine replacement and vineyard removal. To better understand the risk of plant virus related diseases to the Australian viticulture industry and to facilitate the development of management strategies, the prevalence of viruses between 2016 and 2025 was analysed using the diagnostic testing results from all three diagnostic laboratories, covering a vast majority of Australian viticulture.

MATERIALS AND METHODS

More than 13,000 samples of dried, table and wine grape varieties and rootstocks were tested across the three laboratories during the last 10 years. Many samples comprised of pooled leaves, shoots and/or canes of five to ten grapevines. Samples were collected throughout the year, but the majority were collected in autumn and winter. RT-PCR, qRT-PCR or PCR (GRBV) was used for testing (Constable et al., 2013, Kaur et al., 2025), but some RT-PCR assays differed between the laboratories for detection of each virus. If required Sanger sequencing of the amplicon or whole targeted genome sequencing (GRBV only; Kinene et al., 2025) was used for confirmation of virus presence.

RESULTS AND DISCUSSION

Preliminary analysis of the results from CHS (~7500 samples) indicates that viruses remain prevalent and pose a risk to the productivity and sustainability of Australian viticulture. The data will include repeat testing of the same grapevines across multiple years, especially from germplasm, foundation and source blocks, and could overestimate prevalence. Mixed virus infections of three or more viruses were often observed. GLRaV-1, GLRaV-3, GLRaV-4 strain 9, and GVA were detected in 8%, 14%, 15%, and 18% of CHS samples and GLRaV-2, GLRaV-4, GLRaV-4 strain 5 and GVB were each detected in 4% of samples. The combined detection of all GLRaV-4 isolates has not yet been calculated but is likely to exceed 20% of all samples, even though mixtures of strains also occur. The high prevalence of GLRaV-1, GLRaV-3, GLRaV-4, and GVA is concerning as these viruses, alone or in combination, can lead to yield and fruit quality losses in Australia (Krake et al., 1999). The combination of GVA and GLRaV-3 is associated with Shiraz disease, which can result in vine decline in sensitive varieties under certain environmental conditions (Wu et al., 2020). However, further analysis of the annual prevalence of these viruses is required to determine if the

prevalence is changing over time. Prevalence may have increased with the increased along with increasing prevalence of scale insects that has been observed in some winegrape growing regions (Ward et al., 2023). GPGV was present in 25% of samples tested, which is higher than previous reports (~22%, Kaur et al., 2023) and may reflect ongoing spread of the virus in Australian grape growing regions, where the mite vector, *Colomerus vitis*, is also prevalent. The importance of GPGV in Australian viticulture remains uncertain and further work is required to determine if this virus can cause economic losses, although recently mild leaf mottling symptoms were observed in some sensitive and infected varieties. The prevalence of GRBV was >1% as previously reported by Kaur et al. (2025). GFkV occurred in 12% of samples. GRSPaV is most prevalent in Australia and was detected ~80% of samples, which is less than previously reported (90%; Habili, 2015). The lower level of detection may be associated with genetic variability leading to false negative results. Once the data of all three laboratories is analysed, the data will be used to better estimate and inform risk for Australian dried, table and wine grape growers. The data could be overlaid with prevalence data for vectors to evaluate the need for specific management practices at a regional level.
Preliminary interlaboratory comparisons (data not shown) have identified variability between the laboratories in their ability to detect some virus strains. The three laboratories do not always use the same assays and the variability in detection is likely to be associated modified laboratory practices, and with genetic diversity within grapevine virus species in Australia. Therefore, genetic diversity studies are being done, and the data will be used to refine diagnostics assays and harmonise grapevine virus testing amongst Australin diagnostics laboratories.

ACKNOWLEDGEMENTS

The research was funded through the author affiliate organisations and by Wine Australia. Wine Australia supports a competitive wine sector by investing in research, development, and extension (RD&A), growing domestic and international markets, and protecting the reputation of Australian Wine.

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Diversity of grapevine leafroll-associated virus 3 variants and dispersal of vine mealybugs in vineyards of Northern California, U.S.A.

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INTRODUCTION

Grapevine leafroll-associated virus 3 (GLRaV3, species *Ampelovirus trivitis*, genus *Ampelovirus*, family *Closteroviridae*) is widespread and economically damaging in *Vitis* spp. worldwide (Fust et al. 2025, Maree et al. 2013). Secondary spread of GLRaV3 is mediated by soft scales and mealybugs, the most efficient of which is the vine mealybug, *Planococcus ficus* (Hemiptera: Pseudococcidae). Previous work has identified GLRaV3 epidemics often resulting in spatiotemporal aggregations of infected vines along vineyard rows as insect vectors disperse along vine canopies and trellis wires (Hesler et al. 2022, Donda et al. 2023). However, recent observations of temporal random infection patterns of GLRaV3 in Northern California, U.S.A. may be indicative of additional mechanisms of viral spread. To discern possible mechanisms of spatiotemporal infection randomness, a variant level characterization of GLRaV3 infections and a methodology for monitoring the dispersal of vine mealybugs are needed. In this study, an RT-PCR assay combined with restriction fragment length polymorphism (RFLP) was developed to identify major GLRaV3 variants present in vineyards to track inoculum sources and spread. In parallel, a novel marking method of vine mealybugs based on a liquid fluorescent dye was used to track the dispersal of vectors.

MATERIALS AND METHODS

A total of 415 leaf petiole samples was collected in 2024 from symptomatic and asymptomatic vines in nine commercial vineyards in Lodi, CA. An additional 20 leaf petiole samples were collected from GLRaV3-infected and healthy vines in the USDA Cold Hardy Grapevine Germplasm Repository in Geneva, NY to validate *in silico* predictions on the use of AccI, BtgZI, and BglI to digest a 546bp fragment of the heat shock protein 70 homolog (HSP70h) gene for discerning GLRaV3 variants. Total RNA was extracted from petiole samples and used in a two-step RT-PCR assay to amplify a conserved region of the HSP70h gene using specific primers. Amplicons were digested by AccI, BtgZI, and BglI separately and electrophoretic patterns of the digests were compared to the predicted *in silico* restriction fragment lengths to determine GLRaV3 variant identity. In addition, amplicons from a subset of samples exhibiting the expected RFLP patterns were Sanger sequenced to confirm the suspected GLRaV3 variant following sequence comparisons with those of known GLRaV3 variants (Diaz-Lara et al., 2018). The infectious status of samples that did not yield any HSP70h amplicon was tested by a RT-qPCR targeting a conserved region of the 3'untranslated region of the GLRaV3 genome (Diaz-Lara et al. 2018).
Vine mealybugs from a laboratory-maintained colony were marked with a fluorescent dye used in forensics and their behavior was evaluated in the laboratory. Marked insects were then released on selected shoots of a few vines in a commercial vineyard. Their dispersal was monitored at night using a portable UV flashlight.

RESULTS AND DISCUSSION

Petiole samples from vines at the Grapevine Germplasm Repository tested via the RT-PCR-RFLP assay resulted in digested DNA bands with lengths consistent with the *in silico* predicted patterns for GLRaV3 variants I, II, III, and V, as well as an additional variant IX provided by UC-Davis. Sequence analysis confirmed the variant identification determined by the RT-PCR-RFLP assay, thus validating its use for GLRaV3 genotyping in commercial vineyard samples.

The RT-PCR-RFLP assay revealed the presence of GLRaV3 variants I, II, III, V, and IX in both single (77.86%) and mixed infections (22.14%) in vineyard samples from Lodi, CA. This rate of mixed infection is consistent with that reported in other California vineyards, although variants I and II were the most abundant in Lodi while variants II and III have previously been reported as predominant in Napa Valley (Sharma et al., 2011). Further spatiotemporal analysis of GLRaV3 variants will aid in determining inoculum sources and mealybug-mediated dispersion profiles, for the ultimate goal of leafroll disease prevention.

Analyzing the behavior of marked vine mealybugs in the laboratory indicated no substantial effect of the fluorescent dye on insect viability and movement. Marked vine mealybugs released in a commercial vineyard informed active and passive short dispersal at a local scale. Further quantification of vector movement in the vineyard will provide insight into mechanisms of GLRaV3 spread.

ACKNOWLEDGEMENTS

This work was funded through CDFA-PD/GWSS (grant no 25-0370-000-SA) and Cornell Agritech Venture Funds. We are grateful to Drs. Daane, University of California, Berkeley, and Al Rwahnih, University of California, Davis, for supplying vine mealybugs and leaf samples infected with GLRaV3 variant IX, respectively.

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Crop thinning does not improve the impact of Grapevine leafroll and Grapevine red blotch diseases on vine health and fruit composition in the Okanagan Valley, British Columbia

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INTRODUCTION

Grapevine leafroll-associated virus 3 (GLRaV-3) and Grapevine red blotch virus (GRBV) are two important threats to the long term economic sustainability of vineyards (Cieniewicz and Fuchs 2025, Naidu et al. 2014). Both viruses are known to severely impact plant health and fruit quality by reducing vine vigor, total yield, number of clusters, and berry weight, resulting in crop losses of up to 40%. Furthermore, they reduce photosynthetic capacity in the leaves of infected vines, decrease soluble solids (Brix) and increase fruit acidity (Bowen et al. 2020, Song et al. 2021). Currently, no cure is available once grapevines are infected and thus, control strategies are limited to planting with certified "virus-free" clean vines, or a combination of insect vector control and vine removal or 'roguing' (Fuchs 2020). Despite the potential to improve economic losses over time, 'roguing' can be a costly and lengthy process. A short-term strategy to mitigate impacts may temporarily ease the stress of replanting programs on grape growers. Cluster thinning is a common viticultural practice in which fruit is removed from grapevines during the growing season to adjust resource allocation resulting in altered berry composition. Crop thinning up to 55% has shown to be beneficial to fruit composition, and this severity is more crucial than the timing of thinning (VanderWeide et al. 2024). Since reduced sugar accumulation is the most widely reported impact of both GLRaV-3 and GRBV, we hypothesized crop thinning has the potential to mitigate virus impacts on fruit composition. Accordingly, the objectives of this study were to investigate the potential utility of crop thinning to improve vine health and fruit composition of GLRaV-3 and GRBV infected vines in the Okanagan Valley, British Columbia, as well as probe whether viral load influences vine health and fruit composition.

MATERIALS AND METHODS

Two commercial Merlot and Cabernet Sauvignon vineyards were studied over the 2021 and 2022 growing seasons. GLRaV-3(+) and GLRaV-3(-) vines were paired and designated a cropping treatment, either 1.5 clusters per shoot (1.5 C/S) or one cluster per shoot (1.0 C/S) in both Merlot and Cabernet Sauvignon. Similarly, GRBV(+) and GRBV(-) vines were paired in the Cabernet Sauvignon block. Up to 20 vines per infection status:treatment combination were selected. Existing virus infection was identified in the block during a 2020 field survey. Vine leaf samples were submitted to the Grapevine Virus Testing Laboratory in the Cool Climate Oenology and Viticulture Institute (CCOVI) at Brock University, St. Catharines, Ontario, Canada. All samples were tested for GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-4 and GRBV. The infection status of each vine selected in the trial was confirmed each year of the study. Plant physiological measurements, including leaf greenness (SPAD) and leaf gas exchange were measured at four points during the season (bloom, pea-sized berries, veraison, and harvest). Bud hardiness was also measured three times during fall/winter. Fruit composition, Brix, TA, pH, yeast assimilable N (YAN), and skin and seeds components were measured at harvest. Virus titer was measured at the same four phenological stages

during the growing season using Droplet Digital™ PCR (ddPCR). Statistical analyses of the data was conducted as reported in April et al. (2025a) and (2025b).

RESULTS AND DISCUSSION

GLRaV-3(+) vines had increased crop load and titratable acidity, and reduced Brix. 1.0 C/S vines had lower TA and anthocyanins, and increased pH. Thinning GLRaV-3(+) vines significantly increased pH. GLRaV-3 titer was negatively correlated with photosynthesis, stomatal conductance, SPAD, cluster weight, berry weight and yeast assimilable nitrogen, and positively correlated with skin and seed phenolics. GLRaV-3 impacts were mild, and differed by cultivar and year.
GRBV(+) vines had drastically reduced Brix, as well as decreased YAN, and increased TA and pH. Skin and seed phenolic profiles were unaffected by GRBV. Crop thinning decreased yields, and increased pruning weights, resulting in lower crop loads. GRBV viral load was positively correlated with skin anthocyanins, tartartic esters, flavonols, and tannins, and negatively correlated with stomatal conductance, cluster weight, yield, berry weight and titratable acidity. GRBV significantly altered fruit composition in Cabernet Sauvignon and is not remedied by crop thinning.
We found overall no consistent correlation between GLRaV-3 and GRBV titers and adverse vine health or fruit composition. Crop thinning did not improve vine health or fruit composition of infected (+) nor healthy (-) vines. It is estimated that cropping from 1.5 to 1.0 C/S in non-infected Merlot could lose growers CAD$3784.26/ac based on yields from 2021 and $6862.30/ac from 2022. Costs from thinning non-infected Cabernet Sauvignon were $2781.88/ac for 2021 and $4663.47/ac for 2022. This study shows no benefit to thinning vines lower than 1.5 C/S in the Okanagan Valley and thus, growers should keep their fruit to avoid diminishing returns.

ACKNOWLEDGEMENTS

This research was possible thanks to funds provided by Agriculture and Agri-Food Canada, the British Columbia Wine Grape Council and the Canadian grapevine Certification Network under the 2018-2023 Canadian Agricultural Partnership Grape and Wine Cluster.

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RNA as a management strategy for GLRaV3

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INTRODUCTION

Infection by Grapevine leafroll-associated virus 3 (GLRaV-3) is of serious concern in New Zealand, particularly for red-variety growers in areas like Hawkes Bay. Grapevine leafroll disease affects vine physiology, fruit yield and composition, and consequently reduces wine quality. No cure exists for virus-infected vines, and current best practice involves the removal of infected vines, often along with adjacent in-row vines, in a process called 'rogueing'.
The effects of GLRaV-3 infection vary among cultivars. For example, while infected Cabernet Sauvignon exhibits characteristic red foliage, varieties such as Sauvignon Blanc show no obvious foliar symptoms. As a result, the true prevalence of the infection is often unknown. Regardless of symptoms, GLRaV-3 is likely to reduce vine vigor and increase susceptibility to additional biotic or abiotic stresses.
RNA interference (RNAi) is a naturally occurring mechanism in which small RNA molecules mediate sequence-specific silencing of gene expression. Our research expands on a recent study which highlights the potential of RNAi as a tool to manage GLRaV-3 infection (Avital et al., 2021).

MATERIALS AND METHODS

Using climate-controlled growth environments, we applied an encapsulated double-stranded RNA (dsRNA) formulation targeting GLRaV-3 to *Vitis vinifera* cv. Cabernet sauvignon vines. Leaf samples were collected from the vines prior to treatment, and subsequently every two weeks throughout the treatment period. GLRaV-3 titre was assessed via quantitative PCR (qPCR) relative to *Vitis vinifera* Ef1α using previously published primers (Reid et al., 2006; Bruisson et al., 2017).

RESULTS AND DISCUSSION

At the end of the treatment period, despite considerable variation between biological replicates, a statistically significant decrease in GLRaV-3 titre was observed in vines treated with encapsulated dsRNA targeting GLRaV-3 compared to the untreated control vines. While additional analysis is ongoing, these initial results are promising. The potential for an RNA-based approach to revolutionize management strategies for GLRaV-3 and other plant-associated viruses, paving the way for more effective, targeted, and cost-effective strategies to manage viral pressures in the winegrowing industry and beyond, should not be ignored.

ACKNOWLEDGEMENTS

This work was funded by the New Zealand Winegrowers Research Levy and the New Zealand Ministry of Business, Innovation and Employment.

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Flavescence dorée in Switzerland: Current Situation, epidemiology, and varietal susceptibility

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INTRODUCTION

Flavescence dorée (FD) , associated with FD phytoplasmas (FDp, 16SrV group), is one of the most destructive grapevine yellows in Europe It is classified as a quarantine pest which results in strict surveillance and mandatory control measures. In Switzerland, FD was first detected in Ticino (south of the Alps) in 2004 and has there become endemic, while new outbreaks have been recorded north of the Alps in the cantons of Vaud and Valais since 2015, where viticulture is of major economic and social importance. The disease is epidemically transmitted in vineyards by the Nearctic leafhopper *Scaphoideus titanus*, which is present in southern Switzerland since the late 1960s and is now widespread in several winegrowing regions. Current management of FD relies mostly on insecticide applications against the vector and the uprooting of symptomatic grapevines. Nevertheless, these measures have not fully contained the disease. This highlights the need for a better understanding of its epidemiology, the interactions between vectors and host plants, as well as cultivar-specific resistance mechanisms.

CURRENT SITUATION AND EPIDEMIOLOGY

In Switzerland, more than 2,500 winegrowers cultivate around 15,000 hectares of vineyards, producing 100 million litres of wine each year. The regions where FD has been detected represent about 75% of Switzerland's total vineyard area. In 2026, about 3,000 hectares are in outbreak and buffer zones, requiring mandatory quarantine measures and leading to high economic and environmental costs. Regarding the distribution and abundance of FDp and *S. titanus*, the country can be divided into three distinct zones: southern Switzerland where the disease is endemic and *S. titanus* is widespread; western Switzerland where local outbreaks of FD occur, and the vector is widely distributed; and northern Switzerland where both FDp and *S. titanus* are currently absent. Recent studies have revealed that, in addition to the classical *S. titanus*/grapevine pathway, the epidemiology of FD is far more complex and comprises alternative cycles involving different vectors (e.g. *Orientus ishidae*) and deciduous trees, such as black alder (Malembic-Maher et al., 2020). Current research also indicates that gone-wild grapevines in forests of southern Switzerland harbour FDp genotypes (such as *map* M54) associated with FD epidemics in cultivated vineyards nearby (Oggier et al., 2024). This suggests the existence of a complex agro-forest epidemiological system, which makes management particularly challenging. The role of these gone-wild grapevines in the forest compartment should therefore be fully considered by authorities and winegrowers.
The aim of an ongoing national project is to extrapolate the regional findings of FDp epidemiology in southern Switzerland to the whole country. A survey on *O. ishidae* and black alder (*Alnus glutinosa*) in 2023 and 2024 provided valuable insights into their potential role in the FDp epidemiology in regions where FD is currently absent. The identification of genotypes related to FD outbreaks, such as *map* M38 and M50 (as well as new variants), in the natural compartment indicates that cultivated vineyards located near forests with alder trees may be at particular risk. Additionally, an in-depth epidemiological study investigated how FDp was introduced and has spread in vineyards in western Switzerland. Here, we used a combination of established multilocus sequence typing

(MLST) markers and newly developed SNP markers applied to over 700 FDp-positive samples collected between 2015 and 2022 (Cadena et al., in preparation). The data indicate that FDp was most likely introduced via infected propagation material. Following this, both vector dispersal and viticultural practices contributed to its dissemination. Together with Agroscope, phytosanitary services are now combining classical surveillance with molecular epidemiology to trace the sources of infection, manage FDp inoculum reservoirs and vector habitats such as gone-wild grapevines, and refine regional control strategies to slow the further spread of FD into currently unaffected viticultural areas.

VARIETAL SUSCEPTIBILITY

Grapevine cultivars exhibit high variability in symptom severity and phytoplasma load, allowing for a broad classification. Highly susceptible cultivars include ‘Cabernet Sauvignon’ and ‘Sauvignon Blanc’, while ‘Chardonnay’ and ‘Pinot noir’ are considered intermediate cultivars. Less susceptible cultivars include ‘Merlot’ and ‘Syrah’ (Eveillard et al., 2016). Rootstocks also differ, with the most commonly used cultivars showing intermediate susceptibility, and a few, such as Kober 5BB, being considered poorly susceptible. However, important knowledge gaps persist for many cultivars. In Switzerland, we focused on understanding the differential susceptibility between the two principal grapevine varieties, ‘Chasselas’ cv. and ‘Pinot noir’ cv. Field data consistently show that ‘Chasselas’ exhibits significantly lower susceptibility to FD than ‘Pinot noir’, despite its higher abundance in affected regions. However, preliminary greenhouse inoculation experiments failed to reproduce these cultivar differences under controlled conditions. This disparity suggests that defence mechanisms in ‘Chasselas’ may be primarily effective in plant/vector rather than plant/pathogen interactions, prompting investigation into the physiological and behavioural impact of ‘Chasselas’ on the vector *S. titanus*.

A series of laboratory tests and field surveys were conducted to evaluate insect performance and host plant interactions on both cultivars. These included field population monitoring, laboratory dual-choice tests, as well as no-choice assays in which developmental and reproductive parameters such as nymph development, adult lifespan, oviposition, egg load and body mass were measured. Feeding behaviour was also examined using electropenetration graph (EPG) techniques. The results demonstrate that *S. titanus* has a reduced fitness on ‘Chasselas’, with lower population sizes, fewer hatchings, shorter longevity, and fewer eggs laid relative to ‘Pinot noir’. Short-term preference tests revealed no immediate differences among the two cultivars, but longer exposures indicated a marked host preference for ‘Pinot noir’. These findings are consistent with antibiosis-based resistance in ‘Chasselas’, which likely contributes to its lower observed susceptibility in the field. Compared with previous research, which usually found that cultivar susceptibility was consistent across field and laboratory conditions, our results stress the importance of vector-related traits in disease dynamics. Above all, it appears that 'Chasselas' has the ability to reduce the fitness and feeding preferences of *S. titanus*. This opens up new possibilities for a sustainable control of FD: breeding and selecting grapevine cultivars that promote vector impairment, rather than focusing solely on pathogen resistance, could be an effective addition to conventional management practices.

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Evaluating the effects of hot-water thermotherapy on grapevine growth, microbiome composition, and susceptibility to flavescence dorée

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INTRODUCTION

Flavescence dorée (FD) is one of the most damaging phytoplasma diseases of *Vitis vinifera*, transmitted by *Scaphoideus titanus*. It causes severe symptoms including leaf yellowing, shoot dieback, and plant decline, posing a major threat to European viticulture. To prevent its spread through infected propagation material, the EU Regulation 2021/2285 mandates the production of FD-free grapevines, allowing either cultivation in FD-free zones, spatial isolation from infected vineyards, or hot-water thermotherapy treatment of all plant material. Thermotherapy, developed in France and widely used in nurseries, involves immersing cuttings or grafts in warm water to inactivate pathogens (EPPO, 2012). While effective in reducing FD and other pathogens such as fungi of the Esca complex, the method may negatively affect plant survival depending on wood maturity and may disrupt beneficial endophytic microbiota essential for plant health and resistance. Moreover, thermotherapy does not provide lasting immunity, and reinfection can occur in the vineyard. This study aims to assess the effects of thermotherapy on grapevine growth, microbiome composition, and susceptibility to FD under natural vineyard conditions.

MATERIALS AND METHODS

Five hundred grafted plants of two grapevine varieties, Chardonnay and Pinot gris, grafted on SO4 rootstock, were subjected to hot-water thermotherapy at 50°C for 30 minutes (thesis T), while untreated grafted plants (500 per cultivar) were maintained as controls (thesis NT). After treatment, the planting was carried out in a experimental vineyard in randomized plots. Vegetative growth was measured throughout the first two growing seasons with manual measurements of length of shoots and number of internodes and trough digital assessment of leaf area index (LAI). FD susceptibility was evaluated by visual assessment of disease symptoms in vineyard plots over two consecutive seasons, coinciding with natural exposure to the vector *S. titanus*. Symptomatic plants were identified and the presence of FD phytoplasma was confirmed trough molecular diagnostic. DNA metabarcoding techniques was used to analyse microbiome composition on treated and untreated plants of Chardonnay (15 plants per thesis). Samples were collected from root tissues and rootstock wood before planting and at the end of the first growing season to analyze bacterial and fungal community structure, diversity, and relative abundance. Sequence data were processed using standard bioinformatic pipelines, and statistical analyses, including alpha/beta diversity metrics, were conducted to identify treatment effects and organ-specific microbial responses.

RESULTS AND DISCUSSION

Hot-water treatment induced a temporary reduction in early vegetative growth across all varieties, with P. gris showing the most pronounced slowdown during the first growing season (1.66 ± 0.26 and 2.76 ± 0.39 LAI values in T and NT plants, respectively). By the second season, growth

parameters of treated plants recovered to levels comparable to NT, indicating that the initial growth delay was transient and did not compromise long-term development. Microbiome analysis, performed on Chardonnay plants, revealed significant organ-specific responses to thermotherapy. At the rootstock level, hot water treatment caused a temporary disturbance of the resident microbial community, leading to increased biodiversity after one growing season (Fig. 1). This effect is likely related to “priority effects,” whereby the reduction of initially dominant taxa allows the emergence of minor microbial species (Debray *et al*., 2022). Further analysis of bacterial and fungal communities at the order and genus levels confirmed that thermotherapy effectively reduced populations of pathogenic microorganisms such as *Allorhizobium vitis* and wood-associated fungi (*e.g.*, *Phaeomoniella* spp.) linked to grapevine trunk diseases. Conversely, beneficial taxa, including growth-promoting or resistance-inducing genera like *Bacillus* and *Sarocladium*, showed a relative increase in abundance after one season, suggesting that hot water treatment may positively influence the endophytic microbiota of grapevine wood. Root microbiota showed rapid recolonization from soil communities, with alpha diversity returning to baseline levels by the end of the first season (Fig. 2). These findings suggest that hot-water treatment can selectively reduce harmful microbes while allowing the re-establishment of beneficial communities. Preliminary data on FD symptom monitoring revealed no significant differences in disease incidence or severity between T and NT plants (30 T and 26 NT symptomatic plants in Chardonnay; 13 T and 12 NT in P. gris). The absence of increased FD susceptibility in treated plants indicates that thermotherapy does not compromise natural disease resistance. Overall, these results demonstrate that hot-water thermotherapy effectively reduces pathogen load in grapevine grafted plants without causing long-term negative effects on vegetative growth, root or wood microbiomes, or FD susceptibility. The transient growth slowdown observed in the first season may reflect physiological stress induced by high temperatures, but full recovery suggests that the treatment is safe for propagation purposes.

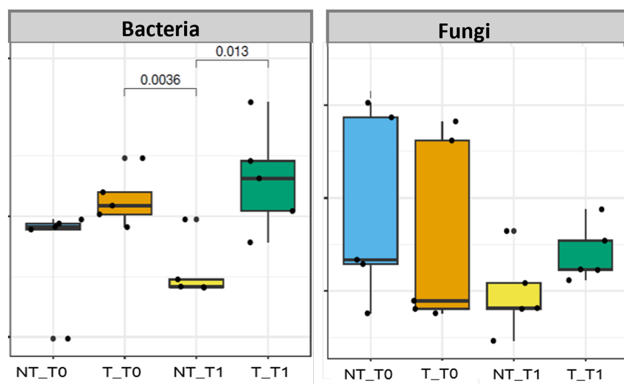

Figure 1: Shannon index in rootstock wood in treated (T) and untreated (NT) samples collected before planting (T0) and at the end of the first growing season (T1).

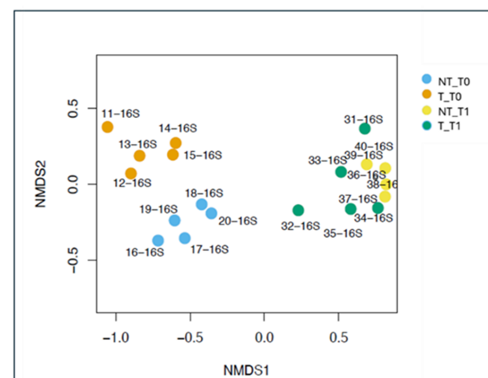

Figure 2: NMDS plot of bacterial community in treated (T) and untreated (NT) samples collected before planting (T0) and at the end of the first growing season

ACKNOWLEDGEMENTS

This work was funded by the Veneto region through the project FD.CONTROL (DGR no. 69, 14 September 2022).

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Applications of plasma activated waters to mitigate grapevine yellows disease

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INTRODUCTION

Grapevine sustainability is threatened by yellows phytoplasma diseases, that in Europe, are mainly “flavescence dorée” (FD) and “bois noir” (BN). With no curative treatments available, current management relies on vector control and uprooting. In this study the possibility of using plasma-activated water (PAW) for the mitigation of grapevine yellows disease was evaluated in vineyard trials. Exposing water to a plasma source results in the formation of solutions with high concentrations of reactive molecule species that act in plants as elicitors, stimulating the immune system (Zambon *et al*., 2019, 2020; Laurita *et al*., 2021). Grapevine plants infected by phytoplasmas and symptomatic in 2023 were treated with different types of PAW in 2024 spring and evaluated during the 2025 season.

MATERIALS AND METHODS

Two vineyards of cultivar Lambrusco di Sorbara and Lambrusco Salamino, respectively (red variety) and one of Pignoletto (white variety), 5 to 7 years old were selected in fall 2023 for yellows symptoms and molecularly tested to verify the presence of phytoplasmas. In 2024 three applications were carried out in symptomatic grapevine plants in May, June and July on 120 plants with PADW (from distilled water), PATW (from tap water), and aquannolyte (from a private company). In the Pignoletto vineyard the farmer cut about 50 cm high the most symptomatic grapevine plants after the treatments. The control plants were treated with distilled and tap water respectively. All applications were performed injecting the liquids using a syringe after drilling small holes in the trunk with a sterile device. Molecular testing was performed on DNA extracted from midribs and cane scrapes collected in July-August 2025 using a CTAB-based method (Angelini *et al*., 2001). Nested PCR amplification on 16S rRNA gene followed by RFLP analyses and/or sequencing allowed the detection and identification of phytoplasmas (Bertaccini *et al*., 2019). Furthermore, phenological traits and symptom severity in treated and control plants were measured and statistically evaluated at a significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

The molecular testing on random sampling in the two cv. Lambrusco vineyards allowed to confirm the presence of 16SrV group phytoplasmas (FD) in all the three vineyards with the number of FD infected plants increased compared to the pre-treatment testing.

