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A comprehensive review of *Grapevine Pinot gris virus* (GPGV)



FINAL REPORT TO WINE AUSTRALIA

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List of Definitions

Asymptomatic:	Atypical symptoms not normally associated with a particular virus
Biological indexing:	Inoculating a sensitive indicator plant (herbaceous and/or woody) with a candidate plant and observing for symptom development
CP:	Coat protein - a protein that forms the capsid which packages the viral genome
Deep sequencing:	A high-throughput method used to determine the nucleotide sequences present in a sample and capable of processing multiple DNA sequences in parallel. (Also known as next generation sequencing, high-throughput sequencing)
ELISA:	Enzyme-linked immunosorbent assay – an antibody based pathogen detection method
Homogenate:	A suspension of cell fragments and cell constituents obtained when tissue is ground in a buffer
Genome:	The complete set of genes or genetic material present in a cell or organism
Germplasm:	A living genetic resource that is maintained for plant breeding, preservation, and other research uses, which may contain grapevines of known or unknown disease status
GPGV:	*Grapevine Pinot gris virus* - a previously undescribed and novel grapevine infecting virus species detected using recently developed deep sequencing technologies
GLMD:	Grapevine leaf mottling and deformation; set of symptoms associated with GPGV infection
LAMP:	Loop-mediated isothermal amplification – a technique for the amplification of DNA at a single temperature
Mothervine planting:	(= foundation planting) A collection of grapevines established from the nucleus collection that are inspected and tested for prescribed pathogens and which are generally used to establish source blocks
MP:	Movement protein - a viral protein that enables movement of a virus from cell to cell
Nucleus collection:	A germplasm of selected grapevine varieties, clones and rootstocks which are true-to-type and in which prescribed pathogens have not been detected
PCR:	Polymerase chain reaction - a method of making multiple copies of a DNA sequence, involving repeated reactions with a polymerase
Phylogenetic analysis:	A means of estimating the evolutionary relationships between species
Primer:	Short fragment of single stranded DNA (15-30 nucleotides in length) that are complementary to DNA sequences that flank the target region of interest for detection and amplification by PCR
Resistant:	Unable to be infected by a pathogen
RdRp:	RNA depended RNA polymerase - an enzyme that catalyzes the replication of RNA from an RNA template

RT-PCR:	Reverse transcription-PCR
Sensitive:	Expressing symptoms when infected with a pathogen
Source block:	(= increase block) - A collection of grapevines ideally established from mothervines that are inspected and tested for prescribed pathogens and from which cuttings are taken for nursery production
Susceptible:	Can be infected with a pathogen
Symptomatic:	Showing symptoms that are associated with, or typical of, a virus infection
Symptomless:	No symptoms shown
Titre:	Concentration of a virus in a plant
Tolerant:	Able to be infected by a pathogen but not expressing disease
Vine Improvement:	The supply and continual improvement of grape vine planting material to deliver optimum vine performance in vineyard production
Virome:	Collection of nucleic acids, both RNA and DNA, that make up the viral community in an ecosystem, such as a grapevine
Virus isolate:	Viral sample that has been obtained from an infected individual and may contain one or more strains
Virus strain:	A virus variant that differs genetically from the reference virus (type variant) that may or may not confer a different, observable, phenotype of infection

Abstract

A comprehensive review of *Grapevine Pinot gris virus* (GPGV) was undertaken to become better informed of the risk of the virus to Australian viticulture. Gaps in the knowledge of GPGV biology and epidemiology and its impact on Australian viticulture were identified. Recommendations were developed for future research in Australia to improve our understanding of GPGV in the Australian environment and development of management strategies that are relevant locally. Preliminary management strategies were identified that can be implemented throughout the Australian viticultural supply chain to minimise risk of GPGV. A small "Preliminary Sampling Trial", which assessed sample type, amount and timing for accurate GPGV detection, identified diagnostics tests to support these management strategies.

Executive Summary

This project was initiated in response to industry concern about the detection of *Grapevine Pinot gris* virus (GPGV) in several wine grape varieties (2016, 2017) and one table grape variety (2018) in three Australian states. The overarching purpose was to become informed and:

- Understand the risk of GPGV to Australian grape and wine industries
- Empower the nursery and vine improvement sector to adopt appropriate management strategies to maintain high-health status of planting material and minimise spread of GPGV
- Empower growers to manage infected blocks and to minimise spread of GPGV
- Identify gaps in knowledge of the biology and epidemiology of the virus and identify priorities for future research and development about GPGV in Australia.

Significant research has been undertaken in Europe, especially in Italy where GPGV was first described and associated with a disease called grapevine leaf mottling and deformation (GLMD) affecting the cv. Pinot gris in 2012. The association between GLMD and GPGV is complex and remains unclear. A review of the literature identified that GPGV infects a broad range of wine, table and rootstock varieties but GLMD is reported to affect only some of these. Some varieties that can be affected by GLMD do not always show the disease, even though they are infected by GPGV. Overseas, different groups of GPGV strains are often associated with either symptomatic or asymptomatic grapevines, but the relationship between GPGV strain and symptom expression does not always hold. Reduced yield and growth is reported in some varieties that are affected by GLMD. GLMD, associated with GPGV, is not known in Australia, although no extensive surveillance has been done for the virus or the disease. Therefore, it is not known if GPGV impacts production or vineyard sustainability in Australia.

Infected *Vitis vinifera* and rootstock propagation material has led to the global dispersal of GPGV and the virus is now known to occur in grapevines in Asia, Europe, North America, South America and Australia. Although GPGV may have been present in a few countries prior to 2003, the movement of GPGV globally, and especially in Europe, is thought to have occurred since 2003. The introduction of GPGV into Australia is unknown, but this event is more likely to have taken place after 2003, in line with the spread into other countries and before 2014, when GPGV diagnostic testing was introduced by Australian post entry quarantine.

Research suggests that GPGV can spread rapidly once it is introduced into a new region or vineyard and it has been demonstrated that grape leaf bud and blister mites (*Colomerus vitis*) can transmit GPGV between grapevines. These mites are prevalent in many Australian grape growing regions and may lead to the broader dissemination of GPGV if they are not adequately controlled. GPGV has been detected in several alternative plant species in Europe that also occur in Australian vineyards, however, the importance of these alternative hosts as a reservoir from which mites can acquire and transmit GPGV to grapevines is unknown.

GPGV is detected using molecular methods such as reverse transcription-polymerase chain reaction (RT-PCR) and many tests have been developed. As a part of this project a “Preliminary Sampling Trial”, which assessed sample type, amount and timing for accurate GPGV detection, showed that reliable GPGV detection by RT-PCR is affected by genetic diversity amongst GPGV strains. The results supported the verification of four RT-PCR assays developed in Italy and these tests are recommended for detection of GPGV in Australia until further work is done to assess the genetic diversity of Australian GPGV strains to inform accurate diagnostic testing. In the “Preliminary Sampling Trial” GPGV was detected in autumn, winter and spring in one variety grown in a cool climate and another

variety grown in warm climate. Further work is required to assess test reliability in summer at both sites. There is also a need to conduct a similar trial over several seasons using more varieties to confirm the most reliable time point to conduct GPGV testing.

Literature on current management strategies for GPGV and GLMD for the vineyard or nursery sectors is limited. However, preliminary management strategies that can be implemented immediately to control spread of GPGV in Australia include:

- Incorporation of GPGV testing in certification schemes to facilitate movement of high-health pathogen-tested material in which important viruses, including GPGV, have not been detected
- The use of grapevine tissue culture and/or an insect-proof screenhouse to hold high-health germplasm can minimise risk of infection
- Virus elimination can be undertaken to produce virus-free mother grapevines
- Bud and blister mite control in all vineyards, including nurseries.

A pest risk analysis was undertaken to assess the risk of entry, establishment and spread of GPGV in Australia. The assessment of consequences step was not completed because there are significant knowledge gaps about GPGV biology, epidemiology and the full impact on production and sustainability both overseas and in Australia. However, the information was used to identify critical control points and control measures within the nursery and vine improvement sector and the broader wine and grape industries for maintenance of vine health and to minimise risk of GPGV.

This review identified the following gaps in the knowledge of GPGV in Australia:

1. The geographic and varietal distribution and prevalence of GPGV and GLMD
2. The most appropriate field sampling strategy and reliable diagnostic methodologies for GPGV detection
3. The genetic diversity of GPGV and its impact on detection and symptom expression
4. Varietal response to GPGV infection on its own and with other viruses
5. The agronomic impact of GPGV and GLMD in Australia
6. The Australian vectors of GPGV and their management
7. Identification and role of alternative hosts
8. Developing GPGV free germplasm.

Future research to address these gaps is required so that adequate management strategies, that are specific for Australia, can be developed to control and minimise risk from GPGV and which can be implemented throughout the Australian viticultural supply chain. In reviewing the Australian response to the Australian GPGV detection it was also identified that there is need to foster and retain current and future knowledge, capacity and capability in pest and disease research for Australian viticulture to support the day to day management of endemic pests and disease and to be able to adequately respond to exotic pest interceptions and incursions.

1.0 Background

Grapevine Pinot gris virus (GPGV) is a relatively new to science virus that was first described in 2012 (Giampetruzzi *et al*., 2012). It was associated with a disease called grapevine leaf mottling and deformation (GLMD) in the cv. Pinot gris in Italy. GPGV was subsequently detected in many countries and in many other grapevine varieties, some of which had GLMD and some which were asymptomatic. Significant yield losses have been observed in GLMD affected grapevines where GPGV was also detected.

GPGV was detected for the first time in Australia in 2016, in several grapevine varieties in New South Wales and South Australia (Wu and Habili, 2017). It was later found in Victorian vineyards in 2017 (F.E. Constable, unpublished) and in further vineyards in South Australia in the same year. In early 2018, the Consultative Committee on Emergency Plant Pests (CCEPP), in consultation with industry, deemed that GPGV was not technically feasible to eradicate and the virus was declared as established and present in New South Wales, South Australia and Victoria. It is no longer regulated at the Australian border.

2.0 Project Aims

The association of GPGV to disease, and the impact on grapevine production in Australia has not been studied. The aim of this review is to identify knowledge relevant to Australia and gaps in understanding on the biology, epidemiology and diagnostic methodology for GPGV.

A preliminary project, "The Preliminary Sampling trial", is also being undertaken in conjunction with this review and is funded by Wine Australia, Vine Improvement organisations, Vine Industry Nursey Association and Vinehealth Australia. It aims to provide initial information about the reliability of GPGV detection in Australia by testing different sample types and sample amounts collected from two different varieties each grown in a different climatic region. The seasonality of detection is being investigated by testing samples collected in autumn, winter and spring of 2018.

3.0 Method

A comprehensive review of published literature and personal communication with individual researchers was undertaken to identify knowledge relevant to Australia and gaps in understanding on the biology, epidemiology and diagnostic methodology for GPGV.

Dr. Constable attended the 19th International Council for the Study of Viruses and Virus-like Diseases of the Grapevine (ICVG) Conference, 9-12 April 2018 in Santiago Chile, and information gathered at the conference, including abstracts and personal communication with international researchers, was used to inform this project.

A risk analysis of GPGV was completed based on information gathered in the literature review, to determine the threat of GPGV to Australian vineyards, with prioritised actions for industry stakeholders (Appendix 5).

A preliminary trial was done to assess effective, reliable sampling strategies to support accurate GPGV detection in propagation material (Appendix 6).

Communications about GPGV and updates on the review and sampling trial were disseminated to the industry (Appendix 1), including:

- An updated GPGV fact sheet with new knowledge on biology, epidemiology and management

- Information for the nursery and vine improvement sector on management of propagation material with respect to GPGV for the 2018 cutting season and beyond
- Industry focussed communications for stakeholders on major findings (E-news articles, website updates, fact sheet, industry journal articles; in production).

4.0 A comprehensive review of *Grapevine Pinot gris virus*

4.1 Introduction

Grapevines with virus-like symptoms were noticed in 2001 on the varieties Pinot gris and Sauvignonasse, in vineyards in western Slovenia (Mavrič Pleško *et al*., 2014). The grapevines had poor growth, shoots with shortened internodes and leaves that were poorly developed with mottling and deformation symptoms. Similar symptoms were noted on Pinot gris in a vineyard in Trentino, northern Italy, in 2003 (Giampetruzzi *et al*., 2012). This disease was called grapevine leaf mottling and deformation (GLMD). In Slovenia and Italy no known pathogens could be associated with the disease using existing detection methods such as electron microscopy, enzyme linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR). However, in 2012 Italian researchers reported the discovery of *Grapevine Pinot gris virus* (GPGV), a previously undescribed and novel grapevine infecting virus species, in Pinot gris affected with GLMD, using recently developed deep sequencing technologies (Giampetruzzi *et al*., 2012). GPGV has subsequently been found in many countries and on most continents (Table 1).

GPGV is a member of the genus *Trichovirus* in the subfamily *Trivirinae,* family *Betflexiviridae* (Giampetruzzi *et al*., 2012). It is a positive-sense single-stranded RNA virus and the type strain (Genbank accession FR877530) is 7275 nucleotides excluding the poly A tail. Like other members in the *Trichovirus* genus, it has three overlapping open reading frames (ORFs): ORF1 encodes a replication protein with conserved methyltransferase, helicase and RdRp domains; ORF2 encodes a movement protein; and ORF3 encodes the coat protein.

4.2 Geographic distribution

GPGV has been detected in many different grapevine varieties and in rootstocks of grape growing regions of many countries in Asia, Europe, North America, South America and in three states of Australia (Table1; EPPO Global Database; Bertazzon *et al*., 2015b; Wu and Habili, 2017; F.E. Constable, unpublished). Movement of the virus within and between countries and continents is most likely through the movement of infected propagation material or by viruliferous vectors.

Table 1. Worldwide distribution of *Grapevine Pinot gris virus* (adapted from the EPPO Global Database, https://gd.eppo.int/taxon/GPGV00/distribution).

Continent	Country	Winegrape, table grape and rootstock variety	Reference
America	Brazil	Many	Fajardo *et al*., 2017
America	Canada: British Columbia Ontario	Syrah, Cabernet Franc, Pinot gris, Riesling, Vidal blanc	Poojari *et al*., 2016; Xiao *et al*., 2016
America	Chile	Grenache 136	Medina *et al*., 2018
America	United States of America	Cabernet franc, Cabernet Sauvignon, Carmenere, Chardonnay, Malbec, Merlot,	Angelini *et al*., 2016

Continent	Country	Winegrape, table grape and rootstock variety	Reference
		Pinot noir, Petit Verdot, Sauvignon blanc, Sauvignon Musqué, Viognier, Zinfandel, as well as other varieties Rootstocks: 1616C, Schwarzmann,	Al Rwahnih *et al*., 2018
America	Uruguay	Tannat	Jo *et al*., 2015
Asia	China: Guangdong, Guangxi, Hainan	Shine Muscat; Beta rootstock, Red Globe, Merlot, Muscat of Hamburg, Cabernet franc, Moldova	Fan *et al*., 2016; Lou *et al*., 2016
Asia	Georgia	Goruli mtsvane, Khikvi, Mtsvane kviteli, Saperavi pachkha, Tavkveri, Korkaula	Casati *et al*., 2015
Asia	Korea, Republic	Tamnara (*Vitis vinifera* x *V. labrusca*)	Cho *et al*., 2013
Asia	Pakistan	Wei-duo-li-ya, Bai-ji-xin, Zi-de qv, Zi-de qv, Gui fei-me gvi and self-rooted grapevines SL12, SL13, SL14, SL45, and SL53	Rasool *et al*., 2017
Europe	Bosnia	Unknown	Bertazzon *et al*., 2016b
Europe	Croatia	Jarbola, Sansigot, Žlahtina	Bertazzon *et al*., 2016b; Vončina *et al*., 2017
Europe	Czech Republic	Chardonnay, Kodrjanka, Laurot, Müller-Thurgau, Pamjati Negrula	Bertazzon *et al*., 2016b; Eichmeier *et al*., 2016b; Glasa *et al*., 2014
Europe	France	Pinot noir, Pinot Meunier, Grenache, Merlot on Gravesac rootstock, Carignan, 94 accessions – 75% of accessions infected, including wild *V. vinifera*	Bertazzon *et al*., 2016b; Beuve *et al*., 2015; Spilmont *et al*., 2018
Europe	Germany	Riesling	Reynard *et al*., 2016
Europe	Greece	Shiraz, Malvasia, Merlot, Traminer, Μαυρόστυφο, Μαυροθηρικό (Maurothiric) Θραψαθάρι (Thrapsatari), Κορινθιακή σταφίδα (Corinthian Raisin) Superior, Ξινόμαυρο (Xinomavro), Πέλλας (Pella)	Bertazzon et al., 2016b; Zikou, 2018

Continent	Country	Winegrape, table grape and rootstock variety	Reference
Europe	Hungary	Olasz rizling – Welschriesling, Chardonnay, Cabernet Sauvignon, Csabagyöngye - Pearl of Csaba, Merlot, Generosa (new variety), Kadarka, Furmint, Purcsin, Balafánt, Sárga Ortliebi, Kék Bakator, Fehér Járdovány, Hárslevelű, Sárgamuskotály Rootstocks: Teleki-Kober 125 and Teleki-Kober 5C (rootstocks)	Czotter *et al*., 2018
Europe	Italy	Ancellota, Black Magic, Cabernet franc, Cabernet Sauvignon, Cardinal, Carmenere, Chardonnay, Fiano, Glera (Prosecco), Incrocio Manzoni 2-15, Malvasia, Merlot, Monica, Moscato, Nasco, Negro amaro, Pinot grigio (gris), Pinot nero (noir) Raboso Piave, Regina, Rossignola, Sagrantino, Sangiovese, Sauvignon blanc, Supernova, Syrah, Tocai friulano, Tocai rosso, Torbato, Traminer, Verduzzo Trevigiano, Vermentino, Vernaccia, Unknown, Rootstocks: Kober 5BB, 110R, SO4, 1103 Paulsen, 420A, Schwarzmann, 101-14, 3309C, 161-49, Rupestris du Lot	Bertazzon *et al*., 2015c; Bertazzon *et al*., 2016b; Bianchi *et al*., 2015; Gentili *et al*., 2017; Giampetruzzi *et al*., 2012; Morelli *et al*., 2014; Saldarelli *et al*., 2015
Europe	Macedonia	Unknown	Bertazzon *et al*., 2016b
Europe	Montenegro	Unknown	Bertazzon *et al*., 2016b
Europe	Poland	Jutrzenka, Solaris, unidentified white and red table grape varieties	Eichmeier *et al*., 2017; Pieczonka 2017.
Europe	Portugal	Unknown	Bertazzon *et al*., 2016b
Europe	Romania	Unknown	Bertazzon *et al*., 2016b
Europe	Serbia	Unknown	Bertazzon *et al*., 2016b
Europe	Slovakia	Veltliner, Dornfelder, Muller Thurgau, Andre, Welschriesling, and two unknown varieties	Glasa *et al*., 2014; Saldarelli, 2015
Europe	Slovenia	Pinot gris, Pinot noir, Muscat blanc, Sauvignonasse	Mavrič Pleško *et al*., 2014

Continent	Country	Winegrape, table grape and rootstock variety	Reference
Europe	Spain	Bobal, Garnacha, Macabeo, Tempranillo, Tintorera	Bertazzon *et al*., 2016b; Ruiz-García and Olmos 2017; Morán *et al*., 2018
Europe	Switzerland	Chasselas	Reynard, 2015
Europe	Turkey	Alphonso Lavalle, Alicante Bouschet, Autumn Royal, Cavus, Chardonnay, Cınarlıkarası, Emir, Gamay, Goguzumu, Hevenk, Kadın parmağı, Kalecikkarası, Kokuluuzum, Lival, Madame Jean Mathias, Michele Palieri, Muscat of Hamburg (Hamburg misketi), Pinot noir, Royal, Syrah, Several unknown Rootstocks: Couderc 1616, Ramsey, Teleki 5C	Elçi *et al*., 2018; Gazel *et al*., 2016; Ulubaş Serçe *et al*., 2018
Europe	Ukraine	Cabernet Sauvignon, Riesling, Rkatsiteli Rootstocks: Kober 5BB, 101-14 and 41B	Bertazzon *et al*., 2016b; Eichmeier *et al*., 2018
Europe	United Kingdom	Pinot noir clone 336 on Gravesac	Silva *et al*., 2018
Oceania	Australia: Victoria New South Wales South Australia	Chardonnay, Fiano, Cabernet Sauvignon, Graciano, Moscato giallo, Malbec, Mataro/1103 Paulsen, Pinot noir, Prosecco, Riesling, Sauvignon blanc, Semillon, Tempranillo, Crimson Seedless Rootstocks: 101-14, Schwarzmann	Wu and Habili, 2017. Constable, unpublished.

4.3 GPGV and disease

The association between GPGV and disease is complex and uncertain. In some grapevines GPGV is found in association with a disease known as grapevine leaf mottling and deformation (GLMD) (Giampetruzzi *et al*., 2012; Saldarelli *et al*., 2015). But it is also found in grapevines that are asymptomatic, even in the same variety. GPGV has significant genetic diversity and it has been reported that there are strains, or groups of strains, that are more likely to be associated to GLMD than others, but this association does not always occur. GPGV is also often reported in mixed infections with other viruses and the implications of mixed infections for disease expression is uncertain. The literature does not always specify if GPGV infected grapevines were symptomatic or asymptomatic and if asymptomatic

grapevines were in fact symptomless (i.e. healthy) or expressing virus-like symptoms that were different to those that define GLMD.

4.3.1 Grapevine leaf mottling and deformation disease

Grapevine leaf mottling and deformation are the symptoms that define GLMD and which have been associated with GPGV infection in some varieties. Symptoms were first observed in Pinot gris and Sauvignonasse in Slovenia in 2001 and then in Italy on Pinot gris in 2003 (Giampetruzzi *et al*., 2012; Mavrič Pleško *et al*., 2014). More pronounced symptoms are observed early in the growing season and, in some varieties, recovery from symptoms and symptomless shoot and leaf growth has been observed after veraison.

Other symptoms that may be associated with GLMD and GPGV infection in some varieties are delayed bud break, shortened internodes, chlorotic mosaic on leaves and stunting of the shoots (Saldarelli *et al*., 2015). Discoloured veins, bunch shatter, desiccated inflorescences, delayed ripening, lack of lignification, millerandage and shoot tip necrosis have also been observed in some varieties showing GLMD and which were also infected by GPGV (Angelini *et al*., 2015, Bertazzon *et al*., 2015a). Reduced yield associated with low bunch weight and bunch numbers in Pinot Gris, Gewürztraminer and Glera (Prosecco) has been reported (Malossini *et al*., 2014, 2015b, Bertazzon *et al*., 2015a).

Ultrastructural modifications were observed in leaf tissues of field-grown GPGV infected grapevines, but this was not related to presence or severity of GLMD (Tarquini *et al*., 2017).

4.3.2 GLMD disease expression can be variable

Many factors may influence expression of virus associated diseases in plants, including GLMD in GPGV infected grapevines. Factors impacting disease expression include varietal tolerance, resistance, sensitivity, susceptibility to viruses, environment, virus strain and titre in grapevine clones or varieties. These factors should be investigated in Australia to determine how GPGV might cause disease in the Australian grape growing regions.

4.3.2.1 Varietal sensitivity

The detection of GPGV in many varieties and rootstocks suggests that most varieties are susceptible to infection; i.e. they can be infected by the virus (Table 2). Whilst GPGV is thought to be a likely cause of GLMD in some varieties, other varieties may be asymptomatic, i.e. displaying atypical symptoms, or are symptomless.

It was reported that GPGV infected Pinot gris, Pinot blanc, Pinot noir, Gewürztraminer, Tocai friulano and Glera in north eastern Italy were more likely to express the typical GLMD symptoms compared to other varieties (Bertazzon *et al*., 2017). More severe symptoms, occurring across the entire grapevine and with shoot tip necrosis, were observed in Pinot gris compared to Glera in Veneto vineyards (Bertazzon *et al*., 2015a). However, in one study Glera only showed one or two affected shoots early in the growing season and recovered later in the season which was attributed to the vigour of this variety (Bertazzon *et al*., 2015a).

GPGV has been detected in grapevines without GLMD but expressing unusual symptoms to which other viruses could not be attributed. For example, GPGV has also been found in the absence of other viruses in grapevines with zigzag growth of shoots with short internodes, abnormal branching and disturbed berry development (Cho *et al*., 2013, Reynard *et al.,* 2016, P. Saldarelli pers comm.). Cho *et al*. (2013) reported a GPGV infection in the *Vitis vinifera x V. labrusca* hybrid Tamnara, which had poor fruit set and fruit with inner berry necrosis but no leaf or shoot symptoms.

Asymptomatic infection, in which grapevines appear healthy or express atypical symptoms, might also be associated with varietal tolerance to GPGV (Bertazzon *et al*., 2017).

4.3.2.2 Time required for GLMD expression

Symptomless GPGV infected grapevines might be a result of a recent infection event and time required for symptom development as has been observed for other grapevine viruses (Constable *et al*., 2012a, 2012b). To measure symptom development with time a small subsample of grapevines, which were free of Italian regulated-viruses (GVA, GVB, GLRaV-1, -2, -3, GFLV, GFkV and ArMV) in symptomatic and asymptomatic vineyards were tested over 3 years by RT-PCR for GPGV (Bertazzon *et al*., 2015 a, 2015b). All symptomatic and asymptomatic (ten each) were positive to GPGV and 4/10 asymptomatic grapevines developed GLMD over seven years. Of the Gewürztraminer grapevines, GPGV was detected in all symptomatic grapevines (eight) and 4/9 asymptomatic grapevines and two of these GPGV infected asymptomatic grapevines developed GLMD during the seven years. This result might be due to slow onset of symptoms after infection but could also be due to re-infection with strains of GPGV associated with disease. Therefore, it is important to understand how disease develops with time to determine visual surveillance and active testing strategies to minimise this risk of false negative results to nursery and to better understand the long-term impact of GPGV infection on grapevine growth and production.

4.3.2.3 Seasonal variation in disease expression

Variability in intensity, or severity, in GLMD expression has been observed from one season to another in Italy, although GPGV could still be detected (Bertazzon *et al*., 2015a, 2015c, Malossini *et al*., 2015a, 2015b, Tarquini *et al.,* 2017). If GPGV is the cause of GLMD, this suggests that environmental factors might influence the host-virus interaction that results in variable disease expression. Similar seasonal variation in other virus associated leafroll disease expression in grapevines has been observed in Australia (Constable *et al*., 2012a, 2012b)

4.3.2.4 GPGV titre and GLMD expression

It was demonstrated that GPGV titre was significantly higher in grapevines expressing GLMD symptoms, compared to grapevines without GLMD symptoms (Bianchi *et al*., 2015; Bertazzon *et al*., 2017; Tarquini *et al*., 2017). In one study, titre dropped during five months from spring (May) and into autumn (September), in both symptomatic and asymptomatic grapevines (Bertazzon *et al*., 2017). No natural resistance to GPGV has been reported in *Vitis* sp. germplasm. However, the lower GPGV titre in asymptomatic grapevines or grapevine varieties might be associated with the defence response of the grapevine host to infection by specific virus strains. Interestingly, Morán *et al*., (2018) did not observe a correlation between virus titre and symptom expression in 22 GPGV infected grapevines, including 17 GLMD affected grapevines and five that were asymptomatic.