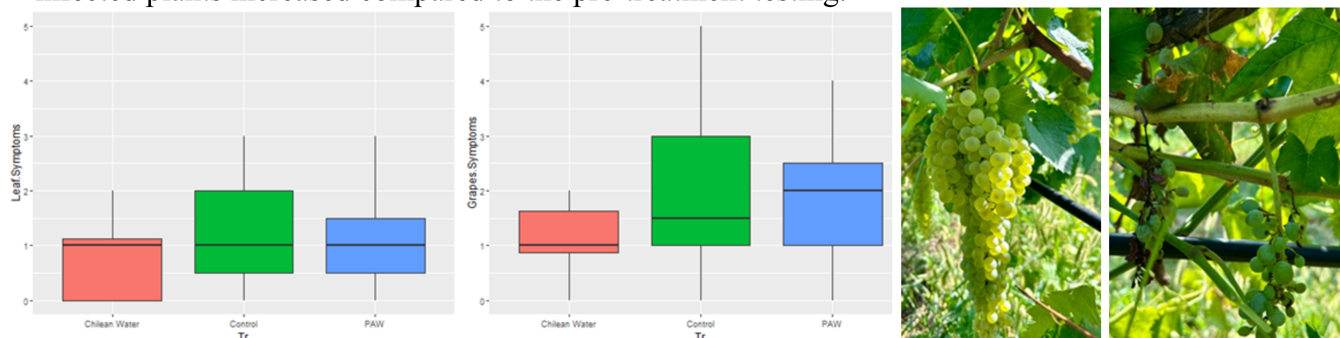

Figure 1. From left: distribution of symptom severity in grapevine leaf and bunches. Aquannolyte, red; control, green, and PAW, blue. Grape bunches from Pignoletto from asymptomatic and symptomatic plants.

In the cv. Pignoletto vineyard the symptomatic plants low pruned in 2024, showed in the large majority, new vegetation and bunches in 2025. However, the bunches from these plants appeared of lower quality and showed insufficient sugar accumulation compared to those produced from the asymptomatic, uncut grapevine plants (Figure 1) and several phytoplasma-negative plants were detected mainly after the aquannolyte treatment. Notably, one uncut plant treated with aquannolyte remained FD-positive in both 2024 and 2025, whereas 8 out of 11 plants that were treated and cut tested negative for phytoplasma presence. From the collection of phenotypic data, only slightly differences were observed in the mean values between the diverse types of water used. In particular SPAD analysis in all treatments showed average values ranging between 32.5 and 34.5. Similarly, the analysis of symptoms observed in the leaves and in bunches did not reveal statistical differences. To highlight the effect of treatments on the phenotype, the data were analyzed grouped as PAW and control (Figure 1). Following treatment with aquannolyte and PAW, a modification in the production of vegetative and reproductive structures was observed. The number of inflorescence and sprouts remained comparable in treated plants or controls, with variations of only a few units. In contrast, the comparison between treatments revealed that, in both cases (number of inflorescences and sprouts) the control plants produced a higher number of such organs (approximately 60) compared to those treated with PAW (≈ 50) and aquannolyte (≈ 35). No significant differences were detected in SPAD values, indicating similar chlorophyll content across all treatment groups. It was possible to observe that the treated grapevines exhibited less severe symptoms compared to the control, both on leaves and grapevine bunches, for both PAWs and aquannolyte. Although the number of samples from the trials was not sufficient to draw statistically reliable conclusions, it appears that pruning alone did not influence phytoplasma containment while in the aquannolyte-treatment some reduction in the symptoms and also in phytoplasma presence was observed. These results indicate that while PAW did not significantly improve grapevine health it showed positive effects to reduce symptoms on leaves and bunches, confirming its promising role as component of integrated disease management (Perez *et al*., 2019). These findings suggest the importance of larger sample sizes and repeated or optimized PAW applications for confirmation and optimization of the system for its scaling up in the vineyards.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Roberta Nannini and Pierpaolo Bortolotti from Servizio provinciale fitosanitario of Modena province and Endrig Alpi Consorzio agrario Ravenna province for their support for the vineyards inspections.

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'*Candidatus* Phytoplasma solani' effectors SAP11-like and SAP54-like influence the expression of genes encoding antioxidative enzymes in the *Arabidopsis thaliana* model

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INTRODUCTION

During the co-evolution of microbes and their hosts, pathogens acquired a wide array of effectors that increase their virulence and facilitate infection. The recent ascent of metagenomic era and new genomic technologies delivered new tools in finding potential effectors in genomes of unculturable pathogens, such as phytoplasma (genus '*Candidatus* Phytoplasma'). As endocellular pathogens, phytoplasmas secrete their effector proteins directly into the host cell, enabling them modulation of developmental and metabolic processes as well as the host immune response disturbance. Moreover, in infected plants oxidative stress conditions often occur, characterized by an increase in the production of reactive oxygen species (ROS). An antioxidative system protecting against damage caused by ROS was evolved by plants, encompassing enzymes such as glutathione peroxidase (GPX), superoxide dismutase (SOD), catalase (CAT) and glutathione S-transferase (GST) (do Carmo Santos et al. 2025, Horváth et al. 2015). Of particular interest to us is '*Ca.* P. solani', one of the most important phytoplasmas in Euro-Mediterranean region which, while associated with widespread grapevine bois noir (BN) disease, also has a broad plant host and insect vector range (Quaglino et al. 2013, Plavec et al. 2024). Previously, the influence of phytoplasma infection on the host antioxidative system was recognized in grapevine infected with both '*Ca.* P. solani' (Rusjan et al., 2012), as well as the grapevine *flavescence dorée* phytoplasma (Davosir et al., 2024a, 2024b). However, the exact mechanisms of these interactions remain unknown. In our previous studies, by sequencing and comparative analyses of several '*Ca*. P. solani' genomes, we identified a number of putative effector genes (Šeruga Music et al. 2025). Our further functional genomic studies on transformed arabidopsis (*Arabidopsis thaliana* (L.) Heynh) overexpressing selected effector genes have shown that SAP11-like and SAP54-like '*Ca.* P. solani' effectors significantly affected plant growth and development. Moreover, interactions of SAP11-like effector with plant transcription factors AtTCP2 and AtTCP4 were also detected, while SAP54-like effector interacted with AP1 and SEP3 (Drčelić et al. 2024, unpublished). In the scope of this study, we aimed to examine how the presence of SAP11-like and SAP54-like effectors influence the gene expression of enzymes involved in oxidative stress in transformed arabidopsis.

MATERIALS AND METHODS

Transgenic arabidopsis lines overexpressing the *SAP11-like* and the *SAP54-like* genes were obtained in our previous studies (Drčelić et al. 2024, unpublished). Total RNA was isolated from tissue of 15-day-old wild-type and transgenic seedlings by using commercial kit NucleoSpin RNA Plant (Macherey-Nagel, Düren, Germany). Subsequently, cDNA was synthesized by reverse transcription by using Maxima Reverse Transcriptase (ThermoFisher Scientific, Waltham, MA, USA) according to the manufacturers' instructions. The expression of the *AtSOD, AtGSTU24, AtCAT1, AtCAT3, AtGPX1, AtGPX4, AtGPX7* and *AtGPX8* genes was examined by qPCR (Mic qPCR Cycler, Bio Molecular Systems, Upper Coomera, Australia) by using already published primers (Horváth et al. 2015, Bi et al. 2017, Lin et al. 2019) and determined using the comparative (ΔΔCt) method.

RESULTS AND DISCUSSION

The gene expression patterns varied between different lines and genes investigated. Among the genes studied, *AtGSTU24* had the highest upregulation in all transformed lines, while the expression of *AtSOD* was not significantly different in the transformed lines, compared to the wild type. Both catalase genes, *AtCAT1* and *AtCAT3*, displayed a slight downregulation in transformed lines. Different gene expression patterns were observed between glutathione peroxidase isoforms analyzed. A general trend of downregulation was observed in the SAP54-transformed lines for *AtGPX1* gene expression, while the SAP11-transformed lines showed a more significant downregulation for *AtGPX4* and *AtGPX7*. The latter was also confirmed by a significant correlation between *AtGPX4* and *AtGPX7* expression levels, pointing to the same regulatory mechanisms during plant-phytoplasma interactions. This is the first study on the influence of '*Ca.* P. solani' effectors on the antioxidative enzyme gene expression, providing preliminary insights into the role of phytoplasma effector proteins in oxidative stress and activation of the antioxidative system in affected plants. Further transcriptomic studies will aim to reveal the putative regulatory networks behind the alterations of the antioxidative system, to aid in the efforts of elucidating the phytoplasma pathogenicity mechanisms in order to reduce their impact on agricultural production.

ACKNOWLEDGEMENTS

This study was supported by Croatian Science Foundation grants IPS-2024-02-3910 "Unravelling the enigma of '*Candidatus* Phytoplasma solani': Interactions of its effector proteins with host plants" (CapsEffect).

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Vector-related resistance traits underlie cultivar differences in flavescence dorée epidemiology

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INTRODUCTION

Identifying resistance traits is crucial to combat incurable vine diseases such as flavescence dorée (FD), associated with Grapevine flavescence dorée phytoplasma and transmitted by the leafhopper *Scaphoideus titanus*. Management currently relies on monitoring, insecticides, and uprooting of infected vines, with major economic and environmental costs. In Switzerland, Vitis vinifera cv. Chasselas is less affected by FD than cv. Pinot noir, even though it is more prevalent in the affected areas (unpublished data). Preliminary laboratory tests suggest no cultivar difference in phytoplasma sensitivity, indicating that the lower susceptibility of Chasselas in the field may result from plant–vector rather than plant–pathogen interactions.

MATERIALS AND METHODS

We studied the interaction between *S. titanus* and grapevine by comparing insect performance on Chasselas and Pinot noir. Field surveys in contiguous vineyards included nymph counts, adult captures on yellow sticky traps, and egg hatchings from 2-year-old canes collected in winter. In the laboratory, we performed no-choice and choice assays. Short and long-term dual-choice tests assessed host preference, and oviposition preference was tested by offering adults wood from Pinot noir, Chasselas, or clematis. Host quality was further evaluated under no-choice rearing conditions by measuring nymphal development, adult lifespan, oviposition, egg load, body mass, and feeding behaviour with electropenetration graph technique (EPG). Finally, phytohormone profiles of each cultivar were analysed by HPLC–MS/MS in uninfested plants and in plants exposed to healthy or FD-infected insects.

RESULTS AND DISCUSSION

Field observations consistently showed higher *S. titanus* populations and greater egg hatching on Pinot noir compared with Chasselas. However, short-term dual-choice assays revealed no clear preference between the two cultivars, indicating that the field patterns are unlikely to result from immediate attraction. Instead, the prolonged exposure choice test suggested a gradual increase in the use of Pinot noir, pointing towards differences emerging over longer interaction periods.
Under controlled no-choice conditions, several life-history traits indicated that Pinot noir acts as a higher-quality host for *S. titanus*, whereas Chasselas tends to constrain insect performance. These trends align with the observation that differences between cultivars become more pronounced with longer exposure, reinforcing the idea that host quality rather than short-range orientation determines vector behaviour. Analyses of oviposition, egg load and feeding behaviour are still underway, but initial patterns follow the same direction.
It is not the first time that FD-susceptible grapevines have been associated with improved *S. titanus* fitness. Ripamonti et al. (2022a; 2022b) showed that *S. titanus* fitness was highest on Barbera, a highly susceptible cultivar, and poorest on Moscato, a less susceptible cultivar with reduced survival, slower nymphal development, and lower egg load. Furthermore, in susceptibility assays performed with laboratory-infected *S. titanus*, Eveillard et al. (2016) observed higher insect mortality on the

three less susceptible cultivars Merlot, Syrah, and Magdeleine. Nevertheless, in these two studies, susceptible cultivars were consistently identified as such both in field or semi-field conditions and in laboratory susceptibility tests, reflecting a higher susceptibility to the phytoplasma itself. In contrast, our preliminary laboratory data show no higher susceptibility of Pinot noir to the phytoplasma compared with Chasselas, while epidemiological data do reveal differences, suggesting that the lower susceptibility of Chasselas may result from its ability to defend against the vector.
Finally, preliminary phytohormone profiling revealed clear differences between the two cultivars. Although mechanistic interpretations remain premature, these contrasting hormonal signatures are consistent with the plant–vector interaction patterns observed across our assays and echo trends described in other plant–insect systems, where shifts in defence-related signalling influence the performance of sap-feeding insects. Overall, the results suggest that cultivar-specific plant traits, and potentially distinct defence signalling profiles, may shape vector performance and contribute to the contrasting epidemiological outcomes observed between Pinot noir and Chasselas.
This research will provide a better understanding of the mechanisms involved in the vine-vector interaction and open prospects for the development of sustainable management strategies for flavescence dorée, targeting the control of its insect vector.

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A single amino acid of grapevine fanleaf virus modifies the root system architecture of a plant host and the virus transmission rate by *Xiphinema index*

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INTRODUCTION

Grapevine fanleaf virus (GFLV) is the main causal agent of fanleaf degeneration disease, which is present in most vineyards worldwide. GFLV severely affects vine health, reduces fruit yield by up to 80% and substantially limits the productive vineyard lifespan (Andret-Link et al. 2004). The ectoparasitic dagger nematode *Xiphinema index* exclusively vectors GFLV in a non-circulative, non-propagative mode. GFLV has a bipartite positive-sense RNA genome that produces eight proteins upon monocistronic translation and proteolytic cleavage (Schmitt-Keichinger et al. 2017). RNA1 encodes the RNA-dependent RNA polymerase (RdRP), which is suspected to be involved in viral RNA replication and determines pathogenicity in herbaceous plant hosts such as *Nicotiana benthamiana*, and RNA2 encodes the coat protein, which forms virions and determines transmission by *X. index* (Schmitt-Keichinger et al. 2017). The simultaneous presence of GFLV RNA1 and RNA2 molecules are required for systemic infection of plant hosts (Schmitt-Keichinger et al. 2017).

Wildtype GFLV strain GHu elicits vein clearing symptoms in apical leaves of *N. benthamiana* plants (Vigne et al. 2013) and a modified root system architecture (RSA) with lessened root tips and increased average root diameter (Roy and Fuchs 2024). Foliar and root symptoms of GFLV-GHu in *N. benthamiana* plants are transient. In contrast, wildtype GFLV strain F13 infection does not lead to any symptoms in leaves or roots of *N. benthamiana* plants (Osterbaan et al. 2019, Roy and Fuchs 2024). Remarkably, a lysine in position 802 of the RdRP of GFLV-GHu is associated with both transient leaf (Osterbaan et al. 2019) and root symptoms (Roy and Fuchs 2024) in *N. benthamiana*. How modified RSA traits of *N. benthamiana* influence *X. index*-mediated transmission of GFLV-GHu is not known. Therefore, we designed novel controlled transmission assays to evaluate and compare transmission rates of GFLV strains GHu and F13, and their respective mutants with a single amino acid change in position 802 of the RdRP. This work provided new insights into the transmission dynamics of GFLV by *X. index* (Roy et al. 2025).

MATERIALS AND METHODS

A transmission assay was performed in a grid pattern where GFLV-inoculated *N. benthamiana* plants were centrally located and healthy recipient *N. benthamiana* plants were placed 10 cm away on the perimeter of a plastic bin (27 cm x 27 cm) filled with a sandy:loess (2:1) soil mixture under greenhouse conditions. Approximately 550 aviruliferous *X. index* from a colony maintained on fig plants were placed on the roots of virus donor plants. Continuous acquisition access and inoculation access periods lasted eight weeks. Transmission assays were replicated at least four times. Plants were inoculated with one GFLV RNA1 of either GFLV strains GHu and F13 or mutants GFLV-F13 $1E^{Pol*/Sd}_{G802K}$ and GFLV-GHu $1E^{Pol*/Sd}_{K802G}$, and RNA2 of GFLV-GHu.
At the completion of the transmission assays, root systems of individual *N. benthamiana* plants were excavated, cleaned and imaged. Images were processed first in ImageJ to extract pixel density and then subject to the Rhizovision Explorer analysis software with custom parameters. Similarly,

nematodes were isolated from soil samples around individual *N. benthamiana* plants, counted, and randomly pooled in cohorts of 3-20 individuals proximal to either donor or recipient plants for GFLV detection by RT-qPCR. Root and leaf tissue of *N. benthamiana* plants were sampled and tested for GFLV infection by DAS-ELISA with specific antibodies or RT-PCR using appropriate primers (Vigne et al. 2018).

RESULTS AND DISCUSSION

Significantly higher transmission rates were documented for wildtype and mutant GFLV-F13 when compared with wildtype and mutant GFLV-GHu but higher transmission rates were obtained with asymptomatic mutant GFLV-GHu $1E_{K802G}$ (33%,16/48) versus symptomatic wildtype GFLV-GHu (26%, 12/47) and with asymptomatic wildtype GFLV-F13 (75%, 30/40) versus symptomatic mutant GFLV-F13 $1E_{G802K}$ (51%, 20/39). No transmission events were detected from mock-inoculated plants, as expected. These results revealed that *X. index*-mediated transmission is influenced by the GFLV strain and the identity of the amino acid in position 802 of the RdRP with a glycine favoring transmission and a lysine reducing transmission (Roy et al. 2025). GFLV transmission was significantly correlated with differences in RSA traits such as the number of root tips, root surface area, and total root length, confirming that GFLV transmission by *X. index* is highly influenced by the configuration and expansiveness of the plant root system (Roy et al. 2025). However, *X. index*-mediated GFLV transmission was not associated with GFLV titer in *X. index* or roots of donor plants. Together, this work suggested that the plant root network may impact GFLV transmission by influencing *X. index* feeding patterns. In the case of asymptomatic infections, the physical proximity of root systems promotes nematode feeding, thus facilitating GFLV acquisition from infected donor plants and subsequent transmission to recipient plants. This scenario applies to GFLV-F13 and mutant GFLV-GHu $1E^{Pol*/Sd}_{K802G}$. In contrast, in the case of symptomatic infections, nematode feeding on donor plants is reduced and the physical distance between root systems of donor and recipient plants limits nematode feeding, thus reducing GFLV transmission from plant to plant by *X. index*. This scenario applies to GFLV-GHu and mutant GFLV-F13 $1E^{Pol*/Sd}_{G802K}$. This is the first report of modified plant host RSA traits via a single viral amino acid of a soildborne virus to influence its transmission by a dagger nematode (Roy et al. 2025).

ACKNOWLEDGEMENTS

This work was supported in part by the California Department of Food and Agriculture, Cornell Venture funds, the Cornell University Einaudi Center, and the Conseil Interprofessionnel des Vins d'Alsace. We are grateful to Sophie Meyer and Claude Gertz for their assistance.

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The ecological influence of a treehopper vector on the epidemiology of a grapevine viral disease

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INTRODUCTION

Grapevine red blotch disease poses a significant economic challenge to the North American wine industry, with infected vineyards incurring replanting and additional management costs estimated between $2,213 and $68,548 per hectare over a 25-year production period (Ricketts et al., 2017). The three-cornered alfalfa hopper, *Spissistilus festinus* [Say, 1830] (Hemiptera: Membracidae), is a vector of grapevine red blotch virus (GRBV), the causal agent of the disease (Flasco et al., 2021, 2023), although it is not a pest of grapevines. Secondary spread of GRBV is often prevalent near vineyard edges where infection hot spots develop (Flasco et al., 2025). No information is available on the biology and behavior of *S. festinus* in a vineyard ecosystem. Therefore, we analyzed plant DNA from the guts of field-collected *S. festinus* individuals to reconstruct feeding histories and identify host use across vineyard landscapes (Hoyle et al., 2025a). This research revealed distinct seasonal feeding patterns of *S. festinus*, potential overwintering hosts, and movement between riparian vegetation and vineyards, providing insight into GRBV epidemiology. Complementary, controlled transmission assays in the laboratory tracked the dispersal of aviruliferous and viruliferous males, females, and mixed groups, and demonstrated sex-specific differences in transmission efficiency and a positive relationship between initial virus prevalence and transmission rate (Hoyle et al., 2024). This work on transmission ecology provided an integrated perspective on *S. festinus* behavior and GRBV epidemiology.

MATERIALS AND METHODS

Plant DNA was extracted from *S. festinus* individuals from more than 70 vineyard parcels in Napa Valley, California, U.S.A., and analyzed via PCR using primers designed in the genomic internal transcribed spacer (ITS) region and the chloroplast trnL-F intergenic spacer (trnF) region. Amplicons were sequenced using the Pacific Biosciences platform, and resulting sequences were used to identify plant taxa present in *S. festinus* gut contents (Hoyle et al., 2025a).
To investigate overwintering behavior of *S. festinus*, 10–20 male and female adults were confined on selected host plants in vineyard ecosystems using sleeve cages and regularly monitored from November through June of the following year.
Dispersal assays were conducted in the laboratory to evaluate the role of viruliferous and aviruliferous *S. festinus* in the transmission dynamics of GRBV. Newly emerged adult males, females, or mixed cohorts were provided an acquisition access period on snap bean plants infected with GRBV isolates NY358 (phylogenetic clade 1) or NY175 (phylogenetic clade 2) for 7–10 days (Hoyle et al. 2025b). Following a 48-hour gut-clearing period on alfalfa, insects were released into arenas containing 30 healthy excised snap bean trifoliates, where dispersal and feeding behaviors were monitored twice daily over a two-week period. At the completion of these experiments, insect and plant samples were collected and tested for GRBV by multiplex PCR and quantitative PCR (qPCR). Additional assays using non-viruliferous *S. festinus* evaluated secondary spread from arenas containing either single or multiple GRBV-infected snap bean trifoliates (Hoyle et al. 2024).

RESULTS AND DISCUSSION

Unique plant genera (23 in 2021 and 108 in 2022) were identified by amplicon-sequencing in the guts of *S. festinus*, with 40 genera shared between the two consecutive years. Feeding hosts included both cultivated grapevines and free-living *Vitis* species (*V. californica* and *V. californica* hybrids) (Hoyle et al. 2025). In both years, a higher proportion of *S. festinus* individuals fed on free-living vines than on cultivated grapevines, a trend that was particularly pronounced in 2022 when favorable environmental conditions promoted diverse vegetation growth beyond irrigated vineyard areas. These findings indicated that free-living vines represent important feeding resources for *S. festinus* (Hoyle et al. 2025a). In addition, prior to the peak visitation of *S. festinus* in Napa Valley vineyards in June, gut content profiles suggested feeding on probable overwintering hosts in riparian habitats, including trees, shrubs, and free-living *Vitis* species. Together, this work characterized the diversity of plant families comprising the diet of *S. festinus*, clarified the extent of its associations with potential overwintering hosts and underscored the ecological connectivity between riparian vegetation and vineyards that may facilitate GRBV dissemination. Furthermore, laboratory experiments revealed that males exhibit greater dispersal distances than females, though both sexes tended to remain near their initial release points (Hoyle et al. 2024). Consequently, GRBV transmission occurred more frequently with males compared to females or mixed-sex groups. Similarly, higher rates of secondary transmission were obtained with males than females and with a higher initial inoculum prevalence (Hoyle et al. 2024). Collectively, these findings provided new insights into key factors that influence GRBV spread dynamics.

ACKNOWLEDGEMENTS

We are grateful to Rodeny Cooper, USDA-ARS Temperate Tree Fruit and Vegetable Research Unit, Monica Cooper, University of California Cooperative Extension, and the network of growers in Napa Valley, California, U.S.A. whose involvement made this project possible. This work is supported by the California Department of Food and Agriculture, Cornell Venture funds, and the Agricultural and Food Research Initiative grant no. 2023-67011-40492 from the USDA National Institute of Food and Agriculture.

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Best practice sampling and virus detection in Australian grapevine propagation assets

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INTRODUCTION

Grapevine (*Vitis vinifera*) is susceptible to infection from 106 viruses (Fuchs, 2023). In Australia, 19 grapevine viruses have been reported, of which, grapevine leafroll-associated virus 1 (GLRaV-1), GLRaV-2, GLRaV-3, GLRaV-4, grapevine virus A (GVA), grapevine Pinot gris virus (GPGV), and grapevine red blotch virus (GRBV) have been associated with a negative impact to grapevine fruit quality and yield in other growing regions (Naidu et al., 2014; Goszczynski & Habili, 2012; Bertazzon et al., 2017; Sudarshana et al., 2015). The proactive testing of these viruses enables effective management to limit subsequent and unwanted spread which can severely limit the productivity and profitability of the Australian Wine Industry.

Reliable grapevine virus testing in Australia remains a challenge due to a complicated landscape of grapevine viruses with variability in their genetic diversity, virus titre, virus localisation within a host, and seasonal and regional dynamics. Laboratory specific factors which further complicate harmony in diagnostics include differences in assay types and specific assays/targets, and sampling differences at the field and laboratory level. The development and validation of effective and robust sampling regimes and diagnostic assays that can be harmonised across laboratories for a range of pathogenic grapevine viruses is integral to a successful long term viral management strategy. This research project aims to provide best practice guidance for successful sampling of grapevines for timing and tissue type and to refresh the diagnostic assays to ensure they are robust against the viral genetic variation present in Australia.

MATERIALS AND METHODS

Interrogation of the extensive existing datasets from previous diagnostic and high throughput sequencing (HTS) of grapevines will be used to identify virus isolates of interest for further analysis, including additional genomic characterisation. The genomic data will be used to guide the molecular assays adopted or developed for future diagnostics.

Grapevine sampling will be assessed from vineyards across several climates (hot, cold, and temperate) that are representative of the Australian grape growing regions with two vineyards for each climate (n = 6). From each climate, Chardonnay and Shiraz grapevines will be selected for sampling. Grapevine sampling will be conducted across two years (CY25-26 and CY26-27) from each season (spring, summer, autumn, and winter) and across different tissue types (shoots pair 1, shoots pair 2, four shoots, and ten leaves) or cane material for seasons during grapevine dormancy. Grapevine samples collected will be tested for viruses using existing and developing molecular assays.

RESULTS AND DISCUSSION

The prevalence and genetic variation of grapevine viruses in Australia will be presented. Preliminary results of virus detection from initial testing across climates, seasons and grapevine tissue types will be presented.

An additional outcome of this work will be the establishment of a well characterised library of grapevine virus isolates to be used as positive controls to ensure the integrity of diagnostic results. Grapevines with viruses and virus combinations of interest, and virus-free controls, from key varieties will be established *in vitro* and molecularly characterised using HTS to be retained long term at Agriculture Victoria Research (AVR). The viral positive control library will support the development and delivery of a grapevine virus diagnostics proficiency testing program that will provide quality assurance of results across participating laboratories and confident decision making for virus management within the viticulture industry. Additionally, the positive control library can be used as a biological resource to support ongoing grapevine virology research in Australia.

ACKNOWLEDGEMENTS

The research presented was supported by funding from Wine Australia. Wine Australia invests in and manages research, development and extension on behalf of Australia's grape growers and winemakers and the Australian Government. We also thank the South Australian Vine Improvement Association, Riverland Vine Improvement Committee and team members, Vine Industry Nursery association, CSIRO and other industry contributors for their support.

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From sample preparation to High Throughput Sequencing: navigating different methodologies for efficient virus diagnostics in grapevine

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INTRODUCTION

Disease management in agriculture has never been more critical, especially when current resources against pests and pathogens do not match environmental standards, nor meet technical and regulation impasses. Thus, investing in clean and sustainable certified clones, supported by highly efficient pathogen detection methods is a priority to face reduction of plant protection chemicals, and emergence of new pathogens. In France, scion and rootstock vines dedicated to multiplication as much as all imported grapevine shoots fall under certification obligations. Current molecular, serological, and indexing methods are limited, targeted, time-consuming, and non-evolutive. These techniques are not suitable nor informative enough to evaluate the sanitary status of a sample, mostly challenged by complex virome and the emergence of new viruses.

High throughput sequencing (HTS) can be used not only in plant research but also in diagnostics, allowing unbiased identification of all sequences in a given sample, (Adams et al. 2016). To date, *Vitis* can host more than 100 viruses: both single or double stranded DNA and RNA types, and HTS has largely contributed to enlarging this collection (Fuchs et al. 2025).

We performed serial experiments to dissect each step of sample preparation (sampling, grinding, nucleic acid extraction, enrichment and library preparation) prior to sequencing, under the Optiseq project. We also tested both short and long read sequencing technologies. This comparative study was carried out on a multi-infected grapevine and its daughter shoots. For each individual plant, multiple organs were analyzed. We aim to develop these protocols to be the most comprehensive for the detection of the virome in grapevine.

First results already enlighten organ choices and critical points in our process in both sample preparation and sequencing level. As other collaborators did before (Bester et al, 2021; Massart et al. 2022), we gathered information to support HTS-guided virus diagnostics in grapevine. We hope to enrich discussion with our results and to propose recommendations to sustain a future certification scheme in France based on HTS methodologies.

MATERIALS AND METHODS

Vegetal resources: super-infected grapevines were obtained by grafting two accessions with known viral status. This scion-rootstock assemblage should host 10 viral species (GVA, GLRaV-1 and -2, GRSPaV, GVT, GFLV, GRVFV, GFkV, GRGV and GSyV-1), and 3 viroïds (GYSVd-1, HSVd and GHVd). From this wood cuttings were produced. In total, six individuals survived and were maintained in greenhouses to minimize further infection.

To assess the importance of seasonality and tissue type for virus detection, as well as test the resulting virus accumulation and localisation, different organs (leaf, wood, root, tendril etc.) were sampled for each plant at different phenological stages.

Samples preparation: over 30 samples were collected. They were ground into powder in liquid nitrogen, either with pestle and mortar or with an impact ball mill (Retsch, Eragny-sur-Oise, France), then aliquoted and stored at -80°C. A variety of extraction methods were tested (e.g. totRNA, DNA CTAB) along with several enrichment methods (e.g. poly(A), ribodepletion, and VANA). Extractions were selected and performed by a number of separate laboratories.