4.3.3 Mixed virus infections and disease expression

GPGV has been found in the absence of other viruses in GLMD symptomatic grapevines (Poojari *et al*., 2016), grapevines with other symptoms (Cho *et al*., 2013) and asymptomatic grapevines which appeared healthy (Malossini *et al*., 2015a; Saldarelli *et al*., 2015). However, in other studies GPGV has been found in association with other viruses in both GLMD symptomatic grapevines and asymptomatic grapevines. Other viruses that were also detected by deep sequencing in the original GLMD affected Pinot gris grapevines, in which GPGV was detected, included *Grapevine rupestris stem pitting-associated virus* (GRSPaV), *Grapevine rupestris vein feathering virus* (GRVFV), Grapevine *Syrah virus 1* (GSyV-1), *Grapevine yellow speckle viroid 1* (GYSVd-1) and *Hop stunt viroid* (HSVd) (Giampetruzzi *et al*., 2012). One or a combination of GRSPaV, GRVFV, GSyV-1, GYSVd-1, HSVd, *Grapevine fanleaf virus* (GFLV)*, Arabis mosaic virus* (ArMV) and *Tomato black ring virus* (TBRV), *Grapevine fleck virus* (GFkV)*, Grapevine viruses A, B and E* (GVA, GVB, GVE), *Grapevine red globe virus* (GRGV), *Grapevine leafroll associated viruses* (GLRaV) -1, -2, -3 and -4 have been found in mixed infections with GPGV in other studies (Al Rwahnih *et al*., 2016; Beuve *et al*., 2015; Eichmeier *et al*., 2016a; Eichmeier *et al*., 2016b; Gazel *et al*., 2016; Glasa *et al*., 2014; Morán *et al*., 2018; Morelli *et al*., 2014; Rasool *et al*., 2017; Reynard *et al*., 2016; Ruiz-García and Olmos, 2017; Saldarelli *et al*., 2015; Vončina *et al*., 2017; Xiao *et al*., 2016). One study excluded

GRSPaV, HSVd and GYSVd-1 as a possible cause for GLMD because they were present in most symptomatic and asymptomatic plants (Bianchi *et al*., 2015). In the same study, GRVFV and GSyV-1 occurred less frequently and were found equally in symptomatic and asymptomatic plants and were also excluded as a cause of GLMD (Bianchi *et al*., 2015). When ArMV, GFLV or TBRV were detected in some diseased and GPGV infected grapevines, symptoms normally associated with these nepoviruses were observed (Al Rwahnih *et al*., 2016; Beuve *et al*., 2015; Glasa *et al*., 2014; Vončina *et al*., 2017). It may be that the fanleaf and degeneration symptoms mask symptoms of GLMD.

Saldarelli *et al*., (2015) demonstrated the presence of different GPGV strains associated with GLMD symptoms and in asymptomatic grapevines in which no other viruses could be detected, and in grapevines co-infected with one or more other grapevine virus species. No studies have investigated the effect of the interaction between specific strains of GPGV and other virus species in association to disease expression.

Koch's postulates have not been shown for GPGV and other viruses as a cause of GLMD symptoms. Therefore, the actual impact of GPGV on its own, and in combination with other viruses and virus strains, is unclear.

In Australia there is a need to:

- Understand the distribution and incidence of GPGV amongst varieties and grape growing regions
- Investigate the association of GPGV and disease in Australian grown grapevines
- Determine the impact on production, including yield, fruit quality, wine quality and pruning weight and quality.

This information will assist in developing appropriate management strategies for the virus and associated disease.

Table 2. Status of symptom expression in *Grapevine Pinot gris virus* infected grapevine varieties, and the country in which symptoms were observed. References as per Table 1.

Winegrape, table grape and rootstock variety	Symptomatic[1] (Country)[2]	Asymptomatic/ symptomless[3]	Not defined[4]
101-14 (rootstock)		X	X
161-49 (rootstock)			X
3309 C (rootstock)			X
'Beta' (rootstock)	CM (Ch)		
1103 Paulsen (rootstock)			X
110 Richter (rootstock)			X
1616 C (rootstock)			X
420 A (rootstock)			X
Alibernet		X	
Alphonso Lavalle			X
Ancellota			X
Andre		X	
Autumn royal			X
Bai-ji-xin		X	
Balafánt		X	

Winegrape, table grape and rootstock variety	Symptomatic[1] (Country)[2]	Asymptomatic/ symptomless[3]	Not defined[4]
Black Magic	CMLD (It)	X	
Bobal	CMLD (Es)		
Cabernet Franc	CM (Ch)	X	X
Cabernet Sauvignon		X	X
Cardinal			X
Carignan	Fanleaf-like symptoms (Fr)	X	
Carmenere			X
Cavus			X
Chardonnay	CMLD ↓yield ↓quality (Cz, It, Tk)	X	X
Chasselas		X	
Cınarlıkarası			X
Csabagyöngye - Pearl of Csaba		X	
Crimson Seedless	CM (mild), SI, SC, zig zag shoots (Au)		
Dornfelder		X	
Emir	CMLD ↓yield ↓quality (Tk)		
Fehér Járdovány		X	
Fiano		X	X
Furmint		X	
Gamay			X
Garnacha	LD (Es)		
Generosa (new var.)		X	
Gewürztraminer	CM, LD, BS, DB, DI, DR, LL, M, SC, SI, ↓ (It)		
Goguzumu			X
Goruli mtsvane			X
Graciano			X
Gui fei-me gvi		X	
Hárslevelű		X	
Hevenk			X
Incrocio Manzoni			X
Jutrzenka		X	
Kadarka		X	
Kadın Parmağı	CM, LD, ↓ (Tk)		
Kalecikkarası			X
Kék Bakator	CM, LD (Hu)		
Khikvi			X
Kober 5BB (rootstock)			X
Kodrjanka			X
Kokuluuzum			X
Korkaula			X
Laurot	mosaic & vein banding (Cz)	X	X
Lival			X
Macabeo	CM, LD (Es)		
Madame Jean Mathias			X

Winegrape, table grape and rootstock variety	Symptomatic[1] (Country)[2]	Asymptomatic/ symptomless[3]	Not defined[4]
Malbec			X
Malvasia		X	X
Malvasia istriana			X
Mataro		X	
Merlot	Fan leaf, CM (Fr, It)	X	X
Michele Palieri			X
Moldova	CM (Ch)		
Monica		X	X
Moscato		X	
Mtsvane kviteli			X
Mueller Thurgau	mild mosaic & downward rolling leaves (Cz)	X	X
Muscat blanc	CM, LD, SI (Sl)		
Muscat Giallo			X
Muscat of Hamburg (Hamburg misketi)	CM, LD, ↓ (Tk)	X	X
Nasco Sagrantino		X	
Negro amaro			X
Olasz rizling – Welschriesling		X	
Pamjati Negrula			X
Petit Verdot			X
Pinot Blanc (Pinot Bianco)	CM, LD, NS, DB, ↓ (It)		
Pinot Gris/Grigio	CM, LD, BS, DB, DI, DR, LL, M, SC, SI, ↓ (It, Sl, Ca)		
Pinot Noir/Nero	CM, LD, BS, DB, DI, DR, LL, M, SC, SI, ↓ (Cz, It, Tk,)		X
Prosecco (Glera)	CM, LD, DB (It)	X	X
Purcsin		X	
Raboso piave			X
Ramsey (rootstock)			X
Red Globe		X	
Regina			X
Riesling	SI, zigzag growth of shoots, disturbed berry development (Ge)		X
Rossignola			X
Royal			X
Rupestris du Lot (rootstock)			X
Sangiovese		X	
Saperavi pachkha			X
Sárga Ortliebi		X	
Sárgamuskotály		X	
Sauvignon Blanc		X	X
Sauvignon Musqué			X

Winegrape, table grape and rootstock variety	Symptomatic[1] (Country)[2]	Asymptomatic/ symptomless[3]	Not defined[4]
Sauvignonasse	CM, LD, SI (Sl)		
Schwarzmann (rootstock)		X	X
Self-rooted SL12, SL13, SL14, SL45, and SL53			X
Semillon		X	
Shine-Muscat	CM (Ch)		
Shiraz			X
SO4 (rootstock)			X
Solaris		X	
Superior			X
Supernova	CM, LD (It)	X	
Syrah			X
Table grapes		X	
Tamnara *(V.. vinifera x V. labrusca)*	inner necrosis of berries and poor fruit set (Ko)		
Tavkveri			X
Teleki 5C (rootstock)			X
Teleki-Kober 125 (rootstock)		X	
Teleki-Kober 5C (rootstock)		X	
Tempranillo		X	X
Tintorera (Alicante Bouschet)	CM, LD (Es)		X
Tocai Friulano	CM, LD, BS, DB, DI, DR, LL, M, SC, SI, ↓ (It)		X
Tocai rosso	CM, LD, BS, DB, DI, DR, LL, M, SC, SI, ↓ (It)		X
Torbato			X
Touriga national		X	
Traminer	CM, LD, BS, DB, DI, DR, LL, M, SC, SI, ↓ (It)		X
Traminer aromatico	CM, LD, BS, DB, DI, DR, LL, M, SC, SI, ↓ (It)		
Unknown varieties	CM, LD (various countries)		
Veltliner		X	
Verduzzo trevigiano	CM, LD, BS, DB, DI, DR, LL, M, SC, SI, ↓ (It)		
Vermentino	CM, LD (It)		
Vernaccia		X	
Vidal blanc			X
Viognier			X
Wei-duo-li-ya		X	
Welschriesling		X	
Zi-de qv		X	
Zinfandel	CM, LD (Tk)		X
Θραψαθάρι (Thrapsatari),			X
Κορινθιακή σταφίδα (Corinthian Raisin)			X

Winegrape, table grape and rootstock variety	Symptomatic[1] (Country)[2]	Asymptomatic/ symptomless[3]	Not defined[4]
Μαυροθηρικό (Maurothiric)			X
Μαυρόστυφο			X
Ξινόμαυρο (Xinomavro)			X
Πέλλας (Pella)			X

[1] CM = discoloured veins, & leaf mottling, LD = small, deformed leaves, BS = bunch shatter, DB = delayed budburst, DI = desiccation of inflorescence, DR = delayed ripening, LL = lack of lignification, M = millerandage, SC = stunted canes, SI = short internodes, SN = Shoot tip necrosis, ↓ = decreased yield and/or quality

[2]Country abbreviations Au = Australia, Ch = China, Cz = Czech Republic, Es = Spain, Fr = France, Ge = Germany, Hu = Hungary, It = Italy, Ko = Korea, Tk = Turkey, Sl = Slovenia

[3]Grapevines reported as asymptomatic and includes grapevines with atypical symptoms (i.e. associated to other causes) or without symptoms (i.e. symptomless)

[4]GPGV positive but symptoms not reported, not observed due to sample timing or the symptomatic and asymptomatic/symptomless varieties not specified in publications.

4.4 GPGV strain variation

4.4.1 Definition of GPGV strains

A GPGV strain is defined by its genetic difference compared to the type variant (GenBank accession FR877530, Giampetruzzi *et al.,* 2012).

Many strains of GPGV occur. Thirty full length or near full length GPGV genomes have been published since genome of the type strain was first characterised. The genome sequences were often deliberately obtained and used to determine their relationship to the type strain (Al Rwahnih et al., 2016; Beuve *et al.,* 2015; Glasa *et al.,* 2014; Gualandri et al.,2017; Poojari *et al.,*2016; Rasool *et al.,* 2017; Reynard *et al.,*2016; Saldarelli *et al.,* 2015 Tarquini *et al.,* 2015), whilst other GPGV genomes have been obtained as a bi-product of the development of deep sequencing tools as diagnostic tools to investigate viromes (Eichmeier *et al.,* 2016b; Czotter *et al.,* 2018). Obtaining full length genome sequence of viruses can be time consuming and expensive, therefore researchers often choose smaller informative regions of the genome, which can easily and cheaply be isolated and analysed to differentiate strains and infer a biological phenotype, such as symptom expression in a host. GPGV strain differentiation amongst isolates has primarily been based on comparisons between regions of the movement protein (MP) and coat protein (CP) gene sequences, although a fragment of the RNA dependent RNA polymerase region in the replicase gene is sometimes used (Saldarelli *et al.,* 2015).

4.4.2 Evidence of strains

A simple phylogenetic analysis was done for this review (Figure 1, F. E. Constable) and shows that the genetic similarity is 95-99% at the nucleotide level amongst 26 of the 30 GPGV strains for which good quality full length or near full length genomes are available. A study using seven near full length genome GPGV sequences indicated that they formed clades associated to GLMD expression and region (Saldarelli *et al.,* 2015). Phylogenetic analysis of the combined partial MP/partial CP gene region, the CP gene only (Bertazzon *et al.,* 2016b; Fajardo *et al.,* 2017; Spilmont *et al.,* 2018; Wu and Habili, 2016) and/or the RNA dependent RNA polymerase (RdRP; Saldarelli *et al.,* 2015) supports the formation of GPGV strains into three or four separate clades, which may have biological phenotypes associated with GLMD symptom expression. The phylogenetic analysis done for this review (Figure 1, F. E. Constable) also shows that the genetic similarity of the 460nt sequence of the combined partial MP/partial CP gene region amongst 130 GPGV strains is 87-99% at the nucleotide level.

4.4.3 Association between GPGV strains and GLMD

Genetic variation within virus species can affect the interaction between a virus and the plant host leading to differences in disease expression (Pallas and Garcia, 2011). Several phylogenetic analyses showed that GPGV strains found in asymptomatic grapevines often formed separate genetic clades, away from those found in the symptomatic grapevines (Bertazzon *et al.,* 2017; Eichmeier *et al.,* 2017; Glasa *et al.,* 2014; Saldarelli *et al.,* 2015). However, Bertazzon *et al.,* (2017) observed that some GPGV strains in each clade were not always obtained from grapevines with the expected disease phenotype. Recently a group of French researchers, again using the same gene region, proposed four clades and also observed that the clear genetic distinction between GPGV isolates from asymptomatic and symptomatic grapevines did not always hold (Spilmont *et al.,* 2018). Therefore, the disease association to specific GPGV strains is unclear and requires further study.

The specific interaction between GPGV and the host to cause disease is uncertain, however some specific genetic motifs and gene regions have been implicated in the expression of GLMD. An initial analysis of four GPGV genomes showed that significant genetic differences occurred in the 5' untranslated region and on ORF1 between three strains from asymptomatic grapevines in Slovakia and Czech Republic and the type GPGV strain (GenBank accession FR877530, Giampetruzzi *et al.,* 2012) from a symptomatic grapevine in Italy and these differences were possibly associated with a recombination event with another virus such as *Grapevine berry inner necrosis virus* (Glasa *et al.,* 2014). Another analysis of the 5'region of ORF1 showed that the type GPGV strain was divergent to 21 other isolates from various regions in Italy and other countries (Bertazzon *et al.,* 2016b). The disease status of the 21 grapevines was not reported but it may be that the larger number of GPGV isolates, collected from a diversity of locations and varieties, with greater genetic similarity in ORF1 might not support the association of this specific region to GLMD expression.

Another study proposed that a polymorphism (T/C) in the stop codon of the MP gene, which should result in a longer protein, was indicative of, but not necessarily responsible for, GPGV strains associated to asymptomatic grapevines (Saldarelli *et al.*, 2015). Consequently, most phylogenetic analyses focus on the combined sequence of the 3' end of the MP and 5' end of the CP gene regions (Bertazzon *et al.,* 2017; Eichmeier *et al.,* 2017; Morelli *et al.,* 2014; Saldarelli *et al.,* 2015; Spilmont *et al.,* 2018; Vončina *et al.,* 2017). Two clades of GPGV isolates were observed, associated with infection in symptomatic and asymptomatic grapevines. Another polymorphism, one codon down from the first discovered polymorphism region MP gene, was identified in Spanish isolates (Morán *et al.,* 2018). These Spanish isolates cluster differently to isolates with the stop codon polymorphism, but the significance of the change is not known.

4.4.4 Variability of Australian GPGV isolates and association with disease

Genetic diversity exists amongst GPGV isolates reported in Australia. An analysis of a 431 nt region of the coat protein gene region of nine GPGV isolates, from different grapevine varieties and clones, indicated that each isolate was unique and that they had 93-99% similarity with strains reported in other countries (Wu and Habili 2017). The disease status of these grapevines was not reported and their relationship to GLMD is unknown.

A phylogenetic analysis of the combined sequence of the 3' end of the MP and 5' end of the CP gene regions done for this review used three GPGV isolates detected from one vineyard in one region of Australia. The analysis shows that diverse strains were present in a single vineyard (Figure 1; F.E. Constable unpublished). The phylogeny also demonstrates the breadth of isolates that have been sequenced worldwide. Australian GPGV isolates 17 and 24 cluster with symptomatic isolate FI3A (99% similarity; GenBank: LN606713.1) and Australian GPGV isolate 14 clusters most closely with the asymptomatic isolate ZA505-3N (99% similarity; GenBank: LN606722). Australian GPGV isolate 14

contains the T/C polymorphism, indicative of asymptomatic strains in other studies, and isolates 17 and 24 do not. Regardless of their association to GPGV strains associated with GLMD and asymptomatic infections, these isolates were found in grapevines that had no obvious GLMD symptoms. It may be that the variety that was infected is not sensitive to GPGV infection.

Although not yet examined in GPGV infected grapevines, mild strain cross protection can occur between virus strains in other plant species, limiting the impact of more severe strains (Zhang and Qu, 2016).

In Australia further work is required to:

- Understand the diversity of Australian GPGV strains and their association to disease in a broad range of varieties
- Develop management strategies for disease control if needed.

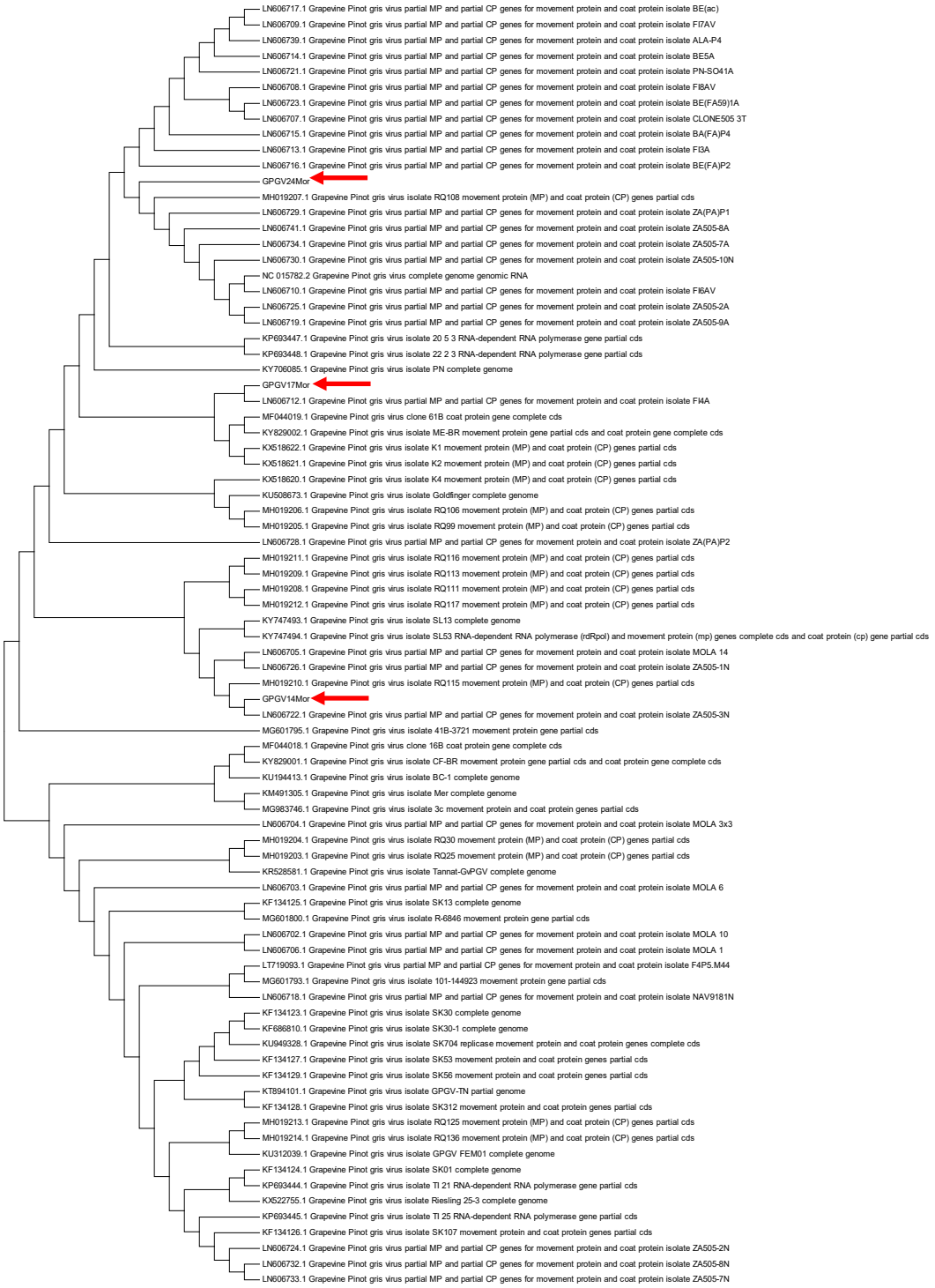


Figure 1. Phylogenetic analysis of *Grapevine Pinot gris virus* isolates by Maximum Likelihood method. A 503 nt region, comprising of the partial movement protein and partial coat protein genes, was used (Saldarelli *et al.,* 2015). Three Australian isolates, from asymptomatic grapevines in one vineyard, are indicated with a red arrow. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). The tree with the highest log likelihood (-2579.5665) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbour-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The analysis involved 82 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There was a total of 503 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar *et al.,* 2016).

4.5 Surveillance and diagnostic capability

4.5.1 Laboratory methodologies for GPGV detection

Methods for grapevine virus detection include visual inspection, biological indexing, the enzyme linked immunosorbent assay (ELISA) and molecular methods; particularly reverse transcription polymerase chain reaction (RT-PCR) and deep sequencing technology (next generation sequencing, NGS; high-throughput sequencing; Martelli, 2014). Of these methods, visual inspection may be unreliable for GPGV detection because some GPGV infected grapevine varieties have been found to be asymptomatic or symptomless, or symptoms expressed might be associated with other biotic or abiotic factors, including the presence of other viruses. Biological indexing, in which characteristic symptoms develop on a sensitive plant inoculated with a pathogen, has not yet been developed for GPGV.

4.5.1.1 Enzyme linked immunosorbent assay

ELISA is a simple serological technique that uses antibodies to detect the presence of an antigen, such as a virus, in a sample (Clark and Adams, 1977). It is useful for high-throughput testing of large sample numbers and results can be gained within 24 hours. An ELISA kit was made commercially available for GPGV in 2018 (Bioreba AG, Switzerland; Poignavent *et al.,* 2018). The company state "*Bark (phloem) scrapings from dormant canes and leaves from young shoots early in the growing season are good tissue sources for testing. It is not recommended to sample mature leaves during summer or in fall*". The reliability of this test to detect strains of GPGV and at different sampling times, has not been determined in Australia. Typically, ELISAs lack the sensitivity of molecular techniques such as PCR, however Bioreba claim equal sensitivity to reverse transcription (RT)-PCR for GPGV detection (Kosfitskas *et al.,* 2018; Poignvent *et al.,* 2018).

4.5.1.2 Deep sequencing

GPGV was first detected using deep sequencing technology (next generation sequencing, NGS; high-throughput sequencing; Giampetruzzi *et al.,* 2012). Metagenomic deep sequencing technology has the capacity to identify multiple pathogens in a single sample. It can be unbiased as it does not rely on pre-designed probes for pathogen detection (e.g. PCR primers etc), which create specificity. It is a useful tool for low throughput virus detection that is rapidly developing, and it is likely to be used more routinely for virus detection and discovery in the future (Al Rwahnih, 2018; Maree, 2018).

4.5.1.3 Reverse transcription polymerase chain reaction

Many RT-PCR methods have been developed for detection of GPGV (Table 3). Continual improvements have been made as more genomic sequence information for GPGV, mainly derived from NGS, has become available. GPGV has an RNA genome and during RT-PCR the RNA is reverse transcribed into copy DNA (cDNA) so that the PCR step can proceed. A thermostable enzyme (e.g. *Taq* DNA polymerase) makes multiple copies of a specific nucleotide sequence from the target DNA or cDNA during PCR. Products generated by end-point PCR are separated on an agarose gel by electrophoresis, stained with a fluorescent dye and viewed under UV illumination: if a product of the correct size is observed a positive result is recorded. Alternatively, real-time (quantitative) PCR (qPCR) techniques have been developed for detection of GPGV that simultaneously amplify and measure the presence of the target in a PCR reaction (Table 3). PCR creates multiple copies of the cDNA and is considered much more sensitive than ELISA, which can be limited by the number of virus particles present in a plant sample (Chevalier *et al.,* 1995; Sefc *et al.,* 2000).

Several labs use a two-step approach to RT-PCR detection of GPGV: making cDNA and adding this to the PCR reaction (Table 3). The reason for this is two-fold: 1) the cost of separate RT and PCR reagents may be cheaper than purchasing one-step RT-PCR kits, particularly when cDNA, generated in one RT reaction, using an oligo (dT) primer and random hexamers, is used in multiple virus PCR tests; 2) it may increase sensitivity because the separate RT step can be optimised to increase cDNA yield for use in PCR

(Al Rwahnih *et al.,* 2017). However, compared to one-step RT-PCR, two-step RT-PCR is more time consuming and the PCR step is more prone to cDNA contamination by aerosol carryover when the RT reaction tubes are opened and when the cDNA is transferred to the PCR reaction, leading to false positive results.

Bioinformatic analyses to compare primers against available GPGV sequences indicate that not all published primers can detect all strains of GPGV (F.E. Constable, unpublished; Al Rwahnih *et al.,* 2017). However, two end-point and two RT-qPCR tests were validated by Italian laboratories (Gentili *et al.,* 2018; Table 3). No significant differences in specificity, accuracy, repeatability and reproducibility were observed between tests developed by Glasa *et al.,* (2014; Method 1), Morelli *et al.,* (2014; Method 2), Bianchi *et al.,* (2015; Method 3), and Ratti *et al.,* (unpublished; Method 4) despite the different equipment and RNA extraction methods that were used by different laboratories (Gentili *et al.,* 2018), although the RT-qPCR tests gave greater analytical sensitivity.

The "Preliminary Sampling Trial" currently underway in Australia (and reported in this final report) to investigate sample type, amount and timing has already raised the importance of using the appropriate molecular test to accurately detect the virus. CHS found that the RT-PCR tests developed by Cho *et al.,* (2013) and Beuve *et al.,* (2015) produce false negative results for some Australian GPGV isolates. This occurred when samples were tested in autumn, but samples of the same grapevines were positive for GPGV with the same tests in summer. Initially CHS diagnosticians suspected that the RNA extraction method was inadequate, and some different processes were tried, but negative results were again obtained using the Cho *et al.,* (2013) and Beuve *et al.,* (2015) RT-PCR tests. When the RT-PCR tests of Bianchi *et al.,* (2015 Method 3) and Glasa *et al.,* (2014; Method 1) were used, the same RNA extracts of the autumn samples were found to be positive for GPGV (F.E. Constable, unpublished). These results demonstrated that the ability to detect GPGV isolates is impacted by genetic variability. It also suggested that the genetic diversity of the GPGV population and titre of specific strains within grapevines might change over time. Further work is required to understand the genetic diversity of GPGV in Australia to improve diagnostic capability and the reliability of RT-PCR. Proficiency testing will ensure laboratories can accurately detect GPGV with the extraction procedures and tests that they use.

The DetF/DetR (Morelli *et al.,* 2014) are the primary set of primers used to generate PCR products to study genetic diversity amongst GPGV strains.