Multiple series of samples (different organs) were sent to our sequencing providers (GenoToul). Extractions were performed on 100 mg with an adapted protocol version of the Spectrum kit (Sigma-Aldrich, Saint-Louis, Missouri, USA) or RNeasy Plant mini kit (Qiagen, Hilden, Germany). RNA ribodepletion using PanPlant riboPools kit (siTOOLs Biotech GmbH, Planegg, Germany), followed by cDNA libraries preparation (TrueSeq mRNA Stranded, 30 Illumina, Inc., San Diego, CA, USA) were prepared then sequenced (2x150 bp) on an Illumina NovaSeq 6000 S4 flow cell by the GenoToul platform (GenoToul, Castanet-Tolosan, France). Alongside our analyzes, these same grapevines were also tested by indexing method, the official and current method of certification in France.

RESULTS AND DISCUSSION

From organ and season perspectives: only the original assemblage contained all 35 viral or viroidal expected sequences. Multiplication through cuttings did provide selection, as a few sequences were missing from each daughter shoot, with no specificity either in species or variants; these exclusion mechanisms remain unknown.

From all the tested organs, wood from winter pruning is the most exhaustive matrix detecting all sequences (virus, satellites and viroids) within each plant. Roots came second with 93 % of the viral sequenced and 100% of the species being detected. Tendrils and inflorescence came next with 80 to 90% of sequences and viral species being detected. Finally leaf with up to 50 % loss in both species and variant sequences seems to be the worst matrix for predicting the complete virome description of a plant. Some species and variants seemed to be specifically excluded from some matrices.

Preparation technics comparison: For Illumina sequencing, ribodepletion was selected over poly(A) enrichment as numerous viruses infecting grapevines are not polyadenylated (e.g., GLRaVs). In collaboration with our sequencing provider, ribodepletion protocol was optimized comparing column to bead probe purification. We improved the remaining rRNA from sequencing, reducing them from 8,15 ± 2,35 to 2,56 ± 0,46 % in favor of bead probes. Both treatments were able to recover all viral sequences, but virus read accumulation was usually higher, whereas lower for viroid and satellites reads with bead method purification methods.

Preliminary results from long read MinION sequencing, show that not only were more raw reads obtained from ribodepleted RNA compare to RNA resulting from VANA, but the reads were also longer.

Multiple protocols, techniques and data analysis pipelines (not discussed here) were implemented in the laboratory, additional experiments are still ongoing to test other's enrichment, and perform library preparation at home, and explore MinION ONT sequencing.

ACKNOWLEDGEMENTS

OptiSeq project was funded by the CASDAR program class of 2023. The authors also acknowledge Maher Al Rwahnih's, Sebastien Massart's and Philippe Roumagnac's teams for their collaborative insights and associated training opportunities.

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Investigating the seasonal variation in grapevine red blotch virus detection, titer, and within-vine distribution using qPCR and sentinel vines

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INTRODUCTION

Significant advances have been made in the past 20 years on the epidemiology of grapevine red blotch disease, caused by grapevine red blotch virus (GRBV; Yepes et al. 2018). Although GRBV infected planting stock has been the source of introduction into many vineyards, the three-cornered alfalfa hopper, *Spissistilus festinus*, is a known field vector of epidemiological significance in northern California (Flasco et al. 2023). Identifying and removing GRBV positive vines, the current management recommendation for preventing secondary spread (Ricketts et al. 2017; Flasco et al. 2025), requires timely and accurate diagnostic methods especially given the knowledge that GRBV can be transmitted by *S. festinus* in June from asymptomatic vines (Flasco et al. 2023). In this study, we used known GRBV negative sentinel vines in a vineyard with high GRBV incidence to determine how soon GRBV can be detected in vines following planting and to quantify the seasonal variation in the number of newly positive GRBV vines detected and the corresponding virus titer and within-vine distribution.

MATERIALS AND METHODS

The vineyard chosen for this study was the Russell Ranch vineyard (RRV), located in Yolo County, CA and maintained by Foundation Plant Services (FPS) at the University of California, Davis (UCD). Approximately 400 Cabernet franc sentinel vines were propagated, tested by qPCR to verify they were GRBV negative, and then planted in RRV in 2020 in the locations where GRBV positive vines had been previously removed in 2017-2019. GRBV incidence in RRV at the time of planting was 18%. The sentinel vines were then tested by qPCR in April or May (spring), August or September (summer), and October or November (fall) in 2021-2023. In 2024 and 2025, vines were tested in May (spring) and September (summer) only. Twelve basal leaf petioles served as samples at all time points except October/November when 12 lignified canes were sampled instead. For newly GRBV positive vines, petioles or canes from the original sample were tested individually to determine GRBV positive incidence with single vines. In addition, GRBV positive vines that were first detected in the spring were used to compare the absolute number of GRBV genome equivalents present in these same vines in summer and fall. Sentinel vines were visually monitored for red blotch disease symptoms at all time points.

RESULTS AND DISCUSSION

The first GRBV positive vine was detected in fall 2021 (Fig. 1) and was showing clear GRBV symptoms. In 2022 - 2025, the majority of newly positive GRBV vines were detected in the spring, with only a few detected in the summer and fall time points in 2022 and 2025 and none in 2023 and 2024 (Fig. 1). By September 2025, a total of 81 sentinel vines were GRBV positive, representing 28% of the 291 vines that were still alive and large enough to sample. Vines testing positive in the spring did not show GRBV symptoms until summer or fall but symptoms were always visible the same year that the vines first tested positive by qPCR. Despite not showing symptoms in the spring, GRBV titer averaged 10^6 genome equivalents, compared to 10^7 and 10^6 average genome equivalents in summer and fall, respectively. GRBV titer in the summer was signficiantly higher than in the spring and fall at $p<0.05$ but GRBV titer was not significantly different between the spring and fall. The average within-vine incidence was 0.99 in infected vines that were first detected in the spring

compared to 0.73 and 0.67 for the summer and fall, respectively. However, only six and two vines were first detected in the summer and fall, respectively, compared to 73 in the spring. Therefore, we are most confident in the spring results, which indicate that GRBV within vine distribution is uniform.

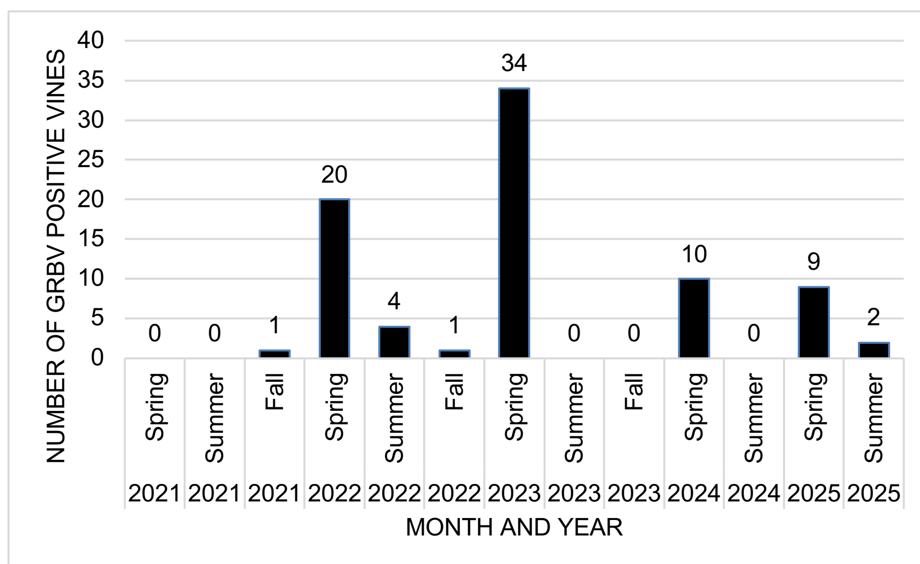

Fig. 1. Bar graph shows the number of newly detected GRBV positive sentinel vines and the month and year in which they were first detected. Spring = testing done in April or May; Summer = August or September; Fall = October or November

In conclusion, the sentinel vine test data from RRV indicates that testing petioles in May using qPCR provides a means of reliably identifying most of that year's newly positive GRBV vines. In turn, these vines can then be removed before *S. festinus* first enters the vineyard in early summer, with the objective of reducing secondary spread.

ACKNOWLEDGEMENTS

This work was supported by funding from the Pierce's Disease and Glassy-Winged Sharpshooter Board and the California Fruit Tree, Nut Tree, and Grapevine Improvement Advisory Board.

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Enhanced sensitivity and detection of grapevine viruses in Mexico using digital PCR in comparison with quantitative PCR

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INTRODUCTION

The economic significance of the grapevine (*Vitis vinifera*) industry requires rigorous disease management. Viral pathogens, which are one of the primary limiting factors for grape production worldwide, cause substantial economic losses. For instance, GLRaV-3 alone is estimated to cause annual losses exceeding $90 million USD in the United States, with vineyard losses potentially ranging from $29,902 to $226,000 USD per hectare. In Mexico, these four RNA viruses—GVA, GFLV, GLRaV-3, and GPGV—are among the sixteen viruses currently documented, posing a continuous threat to regional sustainability.

Accurate and early diagnosis is the cornerstone of control, especially within nursery and certification programs where eradication relies on zero tolerance. While quantitative PCR (qPCR) is the current routine standard, it is fundamentally limited by a lower sensitivity threshold. Low-titer viruses (e.g., GPGV) or seasonal titer fluctuations often cause qPCR to report false negatives, resulting in the unintentional distribution of contaminated plant material. Digital PCR (dPCR), a third-generation PCR technology, achieves absolute quantification without the need for standard curves by partitioning the reaction into thousands of micro-droplets, theoretically achieving significantly higher sensitivity and precision than qPCR.

This research aimed to conduct the first comparative assessment of the newly developed dPCR system against a standard qPCR system for the diagnosis and absolute quantification of four major grapevine RNA viruses (GVA, GFLV, GLRaV-3, GPGV) to determine the methodological superiority and its potential impact on Mexican vineyards.

MATERIALS AND METHODS

The study focused on four positive-strand RNA viruses (GVA, GFLV, GLRaV-3, and GPGV) chosen for their varying titer levels and economic significance. The evaluation utilized a cohort of 45 field grapevine samples collected from different wine, table, and industrial grape-growing regions across Mexico, alongside positive controls and negative controls for limit of detection (LoD) determination. Total RNA was extracted from leaf petioles of the samples using a commercial kit and an automated processing platform to ensure high-quality and consistent nucleic acid yield, critical for accurate PCR detection.

The extracted RNA was subjected to two distinct reverse transcription PCR analyses: RT-qPCR assays were performed on the QuantStudio 5 Real-Time PCR System (Applied Biosystems) using established, validated primers and fluorescent probes for each target. RT-dPCR assays were performed on the QIAcuity One five plex Digital PCR System (QIAGEN); this system utilizes a plate-based partitioning method, followed by thermocycling and fluorescence detection to determine the absolute number of target copies per volume.

The performance metrics, particularly the LoD, were statistically compared. Analysis of Variance (ANOVA) was applied to determine the overall significance of the technique (RT-dPCR vs. RT-qPCR) and the viral target. A subsequent Tukey's Honestly Significant Difference (HSD) post-hoc test was performed to pinpoint specific pair-wise differences and establish the confidence level of the comparative sensitivities.

RESULTS AND DISCUSSION

The ANOVA results demonstrated that both the PCR technique (RT-dPCR or RT-qPCR) and the specific virus (GVA, GLFLV, GPGV, and GLRaV-3) had a statistically significant effect on the LoD comparison results ($p < 0.05$). Furthermore, the high repeatability of the dPCR system was confirmed, as the internal replicates were non-significant, showing robust precision. Finally, the Tukey test confirmed that RT-dPCR achieved a significantly lower LoD than RT-qPCR, establishing a statistically reliable difference with 95% confidence.

The comparative field study on 45 grapevine samples provided the most compelling evidence for RT-dPCR's superiority, especially in detecting low-titer or sporadically distributed infections. The 45 samples were analyzed, and the results from the RT-dPCR were taken as the baseline true positive/negative standard due to its established superior sensitivity.

RT-qPCR generated a high rate of false negative results when compared to the RT-dPCR baseline:

Virus	True positive samples (by RT-dPCR)	RT-qPCR detection rate	False negative rate (by RT-qPCR)
GVA	8	62.5%	37.5%
GLRaV-3	7	85.7%	14.3%
GPGV	18	38.9%	61.1%
GFLV	8	12.5%	87.5%

This study validates the application of the RT-dPCR system as a superior diagnostic platform for major grapevine RNA viruses. The statistically significant difference in LoD between RT-dPCR and RT-qPCR, confirmed by the Tukey test, translates directly into a profound practical advantage in field diagnostics. The ability of RT-dPCR to detect infections that are missed by RT-qPCR (as demonstrated by the high false negative rates, particularly for GFLV and GPGV) is a critical finding for phytosanitary programs.

A missed diagnosis of a GFLV or GPGV infection, due to the high false negative rate of RT-qPCR, allows the propagation of infected planting material and facilitates the spread of the virus by its respective vectors (nematodes and mites), this contributes directly to the economic losses. The successful implementation of dPCR enables a proactive management, further strengthening the defense of the valuable Mexican and global grapevine industry.

ACKNOWLEDGEMENTS

Dr Jimena Carrillo Tripp and Dr Víctor Manuel Rodríguez García, for their valuable time, thoughtful feedback and constant support. CONAHCyT for funding support.

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A nanobody-based ultra-sensitive method for rapid screening of Grapevine leafroll associated virus type 3 (GLRaV-3)

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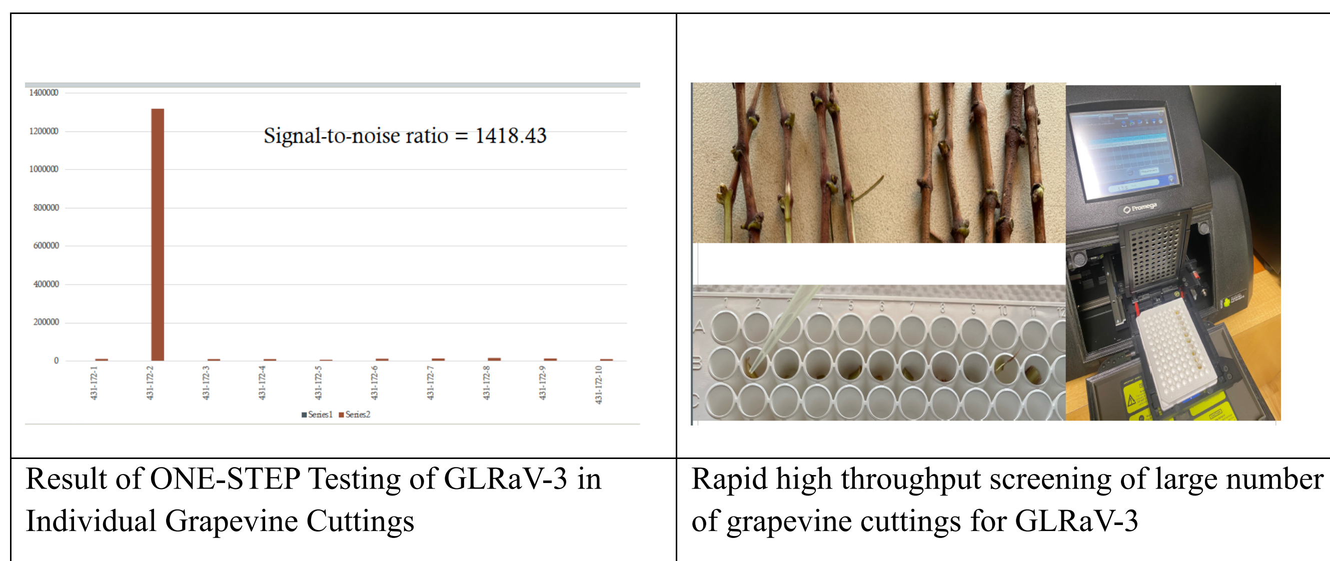
INTRODUCTION. Grapevine leafroll associated virus type 3 (GLRaV-3) is one of the most economically damaging viruses affecting grape production globally. It can lead to up to 50% yield losses due to reduced fruit quality, delayed ripening and lower market value, resulting in estimated economic loss of $25,000 and $41,000 per hectare over the lifespan of a vineyard (Alston et al 2019, Atallah et al 2012). Mitigation strategies include: (1) Use certified grapevines tested to be free of GLRaV-3; (2) Regularly monitor and test to identify infected vines. (3) Control insect vectors (e.g. vine mealybugs) with chemical and biological methods. Current diagnosis requires multiple steps and skilled operators performed in the laboratory. Our objective is to enable growers to conduct testing in the field, in real-time and in large sample size to help improve management outcomes. Here we report a nanobody-based ultra-sensitive the direct direction GLRaV-3 using crude samples without nucleic acid amplification. Specifically, the method uses camelid derived nanobodies against the capsid protein of GLRaV-3 fused with fragments of nanoluciferase. When no GLRaV-3 is present, the reagent has no bioluminescence because the enzyme fragments are inactive. When the GLRaV-3 is present, the nanobodies bind to the capsid protein, bringing the luciferase fragments to proximity to form an active nanoluciferase, producing a bright bioluminescence signal. This method has extremely high signal-to-noise ratio because the enzyme fragments are inactive before binding or when binding non-specifically to the surface substrate. The method also offers high specificity because two nanobodies are required to bind simultaneously to the target to create a signal. By comparison, in conventional ELISA, the enzyme-linked antibody is always enzymatically active whether it's in solution, bound to the target, to substrate surface, or to a structurally similar interferent molecule. A signal-to-noise ratio of over 2200 was demonstrated while conventional ELISA typically has a S/N ratio of 15~20. The method was shown to be more sensitive than RT-PCR and qPCR when compared side-by-side to test GLRaV-3 using serially diluted crude samples.

MATERIALS AND METHODS. Recombinant capsid protein of GLRaV-3 was first obtained by cloning its gene in pET-24a vector, expressed in *E. coli*, purified using 6xHis-tag. Nanobody production protocol (Maass et al 2006) consists of: 1) Immunizing an alpaca with the recombinant antigen. 2) Isolating peripheral blood mononuclear cells (PBMCs) to extract their mRNA and make cDNA. 3 PCR-amplify VHH gene fragments using alpaca-specific primers. 4) creating a VHH phage display library by cloning the VHH genes into the pComb3X phagemid vector (Rader & Barbas 2000). 5) Iterative screening of the library against the immobilized GLRaV-3 antigen. 6) DNA sequencing to identify positive clones. Nanoluciferase (nanoluc) is a small (19.1 kDa), highly stable, and exceptionally bright monomeric enzyme engineered from the deep-sea shrimp *Oplophorus gracilirostris* luciferase. It catalyzes the oxidation of its substrate, furimazine, to produce high-intensity, glow-type blue light at 460 nm in an ATP-independent manner and is 100-150 times brighter than firefly luciferases. Its small size minimizes steric hindrance in fusion proteins. The split nanoluciferase system is an enzyme complementation assay where Nanoluc is divided into two subunits: a large N-terminal fragment (lgLuc, ~18 kDa) and a small C-terminal peptide (smLuc, 11 amino acids). When these two subunits are fused to nanobodies, they are brought into proximity by nanobody-binding to its target allowing the two fragments to reassemble into a functional enzyme. Here, three GLRaV-3 VHH clones (VHH21, VHH51 and VHH111) were used to make 24 (12+12) fusion proteins with lgLuc and smLuc in the general format: VHH-linker-lgLuc, VHH-linker-smLuc.

RESULTS AND DISCUSSION. We screened 12 x 12 = 144 possible fusion protein combinations where first group of 12 fusion proteins contained the small Luc unit and second group of 12 contained the large Luc subunit. In addition to the Luc subunits, each fusion protein also contained a unique GLRaV-3 VHH and a unique linker. Three VHHs and four unique linkers produced 3x4=12 fusions in each group. The signal-to-noise ratio was found to vary between 120- to 2280-fold, depending on the fusion combinations. By comparison, conventional ELISA typically has S/N ratio between 15-20. Using the fusion combination of highest S/N ratio (2280), we did side-by-side comparison of testing a serially diluted grapevine sample infected with GLRaV-3. Comparing to conventional ELISA, RT-PCR and qPCR, the method described herein showed a sensitivity improvement of 250- and 80-fold, respectively. It is envisioned that reagents can be freeze-dried in the wells of 96-well plates, luminescent signals can be read out upon sample addition without the need for additional wash steps. This "mix-and-read" format, when combined with portable luminescence readers, enables rapid and on-site testing in vineyards and/or nurseries. It can also be adapted to handheld luminescence readers for single sample testing. Although GLRaV-3 was used to demonstrate feasibility, this method represents a new ultrasensitive ELISA in future for highly sensitive and specific detection of plant pathogens, infectious diseases, cancer markers and beyond.



Result of ONE-STEP Testing of GLRaV-3 in Individual Grapevine Cuttings	Rapid high throughput screening of large number of grapevine cuttings for GLRaV-3

ACKNOWLEDGEMENTS. We sincerely thank 1) USDA for a Small Business Innovation Grant (SBIR #33610-19533) to develop GLRaV-3 nanobodies; 2) Prof. Julie Dechant at UC Davis School of Veterinary Medicine for immunizing an alpaca under approved IACUC protocol; 3) Prof. Bruce Hammock at UC Davis Dept. of Entomology for wonderful collaborations on nanobody research.

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Implementation of high-throughput sequencing for post-entry quarantine testing of viruses

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INTRODUCTION

The Plant Health and Environment Laboratory (PHEL) is a world-leading laboratory in its application of high-throughput sequencing (HTS) to diagnostic testing (Liefting et al., 2021; Waite et al., 2022; Nunes-Leite et al., 2024). Since becoming the first laboratory in New Zealand to achieve ISO17025 accreditation for Oxford Nanopore Technology HTS testing of plant pathogens, we have sought to expand our HTS offerings into the post-entry quarantine (PEQ) space, to augment our existing test regime with this technology. Plants tested for viruses in PEQ need a method sensitive enough to detect infections at very low titres, similar or better than quantitative reverse-transcription (RT-qPCR). This work demonstrates that Illumina NovaSeq sequencing can achieve detection limits the same as, or better than, RT-qPCR.

MATERIALS AND METHODS

Method development was initially performed using *Fragaria* as a model system, then expanded to include *Vitis, Prunus, Citrus, Rubus, Actinidia,* and *Malus*. Total nucleic acid extracted from positive controls from healthy plants were spiked with DNA and RNA of target viruses at varying degrees of dilution. Comparisons of test sensitivity were performed for each sample using the Illumina NovaSeq sequencing platform and standard RT-qPCR assay for the virus of interest. Recovery of virus sequences from each library was performed by mapping sequences against representative genomes from each virus of interest, and comparing the sensitivity, repeatability, and reproducibility with that of RT-qPCR. Random subsampling of reads generated by NovaSeq sequencing was employed to test the sensitivity of the HTS method at different levels of sequence output.

RESULTS AND DISCUSSION

Comparison of test sensitivity between HTS and RT-qPCR revealed that sequencing to a depth of 40 – 60 million sequences per sample from ribodepleted total nucleic acid was sufficient to yield equivalence between the methods when targeting 1 – 1,000 viral copies per microlitre of extract (Nunes-Leite et al., 2024). Based on these findings, we began offering commercial HTS testing of PEQ samples in late 2024 for commodities where the sensitivity of PCR and HTS had been evaluated. The approach to mapping of regulated viruses is in line with current import regulations but sequence data can be reviewed for a broader search if the plants develop concerning symptoms during quarantine.

The current New Zealand Vitis import heath standard requires mandatory testing for 18 viruses. Although HTS has a higher per-sample cost than RT-qPCR, its ability to test all 18 viruses simultaneously makes it a more cost-effective and flexible option for plant virus testing when there are two or more test samples.

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VITIMINING: Mining public databases in search of grapevine viruses

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INTRODUCTION

One of the technological revolutions of the early part of this century is the exceptional and exponential advances in the performance of sequencing techniques. High Throughput Sequencing (HTS) is based on its capability to produce a large amount of information at once by massive parallel sequencing, producing a wealth of data in record time. While gigabases of *in silico* data are produced daily, for research purposes as well as for targeted clinical applications, only a small portion is being used and analyzed. This is the part of the data dedicated to answering the question for which they were produced. However, a lot of information is yet to be evaluated and uncovered. These methods are not restricted to the animal kingdom, datasets specific to grapevines (*Vitis spp.*) have been in constant progression in the last few years.
Grapevines are the host of many viruses. To date, over one hundred viral species have been identified infecting this crop cultivated for millennia (Fuchs, 2024). More than fifty of these viruses have been discovered within the last decade, thanks to HTS. While some of them have been clearly demonstrated to infect grapevine (Yepes et al., 2018), others are thought to be part of the plant's environment (Hily et al., 2018).
Our project, based on datamining, focuses on most (*i.e.* all usable) publicly available datasets originating from plants belonging to *Vitis* genus, in an attempt to reconstruct viral genomes and identify the virome of each dataset. We aim to determine if datamining could be helpful in adding new insight, information, knowledge, regarding any viral species. Benefits and limitations regarding the use of datamining and publicly available genomic resources will be discussed.

MATERIALS AND METHODS

For large-scale detection of viral sequences in grapevine transcriptomic data, we developed a Nextflow based bioinformatics pipeline that mines publicly available sequencing datasets as input. When operating in datamining mode, the pipeline automatically retrieves relevant public RNASeq datasets from repositories such as the NCBI's Sequence Read Archive (SRA) or the European Nucleotide Archive (ENA) based on a curated version of metadata table of all datasets originating from a *Vitis* sample. These datasets are then processed through a standardized workflow of viral-metatranscriptomic, beginning with quality control and adapter trimming using Fastp. Host-derived reads are sequentially removed through two complementary approaches: first, taxonomic classification with Kaiju to discard reads matching the *Vitis* genus, and second, alignment against a *Vitis vinifera* reference genome using Bowtie2 to remove remaining host sequences. The resulting non-host reads are assembled *de novo* using SPAdes *rnaviral* mode, and contigs aligning to the grapevine reference genome are filtered out. The final set of contigs (> 200 bp of size) is subjected to similarity searches with BLASTn and protein homology searches with DIAMOND BLASTp, enabling the identification and taxonomic classification of known and potentially novel viral sequences. A sample is defined as positive when 60 % of the genome of the virus is covered.

RESULTS AND DISCUSSION

As of August 2024, 30 000 datasets related to *Vitis* were available in the public databases (NCBI, ENA), covering a wide range of sources, methodologies, and protocols (e.g. RNA-Seq, WGS,

miRNA-Seq, amplicon…). Using NCBI's SRA explorer, we retrieved metadata tables in order to filter out non-informative datasets, to keep mostly RNAseq and smallRNAseq data consisting of over 17 800 runs. Each dataset is then downloaded and fed to a Nextflow pipeline developed in-house.
A subset of over 1 000 runs has been analyzed, giving a first overview of the grapevine meta-virome. Interestingly, 96 % of the samples showed signature of at least one virus or more. Between one to twelve viral species were identified in most datasets (figure 1), with all kinds of genomes being found: RNA as well as DNA genomes, single or double stranded, poly or non-polyadenylated.
Out of this work, we were able to expand the known genetic diversity of many viruses, allowing for the development of more exhaustive and unbiased PCR-based assays. We confirmed the presence of viruses in different cultivars than the one they were originally and so far, solely described. Complete genome sequences have been obtained for viruses for which only partial sequences are available. In addition, our effort is dedicated to characterizing the spatiotemporal distribution of grapevine viruses. This knowledge is essential to focus epidemiological surveillance efforts and better understand both the migration and the emergence of pathogens. Current distributions are being enriched by indirect reporting of newly infested territories derived from datamining, relying on metadata associated with datasets.
While datamining cannot replace targeted and dedicated studies, this approach constitutes a key component and a complementary tool for grapevine viral research. Challenges associated with the use of public genomic data, from completeness and accuracy of metadata associated with dataset, misannotations, and potential data contamination will be discussed.

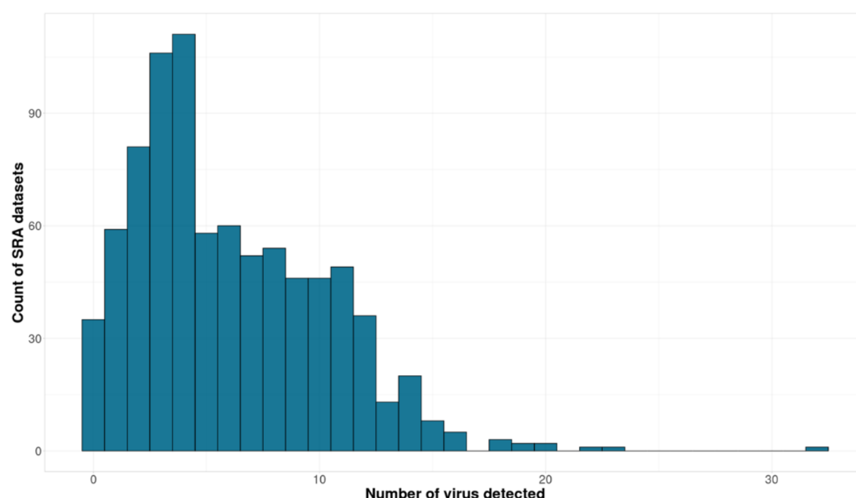

Figure 1: Histogram of datasets with the same number of viruses being detected.

ACKNOWLEDGEMENTS
Vitimining project was funded by the CASDAR program class of 2022, as a continuation of the OPTISEQ project. The authors also acknowledge Amandine Velt for her collaborative insights and help.

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Virome analysis of Russian grapevine germplasm

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INTRODUCTION

Russian ampelographic collections include widely cultivated varieties, as well as unique local varieties, grown in Krasnodar region, Rostov region, Republic of Dagestan, and other regions. These grapevines are important for the selection and conservation of grapevine genetic resources. Unfortunately, grapevine germplasm collections are a reservoir for various pathogens, including viruses that can impact the yield and qualities of new varieties. For this reason, monitoring pathogens in ampelographic collections is crucial.

MATERIALS AND METHODS

Virome analyses was conducted in five ampelographic collections located in southern Russia. During these studies, 222 grapevine samples were collected. Total RNA was extracted from each sample to prepare libraries. Sequencing was performed on a NovaSeq 6000 System (150 bp paired-end reads). Bioinformatics analysis was performed using Geneious Prime software. Contigs were assembled using SPAdes and Geneious assemblers. Annotation of contigs was performed using tblastx against the NCBI Viral RefSeq database. In parallel, the reads were mapped to reference genomes of grapevine viruses. RT-PCR and RT-qPCR were performed to confirm the presence of bioinformatically detected viruses and viroids. Samples in which the virus was identified using both bioinformatics and PCR were considered virus-positive. FASTQ raw sequencing data and the assembled nucleotide sequences of viruses and viroids were deposited in GenBank: PRJNA846715, PRJNA899472, PRJNA1043183, and PRJNA1191642.

RESULTS AND DISCUSSION

As a result of large-scale work, the virome of five main ampelographic collections in Russia was studied (Shvets et al. 2022a, Shvets et al. 2022b, Belkina et al. 2023). A total of 33 species of viruses and 4 species of viroids were identified. A mixed infection was detected in most plants (1-16 viruses and viroids in one grapevine). Between 124 and 512 viruses and viroids were detected in each of the five ampelographic collections. Six viruses and two viroids were found in all collections: grapevine rupestris stem pitting-associated virus (GRSPaV), grapevine virus T (GVT), grapevine pinot gris virus (GPGV), grapevine rupestris vein feathering virus (GRVFV), grapevine fleck virus (GFkV), grapevine red globe virus (GRGV), grapevine yellow speckle viroid 1 (GYSVd-1), and hop stunt viroid (HSVd). The highest percentage of grapevines were infected with GRSPaV (95%), HSVd (86%), GPGV (79%), GYSVd-1 (73%), GFkV (45%), and GRVFV (43%). As a result of bioinformatic analysis, we assembled 776 complete or nearly complete genomes for 29 viruses and 4 viroids.

Grapevine fanleaf virus (GFLV) was identified more frequently than other economically significant viruses (42% of samples). Grapevine leafroll disease was another economically significant disease in the collections. This is a serious threat to vineyards worldwide. Grapevine leafroll-associated virus 1 was detected in 16% of samples, and grapevine leafroll-associated virus 3 was detected in 13% of samples.

In this series of studies, we discover nine novel viruses: grapevine umbra-like virus 1-5, grapevine pararetrovirus, grapevine alphapartitivirus, grapevine secovirus, and grapevine Magarach secovirus. Further research will focus on the biology and damage potential of these viruses.

ACKNOWLEDGEMENTS

These studies were supported by the Russian Science Foundation (grant no. 25-14-20022) and the Ministry of Education and Science of Russian Federation and were performed using the Core Research Facility of Center "Bioengineering" and exper-imental climate control facility U-73547.

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Occurrence and genetic diversity of major grapevine viruses in Oklahoma, USA

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INTRODUCTION

The wine industry in Oklahoma generates close to $1.29 billion in total economic activity for the state, and the history of grape production in Oklahoma dates to the 1800s. But very little is known about the occurrence of major grapevine-infecting viruses in the state except for a MS Thesis that documented the occurrence of grapevine leafroll-associated virus 3 (GLRaV-3) and grapevine red blotch virus (GRBV) in Oklahoma vineyards in 2016 (Wallace, 2016). This study was conducted to address this knowledge gap and to interrogate the genetic diversity of field isolates of GLRaV-3 and GRBV in Oklahoma.

MATERIALS AND METHODS

A total of 85 grapevine samples (leaf petiole or dormant canes) were collected from eight vineyards across six counties in Oklahoma during the spring and fall of 2024. Total RNA extracts from each sample were assayed for 14 major viruses by RT-PCR, including grapevine leafroll-associated virus 1 (GLRaV-1), GLRaV-2, GLRaV-3, GLRaV-4, grapevine fanleaf virus (GFLV), grapevine red blotch virus (GRBV), tobacco ringspot virus, tomato ringspot virus, grapevine fleck virus, grapevine rupestris stem pitting-associated virus (GRSPaV), grapevine virus A (GVA), GVB, GVE, and GVH. Due to their documented negative impacts (Alabi et al. 2016; Cieniewicz et al. 2017 and cited references) and reported genetic variability (Cieniewicz et al. 2017; Diaz-Lara et al. 2018), samples positive for GLRaV-3 and GRBV were further analyzed for genetic diversity assessment of both viruses based on the complete cistrons of the GLRaV-3 coat protein (CP) and heat shock protein 70 homolog (HSP70h) genes and the complete genome of GRBV. The pipeline for virus diagnosis and genetic diversity assessments was essentially as described previously (Ouro-Djobo et al. 2022; 2025; Alabi et al. 2011).

RESULTS AND DISCUSSION

Virus infection was detected in 68.2% of samples, with mixed infections (36.5%) being more common than single infections. Seven of the targeted viruses were detected at varying levels of incidences, including GRBV (48.2%), GRSPaV (40%) GLRaV-3 (20%), GFLV (12.9%), GVE (11.8%), GVA (8.8%), and GVH (3.6%). GRBV variants belonging to both clades I and II were identified in Oklahoma, with the clade II members being more predominant, as previously reported (Ouro-Djobo et al. 2022 and cited references). Multigenic genetic diversity assessments of GLRaV-3 isolates from Oklahoma showed the occurrence of variants belonging to clades I, II and V, with the clade V isolates being more frequently detected in the state relative to results from other U.S. locales. The findings of the study underscore the importance of continued virus surveillance, use of clean plant materials, and targeted management strategies to protect Oklahoma's developing vineyards from the long-term impact of viral diseases.

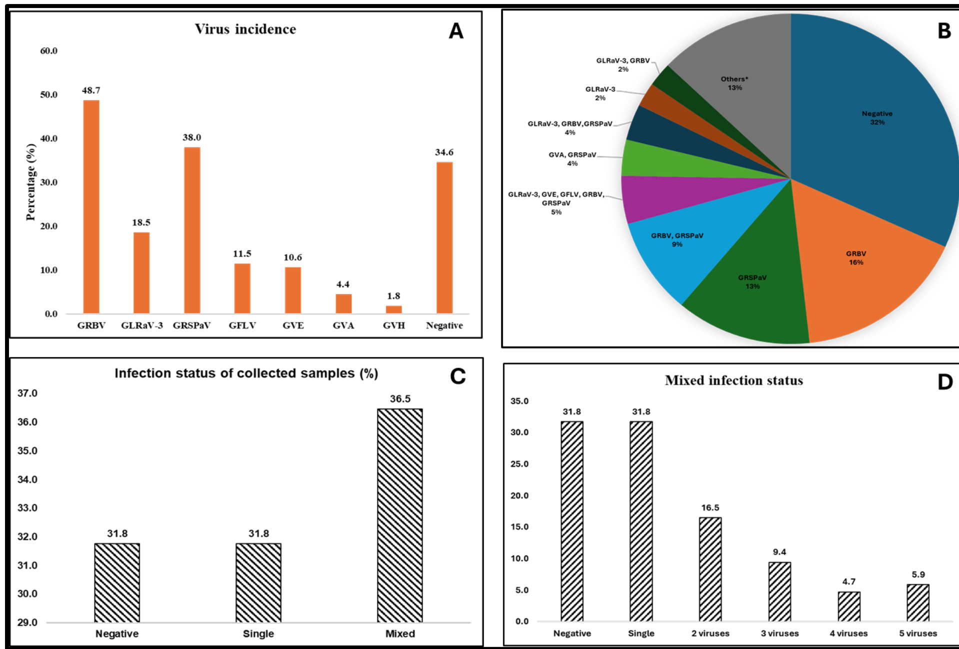

Figure 1. Incidence (A) and infection patterns (B-D) of major grapevine viruses detected in Oklahoma vineyards.

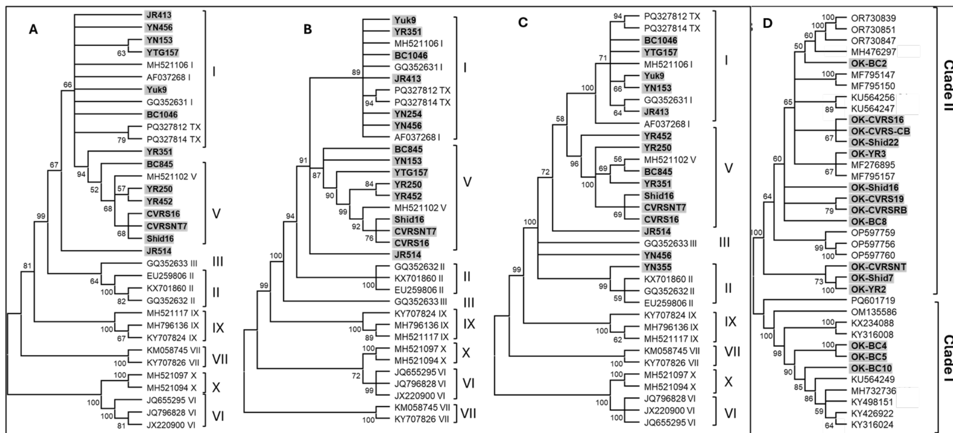

Figure 2. Relationships of field isolates of GLRaV-3 (A-C) and GRBV (D) in Oklahoma (bold, highlighted) with corresponding sequences of virus isolates from other locales. (A) GLRaV-3 CP, (B) GLRaV-3 HSP70h, (C) GLRaV-3 CP-HSP70h concatemers, (D) GRBV complete genome.

ACKNOWLEDGEMENTS

We thank the Oklahoma Department of Agriculture Food and Forestry (ODAFF) through the Oklahoma Viticulture and Enology Fund for funding support and members of the Alabi and Jibrin labs for assistance with sample processing.

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Uncovering the distribution and diversity of grapevine pararetrovirus (GPRV)

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INTRODUCTION

Most historically, known grapevine-infecting viruses have RNA genomes. However, we count ten recently identified DNA viruses, mainly through the use of high throughput sequencing (HTS), belonging to four families: *Caulimoviridae*, *Geminiviridae*, *Amesuviridae* and *Nanoviridae* (Fuchs, 2025). To date, the only one reported in France is grapevine pararetrovirus (GPRV) (Candresse et al., 2025), a double-stranded DNA virus, member of the *Caulimoviridae* family. Its discovery goes back to 2022 in samples from an ampelographic collection in the Republic of Daghestan, Russia (Shvets et al., 2022). Apart from France, it has only been reported in Greece (Mehrvar et al., 2024). As DNA viruses are often associated with severe symptomatology, GPRV detection in distinct geographic areas raises concern and highlights the need for a deeper understanding of its biology, distribution, and dissemination to support risk assessment, surveillance efforts, and to evaluate its potential threat to viticulture. The growing accessibility of HTS in plant research has generated abundant public grapevine genomic data, providing a valuable resource to explore the diversity and spatiotemporal distribution of both known and unknown viruses (Hily et al., 2020), by supplementing datasets, often biased toward outbreak-driven studies rather than continuous monitoring (Richard & Poulicard, 2025). Combining molecular biology and HTS diagnostic with a datamining approach, we aim (1) to assess GPRV presence and its diversity in French vineyards, and (2) to investigate its global distribution and evolutionary history.

MATERIAL AND METHODS

In 2023, HTS analysis was performed on symptomatic grapevine leaf samples (*Vitis vinifera* cv. Gewurztraminer) collected from a commercial vineyard in Alsace, France. The analysis followed the protocol as described in Hily et al., 2024. After total RNA extraction, ribodepletion enrichment, and library preparation, resulting cDNA were prepared then sequenced (2*150 bp) on an Illumina Novaseq 6000. Raw reads were processed using an in-house pipeline, and sequence reconstitution was performed as described in Hily et al., 2018. Primers were designed in the most consensual region of the genome, based on known global diversity. They were used in PCR (or RT-PCR) assays to confirm HTS results and to test for virus presence in 50 symptomatic grapevines collected from French commercial vineyards. For datamining, whole genome sequencing (WGS) datasets were retrieved from the European Nucleotide Archive (ENA) and the Genome Sequence Archive (GSA). Priority was given to datasets from Dong et al., 2023, displaying globally sourced *Vitis* spp. samples with curated annotations. Selected samples were processed using a Nextflow in-house pipeline.

RESULTS AND DISCUSSION

Sequencing yielded 67.1 million paired-end reads, revealing a complex virome from our leaf samples. Two GPRV contigs (8,626 and 8,617 nts) were assembled, with an average coverage of 61 and 19, respectively. Sequences shared 97.25% nucleotide identity between each other and 87.9-88.1% with the Russian sequence (GenBank: OP886324). Among the tested samples (n=50) from symptomatic grapevines, eight tested positive by PCR assay: Alsace (1), Burgundy (4) and Champagne (3), suggesting a widespread of GPRV in French vineyards.

So far, all grapevine-infecting viruses, even those with DNA genomes, have been identified through RNA or small RNA (sRNA) sequencing. In this study, we decided on mining DNA-based datasets, i.e. WGS data. As of October 2025, 2,545 samples have been processed from which five grapevine-infecting DNA viruses were clearly identified: grapevine red blotch virus (GRBV), grapevine geminivirus A (GGVA), grapevine Roditis leaf discoloration-associated virus (GRLDaV), grapevine badnavirus-1 (GBV-1) and grapevine pararetrovirus (GPRV). In addition, our work uncovered five full-length GPRV sequences from five different samples originating from: France (1), Bulgaria (1), Georgia (2) and Azerbaijan (1). This result implies an extensive distribution of the virus to many countries of the Caucasian region where it was first discovered (Shvets et al., 2022), as well as in eastern and western Europe, as described above and in previously cited studies. By capitalizing on available and newly generated sequences, we will investigate the genetic diversity and evolution pattern of GPRV to define its evolutive history. Some works on its biology and symptomatology are underway. Altogether, this work shows that mining WGS datasets is a valuable tool for the detection of DNA plants viruses.

ACKNOWLEDGEMENTS

This work was conducted as part of the LutEnVi project funded by *'Plan National Dépérissement du Vignoble'* (PNDV) program. Thesis funded by PNDV and ANSES *'OptiCQua'* project.

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Are environmental conditions and haplotype diversity key drivers of tomato ringspot virus severity in Quebec grapevines?

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INTRODUCTION

Tomato ringspot virus (ToRSV, species *Nepovirus lycopersici*) has only recently been detected in Quebec vineyards, and many producers are still unfamiliar with the symptoms it induces. ToRSV infection can cause a decrease in fruit production and an inhibition of the maturity of grapes. However, diagnosis can be problematic due to the known genetic diversity of ToRSV strains (Soltani *et al.,* 2021). No curative solution is known to date; preventive measures are the only way to limit spread. Despite its increasing relevance, little is known about the genetic diversity of ToRSV and the factors influencing disease severity in grapevines. However, early observations suggest notable variation in disease severity across regions of Quebec, grapevine varieties, and even within the same variety in a single vineyard. These patterns suggest that distinct ToRSV populations may occur and/or that environmental conditions may contribute to modulate disease severity.

MATERIALS AND METHODS

Plant samples: Samples were collected in fifteen vineyards located in Quebec, Canada, from 2024 to 2025. Mature leaves were collected from various varieties of infected grapevines (six leaves from the bottom, middle, and top canopy). Leaves from asymptomatic grapevines found in proximity to the first sample were also collected using the same protocol (collecting in total at least ten samples per vineyard block). The disease severity was evaluated using a scale of four (0 meaning no symptoms and 4 meaning more than 75% of bunches were affected, Fig. 1A). Four bunches were randomly collected per sampled vine during the harvest period and used for weight and Brix measurements. *Viral nucleic acid extraction and sequencing*: Total RNA extraction was performed with the Spectrum total RNA extraction kit (Sigma Aldrich) modified by Xiao *et al.* (2015), and then a poly-A tailing library was produced using NEB ultra express RNA library prep kit. Sequencing was done by Genome Quebec with Illumina NovaSeq PE100 technology with 3200M reads. *Bioinformatics:* Positive samples were identified using Virtool and SOVAP pipelines. Haplotypes were determined using the variant calling analysis (bcftools and freebayes) and compared against V-pipe pipeline results (BWA aligner). Statistical analyses were used to correlate environmental conditions, haplotypes, and disease severity using mixed linear models and PCA in R Studio (packages: lme4 and vegan, Fig. 2A). Phylogenetic analyses were performed using a consensus multiple sequence alignment, and a maximum likelihood tree (analysis TVM+F+I+R6 substitution model) was inferred with 1,000 bootstrap replicates (IQ-Tree). To root the tree, an outgroup from the family of *Secoviridae* (e.g., *Arabis mosaic virus*) was included as a reference. Visualization was done using the iTOL tool (Fig. 2B).

Figure 1. Scale of disease severity scoring in Frontenac gris used in the study.

RESULTS AND DISCUSSION

Out of 123 samples collected in 2024, 51 symptomatic samples were identified as positive for ToRSV, 6 asymptomatic samples were confirmed negative for ToRSV, 65 asymptomatic samples were positive for ToRSV, and only one symptomatic sample was shown to be negative for ToRSV. This shows that ToRSV infection is present at very high rates in Quebec vineyards and that it is underdiagnosed and overlooked. Mixed linear model and PCA analysis suggested a possible effect of precipitation and growing degree days (GDD) on the severity of the symptoms (Fig. 2A). However, the result indicates that a big proportion of the variance in severity has yet to be explained, suggesting that genetic diversity could also play a role in disease severity. More data are needed for a more conclusive model. Haplotypes identified across vineyards shared several common SNPs (single nucleotide polymorphism), while some SNPs appeared to be plant-specific. SNPs were considered reliable only when supported by a minimum coverage of 10 reads. So far, 37 SNPs have been detected across all samples and are located mostly in untranslated regions (UTR), and the coding regions for the coat protein (CP) and RNA-dependent-RNA polymerase (RdRp). All mutations observed in the CP and RdRP coding regions were synonymous, but could potentially impact the accumulation, translation, or stability of the viral RNAs. UTRs have been shown in other viruses to influence symptom severity, making them particularly interesting targets for further investigation in the ToRSV pathosystem (Fuchs *et al.,* 2017). Interestingly, although most of the sampled vineyards obtained their planting materials from the same supplier in Quebec, a high level of genetic diversity was still observed (Fig. 2B). Phylogeny analysis revealed three main clusters from which the RNA1 sequences appear to have evolved. Those clusters seem to be vineyard specific. More research is needed to understand the factors driving the observed genetic diversity and the emergence of specific haplotypes. The next step would be to correlate haplotype distribution with disease severity to identify the high-risk haplotypes associated with severe symptom expression. This knowledge will support the development of a diagnostic tool targeting high-risk haplotypes, enabling rapid and accurate detection directly in the field.

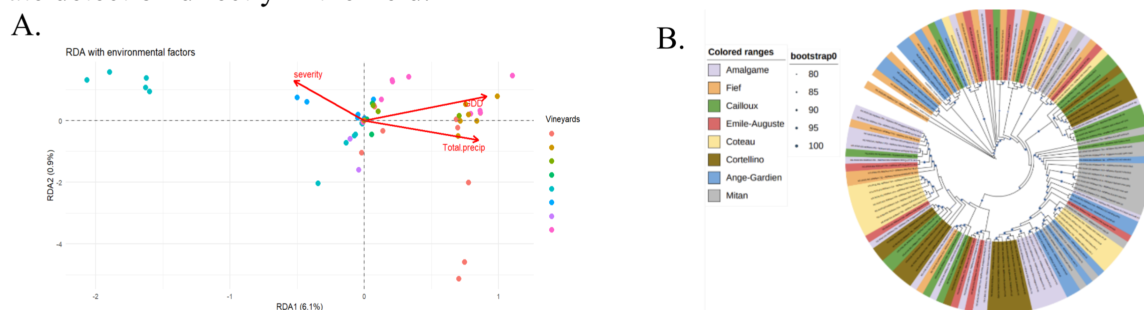

Figure 2 A. RDA analysis of single nucleotide polymorphisms (SNPs) identified in each sample (colour-coded by vineyard) constrained to environmental data and disease severity (arrow). **B.** Phylogenetic analysis of RNA1 with all the samples. Samples are colour-coded by vineyard.

ACKNOWLEDGEMENTS

This work was financially supported by Agriculture and Agri-Food Canada. FM was supported by a student fellowship from the Fonds de recherche Quebec and by a travel award from les Offices jeunesse internationaux du Québec. We are grateful to the producers, agronomists, and interns for their participation in this research project.

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Party of many: The rich social life of GFLV in the vineyard

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INTRODUCTION

Grapevine fanleaf virus (GFLV) is a major agent of vineyard degeneration, widely recognized for its substantial impact on viticulture and wine production across Europe. The current, remarkable geographic spread and genetic diversity of GFLV is likely a result of the massive replanting that followed the phylloxera crisis, when untested, grafted vines, often carrying latent viral infections, were propagated extensively throughout viticultural regions (Ollat et al 2024; Reynolds AG 2017). This episode established a reservoir of diverse GFLV variants and facilitated the complex population structures seen today. GFLV typically possesses a bipartite genome (RNA1 and RNA2), and can sometimes also harbor a third satellite RNA (RNA3), each RNA segment being individually encapsidated. As a result, the genomic segments can segregate and reassort independently, further enriching the molecular landscape of GFLV. Within individual grapevines, GFLV infections are frequently composed of multiple viral variants, with co-occurring haplotypes for both RNA1 and RNA2, and, when present, RNA3 (Kubina et al. 2022).

The virus induces a striking diversity of symptoms on grapevines, ranging from leaf distortion and pronounced yellowing to general vine decline, with variability in symptom severity often observed even among neighboring plants within the same vineyard block. This phenotypic mosaic is influenced by viral genotype composition, host cultivar, and local environmental conditions. Critically, fanleaf degeneration leads to marked reductions in yield, representing a major economic burden. In France, annual losses due to fanleaf disease were estimated at over 1 billion euros (Schmitt-Keichinger & Fuchs 2025).

Here, we analyzed GFLV molecular diversity within a single vineyard marked by severe yellow symptoms, assessing multiple neighboring symptomatic vines that displayed pronounced symptom variability. This study provides insights into the links between molecular heterogeneity and field symptom expression, offering new perspectives for understanding GFLV epidemiology and disease management in modern vineyards.

MATERIALS AND METHODS

Grapevine leaf samples were collected in spring from a single Pinot noir vineyard planted in 1997, focusing on a large infected patch and its periphery that showed intense yellowing and strong plant-to-plant variation in symptom severity, including differences in leaf colour, leaf deformation, and vine vigour. Within this hotspot, 11 vines were first selected to represent a symptom gradient, then 72 additional vines were selected along six transects crossing the patch, sampling one vine per row over the first 12 rows of each transect, yielding a total of 83 vines. For each of the seven libraries, a GFLV-free vine was included as a negative control and, to monitor process specificity, an alien virus control was also added: potato virus Y (PVY) in the first run and different isolates of potato virus S (PVS) in subsequent runs. All samples were subjected to immunocapture with commercial GFLV-specific antibodies or the corresponding virus-specific antibodies for the alien samples (Bioreba), reverse transcription using random primers following the virion-associated nucleic acid (VANA) protocol, and subsequent pooling into multiplexed libraries for Illumina sequencing. Bioinformatic processing included read demultiplexing, quality trimming (Trimmomatic), *de novo* assembly (rnaSPAdes), initial haplotype assignment by BLAST against a curated GFLV reference set, and iterative refinement by remapping and BLAST analysis of unmatched contigs.

RESULTS AND DISCUSSION

High-throughput sequencing generated usable data for 95 of the 96 samples, including seven GFLV-free vines and seven alien-virus controls, with per-sample yields ranging from approximately 10^5 to 10^6 paired-end 150-nt reads. Across the 81 vineyard vines, 69 were GFLV-positive and 15 remained negative, indicating that even within a degeneration patch a substantial fraction of vines did not show detectable GFLV infection. Preliminary *de novo* assemblies and mapping-based analyses revealed at least 20 distinct RNA1 haplotypes and 12 RNA2 haplotypes, as well as a single satellite (RNA3) lineage, with pairwise nucleotide divergence above 5% between haplotypes of a given segment. In line with previous HTS-based studies, most vines carried complex mixed infections, with multiple RNA1 and RNA2 haplotypes co-occurring within the same plant[3], whereas the RNA3 satellite was detected in only 11 of the 69 infected vines and displayed limited genetic diversity (within 2% divergence).

The presence of both GFLV-free and GFLV-infected vines along transects that deliberately extended beyond the symptomatic yellow hotspot indicates that the perceived 'patch' corresponds to a gradient rather than a sharp boundary, with a substantial number of asymptomatic or mildly symptomatic vines already harboring GFLV. The extensive within-plant haplotype diversity revealed here, with numerous RNA1 and RNA2 variants and a single, more conserved RNA3 lineage, strongly supports a scenario of recurrent introductions of divergent GFLV strains over time, consistent with the legacy of large-scale post-phylloxera replanting with infected planting material.

Frequent recombination in this complex viral assemblage, together with the short-read nature of the VANA data, hampers robust phylogenetic reconstruction and makes it difficult to confidently distinguish genuine recombinants from assembly artefacts.

These constraints highlight the need to complement the present short-read VANA approach with long-amplicon sequencing to phase full-length RNA1, RNA2 and RNA3 molecules, resolve recombinant structures with higher confidence, and better link specific genomic constellations to the observed spectrum of field symptoms in this "party of many" GFLV population.

ACKNOWLEDGEMENTS

The authors thank the vineyard owner for allowing access to the vineyard. We are also grateful to Nathalie Dubuis, Abir Ben Salah, Marc Passerat, and Larisa Grosu for their careful work in sampling, testing, and maintaining plant and soil samples.

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Utilising the National Vine Collection: High-Throughput Strategies for Virus Elimination to Achieve High-Health Grapevine Cultivars

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INTRODUCTION

In New Zealand, the New Zealand Winegrowers National Vine Collection (NVC) is maintained as a vineyard planting. It serves as a vital genetic resource for both the wine industry and grapevine nurseries. The collection includes over 400 grapevine cultivars, many of which are maintained in a lower health section due to viral infections. These cultivars are affected by a range of viral pathogens, including grapevine leafroll-associated virus (GLRaV) and grapevine fanleaf virus (GFLV). Infected vines exhibit a variety of detrimental symptoms, such as stunted growth, reduced fruit yield, and compromised resistance to environmental stressors, which pose significant challenges to both research and commercial grapevine production (Maliogka et al., 2015; Chooi et al., 2022; National Academies of Sciences, Engineering, and Medicine, 2025).

Restoring the health status of the NVC is critically important, as importing cultivars with comparable genetic diversity from overseas is both logistically difficult and nearly impossible due to strict quarantine laws and other import restrictions. Therefore, efforts should concentrate on revitalising the health of the existing collection.

In this context, in vitro culture systems present significant potential for manipulating virus-infected plants to regenerate healthy ones. This study examines the application of high-throughput strategies for virus elimination within the NVC. By integrating advanced molecular diagnostics with improved in vitro techniques, along with high-temperature treatments or ultra-low-temperature (-180°C) liquid nitrogen, we aim to produce high-health grapevine cultivars that are free from viral pathogens and suitable for rehabilitation into the NVC. These efforts are expected to enhance the productivity and longevity of the NVC, thereby contributing to the sustainability of viticulture practices.

MATERIAL AND METHODS

Cultivars were selected from the New Zealand Winegrowers National Vine Collection (NVC), and their virus status was confirmed using ELISA and high-throughput sequencing (HTS). In vitro cultures were initiated from the selected grapevine cultivars confirmed to be infected with GLRaV-3 by ELISA, along with multiple other viral infections identified by HTS. Treatment strategies were designed based on the potential of high-throughput approaches to eliminate viruses. After regeneration, plantlets were cultured under standard conditions and subsequently evaluated for virus elimination using Foundation Plant Services Terminal (FPST) RT-qPCR.

RESULTS AND DISCUSSION

Molecular diagnostics confirmed the presence of GLRaV-3 in most cultivars from the lower health section of the NVC. HTS RNA analysis revealed a diverse virome, including GLRaV-3, Grapevine red globe virus (GRGV), Grapevine rupestris vein feathering virus (GRVFV), and viroids, with vitiviruses like Grapevine virus G (GVG) also prevalent across the cultivars.

The treatment pipeline, utilising high- and ultra-low-temperature liquid nitrogen, successfully eliminated GLRaV-3 from 100% of treated plantlets, as confirmed by FPST RT-qPCR. The FPST

RT-qPCR, targeting the 3′ untranslated region of GLRaV-3, demonstrated high sensitivity for detecting divergent viral strains (Diaz-Lara et al., 2018).
These results demonstrate the potential of adopting the developed virus eradication pipeline to rehabilitate virus-infected cultivars at high throughput within the NVC. By integrating this approach with existing molecular diagnostics, we can restore high-health grapevine cultivars, thereby supporting the long-term sustainability and resilience of the NVC.

ACKNOWLEDGEMENT
This work is part of a project funded by the New Zealand Winegrowers levy. We want to thank all the advisers and internal reviewers for their valuable feedback and the time they dedicated to strengthening this project.

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Virus epidemiology in New Zealand vineyard ecosystems

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INTRODUCTION

Mass movement of plant species from their locations of origin and into environments with genetically distinct ecosystems has occurred through human migration and globalization. In Aotearoa New Zealand, the introduction of exotic plants for agriculture has established agro-ecological interfaces where exotic cultivated plants and indigenous plants often grow side by side. These agro-ecological interfaces also facilitate first meetings between plants and microorganisms, including new interactions between plants and viruses. The potential for viral outbreaks at agro-ecological interfaces is high, as naïve plant species may not have evolved strategies to defend against infection from foreign viruses. If the winegrape industry was to promote the use of groundcover, amenity plants or native plants it is essential to assess the viral risks associated with the plant species. The susceptibility of native plants to viral pathogens of grapevines is undescribed in New Zealand.