4.5.1.4 Nucleic acid extraction for RT-PCR

Various nucleic acid extraction procedures have been used to produce RNA for the detection of GPGV by RT-PCR: many use commercial spin column kits including the Spectrum Plant Total RNA Kit (Sigma Aldrich), NucleoSpin™ RNA Plant Kit (Macherey-Nagel™) or the RNeasy Plant Mini Kit (Qiagen) with a modified Guanidine Thiocyanate buffer (MacKenzie *et al.,* 1997). Other methods include manual extraction procedures that precipitate nucleic acid (Demian *et al.,* 2018) or capture RNA to silica beads (Wu and Habili, 2016). In Australia, AWRI Commercial Services use the silica bead RNA extraction method (Wu and Habili, 2016) and Crop Health Services (CHS) use the RNeasy method described by MacKenzie *et al.,* (1997) or a modified version of this method using the automated QIAcube HT (Qiagen) high-throughput liquid handling system (F.E. Constable, unpublished). In a small comparative test using the same grapevine homogenates, both CHS and AWRI Commercial Services produced comparable results for detection of GPGV. AWRI Commercial Services used the RT-PCR test described by Wu and Habili (2016) and CHS obtained best results using Bianchi *et al.,* (2015; Method 3) and Glasa *et al.,* (2014; Method 1). These results and the results of the Italian validation work indicate that several robust testing procedures are available for detection of GPGV and that differences in extraction procedures may not make a difference to GPGV detection if the method is optimised.

Table 3. The primers and probes that have been designed for RT-PCR, RT-qPCR and LAMP detection of *Grapevine Pinot gris virus*. The location of primers and probes on the NCBI GPGV Reference Sequence: NC_015782, the gene region detected, and the size (bp) of the amplified product are listed. The tests validated by the Italian proficiency test are listed in bold.

Forward primer (5'-3') NCBI Reference Sequence: NC_015782	Reverse (5'-3') NCBI Reference Sequence: NC_015782	TaqMan probe (RT-qPCR)	Gene regions (bp)	Reference and Use
GPG-14F AATTGATCCCGTGTAGTGC 22-40	GPG-632R T Outputs arising from the project including the "Comprehensive review of *Grapevine Pinot gris virus"* are freely available to be shared with relevant industry and state and national research, diagnostic and biosecurity agencies. There are no commercialisation or confidentiality issues to report. CCGAGGACGATGAACCTC 642-624		RDRP618 bp	Glasa *et al.,* 2014 Two step random hexamers
PolF1 CGCATTCAGGTATTAGGGAGTTG 382-404	PolR2 CTCTTTTCAATTCTCTCAGGAG 550-529		RdRp 163 bp	Bertazzon *et al.,* 2017
GPgVPozRTF 23 ACCAATCTAATAAAGACTTCAAA 813-835	GPgVPozRTR 18 AGGATCTCCGACTCATAT 916-899	GPgVPozRT probe 24 [FAM]-TCTGTAAGTCTCCACCGTTCTGTC-[BHQ1] 846-869	103 bp	Bianchi *et al.,* 2015
GPGVRepF TGAGGCATTCGATGTTTCCCA 4940-4957	GPGVRepR ACCCAATCAAGCCATGAACCT 5461- 5441		RdRp 521 bp	Saldarelli *et al.,* 2015
GPGVMP1A GGTTTCTGGGAGGATTGCAAC 5604-5624	GPGVMP1B RCTTYGCGATCAGCTCTCTCC 6643-6624		MP1041 bp	Fan *et al.,* 2016

Forward primer (5'-3') NCBI Reference Sequence: NC_015782	Reverse (5'-3') NCBI Reference Sequence: NC_015782	TaqMan probe (RT-qPCR)	Gene regions (bp)	Reference and Use
GPgV5619f TGGGAGGATTGCAACAGGGGCT 5610-5631	GPgV6668r ATTCGCCTCGCTCAACTTTGCGA 6658-6636		MP1049 bp	(Giampetruzzi *et al.,* 2012) Two step, random hexmers
GPG-5637F **ATTGCGGAGTTGCCTTCAAG** **5638-5657**	**GPG-5939R** **CTGAGAAGCATTGTCCCATC** **5940-5921**		**MP302 bp**	**Glasa *et al.,* 2014** **Italian protocol 1**
Pg-Mer-F1 GGAGTTGCCTTCGTTTACGA 5896-5915	Pg-Mer-R1 GTACTTGATTCGCCTCGCTCA 6665- 6645	N/A	MP 770 bp	Beuve *et al.,* 2015
GPGVTaqF: CATTTCTGTGAGGATAGGTGTCATG **6099-6117**	**GPGVTaqR: CAGACATAAGTGAACGGCCAATAT** **6166-6143**	**GPGV: [FAM]-AGGCCTTTCAATTCA-[MGB]** **6127-6141**	**MP – CP** **67 bp**	**Ratti *et al.,* unpublished Italian protocol 4**
DetF-TGGTCTGCAGCCAGGGGACA **6225-6244**	**DetR TCACGACCGGCAGGGAAGGA** **6812 -6793**		**MP/CP** **588 bp**	**Morelli *et al.,* 2014** **Italian protocol 2**
GPGV-6474F TTCTGGTGATCCAATGGTAAAGA 6486-6508	GPGV-6912R ATTGCAAAGGCCGCACACACTTG 6924-6902		MP/CP 438 bp	Morán *et al.,* 2018 Experimental
GPGV-RT-F GTCTAAATCTGGCTGTGCTGAAAATAGTGC 6516- 6545	GPGV-RT-R CCTGAGGTCCCTTCAACTGC 6744-6725	GPGV- genprobe 6-FAM-AGATCAACAGTCAGGAGAGAGCTGATCGC-ZNA4-BHQ1-	MP/CP 228 bp	Morán *et al.,* 2018 Experimental
GPGVMPCP1A	GPGVMPCP1B		MP/CP	Fan *et al.,* 2016

Forward primer (5′-3′) NCBI Reference Sequence: NC_015782	Reverse (5′-3′) NCBI Reference Sequence: NC_015782	TaqMan probe (RT-qPCR)	Gene regions (bp)	Reference and Use
GCTGTGCTGAAAATAGTGCTG 6527-6547	GAAGCCGTGATAGCATTAGTC 7152-7132		626 bp	
GPGV6586F GAYATGTCGATTCGTCAGGAG 6587-6607	GPGV7021R CGACTTCTGGTGCCTTATCAC 7022-7002		CP 435 bp	Xiao *et al.,* 2016
CPF3, TGTCGATTCGTCAGGAGTTG 6591-6607	CPR3 GGGTAAATTGTCTCCCCTGA 6759-6740		CP 170 bp	Bertazzon *et al.,* 2017
GPGVCP1A TGAGATCAACAGTCAGGAGAG 6609-6629	GPGVCP1B GAAGCCGTGATAGCATTAGTC 7152-7132		CP 544 bp	Fan *et al.,* 2016
GPG-6609F GAGATCAACAGTCAGGAGAG 6610-6629	GPG-7020R GACTTCTGGTGCCTTATCAC 7021-7002		CP 411bp	Glasa *et al.,* 2014
GPGV CP-F2 ATAGCAGTTGAAGGGACCTC 6722-6741	CP-R1 GCCTCTCACTTTCGACATG 7124-7109		CP 406 bp	Angelini *et al.,* 2016
GPGV CP-F2 ATAGCAGTTGAAGGGACCTC 6722-6741	CP-R2 AAGCCGTGATAGCATTAGTC 7151-7132		CP 430 bp	Bertazzon *et al.,* 2016b
forward ACCACAATGGTGAAGTGTCATGA 6761-6783	reverse ACGAAAAGTCATTAACTTTATGTCA 6901-6877	probe FAM-TCTTTTAATCCTTCCCTGCCG 6785-6805	CP 140 bp	Al Rwahnih *et al.,* 2016
GPgV504F GAATCGCTTGCTTTTTCATG **7093 - 7112**	**GPgV588R** **CTACATACTAAATGCACTCTCC** **7177 - 7156**	**GPgV CP542 probe [T.RED]-AGACTAATGCTATCACGGCTTCGG**	**CP 83 bp**	**Bianchi *et al.,* 2015** **Italian protocol 3**

Forward primer (5'-3') NCBI Reference Sequence: NC_015782	Reverse (5'-3') NCBI Reference Sequence: NC_015782	TaqMan probe (RT-qPCR)	Gene regions (bp)	Reference and Use
		-[BHQ2] or BHQ3 7131-7154		
ATGTCGATTCGTCAGGAGCTG 6590-6610	CTACATACTAAATGCACTC 7177-7159		CP509 bp	Cho *et al.,* 2013
GPGV7220R: GTTACGTGCTCCTATGAGA 7221-7203			RT primer	Demian *et al.,* 2018 ICVG abstract Amplification using 2-step with GPGV specific RT primer
GPGV10F: CAATTGATCCCGTGTAGTGC 22-40	GPGV2015: CAGGTTCATYTTTGGATTCAAC 2025-2004		5'UTR2005 nt	
GPGV5557F: ACTTATCTGATGGCTCTGATG 5569-5589	GPGV7220R: GTTACGTGCTCCTATGAGA 7221-7203		MP/CP region Ca 1600 nt	
GPGV5578: CAGGTACCATGGCTCTGATGAAGAGGAT 5578-5597	GPGV7177R: TCTAGACTACATACTRAATGCACTCTCC 7178-7156			
159-F3 Forward Outer Primer GATTGTCAAGGTTTTTTGTGAA 159-FIP 5494-5515 Forward Inner Primer (F1c - TTT- F2) GCTATCCTCTTCATCAGAGCCATCCATCTTCTAAAGGGCAAATCC 159-	159-B3 Backward Outer Primer CCTCTCCAGTAAAATTCCGG 5715-5696 BIP Backward Inner Primer (B1c – TTT – B2) GCAACAGGGGCTACTCCAATACCCTTTGAGCGACTTTG	color of the infected samples changes from pink to yellow		Zikou, 2018. LAMP

4.5.2 In-field diagnostics

Loop-mediated isothermal amplification (LAMP) technology for infield detection of GPGV has been developed (Table 3, Zikou, 2018). The technology is similar to PCR, except that the enzymes used to copy DNA do not require changes in reaction temperature to denature the double stranded DNA, allow annealing of primers and subsequent building of a new DNA strand. Results can be measured in real-time, like RT-qPCR, using a small portable instrument. But these devices are not always necessary as a positive result can be identified visually associated with turbidity, a colour change or fluorescence of an intercalating dye in the test tube. Further development of this technology could assist in field diagnosis of GPGV without the need for laboratory testing. However, the requirement for at least four primers

can increase the specificity of the test. Increased specificity could result in false negative results if primers are designed to regions of the GPGV genome with high genetic diversity.

4.5.3 Field sampling strategies for accurate detection

Field sampling can impact on the reliability of ELISA, PCR and NGS methods to detect viruses, including GPGV. Consideration needs to be given to the type and amount of tissue that is collected and the timing of sampling. Viruses can occur in uneven concentration and distribution within a grapevine (Constable *et al.,* 2010, 2012a, 2012b). Tarquini *et al.,* (2017) showed that GPGV particles were located in the bundle sheath cells that surround vascular tissues in leaves and Eichmeier *et al.,* (2016) detected GPGV in phloem scrapings from shoots using NGS. Therefore, vascular tissues, including bundle sheath cells, collected from leaves and shoots are likely to provide the most reliable tissue type for GPGV detection. Jo *et al.,* (2017) found GPGV in analysis transcriptomic data from berry flesh and seeds, however the proportion of GPGV sequence reads was lower than expected in some samples indicating that these tissue types may be unreliable.

The strategy used for field sampling for GPGV testing varies in the literature by type of tissue sampled, number of samples per grapevine and the timing of sampling over the season (Table 4). In many published studies the sampling strategy is not defined and GPGV test results between studies cannot be easily compared. Currently, commercial testing of other grapevine viruses is most commonly undertaken in autumn in Australia, at a time when GPGV titre is likely to be low. The "Preliminary Sampling Trial" in Australia is beginning to investigate the ability to detect GPGV at different phenological stages when virus titre is expected to vary, but more work is needed.

4.5.3.1 Tissue type and location for reliable detection of GPGV

As with other viruses, distribution and titre of the virus within the grapevine may be uneven, thus sampling strategies need to be sufficient to capture an accurate reflection of viral presence. The samples taken in the vineyard for many published studies are cited as either leaves, leaves and petioles, shoots or canes. Canes are sometimes lignified or dormant. The petiole or leaf vein, or the scraped vascular tissue from canes, is then used for molecular testing. Sampling strategies are not well defined in the literature, with little information of the sample number or sample location on the grapevine. Definition of phenological stage of the shoots/canes are often unclear; whether the canes are partially or fully lignified, or the stage of dormancy. The quantity of material per grapevine and location on the vine is rarely outlined. Reynard (2015) cites 3 canes per grapevine, and Bianchi *et al.,* (2015) used 5-20 leaves per grapevine depending on grapevine size. These differences in the literature regarding the type of tissue sampled and method of the field sampling, together with the use of a range of RT-PCR methods, especially if sampling, timing and RT-PCR test used are at risk of returning a false negative result, means that direct comparison of results should be done with caution.

4.5.3.2 Time of sampling

The time of sampling varies amongst the literature: samples have been tested across the season from dormancy, through to spring /summer and some in autumn (Table 4). Although this suggests testing can be done at most times of the year, it has been demonstrated that the titre of GPGV decreases from spring, when titre is highest, until autumn (Bertazzon *et al.,* 2015b., Bianchi *et al.,* 2015). Consequently, GPGV testing may be more reliable and less likely to return false negative results in spring or summer than in autumn.

In Italy, a validated protocol (Barba *et al.,* 2017; Gentili *et al.,* 2018) for sample collection and GPGV detection was developed. Samples can be tested with one of the four validated RT-PCR/RT-qPCR tests (Table 4; Gentili *et al.,* 2018) and recommendations for sampling included:

- Collect samples during dormancy and up to late spring
- Use lignified shoots or leaves with symptoms: collect two woody portions from the basal part of shoots of the same year, ensure material is not affected by other biotic or abiotic factors
- Keep plant material dry and cool
- Ensure arrival to the laboratory within 72 hours
- Store woody material for testing at 4 °C for no longer than 60 days
- Samples can be frozen (-20°C) for one year.

This sampling strategy could be followed in Australia, but it requires validation.

Table 4. Field sampling strategies utilised for *Grapevine Pinot gris virus* (GPGV) monitoring and research.

Location	Tissue sampled	Timing	Paper
USA	Lignified canes	Autumn (October)	Angelini *et al.,* 2016
Italy	Leaves	Monthly from mid Spring (May) to early Autumn (September)	Bertazzon *et al.,* 2015b
Italy	5 -20 leaves & petioles per grapevine	May-June, (Spring/Summer), September, (early Autumn)	Bianchi *et al.,* 2015
Turkey	1-year old canes	Dormancy	Çağlayan *et al.,* 2015
Georgia	Leaves	Autumn	Casati *et al.,* 2014
Czech Republic	Cuttings (canes)	Late Autumn (November)	Eichmeier *et al.,* 2016b
Uruguay	Berries	1.5 & 7 weeks post flowering (Spring/Summer)	Jo *et al.,* 2015
Switzerland	Dormant canes, 3 per grapevine	January (Winter)	Reynard, 2015
Italy	Leaf veins & dormant canes	Sampled 3 times per year and evaluated for symptoms pre-flowering April (Spring).	Tarquini *et al.,* 2017
Australia	Green shoots, petioles, lignified canes, dormant canes	Early and late Spring, early Summer (October, November, December), Autumn (April) and Winter (July)	Wu and Habili, 2017; Preliminary Sampling Trial

4.5.3.3 A "Preliminary Sampling Trial" in Australia

The "Preliminary Sampling Trial" in Australia is an initial investigation into the establishment of a protocol for sample type, number and timing for reliable GPGV detection. In this trial, positive GPGV vines were identified with preliminary testing in 2017 in two grapevine varieties from two climatic regions. In 2018, further samples were collected from these vines three times during the season (post-harvest, dormancy and spring). All grapevines expected to be positive for GPGV, based on an initial detection in summer, were positive for GPGV in autumn, winter and spring, when RNA was extracted using the modified RNeasy procedure for the Qiacube HT instrument and tested using the Glasa *et al.,* (2014; Method 1) and Bianchi *et al.,* (2015 Method 3) RT-PCR/RT-qPCR tests. Another important part of this trial is testing using different tissue types which includes two or four shoots or canes or ten leaves with petioles; the latter only collected in spring and autumn testing. Preliminary results show that GPGV could be detected using two or four shoots or 10 leaves of most grapevines that were tested. In a few grapevine samples that were expected to be positive, GPGV was not detected and this may have

been associated with variability in titre or uneven distribution of GPGV within the grapevine. The "Preliminary Sampling Trial" is limited to two different varieties, each grown in different regions and may not be representative of other varieties and other regions in Australia. To ensure accurate diagnosis, and verify or improve current protocols in Australia, it is recommended to:

- Extend the current trial to include a summer sampling point
- Undertake a similar and larger trial using the same sample time and tissue types but including more varieties and regions and GPGV strains
- Examine the diversity of GPGV in Australia and its effect on diagnostic accuracy
- Track the movement of GPGV through an infected grapevine after an initial infection to inform
    - Sampling strategies, including tissue type and quantity
    - The duration from an initial infection event to reliable detection
- Measure GPGV titre with time in different regions and different varieties to determine
    - The most reliable season for GPGV detection in Australia
    - If GPGV detection in Australia might be impacted by climate and variety.

Reliable testing for GPGV, developed for Australian conditions, with other virus testing should be adopted as an industry standard to protect the productivity and sustainability of vineyards.

There is a need in Australia to develop statistically validated sampling strategies for the number of grapevines to test within a block to obtain best estimate of virus prevalence, including GPGV, and to monitor their rate of spread over time. This information will help grapevine improvement associations, nurseries and growers to more accurately assess the risk that a virus might have on production and assist in decision making for virus management, i.e. vector management, to remove individual grapevines, remove a whole vineyard or sections of vineyards or to persevere with an infected vineyard and top working. It would inform vine improvements and nursery about the use of vineyards for cuttings.

4.6 Impact of GPGV and GLMD on grape and nursery production

The nursery, vine improvement and germplasm sector are a critical link in the supply chain to ensure high-health (clean) status material for vineyard plantings.

In Australia and overseas, high-health certification schemes disseminate pathogen-tested grapevine material to commercial growers (Constable and Drew, 2004). The schemes are ideally underpinned by a nucleus collection, which is routinely tested, and in which prescribed pathogens are not detected. Nucleus material is obtained through importation from other countries or through local breeding or selection programs. Propagation material from nucleus collections is used to create foundation plantings from which increase or source blocks are established for the provision of propagation material to nurseries. Active virus testing may be undertaken at each level of the scheme in Australia. There is one high-health nucleus planting established by the Australian Vine Improvement Association (AVIA) at Dareton in New South Wales, but virus testing frequency has waned over the last few years due to lack of funds. Other Vine Improvement germplasms undertake virus testing but on an inconsistent basis. Virus testing also occurs variably through the remaining propagation supply chain of Vine Improvement.

The impact of GPGV on productivity and sustainability of vineyards, and on the nursery and germplasm sector needs to be considered based on its impact on individual grapevines, incidence and distribution in Australian vineyards, and the potential for spread. It is also important to consider the impact on table, dried and wine grape production on a vineyard, regional and country basis.

4.6.1 Impact on vineyard production*; individual grapevine basis*

Several studies have been done in Italy to monitor and measure production and growth parameters of GLMD affected/GPGV infected vineyards planted with varieties that are thought to be more sensitive to

GPGV infection (Table 5; Bertazzon *et al*., 2015a; 2015c; Malossini *et al*., 2014, 2015a, 2015b). The strain of GPGV and the presence of other viruses was not reported in the trial by Bertazzon *et al*., (2015a, 2015b). The impact of GLMD on yield (bunch weight and/or numbers) was variable, depending upon the variety and symptom severity. In most trials significant reductions in yield occurred in the presence of severe GLMD symptoms. In one of the three Glera vineyards assessed, no significant yield loss occurred, however, 35% and 66% yield loss occurred in the other two vineyards. In a group of severely affected Pinot gris grapevines up to 85% yield loss and significant changes in fruit quality parameters occurred, (Bertazzon *et al*., 2015a). Malossini *et al*., (2014, 2015b) measured yield, grape pruning weight and fruit quality parameters in 10 symptomatic and 10 asymptomatic Pinot gris grapevines and 16 symptomatic and 17 asymptomatic Gewürztraminer grapevines. GLMD symptomatic Pinot gris and Traminer grapevines had 30-43% reduced vigour (pruning weight) and 50-60% reduced yield, due to lower number and weight of clusters. The Ravaz index (yield/pruning weight) was lower in diseased grapevines compared to asymptomatic grapevines. There was no significant difference in total soluble solids, total acidity, tartaric acid, malic acid, pH or assimilable nitrogen between GLMD affected and asymptomatic grapevines.

The above results indicate that significant economic impacts associated with GLMD and GPGV infection may occur in sensitive varieties, on an individual grapevine basis. There are very few other studies about impact to fruit production, fruit quality and cane pruning weight for GPGV, but another study in Prosecco vines, in Veneto, north east Italy, observed that a reduction in bunch quality (detail undefined), led to the removal of GLMD affected plants and subsequent economic loss (Raiola *et al*., 2013). Further studies are required to fully assess the impact of specific GPGV strains on growth and yield of different grapevine varieties. Similar studies are required in Australia to investigate the impact of specific GPGV strains, alone and in combination with other viruses, on individual grapevines by accurately measuring fruit yield, fruit quality, vegetative growth and grapevine sustainability.

Table 5. Effect of Grapevine leaf mottling disease (GLMD) on individual grapevines in vineyards of Pinot Gris, Gewürztraminer and Glera (Prosecco) in North Eastern Italy in two studies over different time periods. (Bertazzon *et al*., 2015a; Malossini *et al*., 2014, 2015b)

Region	Timing	Vineyards & variety	Yield reduction with symptoms	Pruning weight reduction	Grape quality parameters		
					Brix	pH	TA
Reference: Bertazzon *et al*., 2015a GMLD rated at 4 levels of severity, except 2 of the Glera vineyards that had only 2 levels- presence and absence of symptoms: 12 vines measured, 4 reps x 3 vines, per severity level							
Veneto	2013-2014	Glera evaluated in 3 vineyards	Variable: 66%, 35% and 0% (due to bunch wt)	[a] n.r.	[b] n.s.	n.s.	[c] Sig 1 vyd
		Pinot gris evaluated in 2 vineyards	80 - 85% in most severe symptomatic grapevines (due to bunch wt and bunch no.)	n.r.	[d] Sig 1 vyd	[d] Sig 1 vyd	[c] Sig 1 vyd
Reference: Malossini *et al*., 2014, 2015b) GLMD rated at 4 levels of severity							
Trentino	4 years 2009 - 2013	Pinot gris;[e] 20 grapevines of 1053 (2%) measured	50% (due to bunch wt and bunch no.)	30%	n.s.	n.s.	n.s.
	2 years 2009 - 2011	Gewürztraminer; [e]33 grapevines of 1106 (3%) measured	60% (due to bunch wt and bunch no.)	43%	n.s.	n.s.	n.s.

[a]n.s. = not significant; [b]n.r. = not reported

[c] Significant in only one vineyard; [d] Significant in only the most severely affected vineyard

[e] Quality parameters were measured in 10 symptomatic and 10 asymptomatic Pinot gris grapevines and 16 symptomatic and 17 asymptomatic Gewürztraminer grapevines.

4.6.2 Impact on nursery, vine improvement and germplasm sector

GPGV is transferred between vineyards during propagation, via cuttings, rootlings and by grafting, in *V. vinifera* and in rootstock material (Saldarelli *et al.,* 2013 and 2015, Malagnini *et al.,* 2015). Subsequent spread is likely to occur through movement of viruliferous insect vectors.

4.6.2.1 GPGV occurs in germplasm worldwide and is often associated with recently imported material

Establishing the incidence of GPGV in germplasm collections and nurseries has been undertaken in several countries to estimate the risk of dissemination to vineyards with GPGV being detected in multiple accessions and varieties (Al Rwahnih *et al.,* 2015, 2016; Bertazzon, *et al.,* 2016 b; Çağlayan *et al.,* 2015; Eichmeier *et al.,* 2016a; Eichmeier *et al.,* 2016b; Elçi *et al.,* 2018; Fajardo *et al.,* 2017; Vončina *et al.,* 2017).

GPGV was frequently detected in germplasm collections and it was found to infect multiple varieties within many collections. In Turkey, 22/60 samples from a germplasm collection and vineyards, including local and foreign varieties, tested positive to GPGV (Çağlayan *et al.,* 2015). GPGV was detected in 21

certified mother vine plants from four varieties in the Czech Republic (Eichmeier *et al.,* 2016a). GPGV was detected in 36.8% of rootstock grapevines (42/114; including Kober 5BB, 110 Richter, SO4, 1103 Paulsen, 420A, Schwarzmann, 101-14, 3309, 161-49, Rupestris du Lot) tested from nurseries in North East Italy (Bianchi *et al.,* 2015). In Brazil GPGV was detected more frequently in recently imported grapevine material, sourced from many countries, compared to older imports in the same germplasm (Table 5) (Fajardo *et al.,* 2017), suggesting infected propagation material was a primary mode of introduction into the region.

Table 6. Relationship between time of import of grapevine material to Brazil and proportion of the material found to be GPGV positive using RT-PCR methods (Fajardo *et al.,* 2017).

Timing of importation of grapevine material	Total number of samples	Number of samples positive to GPGV	% samples positive to GPGV
Early 1990s imports	172	19	11.2
2000 and 2004 imports	80	3	3.8
2015 imports	46	36	78.3
TOTAL	298	58	19.5

4.6.2.2 GPGV spread can occur after an initial introduction

GPGV can been introduced to germplasm collections through subsequent spread, probably by vectors, within a region. For example, in Italy GPGV was not detected during retesting of archived nucleic acid samples of 34 grapevines from two germplasm collections from 2002, that were found to be of sufficient quality for virus testing (Bertazzon, *et al.,* 2016a, 2016b). But the same grapevines were positive in 2014 suggesting spread of GPGV into and within those collections during a 12-year time frame.

Only one GPGV grapevine (cv. Touriga Nacional), which was asymptomatic, was found amongst the 2,322 grapevines tested in the Classic Foundation Vineyard, Foundation Planting Services, Davis, California. GPGV is thought to have been introduced to the collection through infected planting material. Interestingly, GPGV is prevalent in wine grape vineyards in Napa County but not in other regions (Al Rwahnih *et al.,* 2016; Angelini *et al.,* 2016). It may be that there is an environmental factor, possibly associated with vector activity, that leads to greater spread of GPGV in Napa compared to other regions. The route of GPGV introduction into the Napa Valley is unknown.

The observations of GPGV in newer material in some countries suggest a more recent movement of GPGV throughout the world, associated with planting material. In Australia, GPGV has been detected in some older grapevines (F.E. Constable unpublished), however the first detections, as reported by Wu and Habili (2017), were in material imported into Australia during the last 20 years. Detection in older grapevines in Australia may be due to subsequent spread via vectors, from infected younger vines. Verification of vine age of infected planting material can also prove difficult due to issues such as confidentiality, change of ownership and uncertainty of top-working or grafting history.

4.6.2.3 GPGV requires active testing by laboratory methods

In Italy, it was observed that the lack of testing for GPGV in planting material and thus potential for infection could threaten the currently significant export of nursery material to some countries (Gualandri *et al.,* 2016). Due to the presence of GPGV in asymptomatic material, associated with tolerant varieties and/or asymptomatic GPGV strains, molecular testing of both scion and rootstock varieties is necessary to ensure clean material is available.

GPGV is not reliably detected using standard biological indexing methods for virus detection and which rely on symptom expression on sensitive grapevine varieties after they are chip bud inoculated with a candidate variety. In North East Italy, GPGV was detected in 53% (44/84) symptomless and biologically indexed grapevines that were maintained in an insect proof screen house for 10 years (Bianchi *et al*.,2015). GLMD was not observed at any time during Australian post entry quarantine (PEQ) when imported grapevines were tested by biological indexing using LN 33 rootstock, but it has been detected in approximately 10% of grapevine imports since active RT-PCR testing was introduced in 2014 (DAWR, 2018). This highlights that biological indexing on commonly used grapevine indicators is unreliable for GPGV detection and active RT-PCR or ELISA testing is required. It also highlights the risk to Australia of grapevine material imported prior to active RT-PCR testing for GPGV during post entry quarantine since GPGV would not have been observed during biological indexing. Surveillance of germplasm requires active testing by RT-PCR methods.

To achieve an accurate indication of the incidence and prevalence of GPGV in a vineyard it is critical to sample and actively test a representative population of grapevines by RT-PCR methods, including those that are affected by GLMD and those that are asymptomatic. Visual observation for disease could lead to the broader distribution of planting material with GPGV from asymptomatic grapevine cuttings.

Active testing should be done on all varieties and rootstocks. GPGV can be detected in asymptomatic rootstocks (Table 2) and the role of rootstocks in spread of the virus needs to be explored (Bertazzon *et al*.,2015a).

4.6.2.4 Untested grapevine material in germplasms and certification programs is a risk for the further spread of GPGV

The detection of GPGV in many overseas germplasms highlights the risk that untested germplasm and nursery material pose, in Australia and overseas, for the dissemination of GPGV to industry. It is possible that GPGV was introduced into Australia before the virus was identified in 2012. There is likely to have been spread into Australian germplasm and commercial nurseries and vineyards through propagation material and subsequent spread by insect vectors. This might explain the detection in older grapevines and in commercial vineyards in several grape growing regions in Australia (F.E. Constable and L. Tassie unpublished). Active surveillance of Australian germplasms and nursery material is required to estimate the risk of further dissemination through planting material to industry.

Assuming strains of GPGV do cause disease, spread of GPGV through infected propagation material could result in productivity losses to nursery associated with reduction in cane pruning weights and poor quality propagation material. Furthermore, if affected material is disseminated flow-on effects experienced by commercial growers such as losses in fruit yield and reduced fruit quality could occur.

Further potential impacts to the sector are additional costs incurred from testing the incidence of GPGV in the nursery, source blocks and germplasm, and the subsequent management strategies to mitigate further risk of GPGV infection including grapevine removal and replacement as well as strategies to control vectors and alternative hosts.

In Australia there is an urgent need:

- To screen germplasm collections, mother blocks and table, dried and wine grape vineyards to determine the distribution and incidence of GPGV and GLMD in Australian vineyards to inform the risk to production and management strategies
- For the viticulture industries and propagation sector to come to a consensus on the tolerable level of GPGV in mothervine and source blocks
- For GPGV to be incorporated into routine testing of nucleus collections, mothervines and source blocks until the impact of GPGV in Australian viticulture is understood

- For the industry to communicate with PEQ the need to test for endemic transmissible pathogens, and virus eradication or destruction of infected material
- To encourage
    - Growers to test for GPGV as well as other viruses to inform risk of graft failure or disease if they are considering top working grapevines
    - Growers to ask for evidence of testing for GPGV and other viruses on planting or grafting material they buy so they can make an informed choice about disease risk.

4.7 GPGV Epidemiology

4.7.1 Alternative hosts

In Europe GPGV has been detected in *Ascelpias syricaca* (Common Milkweed – native to Canada,) *Chenopodium album* L. (Fat Hen), *Fraxinius* species (Ash species), *Rubus* species (e.g. Raspberries and Blackberries), *Rosa* species (e.g. Rose), and *Silene latifolia* subsp. Alba (Mill.) (White Campion) (Gaulandri *et al.,* 2016; Demian *et al.,* 2018). Many of these hosts were tested because they showed virus-like symptoms including chlorotic spots and mottling.

In Hungary GPGV strains infecting non-grapevine hosts were very closely related or the same as strains in grapevines of the same vineyard, which suggest that movement between the plant species may occur (Damian *et al.,* 2018). In the same study, only part of the GPGV genome could be detected in *Chenopodium* and *Fraxinus* species, suggesting that the virus was in low titre in these alternative hosts (Demian *et al.,* 2018). This result could be important to the epidemiology of GPGV as low virus titres may lower the efficiency of vectors to acquire the virus for further spread. Further work is required to understand how the virus interacts with alternative hosts and the subsequent implications of these alternative hosts as a reservoir for disease. There is also a requirement to determine if the alternative hosts are a reservoir for a vector-mediated transmission of GPGV to grapevines in Australia.

The presence of other viruses was not reported in any of the GPGV infected weeds and it is unclear if the symptoms in these alternative hosts were caused by GPGV or another agent. Many of these alternative GPGV host plant species also occur in Australia and it is recommended that surveillance is undertaken in Australian GPGV positive vineyards for these alternative hosts and that their virus status is determined. Surveillance of other plant species occurring in or near Australian vineyards is also required to determine their potential as alternative hosts that could act as sources of inoculum and contribute to spread of GPGV.

4.7.2 Vectors

Experimentally GPGV was shown to be transmitted by grape leaf bud and blister mites (*Colomerus vitis*; Malagnini *et al.,* 2016). Two strains of *C. vitis* are reported and associated to the tissue types they infest; blister and bud strains. These strains can also be differentiated genetically but they are morphologically indistinguishable (Carew *et al.,* 2004). Bud and blister mites overwinter under the outer scales of buds (Smith and Stafford, 1948; Whitehead *et al.,* 1978; Buchanan and Amos, 1992; Bernard *et al.,* 2000). After bud burst, bud mites migrate to newly developing buds where they lay eggs and blister mites migrate to leaves and cause erinea, where reproduction takes place. Bud and blister mites return to buds in autumn for overwintering. Several generations may occur in a year (Carew *et al.,* 2004). Differential susceptibility of grapevine cultivars to blister mites is reported (Javadi Khederi *et al.,* 2018). This may influence the rate of transmission of GPGV by mites.

Grapevine bud and blister mites are considered monophagous and have not been reported on other species (Duso and De Lillo 1996). Detection of GPGV in alternative hosts leads to three alternative hypotheses: 1) bud and blister mites might occasionally feed on other plant species, 2) other polyphagous vectors are involved in GPGV transmission, or 3) GPGV is mechanically transmitted between plants. All three hypotheses require investigation. The monophagous nature of bud and blister

mites requires testing using known alternative hosts of GPGV. The ability of bud and blister mites to transmit GPGV to other hosts should also be investigated. Other mites that can infest grapevines should be tested for their ability to transmit GPGV between grapevines and to other plant species. The mite species occurring on alternative GPGV hosts growing in vineyards should be examined, and the ability of mite species occurring on these weeds to transmit GPGV should be examined.

Bud and blister mites may be dispersed through wind and may be dispersed in cuttings (Michalska *et al.,* 2010). Blister mites might be transported through movement of infested leaf materials. Mites could also be dispersed on clothing and equipment. Carew *et al.* (2004) reported genetic variation between bud mite populations and between blister mite populations on different varieties and geographically, even within a vineyard, suggesting they are unlikely to spread rapidly without assistance. The unaided movement of mites is unlikely to lead to rapid spread of GPGV from block to block.

Transmission studies showed that both bud and blister mites were able to transmit GPGV in glasshouse (controlled) and field conditions, but bud mites were more efficient, as more grapevines became infected (Malagnini *et al.,* 2016). Recent research suggested that mites could acquire GPGV during 4 hours of exposure to infected symptomless and symptomatic grapevines (Malagnini *et al.,* 2018).

Other trichoviruses are transmitted in a semipersistent manner by Eriophyid mites (Gispert *et al.,* 1998) – that is the virus remains in the insect for a longer period (possibly in the foregut), does not propagate in the insect and is lost upon moulting. There may be no latent period for transmission of a virus by a mite. The type of transmission for GPGV has not been reported. Further work is required to determine the persistence of the GPGV in mites as this could affect management strategies to control the spread of the virus. A greater investigation of GPGV acquisition and transmission efficiency between bud and blister mites is also required, especially under Australian conditions. There is a need to understand the capacity for diverse strains of bud and blister mites to transmit Australian strains of GPGV. There is also a need to investigate if other vectors of GPGV exist in Australia

Malagnini *et al.,* (2016) hypothesised that larger mite populations in buds led to greater GPGV transmission efficiency. The effect of changing climates on the life cycles and population dynamics of mites is required, especially in relation to GPGV epidemiology. It will also be important to consider the impact of climate/environment on GPGV infection of grapevines, as titre and distribution of the virus within a host may affect virus acquisition efficiency.

In Australia, heavy bud and blister mite infestations in vineyards were linked to 'Restricted Spring Growth' (RSG) in which stunted shoots with zig-zag growth and shortened internodes were observed along with leaf distortion (Bernard *et al.,* 2005). RSG symptoms were eliminated when mites were successfully controlled. In Europe, GPGV has also been associated with leaf distortion, zig-zag growth and shortened internodes and recovery from symptoms is sometimes observed later in a growing season and grapevines might not express symptoms in the following season (section 4.2.3). It may be that these symptoms in Europe are sometimes associated with mite infestations rather than GPGV. Conversely it maybe that RSG symptoms in Australia are sometimes associated with GPGV.

4.7.3 Other transmission modes

Like all grapevine viruses, GPGV can be spread through the movement and exchange of infected propagation material. The virus and the disease are also graft transmitted (Saldarelli *et al.,* 2013, 2015, 2017, Malagnini, 2015). The mechanical transmission of GPGV on pruning or harvesting equipment is not known and requires testing. GPGV could not be transmitted experimentally by mechanical (rub) inoculation to the herbaceous plant indicators *Chenopodium quinoa, Nicotiana benthamniana* or *N. occidentalis,* suggesting that these are not hosts of GPGV, or mechanical inoculation is difficult (Saldarelli *et al.,* 2017).

4.7.4 Rate of incidence and spread of GPGV or the disease GLMD

4.7.4.1 Global movement and introduction of GPGV into grape growing regions

The increased movement of grapevine material on an international basis places a greater risk of importing exotic plant borne pathogens in viticulture. The relatively rapid dispersal of GPGV by propagation material is illustrated by surveys in several countries where incidence of GPGV was higher among recently imported material compared to local material (Table 4; Bertazzon *et al.,* 2016a, 2016b; Fajardo *et al.,* 2017; Zikou, 2018).

Many regions of Italy have been surveyed for GLMD and GPGV. Specifically, research has been undertaken in the areas of north eastern Italy, near the first identification of GLMD and GPGV in Friuli-Venezia Giulia, Trentino-Alto Adige and Veneto regions of north Eastern Italy. These regions are primarily planted with varieties such as Pinot gris, Pinot blanc, Gewürztraminer and Prosecco (Glera), that seem to more readily show GLMD symptoms. Compared to Trentino-Alto Adige and Veneto, Friuli-Venezia Giulia is located nearest to the earlier discovery of GLMD-like symptoms in Slovenia in 2001 (Mavrič Pleško *et al.,* 2014).

It has been proposed that GPGV was introduced to the Veneto region from Eastern European countries after 2002 (Bertazzon *et al.,* 2016a, 2016b). GPGV was not detected in historic grapevine material collected from 37 grapevines in the Veneto region in 2002, but it was detected in 27/34 of the same grapevines in 2014. The same study showed GPGV to be present in material collected from grapevines from some eastern European countries (Czech Republic, Macedonia, Montenegro, Ukraine) and Spain prior to 2005, but absent from grapevine material from Croatia and Serbia and some western European countries (France, Greece, Portugal). GPGV was detected in samples of the same grapevines from Croatia, France, Portugal and Spain collected after 2010. Bertazzon *et al.,* (2016a, 2016b.) reported that there had been an increase in importation of varieties from Eastern Europe in the early 2000's which further supports this theory.

In many other countries GPGV appears to have been introduced through recent importations, possibly within the last 10-20 years. On the Italian island of Sardinia, GPGV was not detected in vineyards older than 10 years and/or planted with local cultivars (Gentili *et al.,* 2017). A survey of counties in California found GPGV relatively widespread only in the Napa County (Al Rwahnih *et al.,* 2018). Foundation Plant Services (FPS), California, determined the presence of GPGV in grapevines from Greece, Canada, and Georgia undergoing testing during the importation process for the USA (Fransom, 2016). In Greece, GPGV was detected more frequently on foreign varieties compared to local varieties and sequence analysis of the PCR products and genomes indicated the virus strains detected had low genetic diversity, suggesting a recent introduction into Greece on foreign varieties (Sassalou *et al.,* 2018).

4.7.4.2 GPGV spread and symptom expression

Once GPGV has been introduced to a vineyard, subsequent vector-mediated spread can occur (Malagnini *et al.,* 2018). Research shows that uninfected plants planted near GPGV infected grapevines can become infected within the first year of planting and most became infected within three years, but symptoms were not apparent during the early stages of infection (Bertazzon *et al.,* 2018). GLMD could take as long as seven years to develop in GPGV infected grapevines and expression is variable from year to year (section 4.2.3; Bertazzon *et al.,* 2015a; 2015b). GLMD is frequently associated with some GPGV strains in some varieties (section 5.3), but similar diseases and associated symptoms may also be associated with other factors such as bud mite damage (Bernard et al., 2005). Therefore, GLMD symptom expression is not a reliable indicator of GPGV infection and should not be used to determine incidence and spread of the virus.

In France a nationwide survey for GPGV was done after its initial detection in Bordeaux (Beuve *et al.,* 2015; Spilmont, 2017). Of the 3000 samples that were tested, 72% were infected with GPGV and it was

detected in both old and young vineyards. A greater infection rate was observed in the south of France (Midi-Pyrenees, Languedoc-Roussillon, Provence-Alpes, Cote d'Azure, approx. 90%) compared to other regions (Bourgogne, Champagne > 50%; Alsace, Bordelais, Val de Loire, < 50%). GPGV was only detected in 2% of clones maintained in a screen house, but a much higher rate was observed in the field. This suggests the potential role of a vector and the reduced risk to high-health material maintained in insect proof facilities. A phylogenetic analysis showed French symptomatic GPGV strains clustering with Italian asymptomatic strains, indicating similar strains are present in both countries and might be attributed to the subsequent movement of GPGV eastwards after its introduction to Italy.

In north east of Italy, the spread and incidence of GLMD was monitored over time in two regions; Trentino and Friuli Venezia. In Trentino, Pinot gris and Traminer were studied over seven and five years, respectively (Malossini *et al.,* 2014, 2015a, 2015b). The percentage of grapevines showing GLMD symptoms increased over time in both varieties (Table 7a) but the proportion of symptomatic grapevines was considerately higher in Pinot gris compared to Traminer. In both blocks, there was approximately a doubling in percentage of symptomatic grapevines; from 13 % to 30% over 4 years for Pinot Gris, and from 3% to 6% for Traminer, but thereafter incidence appeared to plateau. The reason for the plateau in disease incidence is unknown but might be associated with changes in vineyard management to try to mitigate further spread of GLMD, such as vector control. Another study undertaken in Pinot gris but in Friuli Venezia, showed a decrease in GLMD affected grapevines each year of the four years studied ((Tarquini *et al*., 2017), Table 7b) reducing from 64.0% in 2013 to 13.2% in 2016. This trial also categorised GLMD expression into 'severe', 'moderate' or 'mild' and GLMD was categorized as mild in 2013, 2015 and 2016, but in 2014 most affected grapevines had severe GLMD (Table 7b). These results suggest other factors, such as environment and management may play a role in influencing disease expression. Although yield and quality parameters were not measured it is possible that the symptoms of GPGV/GLMD may be moderated in certain environments.

Table 7a. The proportion of Pinot gris and Traminer grapevines affected by leaf mottling and deformation (GLMD) symptoms in a vineyard in Trentino, North East Italy (Malossini *et al.,* 2015a, 2015b).

Variety	Trial size	% of grapevines with GLMD symptoms						
		2009	2010	2011	2012	2013	2014	2015
Pinot Gris	1053 grapevines	13.0				30.0		34.5
Traminer	1106 grapevines			3.0		6.0		6.3

Table 7b. The proportion of Pinot gris grapevines affected by leaf mottling and deformation (GLMD) symptoms in a vineyard in Friuli Venezia, North East Italy (Tarquini *et al.,* 2017).

		% of grapevines with GLMD symptoms			
		2013	2014	2015	2016
Across block		64.0	30.4	14.1	13.2
Symptom class	Severe	12.9	75.0	31.2	20.0
	Moderate	27.3	8.3	18.7	6.7
	Mild	59.8	16.7	50.1	73.3

No association between the incidence of GLMD affected grapevines and grapevine age were found in two surveys in Italian vineyards aged between 2 to 40, or 2 to 60 years old and in a French survey that included 100-year-old grapevines (Angelini *et al.,* 2015; Bertazzon *et al.,* 2017; Spilmont *et al.,* 2018). In Veneto, an increase in the severity of GLMD expression was observed in some varieties grown on tight, clay soils, compared the same varieties grown on other soil types (Bertazzon *et al.,* 2017). GLMD appears to be more widespread (84%) in vineyards in the hill regions of Veneto than in vineyards on the plains (49%); (Angelini *et al.,* 2015). This may reflect the general tendency for greater pathological symptom expression and susceptibility in grapevines under some type of stress, as is common with many grapevine diseases.

Although the GLMD can be widespread amongst vineyards within a region, the incidence of disease within individual vineyards could be low and with no significant impact on production. In one grape growing region of north eastern Italy, 70.9% of vineyards had at least one GLMD symptomatic plant. Most affected vineyards (81.4%) had less than 1% GLMD symptomatic grapevines and only nine vineyards (4%) had more than 10% of symptomatic grapevines (Bertazzon *et al.,* 2017). The impact in many vineyards with low levels of GLMD may be negligible, especially in the absence of a vector, assuming GPGV strains are the cause of GLMD. It is important to observe the rate of spread and development of the GLMD, including measurements of cane pruning weight and fruit yield and quality, to understand the long-term productivity of vineyards. It would be interesting to use data from overseas, including disease incidence, productivity and quality parameters and environmental data to model potential impacts of GPGV and GLMD under Australian conditions.

4.7.4.3 Gaps in the knowledge of GPGV epidemiology in Australia

Although some European findings may be extrapolated to local viticultural regions, further work is required to improve our understanding of the GPGV and GLMD epidemiology in Australia. The distribution of GPGV in Australia is currently unknown as no extensive surveys have been done. It is unknown how quickly GPGV spreads in Australia. It is also unknown how GPGV infected grapevines express disease under Australian conditions, especially given the breadth of environments under which grapevines are grown. It is difficult to confirm specific symptom association with GPGV infection in Australia at this time: some samples were submitted for testing due to symptoms that were unaccounted for, and further work is needed to follow up on these, particularly in the table grape example. Many Australian GPGV positive samples were tested as dormant canes in autumn from sample vines for which no symptoms were observed on leaves during the growing season. Other samples were provided for spring testing as either green shoots without leaves, green shoots with leaves but displaying unclear symptomology or suspect bud mite damage, or green shoots with leaves displaying no symptoms. It may be useful to follow up positive test results with resampling and testing, GPGV

strain identification and field observations for symptom development. Although strain variation amongst GPGV isolates in Australia has been reported (Wu and Habili 2017; section 5.4) further work is required to determine their relationship to disease in grapevines and how Australian GPGV strains compare to strains overseas. Genetic variation may assist in modeling impact of disease in an Australian environment, if it is assumed that some strains are the cause of GLMD. The interaction between GPGV and the host in Australia requires investigation to better understand how the virus might cause disease.

4.8 Current management strategies

Aranda and Freitas-Astúa (2017) outlined requirements for effective management of virus-induced diseases: "Management has to be knowledge-based, and therefore it is important to understand the role of ecological factors in the emergence and development of virus epidemics, as well as acquiring better knowledge on the genetic diversity and evolution of plant virus populations. Ecological factors influencing the behaviour of virus populations within their habitats refer to interactions among viruses, vectors and hosts, and include host range, symptom expression, tissue tropism, transmission modes and mechanisms, virus persistence in the environment, and a diverse range of cultural practices and human activities having an impact in these interactions."

With the potential for more horticultural pest incursions occurring on a global basis, there is increased awareness of the need for efficient responses and management of pest invasions in plant industries generally.

Literature on current management strategies for GPGV and GLMD for the vineyard or nursery sectors is limited. The epidemiology of GPGV is still being investigated overseas, with greater clarity being sought on the symptoms and their expression, the genetic diversity and impact of the virus, differing susceptibility of varieties, possible environmental interactions, detail of vector transmission and the significance of possible alternative host plants.

The two key methods for spread of GPGV are vegetative propagation of material and presence of a likely vector. These should be the focus for primary management strategies for vineyards and the propagation sector, as is the general case for virus control in the absence of any specific and detailed information (Constable and Drew, 2004). Management guidelines for GPGV in Italy include mite management and use of high-health planting material that has undergone pathogen testing and/or virus elimination procedures (P. Saldarelli, pers comm.).

4.8.1 Vector control

Until more is known about the biology of the insect vector *C. vitis*, and other potential vectors, and their interaction with the GPGV, it is difficult to make more accurate recommendations to control vector-mediated spread of the virus. In the absence of information about the epidemiology of GPGV in Australia, management strategies based on control of *C. vitis*, assuming it is a vector of GPGV, could be encouraged as an interim measure to mitigate spread of the virus and disease.

The presence of *C. vitis* and other eriophyid mites is common in Australian grapevines. Management strategies for these mites, including spray programs and predatory mites have been investigated and developed for Australia (James, 1992; Bernard and Hoffman, 2011, Learmonth, 2011). In California, there is reference to mite control, with wettable sulphur applications in September/October, or late autumn, when the erineum mites migrate to buds (Varela & Cooper, in Al Rwahnih, 2017). None of these control strategies were developed considering mites as a vector for virus. Although mite control may suppress GPGV spread, further work is required to understand how mite population density and management, impact GPGV transmission efficiency and epidemics in Australia.

France is considering the establishment of pathogen tested germplasm in an insect proof screenhouse to prevent vector mediated transmission of GPGV and other viruses as well as *Xylella fastidiosa* (Marie

Catherine Dubois, pers comm.). Maintenance of high-health grapevine material in an insect proof screenhouse is a recommendation in the EPPO certification scheme for grapevines (EPPO Standard PM4/8(2), OEPP/EPPO, 2008). The Australian citrus industry has undertaken this approach to protect the high-health status of its germplasm (McMichael *et al.,* 2013). This approach could be emulated in Australia for grapevines, although the cost may be high. Another more cost-effective approach would be to retain high-health germplasm in tissue culture to ensure there is a backup of varieties in case of re-infection by pathogens, including GPGV, in the field and other catastrophic events that might impact the security of field-based germplasm.

4.8.2 High-health certification programs

Certification schemes underpin the productivity, profitability and sustainability of production of viticultural industries through the provision of high-health planting material in which significant pathogens, such as viruses, have not been detected (section 7).

In Europe, GPGV is not a registered virus within the European and Mediterranean Plant Protection Organization (EPPO) certification scheme for grapevines (EPPO Standard PM4/8(2), OEPP/EPPO, 2008), nor is it legislated to be included in country based programs such as the Italian national certification scheme. However, some European countries have recommended that GPGV should be included in the EPPO scheme to facilitate movement of high-health pathogen-tested material in which important viruses, including GPGV, have not been detected to minimise risk of further spread of the virus through propagation material (Gualandri and Saldarelli, 2016; Steinmöller *et al.,* 2016). As a part of the management strategy to control GPGV spread, monitoring of mothervine plantings, eliminating infected plants, and establishing new plantings using pathogen-tested material was recommended (Gualandri and Saldarelli, 2016). Testing mandated through legislation would ensure identification of GPGV infected grapevine material, preventing further distribution and assist nurseries and growers to contain the disease.

The Classic Foundation Vineyard, managed by Foundation Plant Services, University of California, Davis, holds disease-tested grapevine germplasm, which forms the basis for the distribution of commercial high-health propagating stock to industry (http://ncpngrapes.org/Grape_Centers/FPS_UCD/). Although no spread was observed, the detection of GPGV in one grapevine only of this germplasm highlighted the risk of potential further spread in this vineyard and subsequent distribution to the broader industry. As a consequence, GPGV was included in the FPS grapevine virus testing protocol (Al Rwahnih *et al.,* 2016; http://fps.ucdavis.edu/grape2010.cfm).

Ensuring Australian grapevine germplasms are not infected with GPGV will assist in preventing dissemination of this virus, and testing germplasm collections for GPGV is critical. Ongoing, active testing for GPGV in grapevine material in certification programs, including those run by various Australian Vine Improvement associations and nurseries (propagation sector), would assist in minimising the further spread and impact of GPGV to industry. Methodology for accurate detection of GPGV and other viruses in propagation material requires further research including a statistically validated method for the number of grapevines to be sampled as well as timing (season) and sample type.

4.8.3 Virus elimination

Sanitary selection, through accurate detection of viruses, and various elimination techniques can be used to produce virus-free material. Methods that have been used for successful virus eradication in grapevines include thermotherapy, somatic embryogenesis, in vitro chemotherapy, cryopreservation or meristem culture (Constable and Drew 2004, Golino *et al.,* 2017). In Australia, AWRI and Agriculture Victoria offer virus elimination services.

Virus eradication research into production of GPGV-free material via meristem tip culture and/or thermotherapy has been reported by Gualandri *et al.,* (2015, 2016). Traminer showed no GLMD symptoms after six months of treatment. In California, Rieger (2016) reports that GPGV is eliminated by meristem shoot tip culture, which is a standard practice of the Foundation Planting Services (Golino *et al.,* 2017). This process of producing "virus-free" grapevines takes 18-24 months before they can be planted in the field-based foundation blocks. Given the length of time that GLMD may take to be expressed, these procedures require that GPGV be actively tested over several years to ensure success.

Research is required over a minimum of three years to monitor GPGV infection in treated and untreated material to measure the rate of success of the various eradication procedures and undertake a comparison of cost and time involved. The use of clean planting material is the best way to minimise the dissemination, incidence and prevalence of viruses in grape growing regions and improve the productivity and sustainability of vineyards. It is then critical to understand the pathways for GPGV introduction into the nursery supply chain and put in place mechanisms to address these, acknowledging also that private imports can be a pathway for GPGV introduction into Australia. Ensuring planting material is free of GPGV will require testing at all points in the supply chain where likely infection and spread may occur. This may need to be incorporated into accreditation schemes; incurring extra cost and due diligence and impacting on the propagation material sector.

4.8.4 Monitoring and vine removal

In Italy, Gualandri and Saldarelli (2016) recommend monitoring of vineyards to determine the incidence of the GLMD disease and its spread, so that further spread may be controlled with removal of grapevines (roguing). Removing affected vineyards and replanting with clean material is cited as the only sure way to combat GPGV. Gentili *et al.* (2017) noted that a high incidence of GPGV infected asymptomatic grapevines makes control of the virus incidence and spread difficult. The impact and economic benefit of roguing to prevent GLMD and GPGV spread, requires accurate modelling in Australia.