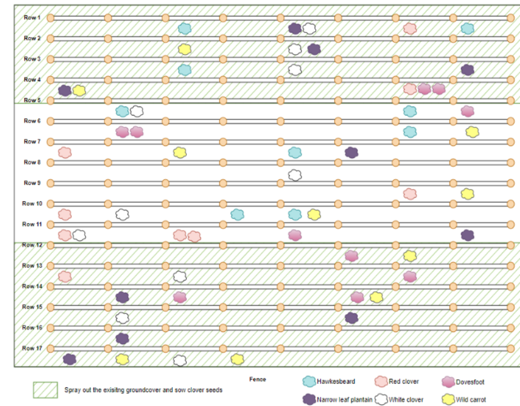
Earlier research showed Grasslands Huia white clover (GHWC) can support large and persistent populations of citrophilus mealybugs, an efficient vector of grapevine leafroll-associated virus (GLRaV-3, Petersen and Charles, 1997). Research under laboratory conditions showed citrophilus mealybug successfully transmit GLRaV-3 to *Nicotiana benthamiana*, but not to GHWC. Feeding of GLRaV-3-viruliferous mealybugs for 20 days on GHWC is sufficient to break their GLRaV-3-transmission to grapevines (Gough et al., 2025). We therefore assessed the virus population of native and groundcover plant species growing in and around vineyards to understand the vineyard virome, and potentially virus-reservoir of beneficial plants.

MATERIALS AND METHODS

In Marlborough, grapevines and groundcover within a vineyard and adjacent native plants were assessed for the presence of virus infections using high-throughput sequencing (HTS) of RNA from plant samples. The presence of identified viruses was confirmed by targeted reverse transcription polymerase chain reaction (RT-PCR) from plant RNA. Note, the plant samples were not washed prior to RNA isolation. Potted sentinel plants of 12 species, selected based on virus and insect vector host records and plant availability, were grown for 25-38 days within and around the vineyard. Upon retrieval these sentinel plants were screened by RT-PCR for five focus viruses, (alfalfa mosaic virus (AMV), red clover vein mosaic virus (RCVMV), soybean dwarf virus (SbDV), white clover mosaic virus (WClMV) and Waikavirus-like virus A (WKV-A)) that were previously detected on site.

In 2022, a commercial vineyard in West Auckland was assessed for GLRaV-3 in grapevines and groundcover plants. The vineyard is one of three in New Zealand that was planted in 2014 with four grapevine cultivars deliberately infected with GLRaV-3 interspersed with 119 GLRaV-3-uninfected buffer and control grapevines. The vineyard had a robust mealybug control programme for its first 3 years, then was left with no insecticide application for a further 6 years, and therefore was ideal to test the potential for viruliferous mealybugs to transmit GLRaV-3 to non-*Vitis* host plants. Prior to groundcover manipulations, the spread of GLRaV-3 into uninfected vines was assessed by ELISA to verify its active transmission by mealybugs. Non-*Vitis* sampling (10 plants from each species) was undertaken including hawksbeard, red and white clover, dove's foot, narrow leaf plantain, and wild carrot and screened for GLRaV-3 by ELISA and RT-PCR. A 'lawnmower' approach followed by

pooled HTS was taken to detect viruses present in groundcover plants. Thereafter, the vineyard groundcover plants were sprayed with herbicide over the outer four rows on each side of the block (Figure, green hatched upper and lower). The groundcover within the six internal rows remained untouched. The outer rows were subsequently cultivated and sown with GHWC seed. After at least 4 months of potential GLRaV-3 transmission to the GHWC plants were sampled and tested by ELISA for GLRaV-3, whereas mealybugs and scale insects collected from GHWC were tested for GLRaV-3 using RT-qPCR.



RESULTS AND DISCUSSION

The HTS from plants in the Marlborough vineyard identified several new virus-plant interactions as well as novel virus species. Grapevines tested positive for AMV but not the other viruses assessed. The RT-PCR of sentinel plants also detected the movement of many viruses. Since none of the plant samples assessed in this study were washed prior to virus identification, these results should be repeated prior to official reporting. Nevertheless, many viruses were detected in or on grapevine, groundcover and sentinel plants demonstrating high pressure from virus reservoirs in this ecosystem.

The West Auckland vineyard had GLRaV-3 spread within grapevines, evidenced by a high transmission rate (32% infection, i.e. 38 positive vines / 118 previously GLRaV-3-free vines). The HTS pools from 'lawnmower' sampling found AMV, RCVNV, WClMV, among other common viruses but not GLRaV-3. No GLRaV-3 was detected in the selected groundcover species tested or in sown GHWC plants. Mealybugs or scale insects (16 individuals) found on GHWC were individually tested for the presence of GLRaV-3 and all were either negative or undetermined (high Cq value, >34).

The West Auckland trial had >63% grapevines infected with GLRaV-3 with evidence of prior GLRaV-3 transmission. Within this high pressure of GLRaV-3, no groundcover plants were detected as infected with GLRaV-3. The purposefully sown GHWC plants were not infected with GLRaV-3, nor were the insect vectors feeding on GHWC plants. This result demonstrates in-vineyard conditions and under high GLRaV-3 pressure that GHWC is not a reservoir for GLRaV-3. Whether GHWC in-vineyard is preventing further transmission by mealybugs of GLRaV-3 to grapevines is yet unproven.

This research demonstrates that vineyard ecosystems in New Zealand maintain a rich virome reservoir. Washing of plant samples is imperative to distinguish virus infection within rather than on plants (Fox et al., 2025). A wide range of groundcover plant species within vineyards do not add a reservoir for GLRaV-3; GHWC plants may trap mealybugs and liberate them from GLRaV-3 and thereby its transmission to new grapevines. Our findings add to our knowledge and methodologies for assessing the vineyard ecosystem, including potential GLRaV-3 management.

ACKNOWLEDGEMENTS

The authors would like to thank the vineyard owners for their contributions to this research.

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RNA–mediated circularization reveals replication complexity in grapevine endophyte endornavirus species

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INTRODUCTION

Over one hundred viral species and viroids have been reported in grapevines, along with several fungal and oomycete pathogens affecting vine health and productivity. Among them, endornaviruses are persistent +ssRNA viruses that replicate as dsRNA in plants, fungi, and oomycetes. They lack a capsid, are transmitted vertically, and are usually asymptomatic, though they can modulate host physiology and interact with other microorganisms. The grapevine endophyte endornavirus (GEEV), first described in fungal-infected grapevines from South Africa, encodes a large polyprotein containing helicase, glycosyltransferase, and RdRP domains. As endornaviruses are not known to associate with satellite RNAs or subviral elements, any such interaction is of particular interest. During a dsRNA-based virome survey of Canadian vineyards, we identified a novel GEEV-related RNA virus consistently associated with an uncharacterized RNA sequence (UCS). Preliminary analyses indicated a possible functional link between GEEV and UCS through RNA circularization. This study aims to (1) characterize the full-length genome of this novel GEEV variant, (2) define its phylogenetic position within *Endornaviridae*, and (3) clarify the molecular role of the UCS in viral circularization and replication.

MATERIALS AND METHODS

Double-stranded RNA (dsRNA) was extracted from grapevine leaves collected across Canadian vineyards using a cellulose-based protocol, purified, and used for Illumina MiSeq sequencing or targeted PCR analyses. Strand-specific cDNA synthesis, poly(A) tailing, and end-specific amplification enabled full-length viral genome assembly and precise mapping of 3′ and 5′ termini. Sequence reads were trimmed, assembled de novo, and annotated using conserved domain searches using an in-house bioinformatic workflow. To investigate potential self-circularization or insertion events, a two-round PCR was designed to amplify GEEV–UCS junctions, followed by gel validation, sequencing, and RNAfold-based prediction of UCS secondary structures and palindromic motifs. Phylogenetic analyses of 84 complete GEEV genomes were conducted using maximum-likelihood methods and sequence identity matrices to define species-level groups at the 75% nucleotide identity threshold.

RESULTS AND DISCUSSION

Eighty-four complete GEEV genomes, including accurately mapped termini, were assembled and analyzed. Phylogenetic reconstruction revealed five distinct species-level clusters, GEV1_G1, GEV1_G2, GEV2_G1, GEV2_G2, and GEEV, exceeding the current three-species classification and indicating greater endornavirus diversity in grapevine (Fig.1). PCR and sequencing consistently detected an uncharacterized RNA sequence (UCS) associated with GEEV. The UCS exhibited stable secondary structures with palindromic motifs predicted to pair with GEEV termini, supporting a role

in RNA circularization. Sequencing validated GEEV–UCS junctions, confirming chimeric circular RNA forms that may function as replication or translation intermediates. A UCS-mediated circularization model is proposed, in which palindromic motifs promote end-to-end ligation of the viral genome, forming circ-GEEV molecules that could enhance translation efficiency or RNA stability. Ongoing studies aim to validate UCS function experimentally and assess its prevalence and biological significance in ours vineyards.

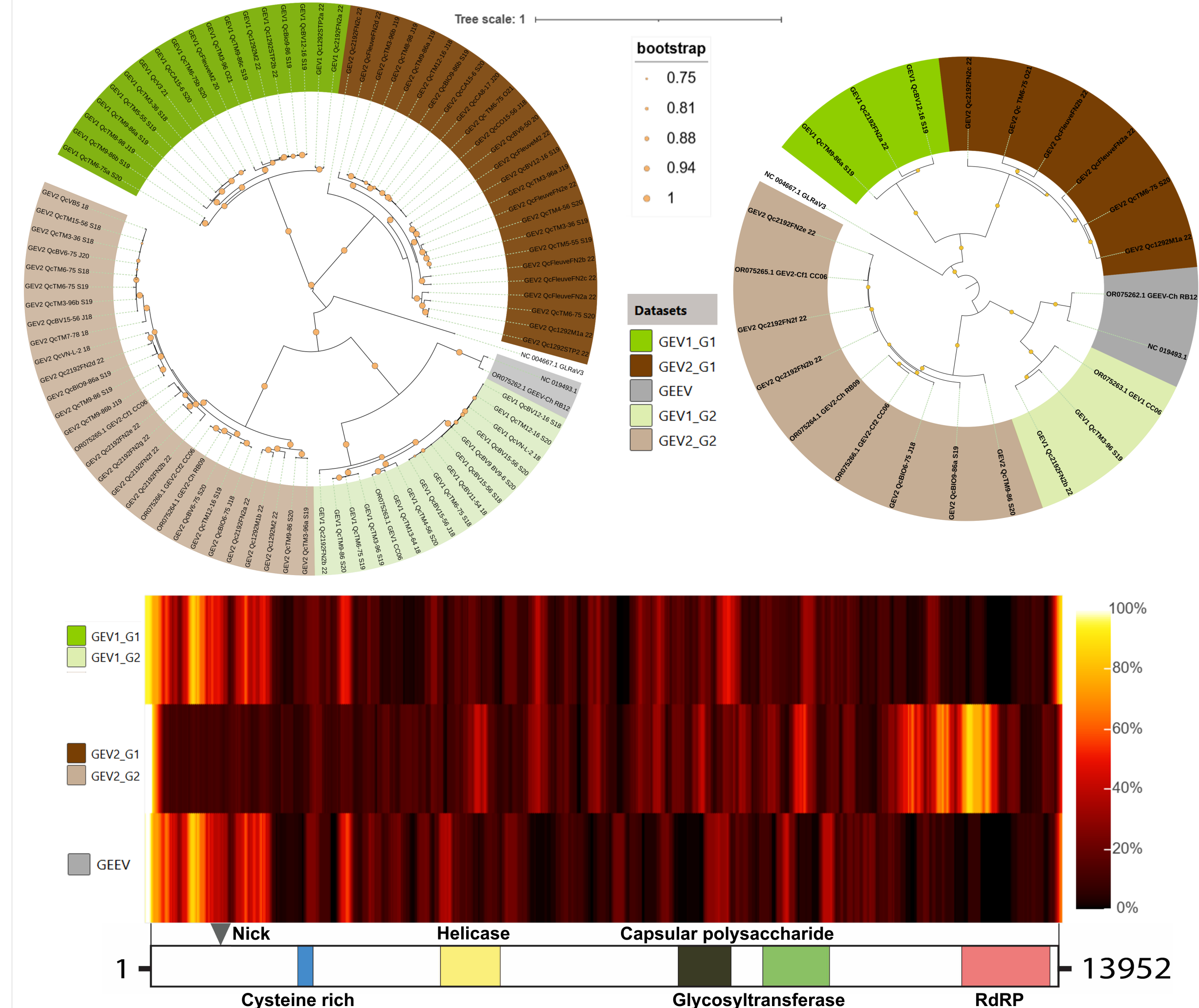

Figure 2. Refined Phylogenetic resolution reveals five distinct grapevine endornavirus species. This figure illustrates the phylogenetic relationships among grapevine endophyte endornavirus species using full-genome data. **A** and **B** display circular phylogenetic trees based on all complete genomes and >95% nucleotide identity clustering, respectively. The analysis suggests the existence of five distinct species-level groups: GEV1_G1, GEV1_G2, GEV2_G1, GEV2_G2, and GEEV, based on the 75% species demarcation threshold. GLRaV-3 (NC_004667.1) serves as the outgroup. **C** presents a nucleotide similarity heatmap across the full genomes of GEV1, GEV2, and GEEV, along with the annotated genomic architecture highlighting conserved functional domains including RdRP, helicase, glycosyltransferase, and others.

ACKNOWLEDGEMENTS

We acknowledge Agriculture and Agri-Food Canada for providing funds and our Bioinformatics and Frelighsburg experimental farm teams for their help and consistent assistance.

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Monitoring the spatiotemporal spread and evolution of grapevine leafroll-associated ampeloviruses in a commercial vineyard of Northen Greece

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INTRODUCTION

Grapevine leafroll disease (GLD) is one of the most economically important diseases of grapevine worldwide. Five viruses of the *Closteroviridae* family (grapevine leafroll-associated virus 1, -2, -3, -4, -7 – GLRaV-1, -2, -3, -4, -7) have been associated with GLD (Meng et al., 2017). GLRaV-1, GLRaV-3 and GLRaV-4 are members of the genus *Ampelovirus,* and they are transmitted in short distances by various species of the families Coccidae/Pseudococcidae, while the infected propagating material contributes to their long-distance spread (Naidu et al., 2015). GLRaV-1, GLRaV-3 and GLRaV-4 are widely distributed in Greek vineyards. Based on this knowledge, a study was carried out to investigate their spread within a commercial vineyard and their presence and evolution in single vines over time.

MATERIALS AND METHODS

A vineyard of Malagouzia cultivar (8 years old in 2021) from Epanomi (Northern Greece) was selected, as it was close to an older one showing typical GLD symptoms. Initial application of high throughput sequencing (HTS) indicated the presence of GLRaV-1, -3 and -4 in both vineyards. A total of 194 vines from the young vineyard were marked and sampled at the end of each growing season for three consecutive years (2021-2023). Most of the selected vines were grown in two adjacent rows in the middle of the field while few vines situated in other rows were also sampled. Total RNA was extracted from phloem tissue (Ruiz-García et al., 2019) originating from the collected samples and tested for the presence of GLRaV-1 and GLRaV-3, using real-time RT-PCR methods (Bester et al., 2014; Panailidou et al., unpublished data), and for GLRaV-4 through conventional RT-PCR (Panailidou et al., unpublished data). To investigate the evolution of the population of GLD-associated ampeloviruses within single vines over time an amplicon deep sequencing approach was applied. More specifically, plant material was collected as mentioned above during 2021-2023 from a single vine of the same vineyard. Total RNA extraction was carried out using the same protocol and three RT-PCR methods were developed for the amplification of a 334bp fragment of the HSP70h of GLRaV-1, GLRaV-3 and GLRaV-4, respectively, using Q5® Hot Start High-Fidelity polymerase (New England Biolabs, Inc.). Three composite samples were formed with the PCR amplicons of each growing season respectively and subjected to amplicon Deep Sequencing in Illumina NovaSeq, SP in Microsynth (Balgach, Switzerland). Bioinformatic analysis of the obtained data was performed in Geneious Prime 2025 v2.2 using a pipeline that included quality check, trimming of the reads and de novo assembly followed by MegaBLAST analysis of the obtained contigs in a local database containing all available GLRaV-1, GLRaV-3 and GLRaV-4 sequences. To normalize the obtained data the RPKM method (reads per kilobase of exon per million reads mapped) was used.

RESULTS AND DISCUSSION

The three viruses were detected in the vineyard during the first sampling (2021) with GLRaV-3 and GLRaV-4 being the most common (Table 1). During the following two years' surveys, there was an increase in the number of infected vines. Interestingly, GLRaV-4 was identified in most tested vines in 2021 and in 2022 all samples were found positive. A significant increase on the presence of GLRaV-3 and GLRaV-1 was also recorded as the rate of infected vines rose from 64,4 to 78,9% and from 13,91 to 29,38%, respectively. Based on the spatial observations, the newly infected vines were mainly identified in adjacent positions from the already infected ones.

The study of the evolution of ampeloviruses in a single vine over the three growing seasons indicated changes in population composition (Figure 1). A GLRaV-3 isolate (3-a), a GLRaV-4 strain 5 isolate (4/5-a) and two GLRaV-4 strain 6 isolates (4/6-a and 4/6-b) (96.27% sequence identity) were shown to be dominant in all three growing seasons. In Oct-2022, a new genotype of GLRaV-3 (3-b) was observed, sharing 87.80% sequence identity with 3-a. In Oct-2023, one more genotype of GLRaV-3 was identified (94.24% and 90.17% sequence identity with 3-a and 3-b, respectively), while the vine was also found infected with a GLRaV-1 genotype and two genotypes of a divergent GLRaV-4 strain 6 (98.31% sequence identity) that were previously identified to occur in the vineyard through HTS. Overall, our findings suggest a possible implication of vectors in the spread of the three viruses in vineyards of Northern Greece. Further studies are underway to identify the vector species involved.

Table 1. Presence of GLRaV-1, GLRaV-3 and GLRaV-4 in the total number of samples collected in each growing season. The number of new infections per virus and year is indicated in parentheses.

Virus	Oct-2021	Oct-2022	Oct-2023
GLRaV-1	27/194	38/194 (+11)	57/194 (+19)
GLRaV-3	125/194	136/194 (+11)	153/194 (+17)
GLRaV-4	188/194	194/194 (+6)	194/194

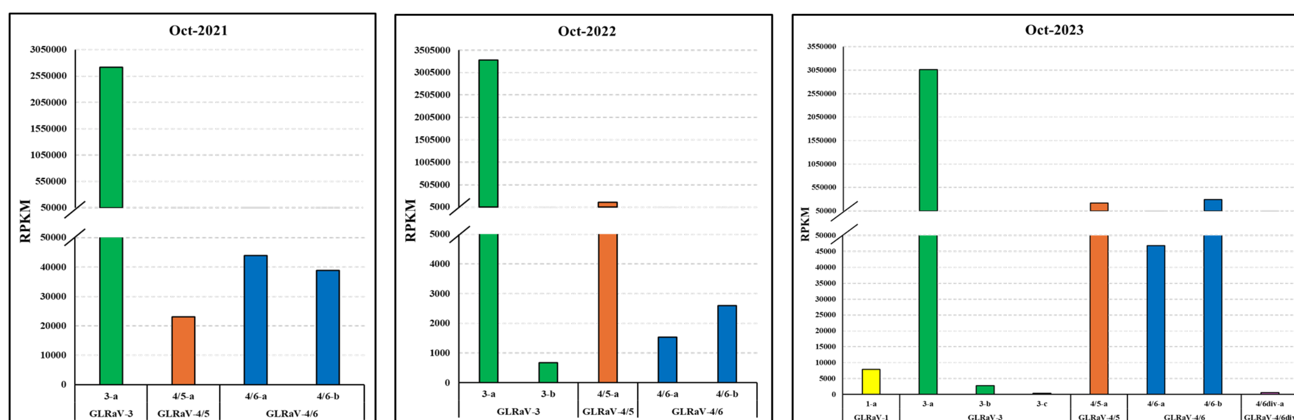

Figure 3. Ampeloviruses populations in a single vine during three growing seasons.

ACKNOWLEDGEMENTS

The research work was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the 3rd Call for HFRI PhD Fellowships (Fellowship Number: 5999).

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Impact of host genotype on grapevine Pinot gris virus infection and symptom expression

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INTRODUCTION

The Grapevine Pinot gris virus (GPGV), a positive-sense RNA virus of the genus *Trichovirus*, is now widespread in most grape-growing regions worldwide (Tarquini *et al*., 2023). Its dissemination occurs mainly through infected propagation material and local transmission by the grape erineum mite *Colomerus vitis*. GPGV is associated with Grapevine Leaf Mottling and Deformation disease (GLMD), which can cause leaf chlorosis, deformation, and reduced yield. However, disease expression is highly variable, as both symptomatic and asymptomatic vines may host the virus. This variability is thought to depend on viral genetic diversity, particularly mutations in the movement protein gene distinguishing virulent (GPGV-vir) and latent (GPGV-lat) variants, together with host genotype and viral load. Furthermore, the severity of GLMD symptoms varies among grapevine cultivars and across growing seasons, with Pinot gris, Traminer, and Glera being among the most sensitive varieties. In this study, we evaluated the susceptibility of 16 *Vitis vinifera* cultivars and 5 rootstocks to GPGV infection, assessing symptom severity, viral population structure, viral RNA accumulation, and the production of virus-derived small interfering RNAs (vsiRNAs).

MATERIALS AND METHODS

Glera plants grown in the same vineyard and exhibiting stable GLMD symptoms for at least three consecutive years were selected as sources of GPGV inoculum. The GPGV populations infecting these plants were characterized by molecular cloning and subsequent Sanger sequencing. Cuttings collected from GPGV-source plants were grafted with GPGV-free scions of different grapevine varieties (50 grafts per combination). The experiment was conducted twice: first in 2015, using 13 *V. vinifera* cultivars, and again in 2018, including 10 of the original cultivars along with 5 rootstock genotypes. Plants were monitored for three years post-grafting to assess the incidence and severity of GLMD symptoms. In June 2020, leaves were collected from three plants per variety from the 2018 grafting experiment, symptomatic when present or asymptomatic otherwise, and GPGV RNA accumulation was quantified following Bertazzon *et al*. (2025). Direct Sanger sequencing of DetF/DetR amplicons, encompassing the 3′ end of the movement protein (MP) gene and the 5′ region of the coat protein (CP) gene, was performed to identify the predominant GPGV variant (Saldarelli *et al*., 2015). In addition, the accumulation of vsiRNAs at 6635-MP and 6992-CP loci was quantified using looped-qPCR according to Tarquini *et al*. (2021).

RESULTS AND DISCUSSION

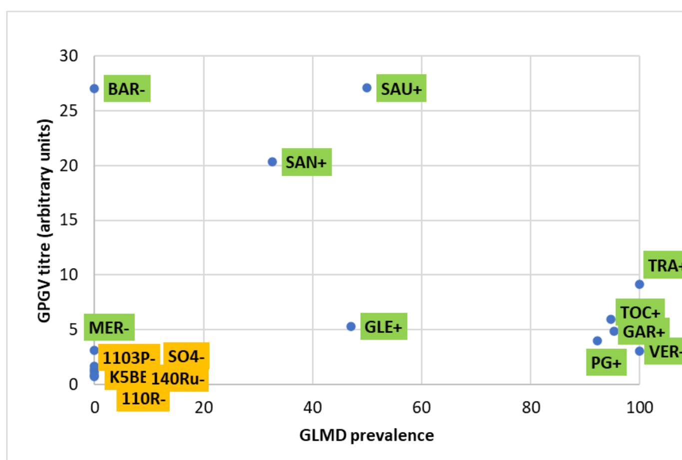
Based on GLMD incidence and symptom severity, the grapevine genotypes examined were classified into three susceptibility groups (Table 1): (1) highly susceptible, showing more than 90% symptomatic plants with severe symptoms (5 *V. vinifera* cultivars); (2) moderately susceptible, with 10-60% symptomatic plants and intermediate symptom severity (5 *V. vinifera* cultivars); (3) scarcely susceptible, including 6 *V. vinifera* cultivars and all rootstock genotypes, which exhibited few or no symptoms. Molecular diagnostics confirmed the presence of GPGV in all analysed plants, demonstrating that none of the tested genotypes prevented virus transmission from the infected Glera rootstock to the scion. This included both rootstocks and 2 *V. vinifera* cultivars (BAR and MER) that remained asymptomatic. Viral RNA accumulation in *V. vinifera* appeared to be independent of GLMD symptom expression; notably, significantly higher GPGV titres were detected in symptomatic leaves

of intermediately susceptible varieties such as SAU and SAN, as well as in asymptomatic leaves of the scarcely-susceptible cultivar BAR (Figure 1). Conversely, rootstocks consistently exhibited low viral loads, suggesting restricted virus replication or movement in these genotypes. The accumulation of vsiRNAs targeting the CP gene was consistent with GPGV RNA levels, whereas vsiRNA produced at the 6635-MP locus showed no correlation with either viral titres or host susceptibility. Molecular cloning followed by Sanger sequencing showed that the Glera plants used as virus sources hosted a mixed GPGV population, with the virulent variant predominating (GPGV-vir 66.7%, GPGV-lat 33.3% of sequenced clones). Two years after grafting, symptomatic plants were mainly infected with the virulent variant, whereas asymptomatic plants of scarcely-susceptible cultivars exclusively harboured the latent variant. Collectively, these findings support the hypothesis that some grapevine genotypes, especially rootstocks, can influence GPGV population dynamics, maintaining consistently low GPGV titres and by selectively favouring specific viral variants within the infected host.

Cultivar name	Abbreviation	Susceptibility to GLMD
Barbera	BAR	low
Cabernet franc	CF	low
Chardonnay	CHAR	low
Garganega	GAR	high
Glera	GLE	intermediate
Lambrusco salamino	LAM	low
Merlot	MER	low
Moscato bianco	MOS	low
Pinot grigio	PG	high
Primitivo	PRI	intermediate
Sangiovese	SAN	intermediate
Sauvignon blanc	SAU	intermediate
Tocai friulano	TOC	high
Traminer aromatico	TRA	high
Trebbiano toscano	TRE	intermediate
Vermentino	VER	high
110 Richter	110 R	low
1103 Paulsen	1103P	low
140 Ruggeri	140 Ru	low
Kober 5BB	K5BB	low
SO4	SO4	low

Table 1: *Vitis vinifera* varieties and rootstocks tested in the two experimental trials (2015 and 2018) and their level of susceptibility to GLMD.

Figure 1: Grapevine cultivars (2018 trial) classified based on GLMD prevalence and the viral titre of the infecting GPGV population. The symbols "+" and "–" after the cultivar abbreviation indicate the presence or absence of symptoms in the analysed plants, respectively.



ACKNOWLEDGEMENTS

The authors thank Vivai cooperativi Rauscedo (VCR) and Vivai Parolin grapevine nurseries for grafting and growing of the plants.

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POSTER ABSTRACTS

New insights on the role of Scaphoideus titanus in the epidemiology of flavescence dorée in vineyards

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INTRODUCTION

Flavescence dorée (FD) is a severe grapevine disease caused by phytoplasmas belonging to the 16SrV ribosomal phylogenetic group. Its epidemiology in European vineyards is primarily associated with the leafhopper *Scaphoideus titanus* Ball, ampelophagous species introduced into Europe from North America. Although additional secondary vectors, such as *Orientus ishidae* Matsumura and *Dictyophara europaea* (L.), have been identified, *S. titanus* remains the most efficient FD phytoplasma (FDp) vector in northern Italian vineyards. It completes its life cycle on the grapevine, where the juvenile stages emerge from mid-May, develop through five larval instars, and then become adults around the beginning of July. Recent investigations have refined our knowledge of *S. titanus* biology. Bocca et al. (2020) demonstrated that adults may survive for up to two months or longer, contrasting with earlier assumptions of a maximum lifespan of 30–40 days. Given that the percentage of individuals infected with FDp increases progressively during the summer (Lessio et al., 2009), this would aggravate the epidemiological role of *S. titanus* in the spread of the disease. This study aims to investigate the risk of FDp spreading in two vineyards in Veneto region (north-eastern Italy) by studying the level of infection in adults of *S. titanus*.

MATERIALS AND METHODS

At the beginning of July 2024, in two vineyards in the province of Vicenza, planted with Chardonnay (BRE 45°42'32.9"N; 11°34'12.7"E) and Merlot (CIM 45°43'09.0"N; 11°33'33.8"E) varieties, 8 and 20 traps were set up, respectively, which were replaced weekly until the end of September (in total 12 weeks), collected and then stored at -20 °C. Subsequently, the specimens of *S. titanus* collected were counted, and DNA was extracted from some of them according to the protocol defined by Rizzoli et al. (2021). The analysis for the presence of FDp was performed using real-time PCR analysis (Angelini et al., 2007). The average number of positive males and females per trap was calculated for each monitoring period and the averages were statistically compared using Student's t-test and ANOVA with p=0.05.

RESULTS AND DISCUSSION

A total of 6430 specimens of *S. titanus* were captured in the BRE vineyard and 2775 specimens in the CIM vineyard between early July and the end of September. In both vineyards, the peak of captures was observed in the third and fourth week of July, respectively, while populations gradually declined until the end of August, and then disappeared in the first week of September. These data were consistent with previous historical observations collected by CREA in 2020, 2021, 2022, 2024, and 2025, with a total of 2283 traps positioned in about 100 vineyards across a wide wine-growing area in the province of Treviso (northeastern Italy) from July to October (Fig. 1). In July, more male specimens were captured than females, while in August the ratio was reversed, confirming what had already been observed by Lessio et al. (2009).

In total 927 *S. titanus* from the BRE vineyard were analysed; among them, 227 tested positive for FDp (24.70%), with a peak of positive samples in the third week of July (84 positive out of 102 analysed, i.e. 82.35%), while the lowest number of positive specimens was identified in the first week of September (4 positive out of 87 analysed, i.e. 4.6%). Similarly, in CIM vineyard 16.84% out of 1568 specimens analysed resulted FDp positive (374 insects), with a peak in the last week of July

(39.08%) and the lowest number of positive cases in mid-September (zero positive specimens out of 12 analysed). Statistically, no significant differences were found between the two vineyards or between the different monitoring periods, due to the high variability between the traps. Moreover, no statistically significant difference was observed between the taxa of infection in males and females. These data (supported by a five-year collection) showed that in the monitored vineyards of the provinces of Treviso and Vicenza, important numbers of *S. titanus* are found mainly in July, while in September the populations are greatly reduced. This trend decreases the risk of FDp spreading in the late season. Furthermore, the highest percentage of FDp positive specimens was detected in both BRE and CIM vineyards in July, while in September there were only a few FDp positive specimens, although the differences were not statistically significant. In summary, the risk of FD spreading by infected *S. titanus* is highest in July and, to a lesser extent, in August, while it becomes minimal in September.