4.8.5 Vine nutrition

One study has shown that the foliar application of fertiliser as a mixture of macro, meso and microelements, with the addition of seaweed (*Ascophyllum nodosum*) can mitigate the impact of GLMD inseverely affected vineyards in some years by improving bunch weight and TSS (Brix), although the changes were not always statistically significant (Bertazzon *et al.,* 2016c). This approach may alleviate the impact of GPGV and GLMD if removal of grapevines is not practical, but it may not mitigate the spread of the virus and disease. Further work is required to determine if this approach is viable under all grape growing conditions.

4.9 Biosecurity

Movement and trade of contaminated plants and plant products threatens plant biosecurity globally and at a local level (Martin *et al.,* 2016). Management in other countries, off-shore, and at PEQ in the importing country can prevent the introduction of new plant pests. Certification programs are designed to mitigate the impact of important pests and disease. These programs depend upon accurate knowledge of the biology and epidemiology of the pest that impact the crops and environment that they were designed to protect.

GPGV was identified and confirmed overseas in 2012 through NGS of GLMD diseased grapevine samples and subsequent development of RT-PCR tests. Australian quarantine authorities updated protocols to routinely test for GPGV in imported grapevine material during 2014 and, since then, approximately 10% of grapevine imports tested positive for GPGV during PEQ (DAWR, 2018). Wu and Habili (2017) detected GPGV in varieties that were legally imported between 4-19 years before the detection in 2016. Therefore, it is possible that GPGV may have entered Australia in propagation material prior to 2012

when there was little or no knowledge about the virus and even up to 2014 before diagnostic techniques became available in PEQ. Although strict regulations and biosecurity controls are in place at the Australian border, it is also possible that GPGV infected material has entered illegally and been disseminated to industry.

4.9.1 Notification of an exotic pest

Management of GPGV, or other invading virus or pathogen, on a national level is a process that is initiated on notification of entry or presence of the virus to that country (Plant Health Australia 2017). In Australia, once the pest is reported, the Australian Chief Plant Protection Officer notifies government organisations and affected industries who are signatories to the Emergency Plant Pest Response Deed (EPPRD) and organises a meeting of the Consultative Committee for Emergency Plant Pests (CCEPP). The CCEPP evaluates firstly whether it can be classified as an Emergency plant pest (EPP), and then the feasibility to eradicate the EPP based on the likelihood of a successful eradication and the cost/benefit of eradication. GPGV was deemed not technically feasible to eradicate because it was detected in several grape varieties, occurred as asymptomatic infection, and was found in a number of unrelated locations.

4.9.2 Developing management plans for GPGV

One of the keys to developing both a successful management strategy for eradication and effective and sustainable longer-term management strategies for industry if a pest cannot be eradicated, is to undertake research activities to better understand the distribution, spread and management options, based on information specific to the country's environment and conditions. However, a starting point would be informed by information gained and evaluated by other countries where the pest is known to occur. Daane (2018) notes "*Modern connectivity now allows greater potential for information sharing from regions where pests are established, to newly invaded regions*" and "*high-quality, research-based information to guide rapid responses to new invasions will be essential to maintain the global sustainability of viticulture*".

An example of effective management was the rapid response in California to the European grapevine moth after assistance provided from a specialist team from Italy, comprising;

1. *"identification of the insect's geographic range,*
2. *development and implementation of detection and eradication programs, including chemical treatment and mating disruption,*
3. *regulation of grape plant movement to minimise dispersal,*
4. *incorporating research-based information into policy decisions, and*
5. *promoting a wide-reaching educational program for growers, the public, and local officials" Daane (2018).*

A similar approach to develop a management strategy for GPGV has been undertaken in Europe (Saldarelli *et al.,* 2017). As discussed in this review several European and Asian countries have identified the regional and national distribution of GPGV and GLMD. Work is ongoing to understand the genetic diversity of the GPGV and the presence of other pathogens in infected grapevines and to relate this to symptom expression and disease. Work is also ongoing to identify vectors and alternative hosts. Acquisition of research-based facts is the only way sustainable and effective longer-term management strategies can be developed for industry. The impact of GPGV on the Australian wine, table and dried grape industries is still unquantified and requires more research to determine the most appropriate response. An estimation of the distribution and prevalence of GPGV in Australian grape growing regions should also be the first stage in developing a management plan for Australia.

Many affected countries are still at the early stages of assessing GPGV incidence. After the initial isolation and identification of GPGV in association with GLMD disease, Italy responded primarily by region, with more intense surveys for the virus and the disease in areas most severely affected by the GLMD, in the north east of the country. A risk analysis (Steinmöller *et al*., 2016) recommended that monitoring in all German wine regions be carried out to determine if GLMD and GPGV distribution was broader than Rhineland and Baden-Württemberg where it was first reported, although the assumption was that GPGV was already widespread in the country. Other countries have taken a national and/or regional approach to surveillance, possibly dependent upon the size of the country, size of the industry and perceived impact of disease. This information is used to prioritise research to identify the most appropriate management strategies to control further spread of disease.

Recommendations have been made that the virus should be included in the European and Mediterranean Plant Protection Organization (EPPO) grapevine certification scheme, although the virus is considered a lower priority compared to other pathogens such as *Xylella fastidiosa* (Delbecque, 2016). Undertaking active testing and surveillance of certified material and germplasm collections allows a greater understanding of the prevalence of GPGV amongst different varieties in different regions and informs risk and management options for international quarantine and trade, the certification programs, nurseries and growers. Similarly, GPGV could be included in testing of Australian nursery certification programs and it is recommended that growers request testing prior to top working vineyards.

In Australia, incidence of GLMD and GPGV as well as prevalence of both symptomatic and asymptomatic GPGV strains is poorly understood. A surveillance plan for both asymptomatic and symptomatic grapevines and GPGV is recommended. After the initial GPGV detection in NSW and South Australia, a mix of passive and active surveillance for GPGV was undertaken in spring 2017 across New South Wales, Queensland, South Australia, Western Australia, Tasmania and Victoria. Most GPGV testing was done on Victorian, New South Wales and South Australian material and resulted in some additional detections. The number of samples tested for GPGV in Queensland, South Australia, Tasmania, Victoria and Western Australia was <230 samples (F.E. Constable, unpublished). GPGV was not detected in "symptomatic" Queensland or NSW samples or from a single asymptomatic sample from Western Australia. However, overall only a very small proportion of vineyards may have been tested Australia-wide and there is a significant gap in understanding of the true distribution in Australian vineyards. At this stage the exact number of samples and the locations of vineyards tested for GPGV in each Australian grape growing region is unknown. Varieties that were positive for GPGV in Australia are listed in Table 1. As previously stated, traceback is often difficult, and extent or source of the GPGV infection is not always clear. Presence in a variety in one location does not necessarily indicate the population of that variety is infected.

In South Australia, an active surveillance plan was developed using Vinehealth Australia's comprehensive register of South Australian vineyards. Blocks with recently imported material were cross referenced against varieties in which GPGV had been previously reported and to identify targeted sampling sites. This exercise confirmed additional cases of GPGV in South Australia, which contributed to the resolution that GPGV was not technically feasible to eradicate. Testing of grapevines from Queensland, Tasmania, Victoria and Western Australia for GPGV was not targeted in the same way. Instead testing was ad hoc and relied on the willingness of growers to submit samples with GLMD-like symptoms or other possible virus-associated disease or to request GPGV testing during routine screening to assess vineyard health. Although this led to the eventual detection of GPGV in Victoria, a more targeted approach might have led to an earlier diagnosis and contributed to a decision about the feasibility to eradicate prior to 2018 by the CCEPP and industry. This highlights the need for a national vineyard database to underpin coordination of surveillance by the industry signatory to the EPPRD

during an exotic pest incursion on a national scale, and for education and outreach to growers. The GPGV incursion provides an opportunity to assess the national response to an incursion of an exotic pest to adjust the process where necessary, so that it is rapid and efficient and inclusive of all stakeholders.

4.10 Ongoing international research

In California, an initial survey for GPGV was conducted of the foundation collections in 2015. Ten Californian regions were subsequently surveyed in 2016 and 2017. A more extensive study to further investigate GPGV has been established and is outlined by Al Rwahnih (2017) below;

"1. Conduct field surveys and investigate the prevalence of GPGV in different California grape growing regions;

2. Assess the causal relationship between GPGV infection and disease symptoms. Assess the extent to which symptoms are enhanced by co-infection by other viruses;

3. Complete a molecular characterization of California isolates of GPGV;

4. Improve the reliability of molecular and serological detection methods for routine GPGV detection;

5. Monitor the natural spread of GPGV in selected vineyards in California; and

6. Circulate research results to farm advisors and growers."

All components of this study have been initiated, and reported on, with some ongoing aspects to increase understanding and develop appropriate management strategies for GPGV (Al Rwahnih *et al.,* 2017).

Various European laboratories, particularly in France, Italy and Hungary, continue to build upon GPGV epidemiological studies about disease association, genetic diversity, alternative hosts and transmission as well as the impact of disease. Many may not have specific research programs about GPGV and GLMD, but they build studies into broader programs about genomics, production of high-health material and vectors and other areas.

In France, a national approach to ongoing GPGV research has been proposed (Spilmont, 2017). It will focus on longer term monitoring in some affected regions in the north and south east of France, vector identification, the role of alternative hosts, an improved understanding of the symptoms associated with GPGV strains and titre in different regions and varieties, the agronomic impact on production, the role of other pathogen and environment.

The grapevine pathology group led by Dr. Elisa Angelini at Council for Agricultural Research and Economics - Viticulture and Enology (CREA-VE), have undertaken and continue much of the research on GPGV and GLMD in Italy. L'Istituto per la Protezione Sostenibile delle Piante (IPSP), Centro di Ricerca per la Patologia Vegetale (CREA-PAV), and other universities and research organisations have also contributed significant and valuable research through the initial identification of GPGV in GLMD affected grapevines, the identification of a vector, plant-virus interactions and monitoring of disease.

There is potential for Australian researchers to link with international laboratories for a cost effective and efficient way to develop and expedite a global approach to GPGV and GLMD control. However, studies should be done to understand the biology and epidemiology within Australia so that management strategies are designed to suit local conditions. An effective management strategy for GPGV requires both a short and long term coordinated research program, critically combined with an extension program and tailored resource development for each stakeholder group including growers, nursery, vine improvement associations, educators, advisers and vineyard staff.

5.0 Outcome/Conclusion

After a review of the current literature and studies on GPGV, the following gaps in Australia were identified:

1. The geographic and varietal distribution and prevalence of GPGV and GLMD
2. The most appropriate field sampling strategy and reliable diagnostic methodologies for GPGV detection
3. Varietal response to GPGV infection on its own and with other viruses
4. The agronomic impact of GPGV and GLMD in Australia
5. The genetic diversity of GPGV and its impact on detection and symptom expression
6. The Australian vectors of GPGV
7. Identification and role of alternative hosts
8. The management of GPGV in Australia
9. Developing GPGV free germplasm

Additional work is also required on the biology, epidemiology and impact of other grapevine-infecting viruses on production in Australia, which should link with work on GPGV.

Recommendations have been developed for future research in Australia to improve our understanding of GPGV in the Australian environment and development of management strategies that are relevant locally.

Management strategies to control and minimise risk of GPGV that can be implemented throughout the Australian viticultural supply chain, given the current level of knowledge have also been identified and recommended.

6.0 Recommendations

On a broad industry scale, recommendations are made to manage the incursion of this virus, by:

1. Adopting an appropriate suite of research strategies to better understand the distribution, spread, biology and epidemiology of GPGV, to allow development of the most appropriate short and long-term management strategies, based on information specific to Australia's environment and conditions.

2. Combining the above with an extension program and development of targeted resources for each stakeholder group.

3. Creating closer and stronger links between industry and across viticulture (wine, table and dried grapes) with the propagation sector, including vine improvement organisations, nurseries and germplasms, to ensure a cohesive, cooperative approach to ensuring high-health planting material as the basis for sustainable vineyards for the industry. This is also critical for future incursion management.

In addition, it is recommended to:

4. Foster and retain current and future knowledge, capacity and capability in:
    a. pest and disease research, in particular vector-transmissible viruses.
    b. industry specialist areas such as virology, entomology and disease epidemiology
    c. grapevine germplasm and planting material
    d. international relationships with pertinent research organisations and personnel.

5. Review the current process of grapevines through PEQ and encourage testing and traceability for endemic pathogens that are controlled in high-health planting material schemes.

6. Undertake a review of the effectiveness of the wine and grape industries' response to GPGV and implement strategies to minimise future loss of production and maximise containment in future incursions.
7. Develop a targeted emergency response plan to assist with the detection and identification of other viruses that are exotic to Australia as well as new to science viruses.

6.1 Future research to improve understanding of geographic distribution

To date, Australia has not carried out a systematic and broad survey for GPGV. To better inform the transition to management strategy for whole of industry there is an urgent need to understand the distribution and prevalence of GPGV in scion and rootstock varieties across all grape growing regions in the Australian viticultural supply chain - including germplasm collections, nurseries, source blocks and commercial vineyards of table grape, winegrape and dried fruit via:

1. A baseline systematic survey undertaken in the coming growing season on vineyards of table, dried and wine grape varieties and all grape growing regions to understand the distribution and incidence of GPGV to determine the future risk to production and benchmarking appropriate management strategies.
2. A coordinated screening of all germplasm collections, mother grapevines and source blocks to assess all cultivars, *Vitis* species and hybrids and, including ornamental species, fruit varieties and rootstocks, to inform the future risk to propagation and production and appropriate management strategies.

6.2 Future research to improve surveillance and diagnostic capability for accurate detection

There is a need to ensure that diagnostic capability for GPGV in Australia is accurate to enable provision of healthy planting material, and optimal top-working in vineyards. Diagnostic capability can be improved via:

1. Investigating genetic diversity of Australian GPGV isolates to ensure specificity and reliability of the diagnostic tests used for their detection.
2. Tracking the location and measuring GPGV titre through an infected grapevine after an initial infection event to inform reliable field sampling strategies to improve GPGV detection by:
    a. developing a quantitative RT-PCR for accurate measurement of GPGV titre, regardless of strain
    b. sampling different tissue types and quantity regularly (monthly) over several seasons
    c. replicating the trials in different regions to assess environmental impact.
3. Use of quantitative RT-PCR to measure titre of various GPGV strains in different fruit and rootstock varieties in different regions to determine if there is a relationship between GPGV strain and titre and symptom expression in Australia.
4. Development of statistically validated sampling strategies, based on this data, for vine improvement associations, nurseries and growers to more accurately assess the incidence of GPGV.
5. Enable proficiency testing to ensure laboratories can accurately detect GPGV with the extraction procedures and tests that they use.

There is a need in the industry for similar work to be undertaken with other viruses, in particular transmissible grapevine leaf roll-associated viruses that have caused considerable disruption to the grape industry in other countries.

6.3 Future research to improve understanding of GPGV infected varieties, symptom expression, strain variation, coinfection with other viruses, and ultimate impact on vineyard production

The impact of GPGV on grapevine production on its own, and in combination with other viruses, is unclear. There is a need to investigate the association between GPGV and expression of disease and its impact on production in the Australian environment via:

1. Studying the reaction of Australian grown table, dried and wine grapevines infected with GPGV in the presence and absence of other viruses over time;
    a. It is recommended studies are done to understand the diversity of Australian GPGV strains and their association to disease in a broad range of varieties.
2. Measuring yield, fruit quality, and pruning weight parameters to assess the impact of GPGV on production in diseased and asymptomatic grapevines.
3. Monitoring the rate of spread of GPGV in Australia.
4. Combining Australian and overseas data (including disease incidence, productivity, quality parameters and environmental data) to model potential impacts of GPGV and GLMD under Australian conditions.

6.4 Future research to improve understanding of GPGV epidemiology: alternative hosts & vectors

GPGV infects other plant species in Europe and the role of alternative host plants in Australia needs to be determined via:

1. Surveillance of known alternative hosts and other plant species commonly occurring in and nearby GPGV positive Australian vineyards for GPGV and mites.
2. Investigating the interaction between GPGV and alternative hosts to understand the role of these alternative hosts as a reservoir for disease.

The role of grape leaf bud and blister mites (*Colomerus vitis)* and other mites that are ubiquitous in Australian vineyards, as vectors of GPGV in Australia needs to be investigated via:

1. Investigation of the persistence, acquisition and transmission efficiency of GPGV by bud and blister mites, and the capacity for diverse strains of these mites to transmit Australian strains of GPGV.
2. Determining if mite population density and control impact GPGV transmission efficiency and epidemics in Australia.
3. Assessing whether:
    a. bud and blister mites might occasionally feed on other plant species
    b. other polyphagous vectors are involved in GPGV transmission
    c. GPGV is mechanically transmitted between plants.

6.5 Developing local management strategies

As information is gathered management strategies to control GPGV in Australia can be developed, improved and updated in the following key areas:

1. Developing GPGV free germplasm through selection and virus eradication
2. Implementation of GPGV test procedures
3. Control of vectors
4. Control of alternative hosts.

6.6 Recommendations for immediate management of GPGV in Australian viticulture

There are strategies that can be implemented now to mitigate the risk of GPGV to the Australian viticulture industry:

1. GPGV added to a standard suite of grapevine virus tests offered commercially.
2. All 'at the border' or 'post border' laboratories in Australia undertaking GPGV testing, participate in proficiency testing, especially where different diagnostic tests are being employed by different laboratories.
3. Management of mites to reduce the risk of GPGV spread.
4. Support from the propagation sector and industry stakeholders to endorse the recommendation of zero tolerance of GPGV infection in mother grapevines or source blocks and to ensure that this is embedded in the certification standards for nurseries and grapevine improvement associations, especially in the current absence of knowledge about GPGV in Australian viticulture.

6.6.1 Management Strategies

A number of management strategies to supply clean planting material to growers are recommended:

1. Investigation of virus eradication in grapevines including thermotherapy, or meristem culture, with research required over several years to monitor GPGV infection in treated and untreated material, and measurement of the rate of success, cost and time of the various eradication procedures to produce GPGV free grapevine material.
2. Development of an understanding of the pathways for GPGV introduction into the nursery supply chain, and a requirement to test all points in the supply chain where likely infection and spread may occur.
3. Measurement of the impact and economic benefit of roguing to prevent GLMD and GPGV spread in Australia.
4. Investigation of containment facilities to hold high-health germplasm
    a. mite and other insect proof screen-housing
    b. tissue culture.

The purpose of this section was to identify and provide recommendations for GPGV research and extension. The authors acknowledge that these recommendations must be prioritised alongside other biosecurity priorities for industry's benefit.

Appendix 1: Communication

STAKEHOLDER ENGAGEMENT

The Grapevine Pinot gris virus project was undertaken in light of limited prior knowledge on the subject. Consequently, this project provided a unique opportunity to inform the nursery and vine improvement sector and wine and grape industries, of management activities important to contributing to the production of high-health propagation material and reducing GPGV spread.

A number of stakeholder communications were delivered as part of this project using a range of mechanisms. These included production of an updated industry GPGV fact sheet, a range of electronic news articles published and delivered by Vinehealth Australia to over 2,300 subscribers, a dedicated GPGV webpage describing the overarching project, and teleconference, meeting and multiple information papers shared with the nursery and vine improvement sector.

Subsequent to the completion of this project, additional e-news articles will be completed, and potentially an industry journal article and a scientific journal article.

A list of stakeholder engagement items herein includes:

1. Wine Australia Grapevine Pinot Gris Virus Fact sheet - draft
2. Vinehealth Australia GPGV webpage
3. Teleconference – Nursery and Vine Improvement Sector – 15 March 2018
4. Australian Grapevine Foundation Planting Service (AGFPS) Meeting – McLaren Vale 14 November 2018
   Project background and update provided by Libby Tassie, Inca Pearce and Suzanne McLoughlin
5. Preliminary information paper to Nursery and Vine Improvement Sector – May 2018
6. Second information paper to Nursery and Vine Improvement Sector – January 2019
7. Vinehealth Australia e-news article May 15, 2018 - GPGV activity to assist growers
8. Vinehealth Australia e-news article 17 April 2018 - Virus knowledge boost
9. Vinehealth Australia e-news article June 12, 2018 - Improved detection of viruses vital

1. Wine Australia Grapevine Pinot Gris Virus Fact sheet - draft

Wine Australia for Australian Wine

Factsheet December 2018

Grapevine Pinot gris Virus

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Key messages

- GPGV is established and present in New South Wales, South Australia and Victoria and has been detected in a number of wine, table and rootstock varieties
- GPGV may be associated with grapevine leaf mottling and deformation but also occurs in symptomless grapevines
- The impact of GPGV on vine health and productivity is variable
- GPGV may occur in mixed infections with other viruses
- GPGV is spread through propagation material and grafting and is vectored by bud and blister mites
- Virus testing is required to confirm the presence of GPGV
- Vineyard planting material should be obtained from an accredited source and diagnostically tested for presence of GPGV
- Controlling mites and alternative hosts in the vineyard and removal of infected vines will also help to prevent the spread of GPGV

Introduction

Grapevine Pinot gris virus (GPGV) was first identified and characterised in Italy in 2012. It was named after the winegrape variety in which it was first detected, but subsequently has been found in many other wine, table and rootstock varieties, and in many countries. GPGV was first detected in Australia in 2016 and is established and present in New South Wales, South Australia and Victoria.

Symptoms

Overseas, GPGV has been associated with 'grapevine leaf mottling and deformation' (GLMD) disease in some winegrape varieties. GPGV has also been detected in grapevines expressing symptoms of other diseases (asymptomatic) and grapevines which appear healthy (symptomless). In some vigorous varieties, recovery from



Figure 1: Chlorotic mottling and leaf distortion in Pinot Grigio, symptoms of Grapevine Pinot gris virus.
Image courtesy P. Saldarelli, University of Bari, Italy.

symptoms and presentation of normal growth has been observed after veraison. Clarification of symptoms and their association to GPGV in a range of wine, table and rootstock varieties and environments is still required. The occurrence of mixed infections of several virus species in single grapevines also presents a difficulty in attributing symptoms directly to GPGV.

Foliage

Leaves on GLMD-affected grapevines may have chlorotic mosaic or mottling symptoms (Figure 1), and may also be distorted. These symptoms are sometimes more pronounced early in the growing season and can resemble symptoms of cold or bud mite damage.

Vine growth

GLMD-affected grapevines may have stunted shoots, due to shortened internodes, and delayed budburst (Figure 2). Less common symptoms include poor lignification, shoot tip necrosis, zig-zag shoot growth and reduced cane weight (Figure 3).

Fruit

Reduced yield due to low bunch weight and bunch numbers has been reported in Italy in GLMD-affected Pinot gris, Gewürztraminer and Glera (Prosecco) that were also infected with GPGV. Bunch shatter and desiccation, millerandage and delayed ripening were also reported. Fruit of affected grapevines may have increased acidity and lower sugar levels can occur in must. The impact of GLMD on fruit yield and quality in other varieties is currently unknown.

Varietal sensitivity

GPGV has been detected in many wine and table grape varieties and rootstocks, which suggests that most *Vitis* spp. are susceptible to infection. Variability in disease expression may be attributed to differences in varietal sensitivity to GPGV infection: some varieties express the more common symptoms of GLMD, while others are asymptomatic or symptomless. Pinot gris, Pinot blanc, Pinot noir, Gewürztraminer, Tocai friulano and Glera (Prosecco) are reported as the most sensitive varieties to GPGV infection in north-east Italy. Genetic variability of the virus is also reported to affect symptom expression. Variability in symptom expression from one season to another can also occur, and may be associated with environmental factors such as temperature and water stress.



Figure 2: Stunted shoots in Traminer, a symptom of Grapevine Pinot gris virus. Image courtesy P. Saldarelli, University of Bari, Italy.



Figure 3: Stunted, zigzag growth and lateral shoot development caused by the bud mite strain of the grape erineum mite on *Vitis vinifera* in early spring. (W. Gartel) In: Compendium Slide Sets A Pictorial Guide to Grape Diseases © 1989 by The American Phytopathological Society Produced in the United States of America.

Biology

GPGV presence worldwide

GPGV is a member of the genus *Trichovirus* in the subfamily *Trivirinae*, family *Betflexiviridae*. Many different strains of the virus have been detected in grape-growing regions of European and Asian countries, USA and Canada in North America, Brazil in South America and in three states of Australia (New South Wales, Victoria and South Australia). Multiple GPGV strains can occur within a single vineyard. Studies have suggested a division of GPGV strains into three or four clades. Some clades of GPGV consist of strains which are more commonly associated with GLMD-affected grapevines, whilst other clades contain strains more commonly associated with asymptomatic/ symptomless grapevines.

Transmission and spread

GPGV is found in phloem tissues. Like similar viruses, it is not known to be mechanically transmitted by touch or *via* pruning and harvesting equipment. GPGV can be spread through the movement and subsequent planting of infected propagation material. The virus and the disease are also graft-transmissible.

Overseas it has been demonstrated that grape leaf bud and blister mites (*Colomerus vitis*) can transmit GPGV between grapevines (Figure 4). Other vectors are not known.

Virus movement within a grapevine

The concentration (titre) of GPGV within a vine can vary across the season, with the highest titre in spring and lowest in autumn. This variability can affect laboratory detection, as false negatives are possible if virus titre is below the level of detection.

In addition, distribution of GPGV within the grapevine can be uneven and sampling strategies need to be sufficient to ensure accurate detection.

Alternative hosts

In Europe, GPGV has been detected in several plant species, including *Asclepias syriaca* (Common Milkweed) and *Chenopodium album* L. (Fat Hen), *Rubus* (Raspberries and Blackberries), *Rosa* species (e.g. Rose) and *Silene latifolia* subsp. Alba (Mill.) (White Campion). However, the importance of these alternative hosts as a reservoir from which mites can acquire the GPGV and then transmit it to grapevines, is unknown.

Disease management

GPGV is spread through propagation material. The nursery, vine improvement and germplasm sector is therefore critical in the management of GPGV. Material held by this sector should be routinely screened through active diagnostic testing and visual inspection for the presence of viruses and virus-associated diseases, including GPGV. Identification of GPGV infection is challenging because of the occurrence of symptomless or asymptomatic propagation material. It is therefore important to confirm the presence/absence of virus through diagnostic testing, which should be done at all points in the supply chain where infection and spread may occur. Such measures will ensure provision of high-health status propagation material (high quality, pathogen-tested) to industry and achieve desired productivity and sustainability for Australian vineyards.

Unpublished research in Australia has shown that a minimum of two shoots, one from each side of a grapevine, or 10 leaves with petioles randomly collected across an entire grapevine, is adequate for detection of GPGV during autumn, winter and spring. As GPGV can be spread by bud and blister mites, grapevines should be continually monitored and actively tested to check for new GPGV infections.

Active control of bud and blister mites, alternative GPGV hosts and rogueing of GPGV-infected vines should be undertaken by the nursery, vine improvement and germplasm sector. The use of grapevine tissue culture and/or an insect-proof screenhouse covered with a fine mesh to hold high-health germplasm can minimise risk of infection through vector activity and should be considered. Virus elimination can be undertaken to produce virus-free mother grapevines.

All vineyards should use high-health planting material obtained from accredited sources for vineyard establishment and vine replacement. Both the scion and rootstock should be virus-tested separately when purchasing grafted vines and in grafting or top-working situations.

GPGV vectors and alternative hosts should be controlled in all commercial vineyards to minimise virus spread. In addition, the incidence of GPGV should be determined through virus testing and removal of GPGV-infected vines considered.

More work is required in Australia to study symptom expression, virus variants, varietal sensitivity and vector and alternative host interactions to better determine commercial impact and inform management strategies.



Figure 4: Bud mite damage. Image courtesy F. Constable.

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Wine Australia for Australian Wine

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2. Vinehealth Australia GPGV webpage

VINEHEALTH
AUSTRALIA



Grapevine Pinot Gris Virus (GPVG) is a new to science pathogen, first described in Italy in 2012 and subsequently reported in many other countries across Europe, Asia and North America. Diagnostic capacity became available in Australian laboratories in 2015. In late 2016 and in subsequent surveillance undertaken in 2017, the virus was detected in Australia in multiple wine grape varieties and one table grape variety across three states.

Fast facts about GPGV

- Now classified as established and present in NSW, Victoria and South Australia.
- Has been detected in more than 30 grape cultivars worldwide.

http://vinehealth.com.au/industry/pests-diseases/viruses/grapevine-pinot-gris-virus/ 1/5

- Infected grapevines may show symptoms or be symptomless.
- The virus can spread through infected propagation material and possibly by bud mites. It is thought to have alternate hosts such as Fat hen (*Chenopodium album*) which is widely naturalised throughout large areas of Australia.
- Symptoms are most evident in spring and include leaf mottling and deformation and can be confused with cold or mite damage.
- Little is known on the effect of the virus on grapevine growth, yield and fruit quality.

Local research

A Wine Australia-funded project titled 'A comprehensive review of Grapevine Pinot Gris Virus (GPGV), including recommendations for future research, development and extension work in Australia' began in April 2018 and is expected to provide significant guidance for the industry. Findings will be communicated at the conclusion of the project, post 31 October 2018.

Additional Information

Refer Vinehealth Australia's Biosecurity alerts:

12 Oct 2017 (http://mailchi.mp/vinehealth/vinehealth-australia-gpgv-biosecurity-alert-october-2017-1495821)

9 Aug 2017 (http://mailchi.mp/vinehealth/grapevine-pinot-gris-virus-biosecurity-alert)

Refer Vinehealth Australia's E-news articles:

May 2018 (http://vinehealth.com.au/2018/05/15/gpgv-activity-to-assist-growers/)

Refer to the AWRI's GPGV factsheet (https://www.awri.com.au/wp-content/uploads/2015/09/GPGV-fact-sheet.pdf)



Symptoms of Grapevine Pinot Gris virus. Image courtesy Dr. Pasquale Saldarelli, Istituto per la Protezione Sostenibile delle Piante, Bari, Italy.

3. Teleconference – Nursery and Vine Improvement Sector – 15 March 2018

Eight pages of minutes taken to inform future actions. A copy of these minutes can be obtained from Vinehealth Australia upon request.

5. Preliminary information paper to Nursery and Vine Improvement Sector – May 2018

GRAPEVINE PINOT GRIS VIRUS

Preliminary information paper for the Nursery and Vine Improvement sectors

May 2018

Authors: Libby Tassie (Tassie Viticulture Consulting)
Dr Fiona Constable (Agriculture Victoria)

Introduction

In early May 2018, the project 'A comprehensive review of *Grapevine Pinot Gris Virus (GPGV)*, including recommendations for future research, development and extension work in Australia', was initiated. This project is principally funded by Wine Australia with additional funding and in-kind support by Vinehealth Australia, Agriculture Victoria, Vine Improvement Associations, Vine Industry Nursery Association and the Australian Wine Research Institute (AWRI).

The project, led by Vinehealth, is being undertaken by Dr Fiona Constable, Senior Plant Virologist from Agriculture Victoria and Libby Tassie, Principal Consultant, Tassie Viticulture Consulting. Dr Nuredin Habili of the AWRI will also provide expert technical advice to the project.

The project will run until the end of October 2018 and aims to:

- Identify knowledge gaps in the epidemiology and diagnostics for GPGV and use this to prioritise requirements for future research and development,
- Better understand the potential impact of GPGV on Australian viticultural industries, including for wine, table and dried grape production and nursery and vine improvement sector,
- Provide preliminary guidelines:
    - to growers for managing infected blocks and to minimise spread of GPGV
    - for field sampling for GPGV
    - for laboratory diagnostic protocols for GPGV
- Empower the nursery and vine improvement sector to adopt appropriate management strategies for the provision of planting material free of GPGV.

Preliminary sampling trial

As part of the project, a preliminary sampling trial has been established to provide the propagation industry with more information about the reliability of GPGV detection in propagation material taken during the 2018 cutting season.

This should assist the propagation industry to:

- Better understand the risk of false negative results associated with taking cuttings in 2018,
- Better understand the sampling required to minimise false negatives, and reliably assess the incidence of GPGV in propagation material,
- Limit the spread of the virus at this stage when the epidemiology and impact of GPGV in Australia is unclear.

Results from previous samplings and testing for GPGV had been variable and false negatives have occurred, that is, GPGV was not picked up when in fact the vines were infected with the virus. Better understanding of sampling timing and methodology may assist in reducing the risk of virus spread.

Given the unknown implications of GPGV across all varieties and all environments, an important step for the industry at this stage is to reduce risk of spread via infected planting material.

The sampling trial tests for GPGV in vines in 2 vineyards in which the virus was previously detected. The first vineyard is in a hot inland region in Victoria and is a tablegrape variety. The second is in a cool climate region in South Australia and is a winegrape variety. For each vineyard the following are variables that will be tested:

- Time of field sampling: 3 times across the season → autumn, winter, spring
- Field sampling technique:
  - 2 shoots per vine – 1 either side of trunk,
  - 4 shoots per vine – 2 either side of the trunk
  - 10 leaves and petioles per vine – 5 either side of the trunk

Interim results and implications

Initial results from autumn testing indicate that it is possible to detect GPGV in late autumn. Positive results indicate that the field sampling technique used in autumn generally did not affect the outcome of the diagnostic test and as few as two shoots, or 10 leaves from a single vine can be used for GPGV detection.

This trial is very preliminary, and does only look at 2 varieties, in 2 regions, with possibly different GPGV symptom expression in each case.

GPGV appears to affect varieties differently, and detection and impact is still unclear.

What does this mean for Vine Improvement and Nurseries regarding the possibility of detecting GPGV in propagation material prior to taking cuttings for the 2018 season?

1. Samples taken in autumn towards the end of the growing season may return a positive result if the virus is present.
2. A negative result in autumn does not guarantee virus-free status– i.e. the vine might be infected but the virus is below the limit of detection at this time of year.
3. Therefore:
   - It is useful to test at this time of year to minimise the distribution of material from vines that return a positive GPGV result.
   - There is a risk that vines tested in autumn which return a negative result may be infected and distribution of propagation material from these vines could result in spread of the virus.
   - To confirm a negative GPGV result it may be useful to re-test in late spring or early summer, when virus concentrations are higher.

Field sampling technique recommendation

The interim recommendation for field sampling in autumn is to select either 2 or 4 shoots/vine or 10 leaves and petioles, as outlined above, to improve the chance of GPGV detection.

Overall comments

- Testing mother vine and source block material for GPGV in autumn could reduce the potential to spread this virus via infected propagation material.
- Eriophyid mites (*Colomerus vitis*; bud mite; blister mite) are potential vectors of GPGV and mite control strategies may reduce spread of the virus within and between vineyards.
- There appear to be varieties that exhibit symptoms to a greater extent than others, but not necessarily show them every year, therefore symptom expression is not an indicator of infection – **active virus testing is required**.
- Both wine and table grape varieties may be infected with GPGV.
- Rootstocks may also be infected with GPGV so testing of those is **highly recommended**.
- Currently, the impact of GPGV on the performance of different varieties is still unknown

Strategies that may minimise the risk of GPGV spread

- Test all mother blocks and all source blocks now – in late autumn
    - Recommend targeting any wine, table and rootstock variety in demand for this cutting season or are to be distributed.
    - If you get a positive GPGV result, alert potential customers → it will be the decision of the nursery and customer as to the distribution and planting of this GPGV infected material. However, consideration should be given to withdrawing this material from distribution until more is known about the impact of GPGV or there is a clean source. It is suggested that you contact the Australian Vine Improvement Association or Vine Industry Nursery Association for specific guidelines as to the distribution of known GPGV infected propagation material.
    - If you get a negative GPGV result in autumn, it is suggested to retest in spring to confirm result (reliability of sampling in winter will be tested in coming months).
- Plan for:
    - control of mites next season,
    - continued monitoring of mother vines, source blocks and other propagation material for GPGV symptom expression in spring (however remembering that not all vines infected with GPGV will express symptoms), and
    - spring testing of other material not yet tested or in demand, as well as the negative confirmation testing.

For all purchasers of propagation material, it is suggested that you request evidence of virus testing on any material purchased – rootstock, wine or table grape variety.

Further information

For further information on the GPGV project or the preliminary sampling trial that forms part of this project, please do not hesitate to contact:

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Wine Australia for Australian Wine

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6. Second information paper to Nursery and Vine Improvement Sector – January 2019

DRAFT: Second Information paper to the Nursery and Vine Improvement sector, on

Preliminary Sampling Trial for *Grapevine Pinot gris virus (GPGV)*

January 2019

Introduction

This is the second communication from the Preliminary Sampling Trial for Grapevine Pinot gris virus, associated with the literature review; "A Comprehensive Review of *Grapevine Pinot gris virus (GPGV)*, including Recommendations for Future Research, Development and Extension Work in Australia".

Summary Recommendations

The following recommendations are advised after consideration of the results from the sampling trial up to now, and from information gleaned from the literature review.

For any wine, table and rootstock variety that may be in demand currently and over the next few years, it is recommended:

- to screen untested mothervines and source blocks for GPGV;
- with the advent of improved diagnostic capability, to retest any mothervines and source blocks if they previously tested negative for GPGV (particularly on dormant material)[1]; and
- if GPGV is detected, consider approach to limiting spread of the virus through planting material – look for alternative uninfected sources from which to propagate and consider treatments for virus eradication.

For all purchasers of propagation material, it is recommended to request evidence of GPGV testing on any purchased rootstock, wine or table grape variety.

Sampling trial

Background

The Preliminary Sampling Trial was undertaken to improve reliability in the detection of GPGV in Australian planting material, and to identify further related research priorities for the wine and grape industries.

Field sampling involved collection of vine tissue using three sampling techniques, from two different grape varieties, located in two climatically-different regions, tested at three times of the year in 2018 – Autumn, Winter and Spring. The same set of vines were used at all testing times at the cooler site. At the warmer site, a different set of vines was used for the Winter and Spring samplings compared to the Autumn sampling.

[1] Recommend contacting laboratory to verify validity of previous results based on laboratory test used at the time of the testing

Learnings from the trial aim to reduce the risk of:

- false negatives that have occurred in previous samplings for GPGV in Australia
- spread via propagation material.

Results

Sample timing

Initially, the Autumn testing results had been variable, and GPGV was not detected in some vines that previously tested positive. Adjustment of the molecular testing methodology followed, and this resulted in the successful detection of GPGV in all vines that were expected to be infected.

Some commercially available GPGV tests have limited specificity to some genetic GVGP variants. Of a range of PCR tests initially used for diagnostic testing, two tests returned the false negative results. This was attributed to the fact that several different genetic variants of GPGV were detected in one of the Australian vineyards (Constable unpublished).

A small comparative test between Crop Health Services (Victoria) and The Australian Wine Research Institute (South Australia) using duplicate samples collected in Autumn, indicated that both diagnostic laboratories have equally reliable PCR assays for detection of GPGV.

The final sample timing trial results indicate:

- that the ability to detect GPGV isolates could be influenced by genetic variability of the virus
- the importance of using the appropriate molecular test to accurately detect the virus in Australia
- GPGV was detected in dormant wood using the appropriate molecular test
- GPGV was detected in Autumn, Winter and Spring.

Sampling methodology

Comparative GPGV detection in different vine material was assessed using three sampling methods in Autumn and Spring (2 green shoots per vine compared to 4 green shoots per vine compared to 10 leaves and petioles per vine) and two sampling methods in Winter (2 dormant shoots per vine compared to 4 dormant shoots per vine).

Very few differences in GPGV detection were observed between the sampling methods compared at each of the three sampling times. Whilst a more extensive trial may be required to confirm an optimum method, this result is, however, positive. It provides both the nursery and vine improvement sector and industry with options for tissue collection when sampling at various times of the year.

Implications for Vine Improvement and Nurseries

Based on overseas reports, testing for GPGV was thought to be more reliable in Spring, when virus titre is likely to be higher. The preliminary trial suggested that GPGV can be detected in infected grapevines in Australia in Autumn, Winter and Spring. Further work is required to assess test reliability in summer at both sites. There is also a need to conduct a similar trial over several seasons, utilising additional varieties to confirm the most reliable time point to conduct GPGV testing.

For both the vine improvement and nursery sector and industry, this trial suggests that diagnostic testing for GPGV can be undertaken at the same time as traditional virus screening in Autumn or Winter.

A negative result does not, however, *guarantee* virus free status:

- virus titre is likely to vary within a grapevine across the season
- viruses, including GPGV, can be unevenly distributed in a grapevine
- GPGV can be present below the limit of detection at any time of year
- re-testing at a later date is recommended to validate a negative result.

Overall comments

Internationally and in Australia, the impact of GPGV on different grape varieties in all environments is not fully understood. Therefore, it is important to reduce the risk of spread of GPGV via infected planting material.

Eriophyid mites (*Colomerus vitis*; bud mite; blister mite) are potential vectors of GPGV, and mite control strategies may reduce spread of the virus within and between vineyards.

GPGV symptom expression may vary between grape varieties, environment and growing seasons, and therefore is not a definitive indicator of GPGV infection. Infected vines can be symptomatic, asymptomatic or symptomless.

Thus, active virus testing is required of wine and table grape varieties and rootstocks, with or without symptoms, to confirm GPGV presence.

Importantly, this preliminary trial has indicated that:

- GPGV has been detected in wine grapes and table grapes in cool and warmer grape growing regions in Autumn, Winter and Spring for the two vineyards tested
- testing mother vine and source block material for GPGV in either Autumn, Winter or Spring could reduce the potential to spread infected grapevine material
- more work needs to be done to understand the genetic diversity of GPGV present in Australia to ensure reliable detection.

Cautionary note:

The above results are based on sampling two grape varieties from two vineyards, in climatically different regions in only one year, where the virus strains present and their titre levels, were not determined. These results need to be validated through a larger trial of more vineyards and varieties.

7. Vinehealth Australia e-news article May 15, 2018 - GPGV activity to assist growers



Symptoms of Grapevine Pinot Gris Virus infection courtesy Dr Pasquale Saldarelli, Istituto per la Protezione Sostenibile delle Piante, Bari, Italy

GPGV activity to assist growers

May 15, 2018 News (http://vinehealth.com.au/category/news/)

Following the initial detections of Grapevine Pinot Gris Virus in Australia, Vinehealth Australia worked in collaboration with Biosecurity SA and vine improvement associations to develop and implement a surveillance plan for GPGV in South Australia in October to December 2017.

The Register that Vinehealth maintains of vineyards in SA proved valuable in identifying high risk blocks for inclusion in this surveillance plan. This surveillance resulted in further detections of GPGV in SA.

These results, together with further detection in Victoria following testing of suspect vines submitted by industry, resulted in a recommendation to Australia's National Management Group for biosecurity that it is technically not feasible to eradicate the virus from Australia. This recommendation was accepted, which means that the Department of Agriculture and Water Resources

http://vinehealth.com.au/2018/05/15/gpgv-activity-to-assist-growers/ 1/8

will no longer regulate this virus at the national border.

GPGV is therefore now categorised as being established and present in Australia in New South Wales, Victoria and South Australia. It has been detected in multiple winegrape varieties and a tablegrape variety. With this categorisation, it is now up to industry to manage this virus.

However, given that GPGV was only recently identified in 2012, work has begun on actively addressing many of the 'unknowns' surrounding this virus, in order to assist grapegrowers in its management and the nursery and vine improvement sector to limit its spread via propagation material.

A new Wine Australia funded project titled 'A comprehensive review of GPGV, including recommendations for future research, development and extension work in Australia' has been initiated, and is supported by the nursery and vine improvement sector, Vinehealth Australia, the Australia Wine Research Institute (AWRI) and Agriculture Victoria.

The project, led by Vinehealth, is being undertaken by Dr Fiona Constable, Senior Plant Virologist from Agriculture Victoria and Libby Tassie, Principal Consultant, Tassie Viticulture Consulting. Dr Nuredin Habili of the AWRI will also provide expert technical advice to the project.

The project will run until the end of October 2018 and is predominantly looking to:

- Identify knowledge gaps in the epidemiology and diagnostics for GPGV and use this to prioritise requirements for future research and development;
- Better understand the potential impact of GPGV on Australian viticultural industries, including for wine, table and dried grape production and nursery and vine improvement sector;
- Provide preliminary guidelines:
  - to growers for managing infected blocks and to minimise spread of GPGV;
  - for field sampling for GPGV;
  - for laboratory diagnostic protocols for GPGV;
- Empower the nursery and vine improvement sector to adopt appropriate management strategies for the provision of planting material free of GPGV.

Vinehealth Australia will continue to provide updates on this project, including outcomes at the conclusion of the project.

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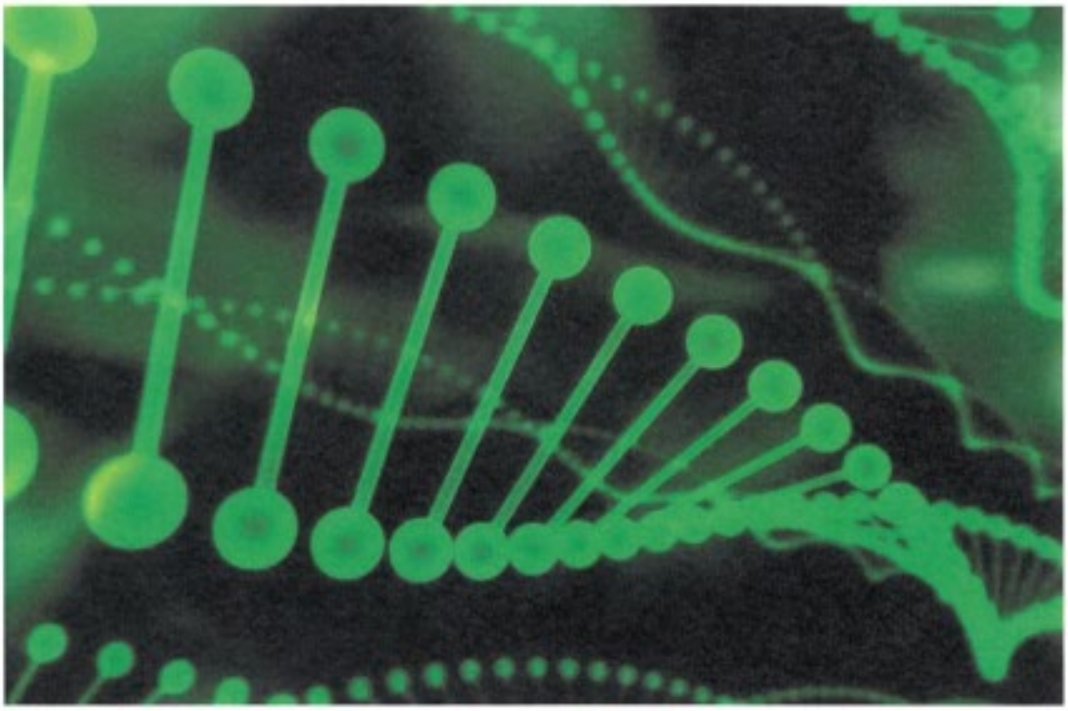
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Focus areas for 2018/19 (http://vinehealth.com.au/2018/05/15/focus-areas-for-2018-19/)

8. Vinehealth Australia e-news article 17 April 2018 - Virus knowledge boost



Virus knowledge boost

April 17, 2018 News (http://vinehealth.com.au/category/news/)

Australia's understanding of grapevine viruses has been given a boost, following a meeting of the best minds in virus research in the world.

Dr Fiona Constable, a senior plant virologist from Agriculture Victoria, attended the 19th Conference of the International Council for the Study of Virus and Virus-like Diseases of the Grapevine (ICVG) in Santiago Chile last week. The conference was attended by more than 100 grapevine and phytoplasma experts from around the world, including Australia's Vinay Pagay, Lecturer in Viticulture at The University of Adelaide.

Major themes of the event included:

- Virus and phytoplasma epidemiology; including spread, vector biology, pathogen diversity and disease distribution;
- Interactions between virus and phytoplasma and their hosts with an emphasis on plant defence responses to infection and vine performance and fruit quality; and
- Diagnostics, including the rapid development of high throughput sequencing technology for virus detection and discovery.

"A highlight of the meeting was the application of many of these themes to improve understanding of the diseases associated with viruses and phytoplasmas including an increased understanding of grapevine pinot gris virus (GPGV) and its role in grapevine leaf mottling and deformation disease," Fiona said.

GPGV was first detected in Australia in 2016, and industry bodies have been working hard to understand its spread and impact.

At the conference, Fiona was able to discuss timing and reliability of testing for GPGV with a number of international virologists and is bringing back new knowledge to inform Australian laboratory diagnostics practices, to reduce the chance of false negative results. Other information gained will help us build a picture of how to best manage GPGV in Australia.

Currently, the GPGV testing service for the grape and wine industries is carried out at AgriBio-Centre for AgriBioscience, the Australian Wine Research Institute (AWRI) and TASAG.

Dr Fiona Constable (BSc Hons, LTU; PhD, UA) is the Senior Plant Virologist in the Plant Microbiology Group and for Crop Health Services diagnostic service of Agriculture Victoria. Fiona has considerable experience in the epidemiology of virus, viroids and phytoplasma associated diseases and development of rapid and reliable molecular diagnostic tests for these pathogens in various crops, including grapevines.

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9. Vinehealth Australia e-news article June 12, 2018 - Improved detection of viruses vital



Improved detection of viruses vital

June 12, 2018 News (http://vinehealth.com.au/category/news/)

Information gathered by Australian delegates at the recent 19th International Council for the Study of Viruses and Virus-like Diseases of the Grapevine (ICVG) Conference held in Santiago, Chile, will improve our ability to manage endemic viruses, undertake virus surveillance and improve our capacity to respond to incursions of exotic viruses.

This conference involved over 80 presentations on grapevine virus, viroid and phytoplasma epidemiology, diagnostics and plant host interactions. Presentations on the latest international Grapevine Pinot Gris Virus (GPGV) research proved very timely, given Australia's recent detection of the virus.

Dr Fiona Constable, Senior Plant Virologist at Agriculture Victoria, attended the conference and shares some initial insights gleaned from presentations and conversations held with individual research colleagues.

GPGV insights

- French researchers have determined that there are now four distinct groups of GPGV strains, with symptoms considered to potentially be associated with specific strains. This reasoning was not always clear, however, as often there was diversity of symptoms observed on infected vines.
- In the USA, researchers noted that vines found to be positive for GPGV were also those found to be positive for Grapevine fanleaf virus (GFLV). Their GPGV isolates were noted to cluster with asymptomatic strains from Europe.
- GPGV vine to vine spread – clustering of vines showing Grapevine leaf mottle disease is indicative of vine to vine spread of GPGV and in some vineyards, spread of GPGV increased from 20% to 77% over three years. Other studies showed a lower rate of spread.
- Mite transfer of GPGV can be relatively rapid – mites under experimental conditions, were found to acquire GPGV from both symptomatic and asymptomatic grapevines in as little as four hours.
- Alternative hosts of GPGV – Hungarian researchers reported finding GPGV in *Chenopodium* species (e.g. Fat Hen), *Rubus* species (e.g. Raspberries and Blackberries), *Rosa* species (e.g. Rose), *Ascelpias syricaca* (Common Milkweed – native to Canada) and *Fraxinus* species (Ash species). Significant work is still to be completed to fully explore and understand the role that these alternative hosts and potential vectors play, if any, in the epidemiology of GPGV in the viticultural context.

Further insights are being gathered and reported on in the Wine Australia-funded project titled "A comprehensive review of *Grapevine Pinot Gris Virus (GPGV)*, including recommendations for future research, development and extension work in Australia."

Other insights

- Delegates were informed that there are now more than 80 viruses known to infect grapevines. However, the association between many newly reported viruses and disease remains uncertain. One recently reported leafroll virus, *Grapevine leafroll-associated virus 13* (GLRaV-13) has though been associated with leafroll symptoms in the field.
- Newly described viruses have mostly been discovered through the use of next generation sequencing (NGS), a rapidly developing and powerful technology that has the possibility to detect all known and novel viruses in one test of a single sample. Of note, NGS has recently been used to identify significant genetic diversity among important grapevine viruses such as *Grapevine leafroll-associated virus 1* (GLRaV-1) and *Grapevine leafroll-associated virus 3* (GLRaV-3), which could impact detection, insect transmission and disease expression. Soon, NGS technologies may be used routinely for plant virus diagnostics to facilitate faster movement of planting material through quarantine and into certification programs. However, challenges in transitioning to NGS technology as a routine diagnostic tool for virus detection were identified. These included quality assurance, harmonizing laboratory and analysis pipelines and cost.
- Some improvements in laboratory-based tools for detection of viruses, including PCR and ELISA, were presented. One example included the use of Camelids (e.g. alpacas) to produce small antibodies for several grapevine infecting viruses including Grapevine Pinot Gris virus. These camelid antibodies have the capacity to significantly improve the sensitivity of ELISA based tests, which are generally simpler and cheaper than PCR.
- Dr Vinay Pagay from The University of Adelaide, presented his Australian research on the use of hyperspectral sensors as an alternative field-based remote sensing detection method of virus associated diseases.
- Phytoplasma research was also presented at the meeting. There are now 18 different phytoplasma species or strains that can infect grapevines, however most are associated with typical grapevine yellows disease. Grapevine phytoplasmas occurring overseas are yet to be reported in Australia. Dr Constable gave a presentation on research that was done to formally classify the Buckland Valley grapevine yellows phytoplasmas that occur in Victoria, Australia. Although the research is yet to be published, a provisional name of *Candidatus Phytoplasma victoriense* was proposed.
- Delegates visited the San Pedro Vineyard and Valentina grapevine nursery where efforts to mitigate the impact of virus diseases in Chilean viticulture, especially leafroll, were presented. Leafroll disease is prevalent in many Chilean vineyards and

the visit highlighted the importance of high-health, virus-tested planting material to improve vineyard productivity and fruit quality.

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GPGV activity to assist growers (http://vinehealth.com.au/2018/05/15/gpgv-activity-to-assist-growers/)

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(http://vinehealth.com.au/2018/06/12/fruit-fly-id-tool/)
Fruit fly ID tool (http://vinehealth.com.au/2018/06/12/fruit-fly-id-tool/)

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Appendix 2: Intellectual property

Outputs arising from the project including the "Comprehensive review of *Grapevine Pinot gris virus"* are freely available to be shared with relevant industry and state and national research, diagnostic and biosecurity agencies. There are no commercialistion or confidentiality issues to report.

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Appendix 4: Pest Risk Assessment



PARTIAL PEST RISK ANALYSIS FOR GRAPEVINE PINOT GRIS VIRUS (GPGV) IN AUSTRALIA

November 2018

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Front cover image: Chlorotic mottling and leaf distortion in Pinot Grigio, symptoms of Grapevine Pinot gris virus. Image courtesy P. Saldarelli, University of Bari, Italy.