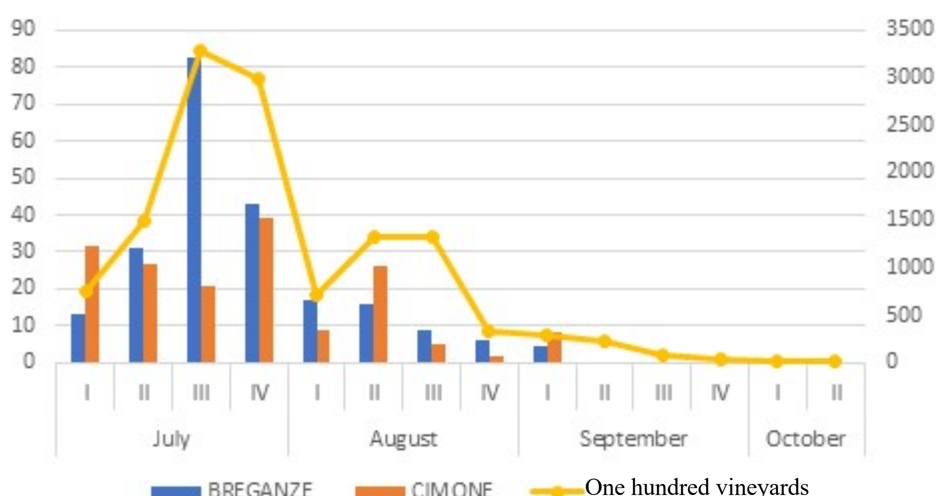

Fig. 1: the histogram shows the number (average per trap) of *S. titanus* specimens collected in BRE and CIM in 2024 from 4 July to 23 September; the continuous line reports the mean number of *S. titanus* specimens collected with 2283 traps in five years from 2020 to 2025 in approximately 100 vineyards in province of Treviso, from July to October.

ACKNOWLEDGEMENTS

The authors wish to express their sincere appreciation to Andrea Saccol, Cezar Dimitrescu, Aurora Signorotto, and Alessandro Di Muzio for their invaluable assistance in the collection of data, which greatly contributed to the completion of this study

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Investigating novel strategies for grapevine virus management in Australia

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INTRODUCTION

The production of high health propagation material supplied through pathogen tested grapevine germplasm, foundation plantings and source blocks is the basis of virus disease management in grape production systems. A range of methods are used to produce pathogen-free germplasm including *in vitro* culture of shoot tips or meristems that are often combined with heat therapy, chemotherapy or cryotherapy (Kaur et al., 2024).
RNA interference (RNAi) plays an important role in plant antiviral systems which mitigate the detrimental effect of viruses through silencing of specific target regions (Rosa et al., 2018). Recently, it was demonstrated that RNAi can be employed to reduce the replication of grapevine Pinot gris virus (GPGV; species *Trichovirus pinovitis*; Family *Betaflexiviridae*) in *in vitro* grown grapevine plantlets through a single application of double-stranded RNA (dsRNA) targeting conserved motifs within the RNA-dependent RNA-polymerase gene encoded by GPGV (Kaur et al., 2024). However, the plantlets were only tested at seven days post application, therefore the durability and long-term effect of the single application was not measured. The impact of this treatment on different strains of GPGV and other virus species was also not investigated. The aim of this study is to investigate the efficacy and durability of the methodology and further optimise application of dsRNA to produce virus-free grapevine propagation materials.

MATERIALS AND METHODS

Excised shoot tips of tissue culture plantlets derived from GPGV infected tissue culture plantlets will be treated with dsRNA or left or untreated as previously described (Kaur et al., 2024). Additionally, the synthetic dsRNA will be applied to grapevine plantlets co-infected with GPGV + grapevine virus A (GVA; species *Vitivirus alphavitis*; Family *Betaflexiviridae*), GPGV + grapevine leafroll-associated virus 3 (GLRaV-3; species *Ampelovirus trivitis*; Family *Closteroviridae*), and GPGV + GVA + GLRaV-3 to evaluate its effects on other grapevine viruses of same and different families. The virus titre of the plantlets will be measured by quantitative RT-PCR before treatment and then at 7, 14, 30, and 60 days. Targeted amplicon sequencing will be used to determine the efficacy of the dsRNA on different variants of GPGV and co-infecting viruses.

RESULTS AND DISCUSSION

The results of this study will be presented and will be used to refine the use of RNAi as an alternative tool for *in vitro* virus eradication. The present study is hoped to provide a practical method for grapevine virus management in the future.

ACKNOWLEDGEMENTS

The research presented was supported by funding from Agriculture Victoria Research and Wine Australia. Wine Australia invests in and manages research, development and extension on behalf of Australia's grape growers and winemakers and the Australian Government. We also thank the South Australian Vine Improvement Association, Riverland Vine Improvement Committee and team members, Vine Industry Nursery association, CSIRO and other industry contributors for their support.

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Tracing the origin and dissemination of flavescence dorée phytoplasma in Western Switzerland using SNP and MLST analyses

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INTRODUCTION

Flavescence dorée (FD) is a quarantine disease of grapevine associated with Grapevine flavescence dorée phytoplasma (FDp) and transmitted by the leafhopper *Scaphoideus titanus*. Since its first detection in 2015 in Swiss vineyards north of the Alps, the disease has continued to spread despite strict control measures. The origin and dissemination routes of FDp remained unclear. This study aimed to elucidate the introduction and spread of FDp across the cantons of Vaud, Valais, and Geneva through high-resolution SNP-based genotyping.

MATERIALS AND METHODS

Symptomatic grapevines were sampled during annual surveillance campaigns. Samples were tested by qPCR for FDp and Bois noir phytoplasmas (Pelletier et al., 2009). A selection of FD-positive samples was subjected to multilocus sequence typing (MLST) using established markers (*map*, *dnaK*, *vmpA*, *malG*) and newly developed markers for PCR amplification and subsequent Sanger sequencing. The new markers were developed based on Illumina-sequencing of geographically and temporally representative samples, followed by variant calling after mapping to the FDp-CH reference genome (Genbank CP097583). The spatial distribution of genotypes was visualised using QGIS 3.34.2-Prizren.

Between 2015 and 2022, more than 4,000 symptomatic grapevines from the cantons of Geneva, Vaud and Valais were tested, with roughly a quarter infected by FDp and about half by Bois noir. MLST analysis of more than 700 FDp-positive samples revealed very low genetic diversity: all isolates belonged to *map* genotype M54, *dnaK* group 1 and *vmpA* group II, with only the *malG* locus distinguishing three profiles showing regional patterns (Figure 1).

The exclusive presence of *map* genotype M54 supports the hypothesis that FDp in western Switzerland was most likely introduced through infected propagation material, as M54 has been mainly associated with the *Vitis vinifera*-*S. titanus* pathosystem (Malembic-Maher et al., 2020). The limited variability observed at these loci contrasts with findings from other countries, where higher diversity of *map* and *malG* has been reported (Kogej Zwitter et al., 2023; Rossi et al., 2019). This reduced diversity likely reflects a small number of introduction events combined with the recent establishment of FDp.

To improve epidemiological resolution, new SNP markers were developed from whole-genome data of representative Swiss isolates. Applying this extended genotyping scheme revealed up to nine distinct profiles showing some regional structuring (Figure 2). Certain areas tended to be associated with one or a few profiles, whereas others displayed a broader mixture, suggesting differences in introduction history and local dissemination dynamics.

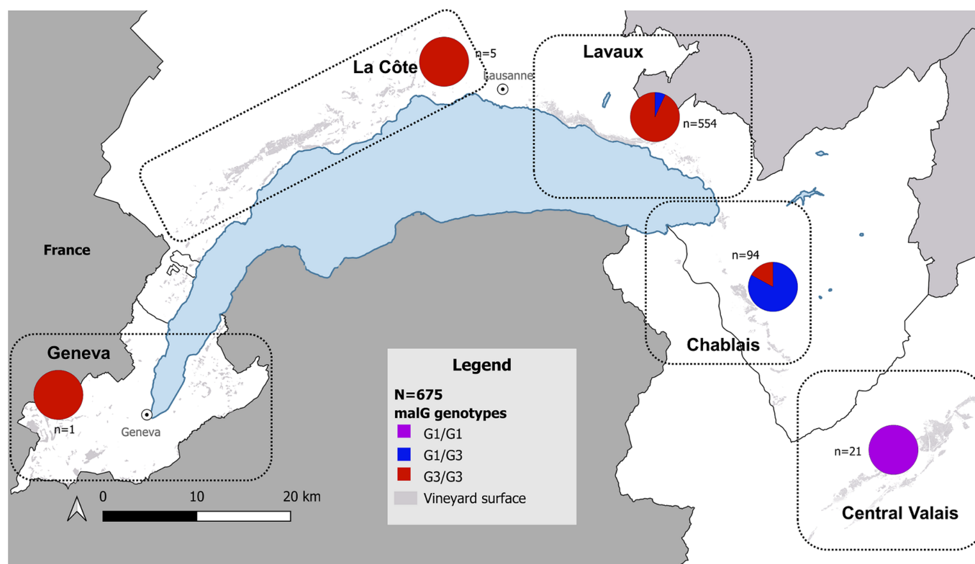

Figure 4 Geographic distribution of *malG* genotypes of FDp in Swiss vineyards north of the Alps. Pie charts show the relative frequency of malG genotypes at each location, based on 675 samples collected between 2015 and 2022. Each pie chart represents samples grouped by geographical area. Sample counts (n) are indicated next to each chart. Vineyard surfaces are shown in light grey.

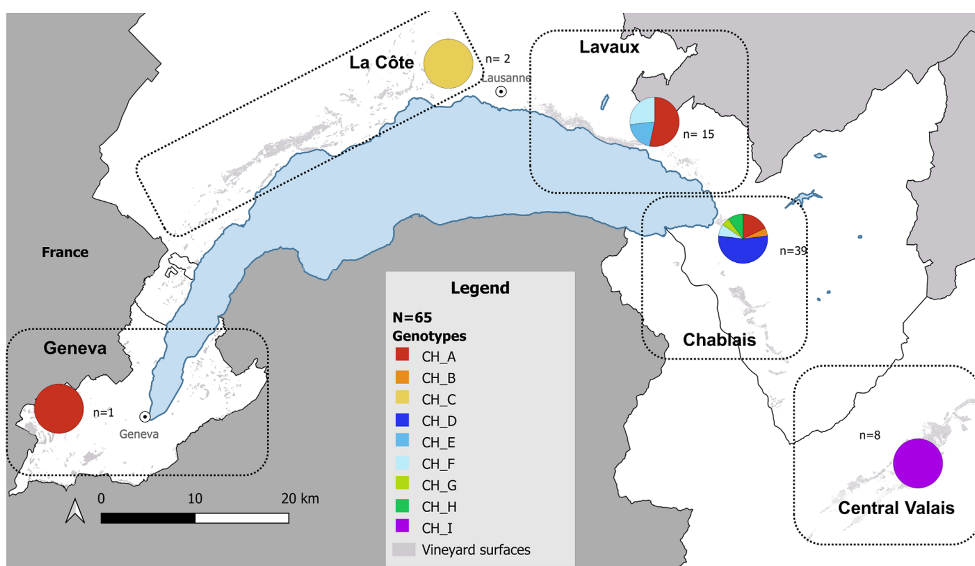

Figure 5 Geographic distribution of multilocus genotypes of FDp in Swiss vineyards north of the Alps. Pie charts show the relative frequency of SNP-based multilocus genotypes at each location. Each pie chart represents samples grouped by geographical area. Sample counts (n) are indicated next to each chart. Vineyard surfaces are shown in light grey.

Overall, the results indicate that FDp in western Switzerland was most likely introduced through infected propagation material, after which both vector dispersal and viticultural practices contributed to its dissemination. The new SNP markers provide substantially improved resolution for tracing FDp spread and form a robust basis for better understanding the regional epidemiology of FD north of the Alps.

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Virome analysis of woodland grape (*Vitis sylvestris)* populations from the Szigetköz region (Hungary)

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INTRODUCTION

The European woodland grapevine (*Vitis sylvestris* C.C. GMEL.) may have been the ancestor of all grapevine varieties, alone or with other species. As a protected species, identifying and preserving its natural populations is a crucial task in nature and biodiversity conservation. Nowadays, only a few isolated populations of *V. sylvestris* remain in Europe, primarily located around floodplains in small, fragmented areas. The Szigetköz area in north-western Hungary is an ideal environment for this grapevine species, as it features numerous floodplain areas. The natural populations of woodland grapevine, which can be a source of viruses, have existed for a long time; the ancestor of the current population dates back before the phylloxera pandemics. There are only a few studies that have reported the virus infection status of *V. sylvestris* in natural populations in Europe. Grapevine rupestris stem pitting–associated virus (GRSPaV - *Foveavirus rupestris*) is one of the most widespread grapevine viruses worldwide (Martelli, 2014; Glasa et al., 2017), and according to previous studies, it also seems to be occurring in *V. sylvestris* (Pacifico et al., 2016; Sabella et al., 2018). Furthermore, during the virus survey of *V. sylvestris* populations, grapevine virus A (GVA - *Vitivirus alphavitis*), grapevine leafroll-associated virus 1 (GLRaV1 - *Ampelovirus univitis*), grapevine virus T (GVT - *Foveavirus tafvitis*), grapevine foveavirus A (GFVA – a distinct form of GVT), and grapevine Pinot gris virus (GPGV - *Trichovirus pinovitis*) were also identified (Sabella et al., 2018; Reynard et al., 2020; Belkina et al., 2025). In this study, we investigated the virome of *V. sylvestris* individuals sampled from natural habitats in Hungary.

MATERIALS AND METHODS

Leaf and petiole samples of *V. sylvestris* were collected at Hedervar-Kimle (3 samples) and Feketeerdo (6 samples) in the Szigetkoz area in 2019. Total RNA was extracted from each sample using an optimised CTAB-based method and subsequently pooled. For high-throughput sequencing (HTS), the small RNA fraction of the pool was isolated, and an sRNA sequencing library was prepared. In parallel, the pool of total nucleic acids was DNase-treated. The sRNA library and the DNase-treated RNA was sent for sRNA and non-coding ribodepleted RNAseq. For the bioinformatic analysis of the sequenced reads, CLC Genomic Workbench software was used. The presence of the viruses in the samples was confirmed with the RT-PCR method using virus-specific primers (GFVA_7486F: 5'-GCTTGTCACCCTAATCCTTGCG-3', GFVA_8531R: 5'-CGTAGCAAGAGTTCCAACTAGC-3'; RSPaV-F/6904: 5'-GAGGCACATTTCATCAAGTCAA-3', RSPaV-R/8445: 5'-TCTGAGCACTTKAACYTCAAAAG-3'). Phylogeny of the identified virus was analysed after Sanger sequencing of the cloned, amplified viral segments.

RESULTS AND DISCUSSION

Bioinformatic analysis of the sRNA and RNAseq data predicted the presence of two viruses belonging to the *Betaflexiviridae* family: GRSPaV and GFVA. No other economically important viruses were detected in the *V. sylvestris* samples. The presence of GRSPaV and GFVA was confirmed in 1-1 out of 9 individuals by RT-PCR. The phylogenetic analyses of the virus, which was originally annotated as GFVA, revealed that it is instead a closely related foveavirus: GVT. GVT is a recently described grapevine-infecting virus (Jo et al., 2017), described in cultivated and wild grapevine, also often in coinfection with GRSPaV (Demian et al., 2021). GVT is widespread in Central Europe and Hungary, and because of its high variability, its multiple introductions have been suggested (Glasa et al., 2018). This could be true, but as we have found it in natural habitats, isolated from grapevine growing regions, some of these introductions could have happened well before the phylloxera pandemics at the end of the XIX. century. The GRSPaV isolate from *V. sylvestris* showed 99% nt. identity to the reference genome (NC_001948.1), while the GVT isolate is most closely related to an Italian GVT isolate (MH674183), showing 93% nt. similarity. Based on our results, the presence of only these viruses and the coinfection of GRSPaV and GVT in the *V. sylvestris* populations reflects the ecological isolation of the Szigetköz and the limited virus spread from cultivated vineyards.

Overall, the virome of *V. sylvestris* appears to be relatively simple, suggesting coevolutionary relationships between *V. sylvestris* and asymptomatic GRSPaV, GVT. To our knowledge, this study is the first description of viruses in woodland *V. sylvestris* from Hungary.

ACKNOWLEDGEMENTS

Our research was funded by the National Research, Development and Innovation Office (NKFIH): K119783, K131679, PD137621. This research was supported by the Flagship Research Groups Programme of the Hungarian University of Agricultural and Life Sciences (MATE): KKCS2024.

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Survey of grapevine viroids in Bekaa Valley in Lebanon

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INTRODUCTION

Viroids are small, circular, non-coding RNA molecules that infect numerous crop plants, including *Vitis vinifera*. A recent global synthesis of grapevine viruses and viroids (Fuchs et al., 2025) reported that eight viroids: *Hop stunt viroid* (HSVd), *Citrus exocortis viroid* (CEVd), *Grapevine yellow speckle viroid 1* (GYSVd-1), *Grapevine yellow speckle viroid 2* (GYSVd-2), *Grapevine yellow speckle viroid 3* (GYSVd-3), *Australian grapevine viroid* (AGVd), *Jasmine viroid* (JGVd), and *Grapevine latent viroid* (GLVd), together with the unclassified *Grapevine hammerhead viroid* (GHVd), are currently associated with grapevines worldwide. According to Di Serio et al. (2017), five viroids belonging to the *Pospiviroidae* are known to naturally infect grapevine: HSVd, CEVd, GYSVd-1, GYSVd-2, and AGVd. HSVd and GYSVd-1 are widely distributed across viticultural regions, while GYSVd-2 and CEVd have been reported sporadically in the Mediterranean Basin (Hajizadeh et al., 2015), and AGVd has so far been detected only in a limited number of countries (Gambino et al., 2014; Di Serio et al., 2017). In a recent study, Grapevine Pinot gris virus (GPGV) was reported for the first time in Lebanon from vineyards in the Bekaa Valley (Abou Kubaa et al., 2020). The same samples collected during that survey were subsequently analyzed in the present work to assess the presence of grapevine viroids using RT-PCR assays.

MATERIALS AND METHODS

A total of 108 grapevine samples were collected from nine vineyards across the Bekaa Valley in (Ammiq, Kefraya, Mansoura, Ferzol, Niha, Qob Elias, and Saghbine), representing both table and wine grape cultivars (Black Magic, Superior Seedless, Crimson, Red Globe, Merlot, Syrah, Chardonnay, and Cabernet Sauvignon). Each sample consisted of young leaf and petiole tissues. The detection of HSVd, GYSVd-1, GYSVd-2, CEVd, and AGVd was carried out by RT-PCR using standard primer sets and cycling conditions previously described in the literature. Amplified products were visualized by agarose gel electrophoresis, and representative amplicons were sequenced to confirm specificity.

RESULTS AND DISCUSSION

In this study, all five viroids (HSVd, GYSVd-1, GYSVd-2, CEVd, and AGVd) were detected in different Lebanese vineyards at varying frequencies (Table 1). HSVd was detected in about 55 % of the analyzed vines and remained the most widespread viroid, while GYSVd-1 was found in approximately 38 % of samples. In contrast, GYSVd-2 and CEVd occurred in fewer than 6 % of the tested vines, and AGVd was identified at a very low frequency (around 3 %), restricted to a few vines from Kefraya and Mansoura. The detection of HSVd and GYSVd-1 in multiple vineyards across the Bekaa Valley indicates their long-term presence in Lebanese grapevine germplasm, though at moderate levels rather than the near-ubiquitous prevalence reported in other countries such as Italy and Iran (Gambino et al., 2014; Hajizadeh et al., 2015). Taken together, this finding represents the first record of grapevine viroids in Lebanon and extends the known distribution of these agents in the Mediterranean. Mixed infections were detected in roughly one-third of the vines (about 29 %), mainly in the central Bekaa region (Kefraya and Mansoura), suggesting that vegetative propagation and local plant exchange networks contributed to their spread. No consistent foliar symptoms of yellow speckle or vein banding were observed, suggesting that most infections are

latent. Sequencing of representative amplicons confirmed viroid identity and revealed high similarity with reference isolates reported worldwide. This study provides the first molecular evidence of HSVd, GYSVd-1, GYSVd-2, CEVd, and AGVd in Lebanese vineyards and adds Lebanon to the list of countries in which grapevine viroids are part of the background virome.

Table 1. Detection of grapevine viroids in vineyards from the Bekaa Valley, Lebanon

Sampling site	No. of samples	HSVd	GYSVd-1	GYSVd-2	CEVd	AGVd	Mixed infections
Ammiq	15	8	6	2	0	0	4
Kefraya	20	11	8	1	1	2	6
Mansoura	15	8	5	0	1	1	4
Ferzol	15	7	5	1	1	0	4
Niha	10	5	3	0	0	0	2
Qob Elias	18	10	7	1	1	0	6
Saghbine	15	10	7	1	1	0	5
Total	**108**	**59 (54.6 %)**	**41 (38.0 %)**	**6 (5.6 %)**	**5 (4.6 %)**	**3 (2.8 %)**	**31 (28.7 %)**

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Advances in shoot tip cryotherapy-based methods for virus and viroid eradication in horticultural crops

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INTRODUCTION

Viral diseases are a major constraint to sustainable agricultural production and are especially problematic in vegetatively propagated crops. Reliable detection and eradication methods are needed to ensure the supply of disease-free plants for industry, breeding, and germplasm conservation programs. Shoot tip cryotherapy, a practical application of cryopreservation for pathogen eradication, has proven effective in eradicating viruses and viroid diseases in many economically important crops. Despite the success of cryotherapy, its efficiency depends on the pathogen location within the shoot tip and the differential survival of cells following cryoexposure. Recent advances in removing difficult-to-eradicate pathogens, particularly those that can infect the meristem, rely on combining thermotherapy and/or chemotherapy with cryotherapy (Li et al., 2025). Understanding when and how to combine these methods is critical for achieving reliable sanitation, especially in crops with mixed infection or those recalcitrant to specific treatment conditions. This abstract outlines recent advances in cryotherapy-based methods for virus and viroid eradication in horticultural plants.

MATERIALS AND METHODS

Diseased plants were established and multiplied *in vitro* to provide shoots for in vitro therapy treatments. Shoot tips were either (i) excised directly from infected in vitro shoots and subjected to shoot tip cryotherapy (Cryo), or (ii) exposed to thermotherapy (Thermo), chemotherapy (Chemo), or a combined thermo-chemo treatment prior to shoot tip excision and cryotherapy (Figure 1). The recovered shoots were evaluated for pathogen presence *in vitro*, and pathogen-free plants were transferred to the greenhouse for retesting for the presence of pathogens.

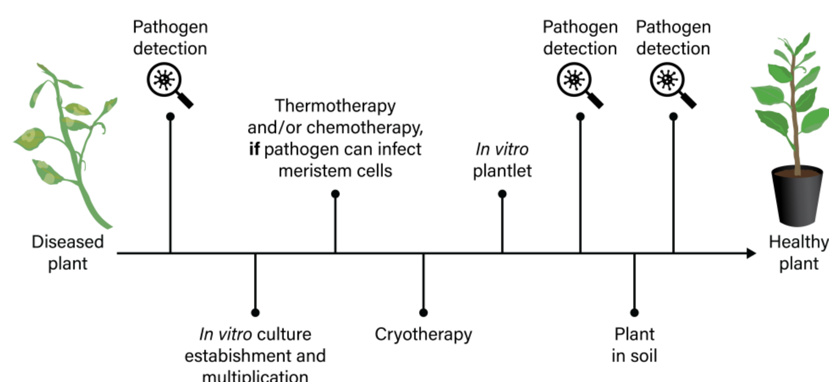

Figure 1. Workflow for the production of pathogen-free plants. Techniques are applied either alone or in combination, depending on plant-virus interactions and in planta pathogen distribution patterns. Figure by Katheryn Chen (Colorado State University, Fort Collins, CO, U.S.A).

RESULTS AND DISCUSSION

The efficacy of cryotherapy alone in producing pathogen-free plants varied depending on the virus or viroid and its infection pattern (Table 1). Shoot tip cryotherapy failed to eradicate some pathogens, likely due to incipient infections in meristematic cells or their ability to reach the apical dome of shoot tips, regardless of plant species. In these cases, successful pathogen eradication or improved efficiency was achieved by combining thermotherapy and/or chemotherapy with shoot-tip cryotherapy. The consistency of these results across crops with single or mixed infections demonstrates the strong potential of these integrated approaches to support the production of healthy planting materials and facilitate the global exchange of germplasm.

Table 1. Plant virus and viroid eradication frequencies (%) using cryotherapy-based methods.

Pathogen	Plant species	Cryo	Thermo + Cryo	Chemo + Cryo	(Chemo + Thermo) + Cryo	References
ACLSV	*Malus domestica*	79	100			Bettoni et al., 2022a
AHVd		0	25-75			
ASGV		0	100			
AcVA	*Actinidia macrosperma*	39	80	53-75	89	Zhang et al., 2024 a, b
AcVB		69	100	73-75	89	
AcCRav		0	60	47-50	56	
AcVA + AcVB + AcCRaV		0	50	25-27	44	
GLRaV-3	*Vitis* spp.	100				Bi et al. 2018
PVA	*Solanum tuberosum*	20	100	100	100	Bettoni et al., 2022b
PVM		0	20	70	70	
PVS		0	29-70	70-100	70-100	
PVS + PVA		0	29	100	70	
PVS + PVM		0	20	70	70	

AcCRaV: *Actinidia chlorotic ringspot–associated virus*; ACLSV: *Apple chlorotic leafspot virus*; AcVA: *Actinidia virus A*; AcVB: *Actinidia virus B*; ASGV: *Apple stem grooving virus*; GLRaV-3: *Grapevine leafroll-associated virus-3*; *PVA*: *Potato virus A*; PVM: *Potato virus M*; PVS: *Potato virus S.*

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Status of Grapevine leafroll and Grapevine red blotch diseases in Baja California, Mexico

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INTRODUCTION

Grapevine leafroll disease (GLD), associated with grapevine leafroll-associated viruses (GLRaVs), is the most prevalent viral disease affecting grapevines, with GLRaV-3 as the most widespread, causing significant economic losses (Naidu et al. 2014, Ricketts et al. 2015). Grapevine red blotch virus (GRBV), the causal agent of grapevine red blotch disease (GRBD), has gained prominence because of its widespread in North America and significant impacts on vine health and fruit quality (Cieniewicz and Fuchs 2025). Primary spread of GLRaV-3 and GRBV occurs through vegetative propagation material, with secondary spread via insect vectors (Naidu et al. 2014, Cieniewicz and Fuchs 2025). In Mexico, grapevine cultivation spans 36,405 ha, producing 477,305 tons of grapes in 2022, with Baja California as a leading winegrape producer, accounting for 85% of national production. Reports of viral diseases, including GLRaV-1, -2, -3, and -4 and GRBV, have emerged in Baja California and Aguascalientes (García-Reséndiz and Carrillo-Tripp 2022, Diaz-Lara et al. 2023). However, these reports lack details regarding distribution, incidence, and vector associations. This study aimed to evaluate the prevalence of these two significant grapevine viral diseases in Baja California, assess the incidence of symptomatic plants in commercial vineyards across primary production areas, and identify factors contributing to the spread of GLD and GRBD. Such research is essential not only to develop effective management strategies to mitigate the economic losses associated with these diseases, but also to ensure the sustainability of grapevine cultivation in Mexico.

MATERIALS AND METHODS

One hundred and twenty one randomly-selected vineyard blocks were surveyed to determine prevalence of GLD and GRBD, based on symptom evaluation in quadrats of 1,000 grapevines per block. An analytical longitudinal study was conducted over a 3-yr study period (2021 to 2023) to monitor the spread of GLD and/or GRBD in five vineyards by assessing quadrats of 25 contiguous vineyard rows with 100 contiguous plant positions per row (2500 plant positions total).
Following a previous methodology (Fú-Castillo et al. 2002), trained technicians determined the percentage of plants infested by the vine mealybug in all prevalence and incidence sites. Within ~10 ha quadrats per vineyard, three rows were assessed, with 25 plants randomly selected per row (75 plants per quadrat). Mealybug surveys were conducted quarterly in sites with vine mealybug presence, and annually in sites without mealybug presence. The vector of GRBV was not considered because the presence of the three-cornered alfalfa hopper had not been confirmed in Baja California at the time of our study (J. Carrillo and D. Schneider, personal communication).
Trained personnel sampled leaf tissue for virus detection (10 leaves per plant per sample). A total of 190 samples (137 symptomatic plants and 53 asymptomatic plants) were taken in October 2022 and 2023. Total nucleic acids were extracted and tested by real-time PCR for the detection of GLRaVs and GRBV. Visual diagnosis of GLD and/or GRBD (symptomatic or asymptomatic) was with results of molecular diagnosis. Statistical analyses of the data were conducted as described in García-Reséndiz et al. (2025).

RESULTS AND DISCUSSION

The average rate of symptomatic plants per site was 20%, with a range of 0 to 92%. No difference in prevalence was found according to cultivar. However, 61% of the vineyard sites were infested by vine mealybug (*Planococcus ficus*), and a significant correlation was found between the percentage of vine mealybug-infested plants and prevalence ($p < 0.01$). The percentage of vine mealybug-infested plants per vineyard ranged from 0 to 28.5%, with an average of 4.1%. Interestingly, vine mealybug presence was not detected in any vineyard evaluated in Valle de Ojos Negros region.