1.0 Introduction

Grapevine pinot gris virus (GPGV) is a new to science pathogen that was first described in Italy in 2012 and which has been subsequently reported in several other countries. In 2016-2018 GPGV was detected in several wine grape varieties and one table grape variety in three grape growing regions in Australia. The impact of GPGV on Australian viticulture, including wine and table grape production, nursery production and fresh fruit export is unknown. The comprehensive review of GPGV epidemiology and diagnosis that accompanies this Pest Risk Analysis (PRA) was done to determine what is known and what is unknown, and this information was used to inform the risk assessment of GPGV for Australian viticulture. The PRA was undertaken using a base template for quarantinable pests (examples include Biosecurity Australia, 2011; DAWR, 2016), to ensure consistency with similar analyses completed for exotic pests.

1.1 Focus

A PRA is generally undertaken to qualify or quantify the potential risk of a particular pest entering and spreading in an area in which it is currently not found, using the best available science, and then identifying necessary phytosanitary measures to manage this overall risk.

In Australia, PRAs are commonly undertaken by the Department of Agriculture and Water Resources (DAWR) to review existing and prospective trade pathways and to implement risk management measures including regulation. PRAs generally address four stages – probability (likelihood) of pest entry, establishment, spread and the consequences of this.

This partial Pest Risk Analysis (PRA) uses qualitative analyses to determine likelihoods of entry, establishment and spread of GPGV. Given that we acknowledge that GPGV is already in Australia, the focus of this PRA will be both retrospective and forward-looking. The likelihood of entry of GPGV in propagation material into Australia is presented over multiple historic time periods: pre-2003, 2003-2014 and 2014-Feb 2018. These relate to varied distribution and incidence of GPGV in the overseas source areas, as inferred by both evidence of GPGV detections overseas and of introduction of GPGV testing at the Australian border in 2014. These time periods may provide importers or purchasers of imported material, a relative indication of likelihood that the material may be infected with GPGV. In addition, the current risk of entry of GPGV-infected propagation material and establishment and spread of this will also be evaluated. At the time of writing this PRA, the risk of entry of GPGV to Australia in propagation material is considered to have increased, with GPGV testing no longer being undertaken in Australian Post-Entry Quarantine (PEQ) facilities. If the current state of management of GPGV at the border remains unchanged into the future, the likelihood of entry, establishment and spread can be considered MODERATE; above Australia's appropriate level of protection (ALOP) of VERY LOW.

The assessment of consequences step was not completed as part of this PRA because there are significant knowledge gaps about GPGV biology, epidemiology and the full impact on production and sustainability both overseas and in Australia. As a result, the level of confidence in assessing the consequences is considered very low. Therefore, an overall risk score for this PRA could not be calculated, and this PRA is DRAFT PROVISIONAL ONLY.

A list of critical control points within both the nursery and vine improvement sector and broader wine and grape industries for maintenance of vine health is also presented.

This PRA will be used to prioritise actions required to address and mitigate further pest entry, establishment and spread phases, and consequences of GPGV in Australian viticulture.

2.0 Background

2.1 Australia's biosecurity policy framework

Australia's biosecurity policies aim to protect Australia against the risks that may arise from exotic pests entering, establishing and spreading in Australia, thereby threatening Australia's unique flora and fauna, as well as those agricultural industries that are relatively free from serious pests (DAWR, 2016).

The risk analysis process is an important part of Australia's biosecurity policies. It enables formal consideration of risks that could be associated with proposals to import new products into Australia, or between parts of Australia. If the risks are found to exceed Australia's overarching ALOP of 'very low', the threat from the particular pest posed by the import of the particular commodity given current controls is considered unacceptable. Risk management measures are then proposed to reduce the risks to an acceptable level. If the estimated risk falls into the 'very low' category or below it, the risk is considered acceptable. This means the commodity will be recommended for continued import without additional restriction in respect of the pest concerned – i.e. further risk management is not justifiable. If it is not possible to reduce the risks to an acceptable level, then no trade will be allowed.

2.2 Existing policy

The Commonwealth Government is responsible for regulating the movement of plants and plant products into and out of Australia. However, state and territory governments are responsible for plant health controls within their individual jurisdiction (DAWR, 2016) and these are documented in Plant Quarantine Standards or equivalent. Where there is no regulation for a pest that is present in Australia, industries may employ voluntary controls through certification programs and development of best-practice management strategies to minimise entry, establishment and spread phases to minimise detrimental consequences.

2.3 Current management of GPGV in Australia

Post February 2018, mandatory GPGV diagnostic testing of imported propagation material into Australia at PEQ, ceased. The nursery and vine improvement sector in Australia is also yet to develop specifications on the production of GPGV free germplasm, including imposing mandatory testing for GPGV of all propagation material sold.

2.4 Knowledge gaps

From the review of the current literature and studies on GPGV, the following gaps in knowledge about GPGV overseas and Australia were identified that could impact management:

- The geographic and varietal distribution and prevalence of GPGV and GLMD
- Varietal response to GPGV infection on its own and with other viruses
- The genetic diversity of GPGV and its impact on detection and symptom expression
- The agronomic impact of GPGV and GLMD (Grapevine leaf mottling and deformation, sometimes referred to as the associated disease)
- Appropriate field sampling strategy and reliable diagnostic methodologies for GPGV detection
- The Australian vectors of GPGV
- Other potential vectors of GPGV
- Identification and role of alternative hosts
- The current management of mites in Australia

Additional work is also required on the biology, epidemiology and impact of other grapevine-infecting viruses on production in Australia, and this work should link in with work on GPGV.

3.0 Scope of this Pest Risk Analysis

This Pest Risk Analysis has been undertaken to:

(i) Evaluate the threat that GPGV poses to the Australian wine and grape industries and
(ii) Where justified, make regulatory and non-regulatory recommendations to reduce the likelihood of this threat.

4.0 Assumptions for this Pest Risk Analysis

The assumptions included in this PRA are:

- GPGV is the cause of GLMD;
- Current industry practice does not require mandatory GPGV testing of propagation material prior to distribution;
- Current industry practice does not restrict distribution of known GPGV-infected material; and
- Mite control is not undertaken as a regular vineyard management activity.

The many unknowns about GPGV biology and epidemiology affect accurate risk assessment and this document should be considered a draft provisional ONLY, until further information can be obtained.

The final risk levels will be compared against Australia's Appropriate Level of Protection (ALOP) of 'very low'. If the estimated risks are rated above 'very low', the threat from the particular pest posed by the import of the particular commodity given current controls is considered unacceptable and regulation is warranted.

5.0 Method for Pest Risk Analysis

This section outlines the method used for the PRA in this report. This PRA has been conducted in accordance with the International Standards for Phytosanitary Measures (ISPMs), including ISPM 2: Framework for pest risk analysis (FAO, 2007) and ISPM 11: Pest risk analysis for quarantine pests (FAO, 2013).

A PRA evaluates biological and other scientific and economic evidence to determine whether a pest should be regulated and the strength of any phytosanitary measures to be taken against it. Two major components of quarantine risk are evaluated in this PRA and then combined to give an overall estimate of risk:

(i) Probability of the pest GPGV entering, establishing and spreading in Australia; and
(ii) Consequences should this happen.

Quarantine risk is evaluated by considering existing commercial practices, incorporating phytosanitary measures currently practiced.

This PRA is conducted in three steps: initiation, pest risk assessment and pest risk management.

5.1 Stage 1: Initiation

Initiation identifies the pest(s) and pathway(s) that are of quarantine concern and should be considered for risk analysis in relation to the identified PRA area.

For this PRA, the 'PRA area' is defined as the whole of Australia.

5.2 Pest risk assessment

In this PRA, pest risk assessment has been divided into the following interrelated processes:

5.2.1 Pest categorisation

Pest categorisation identifies which of the pests with the potential to be on the commodity are quarantine pests (a pest of potential economic importance to an area where the pest has not yet been detected) for Australia and require pest risk assessments.

This pest risk analysis is confined to a single virus as follows:

Identity

Scientific name: *Grapevine Pinot gris virus*

Common name: Grapevine Pinot gris virus (GPGV)

Host range

Vitis sp., *Ascelpias syricaca* (Common Milkweed – native to Canada,) *Chenopodium album L.* (Fat Hen), *Fraxinius* sp. (Ash species), *Rubus* sp. (e.g. Raspberries and Blackberries), *Rosa* sp. (e.g. Rose), and *Silene latifolia subsp. Alba* (Mill.) (White Campion).

Symptoms (refer Literature review section 4.3)

Leaves: mottling, deformation, chlorotic mosaic, discoloured veins

Fruit: Reduced yield associated with low bunch weight and bunch numbers, millerandage, bunch shatter, desiccated inflorescences, delayed ripening

Shoots: delayed bud break, shortened internodes, stunting, lack of lignification, shoot tip necrosis

Some varieties are asymptomatic or symptomless.

Distribution in Australia

New South Wales, South Australia, Victoria

Comprehensive distribution is unknown. The extent of distribution is unknown in commercial vineyards, or germplasm/nurseries /propagation material/source blocks.

Status in Australia

The pest status of Grapevine Pinot Gris virus in Australia is: Established and present in NSW, Vic and SA.

Biology

GPGV is a member of the genus *Trichovirus* in the subfamily *Trivirinae,* family *Betflexiviridae* (refer Literature review section 4.1). Overseas, it is associated with a disease called grapevine mottling and leaf deformation (GLMD) in some grapevine varieties (refer Literature review section 4.3). Symptom expression within a variety can be variable due to GPGV strain variation, environmental impacts and vine vigour. Recovery from GLMD within and between seasons is reported. GLMD does not affect all varieties that are infected with GPGV, regardless of strain. Up to four clades of GPGV strains are known (refer Literature review section 4.3). Some clades of GPGV consist of strains which are more commonly associated with GLMD-affected grapevines and other clades contain strains more commonly associated with asymptomatic/symptomless grapevines.

In Australia the association of GPGV to disease and impact on grapevine production is unclear.

In Europe, alternative host plants of GPGV include *Ascelpias syricaca* (Common Milkweed – native to Canada,) *Chenopodium album* L. (Fat Hen), *Fraxinius* species (Ash species), *Rubus* species (e.g. Raspberries and Blackberries), *Rosa* species (e.g. Rose), and *Silene latifolia* subsp. Alba (Mill.) (White Campion) (refer Literature review section 4.7). They may express virus-associated symptoms when infected by GPGV. It is unknown if these hosts are a source of inoculum for transmission to grapevine.

Movement

GPGV is distributed through movement of infected propagation material, sourced either from overseas or from local material (refer Literature review section 4.7). The increased global movement of grapevine material places a greater risk of importing exotic plant-borne pathogens in the wine, dried fruits and table grape industries. GPGV is transmitted by grape leaf bud and blister mites (*Colomerus vitis*) overseas.

Potential for economic concern

Up to 80% yield loss is reported overseas (refer Literature review section 4.6.1)

Control

Removal of infected vines, virus elimination in germplasm, certification programs – management and distribution of pathogen tested material, mite control and weed control (refer Literature review section 4.8).

Association with entry pathways:

- Major pathway: Infected propagation material
- Minor pathway: Viruliferous mites

5.2.2 Assessment of the probability of entry, establishment and spread

Details of how to assess the 'probability of entry', 'probability of establishment' and 'probability of spread' of a pest are given in ISPM 11 (FAO, 2013). The use of the term 'probability' is limited to the direct quotation of ISPM definitions. In qualitative PRAs such as this one, the term 'likelihood' is the descriptor used for estimates of likelihood of **entry**, **establishment** and **spread**. Methodology used in this PRA is consistent with that of the DAWR, outlined in recent table grape assessments (DAWR, 2016) and (Biosecurity Australia, 2011).

A summary of the process is given below, including a description of the qualitative methodology used in this risk analysis.

Likelihoods are assigned to each step of entry, establishment and spread using six descriptors as outlined in Table 1 below, along with their definitions and indicative probabilities.

Table 1. Nomenclature of quantitative likelihoods (*Adapted from* Biosecurity Australia, 2011) (DAWR, 2016)

Likelihood	Descriptive definition	Indicative probability range
High	The event would be very likely to occur	>700 →1000 times in 1,000 events
Moderate	The event would occur with an even probability	>300 → 700 times in 1,000 events
Low	The event would be unlikely to occur	>50 → 300 times in 1,000 events
Very low	The event would be very unlikely to occur	>1 → 50 times in 1,000 events
Extremely low	The event would be extremely unlikely to occur	>0.001 → 1 times in 1,000 events
Negligible	The event would almost certainly not occur	0 → 0.001 times in 1,000 events

5.2.2.1 Likelihood of Entry

The likelihood of entry for PRAs, considers two components separately – the likelihood of importation and the likelihood of distribution. Both these factors are then combined for each pathway to give a risk rating for likelihood of entry.

5.2.2.1.1 Likelihood of Importation

In this PRA, the likelihood of importation describes the likelihood that Grapevine Pinot Gris virus will enter Australia as a result of distribution and incidence of GPGV in the source areas, commodity form and pest association, relative vulnerability of GPGV during transport or storage and existing controls at the Australian border.

The distribution and incidence of GPGV in the international source areas is presented as four distinct time periods of entry of propagation material to Australia – three historic and once current, based on evidence of GPGV detections over time and likely spread overseas, and of introduction and then ceased GPGV testing at Australian borders:

i) Pre-2003
ii) 2003-2014
iii) 2014- Feb 2018
iv) March 2018 - current

	Factors influencing assessment
Information relevant to all pathways	New varieties imported into Australia before 2002 and between 2014-Feb 2018 have less risk of GPGV infection compared to varieties imported between 2003 and 2014. The risk from March 2018 has increased because GPGV testing is no longer undertaken in Australian Post-Entry Quarantine (PEQ) facilities and GPGV continues to spread overseas. Distribution and incidence of GPGV in the international source areas: • GPGV was first reported in 2012 (refer Literature review section 4.1). • GPGV has been detected in Europe, Asia and North America (refer Literature review section 4.2). • GPGV has been reported as widespread within some countries, within some regions and in some affected vineyards (refer Literature review section 4.7.4). • GPGV has been detected in germplasm, collections and nurseries overseas (refer Literature review section 4.6.2.1). • GPGV has been detected in more than 30 varieties worldwide (refer Literature review 4.2), but primarily in Europe. • Anecdotally, most grapevine varieties/clones imported into Australia have and continue to originate from Europe. • GPGV introduction from overseas into Australia and between foreign countries, was most likely to occur between 2003 to 2014 (refer Literature review section 4.6.2.2). This timing is associated with emergence of the virus internationally, international evidence of significant movement of GPGV post 2002 in Europe, and Australia's diagnostic capability to detect GPGV - which only began in 2014 and continued until Feb 2018; during which time GPGV-infected material into Australia was limited. • GPGV spreads after an initial introduction (refer Literature review section 4.6.6.2).

	• There are no current restrictions in Australia or overseas on distribution of propagation material with GPGV from nurseries or vine improvement associations (refer Literature review section 4.8.2).
	
	Relative vulnerability of GPGV during transport or storage:
	
	• The virus is an obligate parasite and is intrinsically linked to the grapevine, therefore, as long as the propagation material remains viable during transport and storage, GPGV infection inside the propagation material remains viable.
	
	Commodity form and pest association:
	
	• Global movement of the virus between countries and continents is most likely through the GPGV infected propagation material.
	• After establishment within a country or region, movement of the GPGV is most likely through infected propagation material and/or viruliferous vectors.
	• GPGV is associated with asymptomatic and symptomless infection (refer Literature review section 4.2)
	
	Existing controls at Australian border:
	
	• Diagnostic testing for GPGV was introduced into Australian PEQ in 2014 (refer Literature review section 4.9.0).
	• Anecdotally, between 2014 to early 2018, approximately 10% of Australian imports tested positive to GPGV (refer Literature review section 10.0) – unknown if test used detected all strains.
	• GPGV would not have been detected at any time in PEQ using biological indexing on LN33 indicator (refer Literature review section 4.6.2.3).
	• GPGV is no longer considered exotic and therefore not regulated in Australia – PEQ testing no longer required from 2018 (refer Report section 1.0).
	• Testing for viruses present in Australia can be requested by importers during PEQ.
	• Imported material is inspected and treated (Constable and Drew, 2004) during PEQ to control pests including mites:
	  ○ All plant material must be immersed in a broad spectrum miticide/Insecticidal dip containing 100ppm Imidacloprid and 1% Eco-Oil® for 30 seconds.
	  ○ Plant material must be immersed in a sodium hypochlorite (NaOCl) solution of 1% available chlorine for 5 minutes.
	  ○ All plant material is subjected to hot water treatment at 50°C for no less than 30 minutes prior to growth in the closed quarantine facility.
	  ○ Plants must be grown for a minimum of 16 months.
	• Anecdotally, illegal importation of infected material occurs and represents a risk of introduction of GPGV.
	• Screening of incoming passenger, baggage, cargo and mail occurs at ports and mail centres.

	Factors influencing assessment	Assessment
Likelihood of entry – Propagation material from recent imports into Australia (pre-2003)	• GPGV is presumed to have had low prevalence worldwide before 2002, based on testing of historic material (refer Literature review section 4.7.4.1)	*Historic – not being rated*

	Factors influencing assessment	Assessment
Likelihood of entry – Propagation material from recent imports into Australia (2003-2014)	Pest distribution and prevalence: • GPGV has increased in distribution and prevalence worldwide since 2002. • Pest distribution and prevalence of the pest confirmed in source countries for Australian imports. Existing overseas controls: • GPGV testing not done overseas prior to 2012. Existing Controls at Australian border: • Accurate diagnostic testing unavailable prior to 2014 in Australian PEQ. • Anecdotally, GPGV detected in approximately 10% of imports in PEQ after testing introduced (refer Literature review section 10.0).	*Historic – not being rated*

	Factors influencing assessment	Assessment
Likelihood of entry – Propagation material from recent imports into Australia (2014-Feb 2018)	Pest distribution and prevalence: • GPGV detection has increased in distribution and prevalence worldwide since 2002. • Pest distribution and prevalence of the pest confirmed in source countries for Australian imports. Existing overseas controls: • Some germplasm testing to understand incidence of infection to inform need for virus elimination and future distribution of propagation material. • Some vineyard management for mites and alternative hosts. • Some recommendations to remove infected grapevines. • Some virus elimination in grapevines. Existing controls at Australian border:	*Historic – not being rated*

	• Between 2014 and Feb 2018 material that tested positive for GPGV in PEQ was destroyed. It is unknown whether the test used could detect all GPGV strains.	

	Factors influencing assessment	Assessment
Likelihood of entry – Propagation material from recent imports into Australia (March 2018 - current)	Pest distribution and prevalence: • GPGV detection has increased in distribution and prevalence worldwide since 2002. • Pest distribution and prevalence of the pest confirmed in source countries for Australian imports. Existing overseas controls: • Some germplasm testing to understand incidence of infection to inform need for virus elimination and future distribution of propagation material. • Some vineyard management for mites and alternative hosts. • Some recommendations to remove infected grapevines. • Some virus elimination in grapevines. • Continued reporting of new GPGV infections (refer Literature review section 4.2), suggesting likely continued spread. Existing controls at Australian border: • Post Feb 2018, PEQ testing for GPGV ceased and GPGV positive planting material was not destroyed.	**Likelihood = MODERATE (300 – 700 times in 1,000 events)**

5.2.2.1.2 Likelihood of Distribution

The likelihood of distribution describes the likelihood that the pest will be distributed, **as a result of the processing, sale or disposal of the commodity**, in the PRA area and subsequently transferred to a susceptible part of a host.

For this PRA, it means the likelihood of GPGV being distributed within Australia from material that has passed through Australia's borders, for propagation from original illegal ('suitcase') plantings and replanting in other blocks; or from imported propagation material after treatment at PEQ and (i) planted directly into a vineyard as mother vines, or (ii) sent to a commercial nursery or vine improvement for replication and then on-selling commercially, as a result of:

- Dispersal mechanisms of the commodity, including vectors, to allow movement from the pathway to a host
- Host range and accessibility;
- Commodity end-use
- Risks from by-products and waste

	Factors influencing assessment	Assessment
Likelihood of distribution – all pathways	Dispersal mechanisms of the commodity, including vectors, to allow movement from the pathway to a host • The propagation material commodity is produced for the sole purpose of sale to commercial businesses for situations of new planting, top-working and replanting misses as per requirements of the purchasing vineyard businesses. • GPGV is distributed through infected propagation material to the vineyard where it is planted (refer Literature review section 4.7.3). • GPGV might be distributed in propagation material with viruliferous vectors – mites infest buds (refer Literature review section 4.7.2) • Viruliferous vectors may also move GPGV on equipment, clothing, wind and (rarely) animals/birds. • Germplasm is field grown and therefore may be exposed to infection by viruliferous vectors. • There are no current restrictions in Australia or overseas on distribution of propagation material with GPGV from nurseries or vine improvement associations (refer Literature review section 4.8.2). • There is no mandatory testing of propagation material for GPGV prior to distribution which poses a high risk to commercial growers. • When demand for propagation material is very high, sale of non-high-health propagation material may occur. Host range and accessibility: • GPGV can infect scion or the rootstock of *Vitis vinifera* or other hybrids, used for winegrape, table grape or dried fruit production (refer Literature review section 4.2 and 4.3.2.1). • Alternative hosts may be present in vineyards (refer Literature review section 4.7.1). Commodity end-use: • The propagation material is used in the same form as it is produced from the supplier and planted directly into the soil. • Propagation material is used for top-working Risks from by-products and waste • Movement of contaminated fresh prunings is a risk of viruliferous mites.	**Likelihood = HIGH (700-1,000 times in 1,000 events)**

5.2.2.1 Likelihood of Entry (Importation X Distribution)

Likelihoods of importation and distribution are now combined using the tabular matrix in Table 2. This matrix is also used for combining the likelihoods of entry, establishment and spread.

Table 2. A matrix of 'rules' for combining qualitative likelihoods (Biosecurity Australia, 2011) (DAWR, 2016)

	High	**Moderate**	**Low**	**Very low**	**Extremely low**	**Negligible**
High	High	Moderate	Low	Very low	Extremely low	Negligible
Moderate		Low	Low	Very low	Extremely low	Negligible
Low			Very low	Very low	Extremely low	Negligible
Very Low				Extremely low	Extremely low	Negligible
Extremely Low					Negligible	Negligible
Negligible						Negligible

Pathway	Likelihood of Importation	Likelihood of Distribution	Likelihood of Entry (importation x Distribution)
Propagation material from recent imports into Australia (March 2018 - current)	MODERATE	HIGH	MODERATE

5.2.2.2 Likelihood of Establishment

Establishment is defined as the 'perpetuation for the foreseeable future, of a pest within an area after entry' (FAO, 2015). This process assumes that the pest has arrived (it has been imported and distributed) and now focusses on its ability to establish.

Factors influencing the likelihood of establishment include:

- Host range and accessibility
- Survival and reproductive strategies
- Climate and geographic features
- Agricultural practices

	Factors influencing assessment	Assessment
Likelihood of establishment – all pathways	Host range and accessibility: • GPGV infection is persistent in scion and rootstock of *Vitis vinifera* or other hybrids, used for winegrape, table grape or dried fruit production. • Resistance is not reported in the grapevine. • The virus is an obligate parasite and is intrinsically linked to the grapevine. • The virus is an obligate parasite and may be intrinsically linked to alternative hosts. Survival and reproductive strategies:	**Likelihood = HIGH (700-1,000 times in 1,000 events)**

	• As long as the grapevine or other plant host is living the virus is likely to replicate. • Virus distribution and titre is expected to vary within a plant at any one time and during the growing season (refer Literature review section 4.3.2.4). • Rate of replication and titre of GPGV may depend upon strain (refer Literature review section 4.3.2.4). • Virus may persistently or semi-persistently infect mites (refer Literature review section 4.7.2). Climate and geographic features: • Occurs in diverse grapegrowing regions of different climates worldwide including Australia (refer Literature review section 4.2). Agricultural practices: • Virus infection is persistent in grapevines – no antiviral control for propagation material and established grapevines. • Use of pathogen tested material. • Active mite control.	

Likelihood of Entry and Establishment

Likelihoods of entry and establishment are now combined using the tabular matrix in Table 2.

Pathway	Likelihood of Entry x Establishment
Propagation material from recent imports into Australia (March 2018 - current)	MODERATE x HIGH = **MODERATE**

5.2.2.3 Likelihood of Spread

Spread is defined as 'the expansion of the geographical distribution of a pest within an area' (FAO, 2015). The likelihood of spread considers the factors relevant to the movement of the pest, after establishment on a host plant or plants, to other susceptible host plants of the same or different species in other areas. This process is based on a comparative assessment of biological information from the source area and the pest risk area as this relates to the ability of the pest to disperse. For this PRA, it means the likelihood of GPGV being spread from one vineyard block to another or from the nursery to a commercial vineyard.

Categorisation of likelihood of spread also uses Table 2 and is independent of the mode of entry into Australia.

Factors influencing the likelihood of spread include:

- Availability of hosts
- Minimum population requirements
- Pest mobility
- Barriers to pest spread
- Existing controls including industry awareness

	Factors influencing assessment	
Likelihood of spread – all pathways	Availability of hosts: • GPGV has been detected in more than 30 varieties worldwide (refer Literature review section 4.2), but primarily in Europe. • Anecdotally, most imported grapevine varieties in Australia originate from Europe. • Alternative hosts may be present in vineyards (refer Literature review section 4.7.1). • Grapes are grown in all States of Australia. • The largest States by production are South Australia, Victoria, and New South Wales. • Total vineyard area in Australia is 135,133 hectares (Wine Australia, 2017). Minimum population requirements: • Virus titre might be impacted by strain and host interactions. • Virus titre in a host may affect vector acquisition – low titre may reduce acquisition efficiency. • Spread of GPGV in a vineyard is likely to be improved through presence of a vector. • *Colomerus vitis* is a vector – bud mites are more efficient vectors than blister mites (refer Literature review section 4.7.2). • *Colomerus vitis* is ubiquitous in Australian vineyards, with higher levels seen in some seasons. • Knowledge of other vectors is currently unknown. • High vector populations might improve transmission efficiency. • Persistence of the vector might be improved through presence of alternative host plants, if *Colomerus vitis* or other vector can fulfil part or all of a lifecycle on these hosts – *Colomerus vitis* is said to have high specificity for grapevine. • Acquisition by a vector may be affected by the plant host – some may be dead end (i.e. vector acquisition does not occur), but little is known. • A single infected grapevine can lead to transmission to neighbouring grapevines (refer Literature review section 4.7.4.2). • Uninfected vines planted near GPGV infected grapevines can become infected within the first year of planting (refer Literature review section 4.7.4.2) and the rate of spread determined by level of GPGV infection, level of vector	**Likelihood = HIGH (700-1,000 times in 1,000 events)**

	presence and rate of spread, and level of vector control undertaken. • The impact of variety on the rate of transmission is unknown but the differential susceptibility of grapevine cultivars to mites influence the rate of spread of GPGV (refer Literature review section 4.7.2). Pest mobility: • GPGV is distributed in propagation material. • GPGV is transmitted by grafting material. • GPGV spread occurs in the presence of the vector. • Currently the known vector (*Colomerus vitis*) is widespread and prevalent in Australian vineyards. • At present there is no evidence to support the transmission of the virus mechanically on pruning or harvesting equipment. Barriers to pest spread: • Removal of infected grapevines. • Control of alternative hosts – some hosts are common in Australian vineyards e.g. *Chenopodium album* L. (Fat Hen), *Rosa* species (e.g. Rose). • Dispersal of high-health propagation material in which GPGV is not detected. • Active vector control. • Vector resistant varieties. • Low virus titre. Existing controls: • Some movements of grapevine material between states requires hot water treatment (54°C ± 1°C for 5 minutes; or 50°C ± 1°C for 30 minutes) to control some pests and which might control viruliferous mites. • Some growers apply early sulphur applications for mite control but this is not a widespread practice. • Efficacy of the applied sulphur applications may also be questionable in cooler climates when temperature during application limit volatilisation of the sulphur. • Existing general biosecurity practices in terms of cleaning machinery of soil and plant material between vineyards – only partial uptake. • Some testing taking place by the nursery industry on source blocks that supply cuttings for current season – assuming infected material is not distributed. Very limited surveillance on all source blocks and on germplasm.	