In the five sites monitored, the average increase in symptomatic plants per site was 1.7% in 2022 and 9% in 2023. All sites experienced a greater number of new cases from 2022 to 2023, compared to the increase observed from 2021 to 2022. Site 3 exhibited the highest number of new cases between 2021 and 2023, totaling 594, followed by site 2 with 271, site 1 with 187, site 4 with 86, and site 5 with 57. The increase in new cases per quadrat ranged from 2 (site 5) to 24% (site 3).

Of the 190 tested samples, 57% tested positive for at least one virus. The viruses detected in symptomatic plants were GLRaV-1, -2, -3, and GRBV. Asymptomatic samples that tested positive for viruses were associated with GLRaV-2, -3, or GRBV. The frequency distribution comparing visual diagnosis to molecular virus detection revealed differences, indicating a dependency between variables. Although false positives and false negatives were found, visual diagnosis demonstrated a sensitivity of 91%, a specificity of 52%, a PPV of 72%, and an NPV of 81%. These metrics indicate that visual diagnosis is effective at identifying symptomatic plants that test positive for at least one virus.

ACKNOWLEDGEMENTS

This work was supported by funds granted to Dr. Carrillo Tripp [CONAHCYT (FOP02-2021- 4 Grant 316602), CICESE institutional project (683210)], and to CESVBC (SADERBC, Proyecto de Manejo Fitosanitario de la Vid). We express our gratitude to CONAHCYT for the doctoral scholarship awarded to Karen García-Reséndiz.

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Impact of Grapevine Variety on Vector-Mediated Flavescence Dorée Transmission

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INTRODUCTION

Flavescence dorée (FD) is an economically important disease of grapevine caused by phytoplasmas (FDp), transmitted in a persistent propagative manner by the leafhopper *Scaphoideus titanus* and regulated organism in the EU [ESFA,2020]. Control of the disease relies on planting of healthy material, roguing of infected plants and compulsory insecticide treatments against the vector, these latter having raised concerns about non-target effects and human health. Grapevine genotypes in the field show different susceptibility to FD and affect the feeding behavior of the vector [Galetto et al., 2016; Ripamonti 2022a]. In the FD-highly susceptible variety the insect longevity and fecundity are higher and the acquisition of phytoplasmas by *S. titanus* is more efficient than when the vector feed on FD-poorly susceptible variety [Bressan et al. 2005; Ripamonti 2022b]. Considering that the probability of FD infection increased with the proportion of land covered by susceptible cultivars [Adrakey et al. 2022], the aim of the present research was to evaluate the impact of some typical cultivars from the Italian north-eastern regions on phytoplasma acquisition and transmission by the vector *S. titanus*.

MATERIALS AND METHODS

For the inoculation access period, individuals of *S. titanus* at juvenile stages L3–L5 were confined in on FD-positive plants: grapevine Chardonnay in the first year, and on *Vicia faba* in the second year. After 7 days, the individuals were recovered and transferred onto healthy confined *V. faba* plants for 2 weeks (latency access period), then transmission was conducted on *V. vinifera* potted plants. Two typical cultivars from Veneto Region (Garganega and Corvinone) were compare with Chardonnay (FD-highly susceptibility cultivar and Merlot (FD-poorly susceptibility cultivar), under controlled conditions in a greenhouse.
Five *S. titanus* individuals were confined on each grapevine plant using individual sleeves. For each cultivar, 6 and 10 grapevines were used in the first and second year respectively. After 4 weeks, the leafhoppers were collected, and percent of survival was recorded for each plant. The specimens were preserved in ethanol and stored at –20°C.
To assess the infection rate, we sampled 10 individuals pre-acquisition, 10 post-acquisition and 28 post-latency were sampled.
For the Nucleic acid extraction the insects were ground in 400 μL of CTAB buffer and the extraction was performed as reported in [Jangra & Ghosh, 2022]. Genomic DNA from the source plants used for inoculation and from the plants employed in the experiment was extracted following Doyle & Doyle (1990). The plants involved in the transmission trial were sampled pre-infection and after 12 weeks. Healthy grapevines were used as negative controls in each series of extractions.
To detect the presence of phytoplasmas a nested PCR was performed, as described in the EPPO protocol [Bulletin (2016)]. This analysis involves two successive amplification cycles aimed, respectively, at detecting the presence of the phytoplasma and determining whether it belongs to group V. These specific primers drive the amplification of a final 1100 bp product, which was visualized by electrophoresis on a 1% agarose gel.

RESULTS AND DISCUSSION

Regarding the source of acquisition, a markedly different rate of insect positivity was observed across the two years. In particular, acquisition from *V. vinifera* resulted in approximately 26% of insectstesting positive, whereas acquisition from *V. faba* allowed nearly all specimens to be infected. This confirms that the acquisition potential varies depending on the plant species and tissue [Alma et al., 2018].

A different *S. titanus* survival rate was observed at the end of the transmission trials. In particular, Chardonnay appeared to be the most preferred cultivar, showing an average survival rate of 45%. On Merlot, Corvinone and Garganega survival decreased around 10%. Comparing the mortality in FD-highly susceptible variety (Chardonnay) and in FD-poorly susceptible variety (Merlot), the data confirm that insect fitness is strongly influenced by the FD- susceptibility on which the insects were confined.

According to the molecular analyses, the varieties most frequently infected were Chardonnay and Garganega, with 50% and 33%, respectively. In Corvinone, only 10% of plants tested positive, while none of the Merlot plants were found positive, thus indicating different susceptibilities among the tested genotypes. These data may be explained by differences in the insect's feeding behavior, its ability to reach the phloem vessels, or the presence of antimicrobial secondary metabolites, or a reduced ability of phytoplasmas to multiply in some cultivars.

Since poorly susceptible varieties are a poor source of infection for *S. titanus*, susceptibility is likely to have a major impact on the infection pressure and on FD spread at the vineyard level. The information on Garganega and Corvinone will be used to design a proper and sustainable disease management.

ACKNOWLEDGEMENTS

This research was funded by BIORES, Bando PRIN 2022 codice CUP B53D23017600006) and by project Regione Veneto "Studi Epidemiologici sulla Flavescenza dorata: valutazioni sull'efficienza di trasmissione di Scaphoideus titanus in diversi genotipi di vite e ruolo dei potenziali vettori" – CUP: H17G22000310002.

We thank Dr. Vally Forte (CREA-VIT) for the *S. titanus* specimens and Dr. Cristina Marzachì (IPSP) for the supply of infected *V.faba*.

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Host variety and phytoplasma strain influence the grapevine response mechanisms

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INTRODUCTION

Phytoplasmas (genus '*Candidatus* Phytoplasma') are plant-pathogenic bacteria that pose a major threat to global agriculture by causing significant yield losses in many crops. These bacteria exhibit remarkable genome plasticity, leading to considerable diversity in effector composition and, consequently, in pathogenic behavior among strains. *Flavescence dorée* phytoplasma (FDp; ribosomal group 16SrV) is one of the primary causal agents of grapevine yellows disease, which severely affects European viticulture. Previous research has shown that FDp populations comprise genetically diverse strains, with variations in virulence and host interactions. Grapevine responses to FDp infection involve several metabolic adjustments, including alterations in the production of specialized metabolites such as flavonoids, which are known markers of biotic stress. This study aimed to evaluate how two distinct FDp genotypes (M38 and M54) influence the physiology and metabolism in *Vitis vinifera* L. var. 'Pinot gris', and to further compare the host response to FDp-M38 infection in 'Pinot noir' to assess the potential influence of grapevine variety on disease development.

MATERIALS AND METHODS

Leaves of symptomatic and asymptomatic grapevine (Vitis vinifera L. var. 'Pinot gris' and 'Pinot noir') plants were sampled throughout the development of phytoplasma infection (June-September) at three time points from a vineyard in central continental Croatia (Sveti Ivan Žabno). Using a real-time PCR assay (Pelletier et al., 2009), we confirmed the infection with the FDp in symptomatic plants. Additionally, amplification, sequencing and phylogenetic analyses of *map* gene amplicons were performed as previously described (Plavec et al., 2023). For physiological and metabolic analyses, extracts were prepared from leaves using a previously developed methodology (Davosir & Šola, 2023), optimized for grapevine samples. Analyses of physiological parameters were performed by UV/Vis spectrophotometry, while the metabolic data was obtained by both spectrophotometry and chromatographic (HPLC, GC-MS) methods.

RESULTS AND DISCUSSION

Two FDp genotypes, M38 and M54, both belonging to the mapFD2 cluster, were identified in symptomatic 'Pinot gris' leaves, while M38 infection was confirmed in the 'Pinot noir' variety. Preliminary analysis revealed putative response molecules among the identified compounds, up- or downregulated in infected plants compared to the uninfected controls. The epidemiologically more aggressive strain M54 induced stronger downregulation of phenolics' accumulation at the beginning and higher upregulation by the end of the season than the less aggressive M38 strain in 'Pinot gris'. Increased malondialdehyde levels pointed to the oxidative stress in infected leaves, and highly correlated with the activation of *L*-ascorbic acid synthesis. Levels of hydrogen peroxide were reduced in infected leaves, possibly as an FDp mechanism to avoid plant-derived oxidative damage. Genotype M54 was associated with a lower accumulation of soluble sugars and lower damage to photosynthetic pigments while retaining a higher titre than M38. Therefore, pronounced phytoplasma genotype-dependent changes in grapevine physiology, potentially caused by the differences between

M54 and M38 on the level of the efficiency of their effectors should be further investigated. Correlations were established between parameters evaluated in ‘Pinot gris’ and ‘Pinot noir’, pointing to similar regulation of metabolic pathways in different varieties. The exogenous application of identified compounds on FDp-infected leaves could impact the disease progression, however, additional functional testing is needed to confirm the role of specific compounds in disease development. Further research will include analyzing a wider range of putative response biomarkers to compare the patterns between different varieties. Overall, the data indicate that both FDp strain variability and host genotype contribute to the modulation of grapevine metabolic responses, highlighting the need for integrated strain–host studies in phytoplasma research.

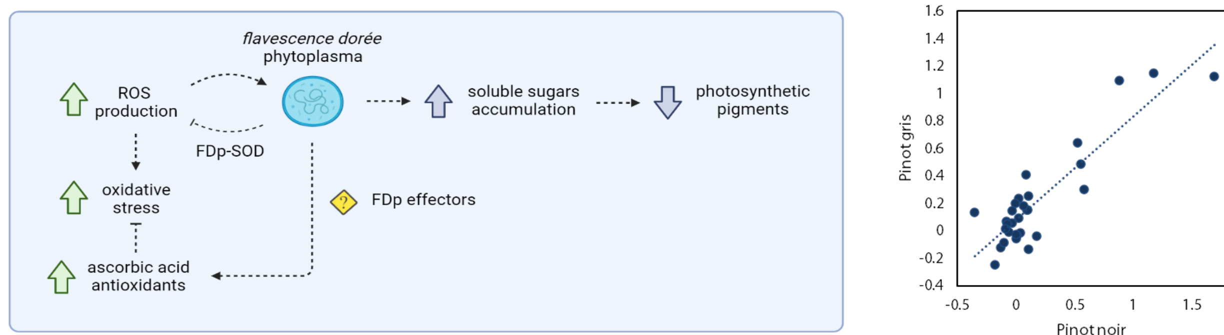

Figure 1A Schematic representation of hypothesized interrelationship between physiological parameters in FDp-infected grapevine based on our currently obtained data**. 1B** Correlation of all parameter values grouped for the ‘Pinot noir’ and ‘Pinot gris’ varieties (r = 0.90899).

ACKNOWLEDGEMENTS

This study was supported by Croatian Science Foundation grants IPS-2024-02-3910 “Unravelling the enigma of ‘*Candidatus* Phytoplasma solani’: Interactions of its effector proteins with host plants” (CapsEffect), IP-2019-04-2469 “Strategies of phytoplasma pathogenicity: effectors, virulence factors and mobile genetic elements” (PathoPhyto) and University of Zagreb financial support.

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Evaluation of GFLV transmission risk in vine nursery plants under exposure to *Xiphinema index*-contaminated soils

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INTRODUCTION

Grapevine fanleaf disease, caused mainly by grapevine fanleaf virus (GFLV) and Arabis mosaic virus (ArMV), poses a significant threat to viticulture worldwide and in Switzerland. The disease, transmitted by *Xiphinema* nematodes and infected plant material, results in substantial yield losses and reduced vine longevity (Schmitt-Keichinger & Fuchs 2025). *Xiphinema* can persist in soil for several years in a quiescent state, surviving harsh conditions after vineyard removal, and remain viruliferous; when environmental conditions favour activity, leading to reinfection even after long fallow periods (crop-free periods or rotations without grapevine) (Demangeat et al. 2005). As a result, stringent certification schemes require nurseries to ensure soils are free from virus-transmitting nematodes, yet current soil analysis techniques are costly and prone to false negatives due to the aggregated, patchy distribution of nematodes.

Pre-cultivation testing is a simplified procedure to full soil analysis that assesses pathogen presence and site history before nursery planting. It's an option for nursery vines, which are in soil only from late spring until late autumn for acclimatization and root establishment. Regulations require no *Vitis* plantings on the site for the previous 5 years. These constraints create significant obstacles for the nursery sector, especially in regions where land availability is limited.

The sector is vulnerable, with a marked decline in new plantings in recent years. Current practice, which is largely derived from vineyard studies, may not adequately reflect the conditions faced by nurseries. This underscores the important need for real, actual data on the risk of GFLV infection in vine nurseries to support sustainable nursery production in Switzerland and beyond.

MATERIALS AND METHODS

This multi-site study aims to assess the risk of grapevine fanleaf virus (GFLV) transmission to nursery plants grown in soils known to be infested with viruliferous *X. index* and to evaluate the effect of increasing fallow duration. GFLV distribution was mapped to identify a 500 m² plot with high infection levels. Comprehensive soil sampling and nematode analyses are underway, with thirty samples to be collected per plot: twenty from the 20-60 cm layer and ten from the 60-100 cm layer. Conducted by nursery staff, to professional nursery standards, vines will be planted each spring over four years on different sections after progressively longer fallow periods: 200 plants in 2026, 600 in 2027, 600 in 2028, and 200 in 2029 per plot. Plants will be lifted in autumn and stored in a cold room. In the following spring, they will be potted and tested during the growing season (year + 1). Plants from the first batch (planted in 2026) will be maintained in pots for two consecutive years to assess latency and identify infections not detected after the first dormancy.

PRELIMINARY RESULTS AND DISCUSSION

The initial phase of the trial has begun with the successful identification and mapping of three experimental vineyard blocks located in distinct Swiss cantons: Block 1 (Geneva, Chardonnay, 30 years old), Block 2 (Vaud, Chasselas, 40 years old), and Block 3 (Neuchâtel, Chasselas, 50 years old). Grapevines in each block have been individually tested by ELISA for the presence of GFLV.

Block 1 had 352 vines with a combined infection rate was 92%, with characteristic leaf malformations and fan-shaped leaves.
Block 2 comprised 352 vines, of which 218 were tested. Over 90% of mature vines were GFLV-positive, and more than 60% of very young replacements (<3 years old) also tested positive, indicating ongoing active transmission. Typical symptoms in mature vines were pale or white patches on older leaves, which are residual signs of the bright yellowing seen in early summer.
Block 3 had 457 vines tested in winter with a 92% infection rate; the selected zone had over 98% infection rate. No symptoms were recorded due to winter assessment.
This project will generate the first systematically collected data on GFLV transmission dynamics in nursery settings cultivated on *Xiphinema*-infested soils in Switzerland. By providing robust, context-specific information on infection risk under actual nursery management practices, these findings may allow more tailored and cost-effective approaches to nursery production. Ultimately, the research aims to support the Swiss vine-nursery sector and to adequately inhibit GFLV spread in Switzerland and similar European contexts.

ACKNOWLEDGEMENTS
The authors would like to thank the nursery owners and vineyard owners who have generously contributed their time and resources to this project. We are also grateful to Nathalie Dubuis, Justine Brodard, Isabelle Kellenberger, Marc Passerat and Léo Bernard, for their meticulous work in the sampling and testing of plant and soil samples.

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Detection of mycovirus-related RNA viruses from grapevine plants by high-throughput sequencing

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INTRODUCTION

Mycoviruses associated to metagenomic studies done on grapevine leaves are well documented since Al Rwahnih (2011). Studies on whole grapevine phylloplane or more detailed analysis on grapevine biotrophic fungi associate mycoviruses describe a wide spectrum of families and genera (Pandey 2018; Moran 2023; Chiapello 2020). The genomic information about these viruses and their biology could be useful when considering their potential application as biocontrol tools against diseases caused by the fungal hosts on a major crop as grapevine (Kondo, 2022; Hough, 2023). Moreover, with the refinement of the knowledge about the biological behaviours of these new viruses, it could be possible the discovery of a double lifestyle of some of them, affecting either the fungi or the plant host. The metagenomic studies in the present work successfully identified several genomic features of recently described mycoviruses, underlining the role of updated information that can allow the retrieval of a correct taxonomic attribution to a wide series of assembled (viral) genomes.

MATERIALS AND METHODS

Plant material and RNA extraction

Ten field-grown symptomatic and non-symptomatic grapevine plants (cv. Cabernet franc) were used in this study for grapevine virome analysis. Total RNAs were extracted from leaf petioles and midribs using PureLink Plant RNA Reagent (Ambion, Life Technologies, USA). RNAs were then cleaned, concentrated and treated with DNase (RNA Clean and Concentrator, Zymo Research, USA).

Metagenomic sequencing and validation

Total RNA preparations were outsourced for high throughput sequencing. Construction of mRNA libraries was using the TruSeq mRNA Library Prep Kit protocol and sequenced on an Illumina apparatus in a paired-end 2 × 100 bp format. Raw reads were quality checked and processed to remove low quality reads (Qscore < 20) and Illumina adapters with cutadapt v 1.8 (Martin, 2011). Quality filtered reads were assembled using SPAdes (Bankevich et al., 2012) with a multiple kmer approach. Annotation of the *de novo* contigs was obtained using DIAMOND v2.1.7 (Buchfink et al., 2021) through a local BlastX search against the NCBI non-redundant protein database. Cross-confirmation of the identified viruses were obtained through a BlastN online search against nucleotide. On these extracts, RT-PCR with specific primers designed on the main contigs produced amplicons that were subsequently sequenced.

Phylogenetic tree

A multiple alignment of the selected assembled genomic sequences was obtained using MAFFT (Katoh et al., 2013). The best fitting substitution models was found using ModelFinder implemented in IQ-tree2 (version 2.3.6; Minh et al., 2020). The substitution model obtained was LG+G4. Maximum likelihood (ML) phylogenies were then inferred using the IQ-Tree2 software. Bootstrap values were calculated using 1,000 replicates. Trees were visualized in FigTree (v1.4.4; https://github.com/rambaut/figtree).

RESULTS AND DISCUSSION

Among the sequences retrieved, which mapped with mycovirus genomes in BlastN or BLastX analysis, several major contigs were identified. We have found a striking identity (>96%) of the contigs N6_ctg355 and the consensus supercontig *def_En-like* with the RNA1 segment (containing V-methyltransferase and RdRp domains) of *Erysiphe necator*-associated ssRNA virus 2, mirrored by the homologous *Plasmopara viticola* lesion-associated vivivirus 3. Also, the RNA 2 of the same viruses (featuring V-methyltransferase and Viral RNA helicase domains) was highly matching (again > 96%) with two other independently assembled contigs (N3_ctg 261 and N4_ctg260). Both these groups of contigs show similarity, but at a much lower degree of aa identity (29%), with the ilarvirus tobacco streak virus, in the same methyltransferase region.
Another group of contigs, represented by N6_ctg7 and N9_336, recognize as BlastX the genome of Plant associated virga-like virus 2 (domains: methyltransferase, RdRP; UTQ50814, UTQ50939) and the helicase domain of *Erysiphe pisi* associated puccinivirus 1 (DBA64267). The extent of identity of these contigs for BlastN (72% for the former virus and 68% for the latter one) and BlastX (76% as aa), suggests that this sequence could belong to a new similar but distinct viral species (~28% similarity with the V-methyltransferase domain of Grapevine leafroll-associated virus 4).
Primers specifically designed on more conserved stretches on the consensus sequences successfully amplified PCR products from the same RNA extracts used for HTS sequencing. The obtained Sanger sequences from those amplicons matched at least as 95% with the original related contigs.
An ML phylogenetic tree was reconstructed on the multiple alignment of the obtained mycoviral proteins, suggesting the presence of three different clade blocks of sequences among the analysed genomes. The RNA2 of the vivivirus seemed to be a basal cluster with the reported methyltransferase and helicase domains, the two more distant clusters of vivivirus RNA1 and replicase ORF of virga-like related sequences are distinct. Finally, a drastic split of one genomic fragment of *E. pisi* associated puccinivirus 1 (coding for V-methyltransferase/viral helicase (DBA64268) was observed.

ACKNOWLEDGEMENTS

This research was granted by Research fund of TUBITAK (Project No: 124 O 041).

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New records for plant viruses, viroids and liberibacters from New Zealand: update 2016-2025

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INTRODUCTION

The Plant Health & Environment Laboratory (PHEL) is responsible for the surveillance of pests and diseases in New Zealand (NZ). In recent years, PHEL has been a leader in its application of high-throughput sequencing (HTS) for plant virus diagnostics (Liefting et al., 2021; Waite et al., 2022; Nunes-Leite et al., 2024), and the first laboratory in NZ to achieve ISO17025 accreditation for using HTS with Oxford Nanopore Technology (ONT) for plant pathogen testing. PHEL published a report on new NZ plant virus, viroid and liberibacter records in 2015 (Veerakone et al., 2015). The data presented here aims at providing information covering new detections since this last publication, up to December 2025.

MATERIALS AND METHODS

Symptomatic plant samples were submitted to the laboratory as part of general surveillance, high-risk site surveillance (HRSS), and domestic testing (e.g. samples submitted as part of high health scheme) activities. For molecular detection, total nucleic acid was extracted using a Kingfisher® mL semi-automated system (ThermoFisher; Waltham, MA, USA) with magnetic beads technology. Detection and validation of new records over the last 10 years were achieved using a range of techniques including transmission electron microscopy, herbaceous indexing, ELISA, PCR/RT-PCR (endpoint and real-time), Sanger sequencing, and HTS. In the last 5 years, detections in symptomatic samples have been achieved mostly with HTS protocols using the ONT platform, followed by validation using PCR/RT-PCR (endpoint and real-time), and Sanger sequencing.

RESULTS AND DISCUSSION

Since our last publication in 2015 to December 2025, PHEL identified 68 new pathogens (64 viruses and 3 viroids, 1 liberibacter) that were reported for the first time in NZ, and there were 43 new host associations for viruses already known to be present in NZ.
Most of the records were associated with samples collected through the HRSS programme, and the remaining samples were received from general surveillance and other domestic testing. Thirty-two new NZ records were identified from ornamental plant species, seven from weed species, seven from native flora, and the remaining 22 from crop species. Five new records (4 viruses and 1 viroid) were identified from grapevine samples, an additional virus detected from an ornamental species can potentially infect grapevine, though there has only been a single report globally of a natural infection in asymptomatic grapevine from 1989. No new records affecting grapevine were made in the last five years, even though HTS has been increasingly used for pathogen identification. Overall, 12 new-to-science viruses were identified in the last 10 years, several of which are yet to be fully characterised. Of the 43 new host records identified, five were novel virus-host associations reported for the first time worldwide. None of these novel finds were associated to grapevine.

ACKNOWLEDGEMENTS

PHEL thanks members of the public and growers for submitting samples.

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Exploring HIPLEX PCR for simultaneous detection of grapevine leafroll-associated virus 3 and grapevine red blotch virus

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INTRODUCTION

Grapevine leafroll-associated virus 3 (GLRaV-3) and Grapevine red blotch virus (GRBV) are among the most important viruses affecting grapevine worldwide (Fust et al., 2025; Cieniewicz and Fuchs, 2025). Traditional diagnostic methods, such as conventional and Realtime PCR, remain reliable but are limited in throughput, cost, and their ability to detect divergent or emerging variants, particularly within the genetically diverse GLRaV-3 complex (Diaz-Lara et al., 2018). HiPlex PCR is an advanced high-multiplex, amplicon-based approach that enables the simultaneous amplification of dozens to hundreds of genomic targets in a single reaction, followed by high-throughput sequencing (HTS) of the resulting amplicons (Costa et al., 2024). To address the limitation of PCR to detect divergent virus strains, a HiPlex PCR approach is under development to enable simultaneous detection of GLRaV-3 and GRBV in a single reaction.

MATERIALS AND METHODS

Comprehensive reference genomic datasets of GLRaV-3 and GRBV were created from the public NCBI database and the internal Foundation Plant Services (FPS) metagenomic data. Based on multiple sequence alignments, a virus-specific primer panel was constructed comprising 56 primer pairs targeting GLRaV-3 and 8 primer pairs targeting GRBV, with degenerate bases and overlapping coverage to ensure inclusivity across viral variants. Additionally, 10 internal control primer pairs targeting *Phaseolus vulgaris* endornaviruses 1 and 2 (PvEV-1, PvEV-2) present in Black Turtle Soup bean plant material spiked into each sample, were added to monitor extraction and amplification performance of the assay (Kesanakurti et al., 2016). The HiPlex PCR strategy followed the multiplex amplicon-based sequencing workflow previously published for fruit tree viruses and viroids (Costa et al., 2024), with assay parameters adapted for grapevine pathogens. A set of 40 grapevine accessions previously characterized by qPCR and/or HTS, including positive, negative, mixed infections, and water controls was used to evaluate the HiPlex PCR assay. Three cDNA replicates were generated per sample. HiPlex PCR reactions, barcoding, pooling, library preparation, and sequencing, were performed by Floodlight Genomics LLC (Knoxville, TN).

RESULTS AND DISCUSSION

HiPlex sequencing generated high and consistent read outputs across the test panel of 40 samples, with median pass-filter counts exceeding 5×10^5 per sample, indicating that the assay performs reliably under highly multiplexed conditions and supports downstream analysis without loss of sensitivity. Among qPCR-negative samples, low read counts were detected, and their distribution allowed us to define a provisional threshold of $\sim1.5\times10^3$ reads to help distinguish background noise from potential viral signal.

In this study, the HiPlex panel successfully detected 28 out of 29 GLRaV-3-positive vines, spanning diverse phylogenetic groups, available in the FPS collection, with one borderline sample (1,279 reads; CT = 19) falling just below the provisional cutoff. For GRBV, all 11 qPCR-positive vines were accurately detected, while one qPCR-negative sample produced a low-level signal (2,156 reads) slightly above the threshold. Given both cases closeness to the threshold, they were interpreted as a borderline case rather than a true false negative/positive cases. Ongoing work includes evaluating the assay across additional dilution levels to refine detection limits, improve primer balance, and further

validate performance under diverse conditions. While optimization is ongoing, these preliminary data support HiPlex PCR as a promising platform for the simultaneous detection of multiple grapevine viruses. With its capacity to test hundreds of samples per sequencing run at a markedly lower cost than multiple qPCR assays on single samples, the method provides a practical and scalable solution for large-scale vineyard testing.

ACKNOWLEDGEMENTS

This research was supported by the California Department of Food and Agriculture (CDFA), Pierce's Disease and Glassy-winged Sharpshooter Board, under Agreement Number 24-0326-000-SA.

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Grapevine virus F, emerging vitivirus in autochonous grapevine cultivars in Turkiye

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INTRODUCTION

The advent and application of high-throughput sequencing (HTS) technologies have allowed the discovery of many new viruses infecting grapevine (*Vitis vinifera* L.). HTS platforms have made feasible the identification of divergent isolates and sequence variants, which, in the case of the grapevine-infecting vitiviruses, have frequently proven to represent novel taxonomic entities. Grapevine vitiviruses are common components of viral populations in Turkish vineyards, with grapevine virus A (GVA), grapevine virus B (GVB), and grapevine virus D (GVD) and grapevine virus L (GVL) having been reported previously (Cigsar et al., 2002; Buzkan et al., 2017, Ilbagi et al., 2021). Nothing is known about the presence of other newly emerging vitiviruses on grapevine in Turkiye so far. During a research programme regarding etiology of leafroll and rugose wood diseases in Turkiye, the application of HTS approaches has led to the detection of grapevine virus F (GVF, gen. *Vitivirus*) in autochthonous grapevine varieties. Therefore, this research was undertaken to investigate GVF prevalence and intraspecific variability on the major grapevine growing areas in Turkiye.

MATERIALS AND METHODS

Four plants of autocthonous cultivars showing viral symptoms were selected for smallRNA enrichment. RNA extraction was obtained according to Gambino et al., (2008) protocol, low molecular weigth RNAs enrichment using PEG (20%, 20mM NaCl) and small RNA portion isolated through acrylamide gel fractioning (Giampetruzzi et al., 2018). Eluted smallRNAs were then quantified, normalized and pooled in pairs. The obtained pools were finally used for the synthesis of two smallRNA libraries according to Illumina TruSeq Small RNA Library protocol. Libraries were sequenced in a 50-base single read run on a HiScan TM SQ (Illumina Inc., USA) platform. Quality checks and adapter trimming of reads were performed using Fastx toolkit (Hannon Lab, Cold Spring Harbor Laboratory, USA). Cleaned reads were used for *de novo* assembly in VELVET (Zerbino and Birney, 2008) and obtained contigs searched for homologies with BLAST(n/x) algorythms (Altschul et al., 1990). A survey was conducted in Turkish grapevine-growing regions to investigate the presence of the virus, collecting leaves from one-year-old shoots. Total RNAs were extracted from 0.2 g of petioles/midribs from infected grapevine leaves according to Rott and Jelkman, (2001). All samples were tested by RT-PCR using specific primers (Panailidou et al., 2019). Obtained amplicons were Sanger sequenced in both directions (Macrogen, The Netherlands).

RESULTS AND DISCUSSION

A total of 4,904,424 and 24,578,645 raw reads were obtained from the two libraries. Quality controls yielded a total of 4,073,246 and 19,891,325 high quality reads, respectively. QC reads were *de novo* assembled by VELVET obtaining a total of 675 and 5281 contigs, respectively. The BLAST(n/x) analysis of the assembled contigs revealed sequences of GVF, among other grapevine infecting viruses (i.e. GVA, grapevine fleck virus, grapevine leafroll-associated virus-1 and -3). A total of 138 samples of autocthonous grapevine varieties from Eastern Mediterranean and Southeast Anatolian vineyards was tested for the presence of GVF with the primer pairs amplifying a 670 bp-fragment

from the RNA-dependent RNA-polymerase gene. GVF was detected in approximately 11.5% of all tested samples. Phylogenetic analysis using the maximum likelihood method, which included the six Turkish isolates and other GVF sequences from GenBank, revealed that the Turkish isolates are closely related and form a distinct clade. Sequenced amplicons showed an identity between 96 to 100% with the isolates from Greece (100%) (MK_490831), South Africa (96%) (KP_114220) and North America (98%) (NC_018458). Notably, the isolate GVF-12 branched separately, positioning itself as an outgroup due to having nucleotide variations from other Turkish GVF isolates.

According to our knowledge, this is the first report for the presence of GVF in grapevine in Turkiye.

ACKNOWLEDGEMENTS
This research was granted by Research fund of TUBITAK (Project No: 124 O 041).

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Molecular investigation of satellite RNAs in grapevine fanleaf virus isolates associated with ‘yellow mosaic’ and ‘infectious malformation’ symptoms in Italy

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INTRODUCTION

Grapevine fanleaf virus (GFLV, *Nepovirus*; *Secoviridae*) is a major cause of grapevine degeneration disease and is known to cause two distinct symptoms on grapevine*, i.e.*, malformation (MF) and yellow mosaic (YM), which are produced, respectively, by distorting and chromogenic strains (Martelli *et al.,* 2001). GFLV genome typically contains two genomic RNA segments (RNA1 and RNA2) responsible for replication, movement, and encapsidation. In addition to these, the genome of some isolates may include a third RNA segment, *i.e*., a satellite RNA (satRNA), ranging from 1094-1140 bp in length, encoding a 37 kDa protein (P3) (Quacquarelli *et al*., 1976; Gottula *et al*., 2013). In GFLV, satRNA molecules show intermittent occurrence and genetic variability among viral strains; however, in contrast to other plant viruses (tobacco ringspot, cucumber mosaic, and Arabis mosaic) studies so far have not established a clear correlation between satRNA presence and symptom expression such as MF or YM (Garcia-Arenal *et al*., 2001; Hily *et al.,* 2018).
This preliminary study aims to investigate whether satRNAs occur preferentially in GFLV-infected YM and/or MF grapevines, to determine their concentration in both infected phenotypes, and to analyze their sequences; providing a basis for further investigation into how satRNAs might contribute to symptom variation and molecular pathogenesis in GFLV infections.

MATERIALS AND METHODS

In May 2021, 60 apparently GFLV-infected grapevine leaf samples showing YM or MF symptoms were collected from eight vineyards in Apulia, Italy. GFLV detection and quantification were carried out using DAS-ELISA, RT-PCR, and RT-qPCR allowing comparison of viral load between different symptom types through statistical analysis. To enable universal detection of GFLV satRNAs, 158 known satRNA sequences from different GFLV-infected grapevine of different varieties and origins were retrieved from the GenBank and aligned, leading to the design of a couple of degenerate primers (named SatUni-s and SatUni-a) able to amplify in RT-PCR a DNA fragment of 866 bp, representing 78% of the satRNA genome. Amplified satRNA fragments were cloned into *E. coli*, sequenced, and analyzed using CLUSTALX, BlastX, and BlastP to determine nucleotide and protein homologies. Sequences of satRNA haplotypes identified in this study were deposited in the GenBank under the accession numbers: OM303023-OM303037) and were compared with 158 known sequences. A phylogenetic tree was generated in MEGA X (Neighbor-Joining, 1000 bootstrap replicates) (Kumar *et al*., 2018) (data shown in Poster).

RESULTS AND DISCUSSION

In the initial serological screening, DAS-ELISA identified 40 grapevine samples infected with GFLV. In contrast, the molecular assays (RT-PCR and RT-qPCR) detected a total of 52 infected samples, with complete concordance between the two methods. Among the GFLV-positive vines, 32 exhibited YM, 15 showed MF, and 4 displayed both symptom types. Using the newly designed satRNA-specific primers, RT-PCR analyses yielded satRNA amplicons in 16 samples, all originating from vines exhibiting YM symptoms. No satRNA was detected in MF-only samples. These findings

suggest a potential association between the presence of satRNA and the expression of YM-type symptoms in GFLV-infected grapevines. Alignment of 15 different haplotypes of sat-GFLV sequences highlighted relevant variance within the isolates, in particular a divergence in size of up to 18 nucleotides, due to certain deletion and/or insertion of sequences in two positions, and a divergence in nucleotide composition. The intra-population comparison yielded an identity of 82.5%-99.8% at nucleotide level and 98.4%-77.1% at amino acid level. The satRNA sequences identified are deposited in the GenBank under the following accession numbers: OM303023-OM303037. The satRNA sequences showed the highest similarity to the Slovenian isolate RefKE1-10 (KR014642), with 96% nucleotide and 94% amino acid identity, and the lowest similarity with the Italian clone P22 (KR014601) by 84% at nucleotide level and the Slovenian isolate Sau_h (KR014645) by 75% at amino acid level.
A phylogenetic analysis based on 75 representative satRNAs sequences retrieved from the GenBank and the 15 satRNAs sequences obtained in this study revealed two main clades, clade II clustering French, South African, and American isolates, and clade I containing sequences from other regions, with the satRNAs from this study distributed across various sub-clades within clade I, showing no specific geographic grouping as previously reported by Čepin *et al.* (2016).
Quantitative PCR analysis of GFLV from both YM and MF phenotypes in symptomatic and asymptomatic leaf tissues showed mean Cq values for the former type ranging from 25.75–34.70 corresponded to 2.86E-02 to 7.15E-05, and Cq values (26.77–37.77) corresponded to concentrations of 1.44E-02 to 9.60E-06 for the later. Further the linear relationship between Cq values and log plasmid DNA ($R^2 = 0.96$) showed a primer efficiency of 95.1%. Statistical analysis revealed no significant difference in GFLV concentration between symptomatic and asymptomatic tissues ($p = 0.801 > 0.05$).
This study highlights the presence of satRNAs exclusively in YM-affected vines (15 out of 32 tested) from different vineyards, and never in MF-affected vines, suggesting a possible relationship between satRNAs and the YM phenotype, although this association remains to be confirmed. The absence may reflect seasonal fluctuations in satRNA accumulation**,** a phenomenon reported for several plant satellite RNAs, although it could not be investigated further within the constraints of this study. Longitudinal sampling and additional analyses are currently underway to clarify whether temporal variation in satRNA levels may account for these discrepancies and to further assess their potential association with the YM phenotype. The analysis revealed no significant differences in GFLV concentration between symptomatic and asymptomatic tissues within the same infected vine, nor among tissues exhibiting different GFLV-related symptoms. These findings indicate that symptom expression is influenced by factors other than viral concentration, consistent with previous research (Vigne *et al*., 2013).

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This One Is Real! Concrete Evidence of BLMoV in Grapevine

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INTRODUCTION

About fifteen Nepovirus species have been reported in grapevine, including grapevine fanleaf virus (GFLV) and Arabis mosaic virus (ArMV), major agents of fanleaf disease, as well as less frequent and often poorly characterized members such as grapevine Bulgarian latent virus (GBLV) and blueberry leaf mottle virus (BLMoV). BLMoV (*Nepovirus myrtilli*) was first described on highbush blueberry in the USA[1]; it is unusual in being transmitted by pollen via bees, with no confirmed nematode vector[2]. Detection of BLMoV in grapevine has been reported from the USA on *Vitis labrusca*[3] and more recently from Korea[4]. Yet, until now no BLMoV sequence from grapevine was publicly available. In addition, an unresolved question remains concerning GBLV isolate CM112[5], which was reported to be serologically distantly related to both GBLV reference isolate and BLMoV, suggesting the existence of further, as yet uncharacterized, diversity within this virus complex. This study provides the first complete genomic characterization of BLMoV in European grapevines, comparing a historical Swiss collection accession originating from Portugal and isolates obtained in France from grapevine material of Portuguese origin.

MATERIALS AND METHODS

Three independent sources were analysed. The first, labelled 'Bulgarian latent' (EAN64), was obtained from O.A. De Sequeira (Estaçao Agronomica Nacional, Oeiras, Portugal) and transferred to Switzerland in 2002. Subsequent microsatellite analysis (December 2024) identified this accession as cultivar Borracal, the same cultivar in which GBLV-CM112 was originally reported. A historical sample (EAN63), sent in 1988 from the same institute as dried *Chenopodium quinoa* tissue, was provided by the same author and was accompanied by a letter describing it as a distant serotype of Bulgarian latent virus. Genome sequencing demonstrated complete identity between EAN63 and EAN64. Based on provenance, cultivar identity, and accompanying historical notes, we consider EAN63 to be the same isolate originally described as GBLV-CM112. The second source comprises two Loureiro blanc accessions (E5 and E6) originally collected in 2016 from a Portuguese vineyard showing fanleaf-like symptoms, both acquired in France by IFV[6]. The third source includes two isolates (TT2016 series), having a common origin but distinct from the E5-E6 isolates. Genome characterization was carried out by HTS. Host range and mechanical transmission experiments used the EAN64 isolate, with infection confirmed by RT PCR and symptom assessment on diverse herbaceous hosts. Inoculation from herbaceous hosts to *Vitis* and *Vaccinium* via *Cuscuta europaea* (dodder) connection is under way to assess transmission under single infection conditions.

RESULTS AND DISCUSSION

Upon mechanical inoculation the EAN64 virus successfully infected *C. quinoa* (local lesions and deformation) and *C. amaranticolor* (asymptomatic), as well as several *Nicotiana* species, including

N. benthamiana, *N. clevelandii*, *N. tabacum* (cv. Xanthii and cv. White Burley), *N. occidentalis* and *N. megalosiphon*, displaying variable symptoms intensity. *N. tabacum* (cv. Samsun), *Cucumis sativus*, and *Phaseolus vulgaris* remained uninfected. Inoculation experiments using *Cuscuta* as a transmission vector are ongoing and will be used to assess the symptoms induced by BLMoV in grapevine in the absence of coinfecting viruses.
Maximum likelihood phylogenetic analysis of Pro-Pol regions demonstrated that all BLMoV isolates identified from grapevine in this study group tightly with the BLMoV reference sequence from blueberry, forming a robust and well-supported cluster within subgroup C of the nepovirus genus. Grapevine isolates share >95% amino acid identity among themselves and approximately 92% with blueberry BLMoV in the Pro-Pol region, confirming their close relationship as members of the same virus species. GBLV, while more distantly related (~77% amino acid identity in Pro-Pol), is positioned within the same subgroup C branch, representing a related but distinct species-level lineage. Similar patterns are observed in the capsid protein (CP), where grapevine BLMoV isolates share >95% amino acid identity among themselves and around 90% with blueberry BLMoV; GBLV displays ~70% identity to BLMoV in CP.
In the Loureiro blanc isolates (E5 and E6), a large RNA satellite of about 1.3 kb was detected, displaying partial homology to the large satellite RNA of Chicory yellow mottle virus (ChYMV). The TT2016 series contained a smaller circular satellite (~450 nt) related to the corresponding ChYMV circular satellite RNA. Notably, the original GBLV-CM112 publication (EAN64) described the presence of a third RNA species, which likely corresponds to the large satellite RNA. However, this satellite RNA was not detected in the sequences obtained here.
These findings provide the first fully characterised genome sequences and authenticated isolates of BLMoV from European grapevine, based on samples from several Portuguese cultivars. While there were a few very early reports of BLMoV presence in grapevine, this study clarifies the historical presence and distribution of BLMoV in Europe and enables further scientific investigations of this quarantine virus in grapevine.

ACKNOWLEDGEMENTS
We thank Laetitia Maillard for performing the microsatellite analysis, Bastien Grolimund for his assistance with mechanical inoculations, Marc Passerat for his help in the *Cuscuta* transmission experiments and Paul Gugerli for initiating the historical work in Switzerland, as well as for his exceptional record keeping, recollection of key details, and enduring enthusiasm for grapevine virus research.

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Metabolic biomarkers of flavescence dorée phytoplasma colonization in symptomless grapevine rootstocks

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INTRODUCTION

Flavescence dorée (FD) is a severe phytoplasma-associated disease affecting *Vitis vinifera* and represents one of the major threats to viticulture in Europe. Most cultivated grapevine varieties are highly susceptible, showing rapid symptom development and mortality upon infection. In contrast, non-grafted rootstocks and certain wild *Vitis* species often exhibit few or no visible symptoms, despite being susceptible to FD phytoplasma (FDp) colonization. Several studies have shown that some of these asymptomatic accessions can harbor FDp, which can be transmitted to scions through grafting, thus acting as potential silent reservoirs of the pathogen (Caudwell *et al*., 1994; Eveillard *et al*., 2016). Understanding the physiological and metabolic responses of rootstocks to FDp colonization is therefore critical to assess both their potential as pathogen reservoirs and their intrinsic defense mechanisms. However, the early detection of FDp in asymptomatic plants remains challenging, as traditional symptom-based diagnostics often fail to capture latent infections confined to woody tissues. In the present study, in addition to symptom observation and molecular diagnostics, leaf-level hyperspectral, biochemical, and UHPLC/QTOF analyses were performed to identify potential secondary metabolites as biomarkers of FDp colonization and to characterize rootstock-specific metabolic responses in infected asymptomatic plants.

MATERIALS AND METHODS

A controlled greenhouse experiment was conducted using 50 one-year-old self-rooted cuttings of two rootstocks, 140 Ruggeri (140RU) and *Vitis rupestris* du Lot (RUP). In summer 2023, *S. titanus* specimens collected from a heavily FD-infected vineyard were caged on the basal section of one-year-old shoots. FDp inoculation was also performed on grafted Chardonnay as a control. Plants were monitored for symptom development over three growing seasons (2023–2025) and potentially inoculated plants were sampled in June 2024 and January 2025 to assess FDp presence in woody organs (two-year-old cane, trunk, and roots) via RNA extraction. In June 2025, eight healthy (140RU_H, RUP_H) and eight FD-infected (140RU_FD, RUP_FD) plants per rootstock were selected. Spectral data were collected from the fifth and sixth leaves using a SpectraVue Leaf spectrometer (360–1100 nm) and a SPECTRO xSORT X-ray fluorescence (XRF) analyser to quantify vegetation indices and the concentrations of essential macro- and micronutrients, respectively. The same leaves were then ground using liquid nitrogen for hydrogen peroxide quantification (Gambino *et al*., 2013) and UHPLC/QTOF metabolomic analysis (De Rosso *et al*., 2025).

RESULTS AND DISCUSSION

All inoculated highly susceptible Chardonnay plants tested positive for FDp in both leaves and woody tissues, developed symptoms, and died within one year. For the rootstocks 140RU and RUP, FDp was detected in woody organs or roots of 23 plants (11/16 and 12/18 inoculated, respectively), 10 of which remained asymptomatic. During the 2024 growing season, only four plants (two per

variety) displayed leaf symptoms associated with FDp, while 15 showed desiccations of two-year-old canes, with asymptomatic shoots emerging from the upper trunk.
In June 2025, plants colonized by FDp in woody organs or roots, which did not show symptoms, were analyzed to identify potential biomarkers of FD infection.
Leaf hydrogen peroxide content, a known recovery marker in *Vitis vinifera*, did not differ between healthy and FD-infected rootstock plants. In contrast, UHPLC/QTOF metabolomic profiling distinguished both rootstock varieties and, within each, healthy from FD-infected plants. Targeted identification provided around hundred leaf secondary metabolites with i.d. score >80%, belonging to the chemical classes of flavan-3-ols/procyanidins, benzenoids, flavones/flavonols/flavononols, terpenols, norisoprenoids, stilbenes, and hydroxycinnamyltartaric acids. Potentially, these compounds are involved in plant defense. The metabolomic profiles had distinct patterns between the two rootstock genotypes and differentiated between healthy and FD-infected plants. In particular, in 140RU the infected plants had higher signal intensities of main terpenoids (nerol, geraniol, and linalool glycosides). In contrast, RUP_FD samples displayed a reduced abundance of several phenolic compounds, particularly hydroxycinnamic acids, flavan-3-ols, and procyanidins, compared with healthy plants. This inhibition of phenolic metabolism, also observed by Davosir *et al.* (2024) during early FD infection when pathogen titres are low and symptoms absent, is consistent with the asymptomatic state of RUP_FD leaves, where FDp remains confined to woody tissues.
Such contrasting metabolic responses suggest genotype-specific defense strategies and provide insights into the mechanisms underlying asymptomatic FDp colonization. These findings underscore the importance of integrating molecular, physiological, and metabolomic approaches to understand latent FD infections in rootstocks and their implications for disease management in vineyards.
The combined interpretation of the two spectral analyses in leaf samples reveals a consistent differentiation between the rootstocks 140RU and RUP, the PCA based on leaf spectral indices being able to explain more than half of the total variance. More focused elaboration in the sanitary status are ongoing.

ACKNOWLEDGEMENTS

This work was funded by the Veneto region through the project FD.CONTROL (DGR no. 69, 14 September 2022).

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Grapevine Pinot gris virus (Trichovirus pinovitis) - a new threat to Portuguese wine-regions

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INTRODUCTION

Grapevine Pinot Gris Virus (GPGV), in the species *Trichovirus pinovitis* is an elongated virus that invades and multiplies in the vascular tissues of grapevines and has a linear, single-stranded, positive-sense RNA genome of approximately 7,259 nucleotides. The first observations of symptoms such as late budding, short internodes, stunting, deformation, and chlorotic spots on the leaves of the Pinot Gris variety occurred in vineyards in Slovenia in 2001 and in vineyards in Trentino, Italy, in 2003, causing a disease called "Grapevine leaf mottling and deformation disease" (Saldarelli et al 2017). The virus is transmitted between vines by grafting, the circulation of vegetative propagation material appears to be the main means of long-distance virus dispersal. Similar to the transmission method of other *Trichovirus*, mite galls/nests were collected from grapevine buds and leaves, identifying the eriophyid mite *Colomerus vitis,* thus suggesting that this mite is a potential vector of GPGV in grapevines (Malagnini et al.,2016).
The presence of GPGV in samples collected during a screening in Portugal in 2014 is referenced by Bertazzon et al. (2016). The same study reports that samples collected in the same type of screening in Portugal in 2005 were not infected with the virus. In this work we present de results of the detection of GPGV in samples collected in different wine regions of Portugal.

MATERIALS AND METHODS

All plant material was collected in the Spring of 2025. Leaves were collected from two different clones (I-VCR-478 and MDR2) of the grapevine variety Aragonêz (also known as Tempranillo) showing deformation and chlorotic spots in a two-year-old vineyard in the “Alentejo” wine region. Samples with similar symptoms were collected from the Loureiro variety in the “Vinho Verde” wine region and from the Pinot Noir variety in the “Lisboa” wine region. Asymptomatic samples were also collected from old vineyards in the “Douro” wine region and from Riesling in the “Lisboa” wine region. This later variety even though without leaf symptoms had a historical of very low production. All samples were tested for GPGV by ELISA (enzyme-linked immunosorbent assay) with the antisera provided by Bioeba,AG (Switzerland). All samples were also tested by ELISA for 10 viruses of the infectious degeneration complex of the genera *Nepovirus* and *Stralarivirus* (family *Secoviridae*) due to the possible similarity of symptoms with antisera provided by Agritest SA (Italy) and Bioreba AG (Switzerland).
The presence of GPGV was verified through by end poit Polymerase Chain Reaction (PCR) with virus-specific primers published by Saldarelli et al (2015). Total plant RNA was extracted with the E.Z.N.A.TM Plant RNA Kit (Omega Bio-tek, USA). Synthesis of cDNA using a iScript cDNA First Strand Synthesis KitTM (Bio-Rad, USA) was done according to the manufacturer’s protocol. PCR reactions were performed with NZYTaq II 2x Green Master Mix (NZYTech, Portugal).

RESULTS AND DISCUSSION

The plants tested were positive by ELISA and PCR for GPGV. All plants were negative by ELISA for the 10 viruses tested of the infectious degeneration complex of the genera *Nepovirus* and

Stralarivirus. The results presented in this work were the first detection of Grapevine Pinot Gris virus in the Plant Virology Laboratory from the INIAV-Portugal.
The symptoms of GPGV are more pronounced at the beginning of the vegetative cycle but from the end of May to the beginning of June, the leaves develop normally. There is a clear difference in symptom expression between varieties (Figure 1) and within these differences between clones of the same variety (Figure 1-A, B). The acidity level of grapes from infected vines is higher compared to grapes from disease-free plants and so asymptomatic plants can be a strong clue to identify GPGV as was the case with the Riesling in the “Lisboa” wine-region. Samples collected in four-key wine-regions of Portugal were positive for GPGV, thus showing that the virus is presence in the most important wine-producing regions of Portugal. However, a previous survey of 5% of all the clones certified in Portugal in the year 2019 tested negative for GPGV giving some assurance to the selected process of obtaining certified grapevines. Nevertheless, the common presence of feral *Vitis* hybrids (Diaz et al., 2021) as well as other occasional hosts like herbaceous plants (*Asclespias syriaca*, *Chenopodium album*, *Rosa*, *Rubus*, and *Silene latifolia*) and trees (*Aillanthus altissina*, *Crataegus*, *Fraxinus*, and *Sambucus*) (Gualandri et al., 2016) in the flora of Portugal constitutes an increased risk of contamination with GPGV.

Figure 1. Grapevines with GPGV. A) Aragonez (Tempranillo) clone I-VCR-478 with severe symptoms like stunting, deformation, and chlorotic spots on the leaves. B) Aragonez (Tempranillo) clone MDR2 with asymmetric display of symptoms C) Riesling asymptomatic.

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Prevalence and molecular characterization of grapevine satellite virus in Greece

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INTRODUCTION

Grapevine satellite virus (GV-Sat, genus *Virtovirus*, family *Tomosaviridae,* order *Tombendovirales*) is an agent infecting grapevines in mixed infections with other viruses first identified by HTS in the USA in 2013 (Al Rwahnih et al., 2013). Its presence has been confirmed in several countries, including Iran, Slovenia, Hungary and Russia, and until now it has been considered to occur at very low rates (Gholampour et al., 2024; Shvets et al., 2022). Grapevine leafroll-associated viruses (GLRaVs) are believed to have helper virus function for GV-Sat at least for its replication (Miljanic et al., 2021). Its small linear ssRNA genome (1060 nt for the reference sequence NC_021480.1) encodes two open reading frames (ORFs), one of which encodes for the coat protein (CP), used in the phylogenetic analyses for GV-Sat characterization. During an extensive study of the grapevine virome in Greece by HTS, GV-Sat was among the most frequently identified viruses. This observation prompted us to further confirm the HTS obtained data by RT-PCR and Sanger sequencing. Phylogenetic analyses followed, as well as an attempt to correlate its presence with GLRaV-1 on 229 samples.

MATERIALS AND METHODS

Grapevine samples from different regions of the country were collected during the last six years. Total RNA was extracted with a CTAB based protocol from leaf tissues and cambial scrapping cuttings. Samples originating from either individual or pooled vines were selected and further analyzed by RNA-Seq using standard procedures by the Greek Genome Center (BRFAA) or by Macrogen Inc. (Seoul, S. Korea). De novo assembly of the obtained reads was performed with rnaSPAdes and BLASTn algorithm was used for the annotation of the obtained contigs. HTS data for GV-Sat presence were confirmed by RT-PCR with primers GVsat F1 - 5'-CTGAAAGAGATTGCGTTGTG-3', GVsat R1 - 5'-ACGGATGTGTAAATGCTAC-3' and GVsat R2 - 5'-ACGGGTGAGTTAAGACGAC-3' generating a 544 bp amplicon. Construction of the phylogenetic tree was achieved with MEGA11. Detection of GLRaV-1 was obtained by RT-qPCR (Morán et al., 2023, Panailidou et al., unpubl. data).

RESULTS AND DISCUSSION

GV-Sat was identified in 20 out of 40 HTS analyses. Table 1 shows the results obtained by HTS, RT-PCR and Sanger sequencing. GLRaV-1 was the virus that showed the strongest correlation with GV-Sat being present in 19 out of the 20 HTS libraries.

Table 1. Grapevine sample origin, HTS data, Sanger confirmation and GLRaV-1 presence

No	Region	Cultivar	Library code	Best Match	Coverage (%)	RT-PCR	Sanger seq. & identity with HTS (%)	GLRaV-1
1	Thermi	Cardinal	**Ag10**	ON567245	100	+	100	+
2	Amyntaio (1)	Limniona	**AMN22**	PQ736719	100	+	100	+
3	Amyntaio (2)	Limniona	AMN24	PQ521021	100	+	100	+
4	Goumenissa	Assyrtiko	X17	PQ521022	100	+	100	+
5	Crete	Vidiano	NTV-66	MH802034	94	+	100	-
6	Crete	Tsardana	**NTV-77**	PQ736719	100	+	100	+
7	Crete	Kotsifali	**NTV-83**	MH802034	78	+	100	+

8	Samos	Muscat	NTV-94	MH802035	100	+	100	+
9	Nemea, Leontio (1)	Agiorgitiko	A8	PQ521020	99.7	+	ND[a]	+
10	Nemea, Leontio (2)	Agiorgitiko	**C3**	PQ736719	100	+	ND	+
11	Nemea, Leontio (3)	Agiorgitiko	**POOLB**	ON567245	99	+	99,4	+
12	Nemea, Koutsi	Agiorgitiko	**D2**	PQ521020	100	+	100	+
13	Nemea, Asprokam. (1)	Agiorgitiko	G7	PQ521020	84, 9[b]	-	ND	+
14	Nemea, Asprokam. (2)	Agiorgitiko	H8	PQ521020	100	+	100	+
15	Old Nemea (1)	Agiorgitiko	**K5**	PQ521020	100	+	100	+
16	Old Nemea (2)	Agiorgitiko	L6	PQ736719	100	+	ND	+
17	Mantinia, Zevgolatio	Moschofilero	N1	ON567245	98	+	ND	+
18	Mantinia, Agiorgitiko	Moschofilero	Q7	MH802034	73, 9[b]	-	97,7	+
19	Mantinia, Milia	Moschofilero	S1	ON567245	99	+	100	+
20	Mantinia, Parorio	Moschofilero	M5	PQ736719	100	+	100	+

[a] Not determined, [b]2-3 contigs only

Interestingly, eight of the above libraries (in bold, Table 1) contained a second GV-Sat variant/isolate. Phylogenetic analysis (Fig. 1) of the CPs (longest translated protein) of the 18 nearly/complete GV-Sat Greek isolates showed considerable sequence variation. The accompanying variants (Library code_b) all clustered together in the same clade as two GV-Sat isolates (Acc. numbers MZ291913, MH802033) from Canada.

A further survey to estimate the prevalence of GV-Sat and its correlation with GLRaV-1 in Greek vineyards was conducted and RT-PCR/qPCR results are shown in Table 2.

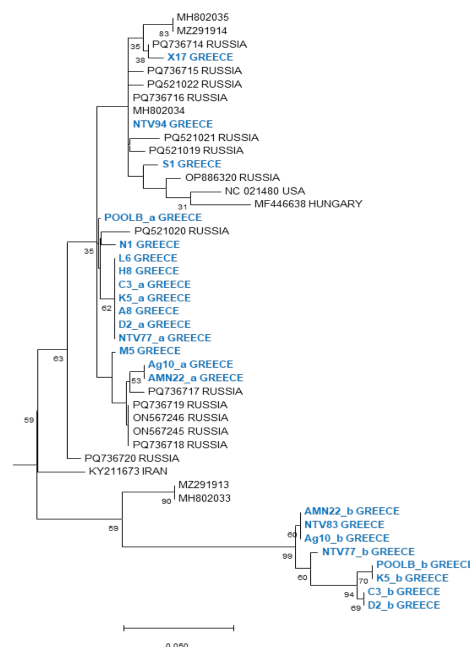

Fig.1. Maximum likelihood tree obtained with Greek (in blue) GV-Sat coat protein amino acid sequences and all currently available sequences in GenBank. Satellite tobacco mosaic virus (STMV, *Virtovirus tabaci*) was the outgroup (Acc. number M25782, not shown).

Table 2. GV-Sat prevalence in Greek grapevines and correlation with CLRaV-1.

Region	GV-Sat	GLRaV-1	Coinfection in GV-Sat^{+}	Coinfection in GLRaV-1^{+}
Thermi	1/31	4/31	1/1	1/4
Amyntaio	6/27	14/27	6/6	6/14
Crete	12/20	12/20	12/12	12/12
Naoussa	0/15	0/15	-	-
Nemea	22/29	26/29	22/22	22/26
Mantinia	11/27	16/27	11/11	11/16
Santorini	2/19	2/19	2/2	2/2
Epanomi	0/9	0/9	-	-
Tyrnavos	0/6	4/6	-	0/4
Xanthi	2/4	2/4	2/2	2/2
Goumenissa	1/5	2/5	1/1	1/2
Samos	7/17	7/17	7/7	7/7
Serres	2/20	1/20	1/2	1/1
	66/229 (28.8%)	**90/229 (39.3%)**	**65/66 (98.5%)**	**65/90 (72.2%)**

Prevalence of GV-Sat in Greek grapevines was estimated at 28.8%, considerably higher than what is reported so far from other countries. Correlation with GLRaV-1 was achieved for 98.5% of GV-Sat positive samples indicating that GLRaV-1 may act as the helper virus of GV-Sat. Furthermore, 72.2% of GLRaV-1 positive samples were coinfected with GV-Sat. Further research is needed to accurately define the potential impact of GV-Sat on grapevines.

ACKNOWLEDGEMENTS

This work was part of the research program "Emblematic Roads of the Greek Vineyards" and it was funded by the General Secretariat for Research and Innovation. Moreover, it was partially funded by Nativine-TAEDK-06183 carried out within the National plan Grece 2.0 funded by EU-NextGeneration EU under the call Research-Create-Innovate.

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