	• Distribution of natural enemies: Work done on predators of mites in Australia. Efficiency of predators to control vectors and prevent initial GPGV transmission unknown. • Publication and distribution of industry awareness articles.	

Likelihood of Entry, Establishment and Spread

Likelihoods of entry, establishment and spread are now combined using the tabular matrix in Table 2.

Pathway	Likelihood of (Entry x Establishment) x Spread
Propagation material from recent imports into Australia (March 2018 - current)	MODERATE x HIGH = **MODERATE**

5.2.3 Assessment of potential Consequences

The objective of the consequence assessment is to provide a structured and transparent analysis of the likely consequences if the pests or disease agents were to enter, establish and spread in Australia.

As GPGV is a relatively new virus and impacts are yet to be described in full overseas, estimating consequence of GPGV in Australia is very difficult without this knowledge. As a result, the consequence assessment will not be completed. Instead, the focus of the PRA will be to summarise risk points with entry, establishment and spread and what prioritised activities need to be completed to reduce these risks.

6.0 Summary of risk points with entry, establishment and spread of GPGV into and within Australia based on current knowledge, and activities that need to be completed to reduce these risks.

Definitions according to HACCP (Constable and Drew, 2004):

- Critical control point - a step at which control can be applied and is essential to prevent or eliminate a (food safety) hazard or reduce it to an acceptable level.
- Control measure - any action or activity that can be used to prevent or eliminate a (food safely) hazard or reduce it to an acceptable level.
- Critical limit - a criterion, which specifies the acceptability and unacceptability.

Refer also "A comprehensive review of Grapevine Pinot gris virus (GPGV), including recommendations for future research, development and extension work in Australia" for additional definitions and Constable and Drew (2004) for further explanation of risk points as required.

Table 3: Control points, control levels, critical limits and additional control measures relevant to the production of high-health propagation material by the nursery and vine improvement sector as reported by Constable and Drew (2004) and updated as part of this report, to facilitate production of GPGV-free propagation material.

Material	Control point	Control measure	Critical limit	Additional control measure
Imported material	Post entry quarantine (PEQ), prior to release.	Testing for all quarantinable pathogens, with any material containing such pathogens to be destroyed. Plants must be grown for a minimum of 16 months to enable full evaluation of disease status. Inspection for pests including mites and sterilisation as follows: Immersion in a broad spectrum miticide/insecticidal dip containing 100ppm Imidacloprid and 1% Eco-Oil® for 30 seconds. Immersion in a 1% active sodium hypochlorite (NaOCl) solution for 5 minutes.	Nil quarantinable pests and pathogens (with GPGV no longer being in this category).	Obtain material from international high-health schemes. Ensure strong border control to reduce chance of illegal ('suitcase') imports entering Australia. Develop and offer a sponsored program to general industry for the importation and maintenance of mothervines, to reduce temptation of illegal ('suitcase') imports. Additional testing and treatment for pests and pathogens already present in Australia including GPGV, which are considered undesirable for high-health propagation

Material	Control point	Control measure	Critical limit	Additional control measure
		Hot water treatment at 50°C for at least 30 minutes prior to growth in the closed quarantine facility.		material. Lobby for and implement reintroduction of GPGV (and other pathogens such as GLRaV-1 and GLRaV-3, GVA, GVB) testing at PEQ.
Nucleus collection	Establishment	Pathogen testing including for GPGV and planting only using material which tested negative. Use of virus removal techniques if any GPGV detected (very minimally practiced at present). All material to have been hot water treated prior to planting at 50°C for at least 30 minutes.	No detections of prescribed or non-prescribed pathogens (including GPGV) or known vectors (except Rupestris stem pitting-associated virus (RSPaV)) or viroids in the propagation material (refer Constable and Drew, 2004).	Prior to the establishment of a collection the site should be appropriately prepared - soil tested, and treated if necessary, for potential pests or pathogens that could affect establishment, this includes weed control. Site selection should be where grapevines have not been previously planted, preferably virgin soil, and established away from possible sources of infection. Consideration should be made to enclosing in insect-proof screenhouse.
	Maintenance	Regular pathogen testing (biological once every three years, serological and/or molecular yearly)- GPGV and GLRaV1, 3, *5,* 9, GVA, GVB, Petri disease, crown gall, phytoplasma, nematodes and trunk disease pathogens. Visual inspection should be conducted regularly for disease, and trueness to type confirmed for all plants via a DNA test in first year. Regular spray application should be undertaken for pests, disease and weeds as per commercial vineyard management.	No detections of prescribed or non-prescribed pathogens (including GPGV) or known vectors (except RSPaV) or viroids (refer Constable and Drew, 2004).	Maintained in pots in an insect-proof screenhouse and/or tissue culture plantlets – in addition to field planting. Rapid removal of any pathogen-infected plant and subsequent testing of nearest neighbours and all other plants of the same clone/variety to determine incidence; replacement vine produced from uninfected clone, new import or through heat therapy.

Material	Control point	Control measure	Critical limit	Additional control measure
		Appropriate labelling of cuttings taken from the plantings and used to establish mother plantings to be employed, for disease status and trueness to type (i.e. certified or non-certified) i.e. QA system in place.		Sterilisation of pruning tools in 2% active sodium hypochlorite solution for 60 seconds. Strict biosecurity measures to be employed to avoid pest entry and spread via people (clothing and footwear), machinery and equipment.
Mother vine plantings	Establishment	Established with material only from the nucleus collection. Hot water treated at 50°C/30min for cuttings or rootlings used for establishment (as per some state import requirements).	No detections of prescribed or non-prescribed pathogens (including GPGV) or known vectors (except RSPaV) or viroids in the propagation material (refer Constable and Drew, 2004).	Prior to the establishment of a collection the site should be appropriately prepared - soil tested, and treated if necessary, for potential pests or pathogens that could affect establishment, this includes weed control. Site selection should be where grapevines have not been previously planted, preferably virgin soil, and established away from possible sources of infection (e.g. considerable buffer to neighbouring vineyard).
	Maintenance	Visual inspection should be conducted regularly during the growing season for disease; trueness to type confirmed via DNA test. Regular spray application should be undertaken for pests, diseases and weeds as per commercial vineyard management, paying particular attention to mite control and control of alternative host plants for GPGV. Pathogen testing should be undertaken routinely - every vine should be tested every	No detections of prescribed or non-prescribed pathogens (including GPGV) or known vectors (except RSPaV) or viroids (refer Constable and Drew, 2004).	Any diseased mothervine should be removed, and neighbouring vines actively tested for spread, or removed. Consider undertaking virus elimination to replace mothervines. Sterilisation of pruning tools in 2% active sodium hypochlorite solution for 60 seconds. Frequency as per operational management.

Material	Control point	Control measure	Critical limit	Additional control measure
		third year for pathogens that are most likely to spread aerially, mechanically or via viruliferous vectors e.g. GPGV, GLRaV1, 3, *5,* 9, GVA, GVB, Petri disease, phytoplasmas, crown gall, nematodes and trunk disease pathogens. Appropriate labelling of cuttings taken from the mothervines and used to establish source blocks, for disease status and trueness to type (i.e. certified or non-certified) i.e. QA system in place.		Strict biosecurity measures to be employed to avoid pest entry and spread via people (clothing and footwear), machinery and equipment.
Source blocks	Establishment	Established with material only from nucleus collections or mothervines. Hot water treated at 50°C/30min for cuttings or rootlings used for establishment.	No detections of prescribed or non-prescribed pathogens (including GPGV) or known vectors (except RSPaV) or viroids in the propagation material (refer Constable and Drew, 2004).	Prior to the establishment of a source block, the site should be appropriately prepared - soil tested, and treated if necessary, for potential pests or pathogens that could affect establishment, this includes weed control. Site selection should preferably be where grapevines have not been previously planted.
	Maintenance	Visual inspection should be conducted regularly during the growing season for disease and within the first four years of establishment for trueness to type. Regular spray application should be undertaken for pests, diseases and weeds as per commercial vineyard management, paying particular	No detections of prescribed or non-prescribed pathogens (including GPGV) or known vectors (except RSPaV) or viroids (refer	Any diseased grapevine to be grubbed, and surrounding grapevines actively tested for spread. Diseased grapevine tissue must not be used for cuttings. Sterilisation of pruning tools in 2% active sodium hypochlorite solution for 60

Material	Control point	Control measure	Critical limit	Additional control measure
		attention to mite control and control of alternative host plants for GPGV. Pathogen testing to be conducted by random sampling throughout the source block every year for GPGV, GLRaV1, 3, *5,* 9, GVA, GVB and phytoplasmas. Any vine displaying disease should be tested. Appropriate labelling of cuttings taken from the source block, for disease status and trueness to type (i.e. certified or non-certified) i.e. QA system in place.	Constable and Drew, 2004).	seconds. Frequency as per operational management. Strict biosecurity measures to be employed to avoid pest entry and spread via people (clothing and footwear), machinery and equipment.
Nurseries	Acquisition	Material obtained from certified high-health mother vines.	No detections of prescribed or non-prescribed pathogens (including GPGV) or known vectors (except RSPaV) or viroids (refer Constable and Drew, 2004). *Mandatory GPGV testing is key at this point to limiting spread and distribution of GPGV in Australia.*	Kept separate from non-high-health material where carried by the nursery, according to QA controls.
	Treatment	Cuttings hot water treated at 50°C/30min.	Appropriate disinfestation of nursery equipment	Monitoring for pests and diseases including insect pests and vectors. Appropriate early control if present.

Material	Control point	Control measure	Critical limit	Additional control measure
		Appropriate labelling for disease status and trueness to type (i.e. certified or non-certified) i.e. QA system in place. Maintain certified material away from non-certified. Regular spray application should be undertaken for pests, diseases and weeds as per commercial nursery management, paying particular attention to mite control and control of alternative host plants for GPGV.	to prevent possible contamination by fungi, bacteria, viroids and insects that could affect the establishment of material in vineyards.	
Commercial vineyards	Establishment	Established with high-health status propagation material (with proof of virus status from both scion and rootstock for grafted rootlings) and for top working.	No detections of prescribed or non-prescribed pathogens (including GPGV) or known vectors (except RSPaV) or viroids in the propagation material (refer Constable and Drew, 2004).	Appropriate site preparation including soil testing, and treated if necessary, for potential pests or pathogens that could affect establishment, this includes weed control.
	Maintenance	Visual inspection should be conducted regularly during the growing season for disease. Any vine displaying disease should be tagged and tested. Virus testing undertaken to include GPGV, GLRaV1, 3, *5,* 9, GVA, GVB and phytoplasmas.	No detections of prescribed or non-prescribed pathogens (including GPGV) or known vectors	Consideration of grubbing of any diseased vines, with maintenance of testing to ascertain spread. Diseased grapevine tissue must not be used for cuttings for top-working or other propagation. Limit movement of fresh

Material	Control point	Control measure	Critical limit	Additional control measure
		Regular spray application should be undertaken for pests, diseases and weeds, paying particular attention to mite control and control of alternative host plants for GPGV.	(except RSPaV) or viroids (refer Constable and Drew, 2004).	prunings between blocks as potential hosts of mites. Sterilisation of pruning tools in 2% active sodium hypochlorite solution for 60 seconds. Frequency as per operational management. Best-practice biosecurity measures to be employed to avoid pest entry and spread via people (clothing and footwear), machinery and equipment.

7.0 References

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Appendix 5: Sampling for detection of *Grapevine Pinot gris Virus* (GPGV) in Australian grapevines: a preliminary field trial

Introduction

Grapevine pinot gris virus (GPGV) was first described in Italy in 2012 and was associated with a disease called grapevine leaf mottling and deformation (GLMD) in the cv. Pinot gris (Giampetruzzi *et al*., 2012). GPGV has since been reported in many varieties and in many other countries, sometimes in association with GLMD and sometimes in grapevines that are asymptomatic or symptomless (Saldarelli et al., 2017; Constable *et al.*, 2018). Significant yield losses have been observed in some individual GLMD affected grapevines in which GPGV was also detected.

In 2016-18, GPGV was detected in several winegrape varieties and one table grape variety in three grape growing regions in Australia (Wu and Habili, 2017, F.E. Constable, unpublished). The impact of GPGV on Australian viticulture, including wine and table grape production, nursery production and fresh fruit export is unknown.

In Australia, virus testing for propagation material has traditionally been undertaken at the end of the growing season, however, the reliability of detection of GPGV at this time of the growing season was uncertain. It was thought to be linked to variability in virus titre associated with the time of year (Bertazzon *et al.,* 2015, Bianchi *et al.,* 2015) and variable distribution within the vine and tissue type, which affect sampling methodology. Preliminary results suggested that GPGV diagnostic test reliability in Australia was greatest in green tissue during the growing season, possibly up to veraison. Detection in dormant tissues was considered uncertain, thus conceivably leading to false negatives; not picking up the virus in plants that are infected.

Sampling strategies for GPGV are not well defined in the literature, with little information of the sample number or sample location on the grapevine. Definition of phenological stage of the shoots/canes collected is often unclear; whether the canes are partially or fully lignified, or the stage of dormancy. The quantity of material collected per grapevine and location on the vine is rarely outlined. In addition, the timing of sampling varies amongst the overseas literature: samples have been tested across the season from dormancy, through to spring /summer and some in autumn.

This project arose because there was an urgent need to supply the nursery and vine improvement sector with information about sampling and testing strategies for the 2018 cutting season to minimise the risk of spread of the virus to grape growers.

Project aim

The overall aim of this preliminary project was to improve the reliability in Australia of assessing the presence of GPGV in propagation material through both field sampling and diagnostic testing evaluations. Achieving this aim could reduce the risk of false negative results and thus the spread of GPGV via infected propagation material. It could also reduce unnecessary vine removal based on false positive results. This is important given that epidemiology and impact of GPGV in Australia is unclear.

This preliminary trial represents a first step towards establishment of a protocol for field sampling, in terms of tissue type, volume and timing, for reliable GPGV detection in Australia.

In order to maximise reliability of the sampling protocol, the trial was carried out in two climatically distinct regions, in two grape varieties (one wine and one table), using three tissue sampling methods at three time points during 2018. Diagnostic testing and detection was carried out at Crop Health Services (CHS), with an initial cross-reference to Australian Wine Research Institute's (AWRI) Commercial Services.

Materials and Methods

Vineyard sampling strategies

Study sites

In 2017, positive GPGV vines were identified with preliminary testing in two grapevine varieties from two climatic regions, cool (Adelaide Hills, SA) and warm (Robinvale, VIC). At the cool climate vineyard, samples were collected from nine year old vines from a winegrape variety (variety undisclosed at grower's request) planted on own roots, spur pruned and grown on a VSP trellis. At the warm climate vineyard, samples were collected from approximately eight year old Crimson Seedless table grape vines, grown on various unknown rootstocks, cane pruned to 4 to 6 canes dependent on vigour and grown on a T-trellis.

Sample vines

In 2018, tissue samples were collected from a minimum of 15 sample vines at each study site.

At the cool climate site, these sample vines were known positives for GPGV, based on 2017 testing. In this same screening, vines also tested positive for GRSPaV. An additional grapevine in which GPGV had not been detected prior, was included as a control.

At the warm climate site, 15 sample vines were selected for the trial, of which only three were known positives for GPGV based on previous testing. A virus screen including GPGV, GVA, GVB, GFkV, GRSPaV and GLRaV-1, -2, -3, -4, -4 strain 5 and -4 strain 9 was carried out on these three vines. In addition to the presence of GPGV, GRSPaV, GFkV and GLRaV-4 strain 5 were also confirmed. Analysis for other pathogens, pests or nutritional deficiences was not carried out. Subsequent GPGV testing proved that at least one of the vines was confirmed negative for GPGV. The initial vineyard block used for the autumn testing was removed by the owner during winter 2018, evidently due to poor performance. A second set of 15 sample vines was then selected in a nearby vineyard block of the same variety for the winter and spring sampling times, after the presence of GPGV was confirmed in that block.

Sampling

Sampling was performed to test the effects of tissue type and titre contribution to GPGV detection. Samples were collected at three different time points in the season: postharvest in autumn 2018 (shoots lignified), winter 2018 (shoots dormant) and spring 2018 (shoots green). Three different tissue types were collected per vine: two (sample a) or four shoots or canes (sample ab), or ten leaves with petioles (sample c). Two shoots and five leaves with petioles were collected from each side of the vine canopy. The shoots were collected at random intervals along each cordon. The leaves were collected from random shoots along the cordon, apart from the sampled shoots (comprising samples a or ab) as basally as possible. Sample "a" comprised of one shoot collected per cordon, and these two shoots were pooled with an additional two shoots collected from either side of the vine to create sample "ab". The leaves and petioles were only collected in the autumn and spring timings.

Grapevine 15 at the cool climate site was not tested in spring. Grapevine 16 at the cool climate site and grapevine 3 at the warm climate site, which was tested in winter and spring, were included as negative controls because they had previously tested negative (Table 1; Table 2).

In addition, to assess the distribution of GPGV within the shoots and leaves of an individual vine, each of the four shoots and 10 leaves collected from two vines of known positive GPGV-status (Grapevines 14 and 15) at the cool climate site were tested individually (Table 3). Leaves were collected randomly from between node positions four to eight from the base of the shoots.

GPGV detection

Nucleic acid extraction

RNA extraction from grapevine samples was done using modified version RNeasy method described by MacKenzie *et al.,* (1997) with the automated QIAcube HT (Qiagen) high throughput liquid handling system (F.E. Constable, unpublished). Four microlitres of RNA were used in a reverse-transcriptase polymerase chain (RT-PCR) reaction.

RT-PCR

Primers for the detection of NADH dehydrogenase ND2 subunit (ndhB gene, NAD) messenger ribonucleic acid (mRNA; Thompson *et al.,* 2003) were used to determine the quality of the extracted RNA. These primers were used to ensure that nucleic acid was present and that there were no inhibitors in the nucleic acid extracts that retard the activity of the RT enzyme or DNA polymerase during the RT-PCR reactions.

Published RT-PCR assays were used for the detection of GPGV and included the endpoint assays described by Cho *et al.,* (2013; endpoint), Beuve *et al.,* (2015; endpoint) and Glasa *et al.,* (2014; endpoint) and the real-time assays described by Bianchi *et al.,* (2015; real-time) and Ratti *et al.,* (unpublished; real-time). The specified cycling conditions and primer and probe concentrations for each assay were followed. Prior to this study a selection of grapevines from the warm climate site were also tested using RT-PCR for *Grapevine virus A* (GVA), *Grapevine virus B* (GVB), *Grapevine fleck virus* (GFkV), *Grapevine pinot gris virus, Grapevine rupestris stem pitting associated virus* (GRSPaV) and Grapevine leafroll associated viruses (GLRaV-) 1, 2, 3, and 4 and GLRaV-4 strains 5 and strain 9 (Constable et al., 2010). A selection of the grapevines (11-15) at the cool climate site had previously been tested for GPGV, GVA, GLRaV-1, GLRaV-3 and GRSPaV by Australian Wine Research Institute (AWRI) Commercial Services.

The SuperScript™ III One-Step RT-PCR System (Thermofisher Scientific) was used for endpoint RT-PCR detection of NAD mRNA and GPGV according to the manufacturer's instructions, except that 0.5µl of SSIII/ Taq enzyme mixture was used in each RT-PCR reaction and the total reaction volume was 25 µl.

The RNA UltraSense One-Step Quantitative RT-PCR system (Thermofisher Scientific) was used for real time RT-PCR detection of GPGV detection of NAD mRNA and most viruses. One step RT-PCR was done according to the manufacturer's instructions and the total reaction volume was 25 µl.

Comparative testing

A small comparative test between Crop Health Services (CHS) and AWRI Commercial Services was done using the same autumn grapevine homogenates of 16 samples to determine if different extraction procedures and RT-PCR assays produced comparable results for detection of GPGV. AWRI

Commercial Services used the extraction procedure and RT-PCR test described by Wu and Habili (2016). CHS used the procedures described above.

Results and Discussion

Symptoms observations

The GPGV infected grapevines selected for testing at the cool climate site had good growth, vigour and yield. There were no obvious visual symptoms of GLMD associated with GPGV. The GPGV strain present in each of the sample vines at the cool climate site was not determined as part of this trial. However, additional diagnostic testing undertaken in this vineyard block, but not as part of the trial, found that several GPGV strains were present, and that these strains cluster with strains associated with both GLMD and asymptomatic grapevines overseas (F.E. Constable unpublished). Assuming that GPGV is a cause of GLMD or other virus symptoms, and the vineyard block currently appears symptomless for GLMD, the clustering results suggest that perhaps the detected strain associated with GLMD is of much lower titre or prevalence compared to the detected strain associated with the symptomless presentation.

At the warm climate site, both blocks of Crimson Seedless used for the trial had been identified as underperforming by the grower, with some unexplained visual symptoms that reportedly appeared most obvious in spring. These symptoms included stunted shoot growth, some with zig zag shoots, some distorted, mottled leaves and fewer and smaller bunches (Figure 1). Symptoms as described were distributed across both the table grape blocks used for this study. In spring 2018, none of these symptoms were obvious in Grapevines 3, 4, 13, 14 and 15 but other grapevines sampled in this study had one or a combination of these described symptoms. If GPGV is a cause of these observed symptoms, despite the presence of other viruses, it is possible that variable symptom expression noted is associated with GPGV strain present. However, this concept requires further study to prove.

RT-PCR accuracy

The RT-PCR assays of Cho *et al.,* (2013) and Beuve *et al.,* (2015) did not detect some Australian GPGV isolates in this study. GPGV was not detected in some autumn 2018 samples of the grapevines from the cool climate site that had previously tested positive for GPGV in 2017 using these RT-PCR assays and the assays described. On the other hand, when the RT-PCR assays of Bianchi *et al.,* (2015), Glasa *et al.,* (2014) and Ratti *et al.,* (unpublished) were used on RNA extracts of the same autumn samples, they were found to be positive for GPGV. Therefore, the RT-PCR assays of Cho *et al.,* (2013) and Beuve *et al.,* (2015) cannot be reliably used as a diagnostic tool for detection of GPGV in Australia. These results also indicate that genetic variability of GPGV exists within Australia and the genetic variability amongst GPGV isolates affects the reliability of some RT-PCR assays. It is also possible that the genetic diversity of the GPGV population and titre of specific strains within grapevines might change over time. Further work is required to understand the genetic diversity of GPGV in Australia to improve diagnostic capability and the reliability of RT-PCR.

The RT-PCR assays of Bianchi *et al.,* (2015), Glasa *et al.,* (2014) and Ratti *et al.,* (unpublished) are equally accurate for detection GPGV as each assay gave the same positive or negative result in each sample in autumn, winter and spring. This supports the verification of these tests done by Italian laboratories which also showed that they were equally reliable (Barba *et al.,* 2017; Gentili *et al.,* 2018). Therefore, these tests are recommended for detection of GPGV in Australia. However, it is important

to review the assays as new information on the genetic diversity of GPGV becomes available to ensure they detect all strains.

Seasonal and within vine detection of GPGV

The results of this study suggest that GPGV can be detected in autumn, winter and spring as most grapevine samples with known status from both regions returned positive results at each sampling time point (Tables 1 and 2). At the cool climate site GPGV was detected in each grapevine that was expected to return a positive result (grapevines 1-15) in each season. The GPGV infection status of sample vines in both warm climate blocks was not known before the trial began, although GPGV was known to occur in some individual grapevines. Each of the 15 grapevines from the original block chosen for this study tested positive for GPGV in autumn, based on results from at least one tissue type. In spring in the second block used at the warm climate site, GPGV was detected in at least one tissue sample of vines that tested positive in winter. Vines from the second block had not been tested in autumn. At the cool climate site, 45/47 (96%) samples each representing a single vine and tissue type, returned a matched GPGV result across the two or three timepoints of testing (Table 1). In every occasion where three time points of testing was undertaken for a particular tissue type from a sample vine at the cool climate site, there was 100% correlation of GPGV results across the three timepoints (Table 1). At the warm climate site, 30/30 samples (100%) achieved matched GPGV results between winter and spring samples undertaken on the same vineyard block (Table 2). These results suggest that there was no seasonal impact on GPGV detection in 2018.

Across the two sites, there was no clear evidence to suggest that one tissue type provided more consistency in GPGV results than another, as the small number of unexpected negative results that were obtained, were from both leaf and shoots samples. GPGV was detected in at least one sample of each infected grapevine at each sampling time point at both study sites, however in a small number of grapevines the virus was not detected across all tissue types at the one time.

At the cool climate site, in spring, GPGV was not detected in “sample a” (two shoots) and “sample c” (leaves) of grapevine 14 and in autumn, it was not detected in the combined shoots sample “ab” in vine 1, and the 10 leaves “c” in grapevine 12 (Table 1). At the warm climate site, in autumn, GPGV was detected only in “sample a” (two shoots) of grapevines 10 and 15, with the other tissue types negative and in grapevine 14 “sample a” (two shoots) was negative but GPGV was detected in the combined shoots “sample ab” and “sample c” (leaves) (Table 2). All other samples collected in autumn from this site were positive (Table 2). These results suggest that GPGV can have an uneven distribution and/or titre within the grapevine. This hypothesis is further supported by the variable positive and negative GPGV test results of individual shoots and leaves from both grapevines 14 and 15 (cool climate) in autumn (Table 3), which indicate that GPGV can occur in uneven distribution and/or titre within the canopy of a grapevine at this time. Across both sample vines, there was 75% consistency (15/20) in GPGV results from the individual leaves tested, compared to 87.5% consistency (7/8) from individual shoots tested. Further work is required to examine the distribution of GPGV in individual shoots and leaves at different times of the year and different climates to inform accurate sampling for GPGV detection. It is possible that some GPGV negative results were due to co-extraction of compounds that inhibit the RT-PCR reaction. However, all samples were positive for the NAD RT-PCR assays, indicating that they were of sufficient quality for detection of GPGV by RT-PCR.

At the warm site, for the second block’s winter and spring sampling times, grapevines 3 and 4 both had no obvious symptoms of zig zag shoots in the winter, and grapevine 3 had previously tested negative to GPGV. GPGV was not detected in the winter samples nor in the spring shoot samples “a” and “ab”. However, GPGV was detected in “sample c” (leaves) of both grapevines in spring 2018.

These results also suggest that GPGV was in uneven distribution and/or titre in the grapevines and it may be that the detection of GPGV only in the leaves is an indication of a recent infection event. In Australia, other grapevine viruses can be undetectable in grapevines due to uneven distribution and titre after a recent infection event and virus titre as well as symptom expression can take up to two years to build to detectable levels (Constable *et al.,* 2012a, 2012b). If GPGV is the cause of the symptoms observed in the warm climate vineyard blocks, the lack of symptom expression in grapevines 3 and 4 might be associated with virus titre and timing of an infection event.

European studies suggest that the titre of GPGV is highest in spring then decreases until autumn (Bertazzon *et al.,* 2015., Bianchi *et al.,* 2015). In Australia, the reliability of GPGV detection in summer has not been examined and virus titre has not been measured throughout an entire season. No studies have been done, in any country, to examine test reliability by measuring virus titre over several years. To refine sampling strategies to prevent false negative GPGV test results, further studies are required to improve understanding of the seasonal impact on GPGV detection in Australia including frequent testing (e.g. monthly) of GPGV infected varieties of different vigour, in different regions and over several years. Measuring virus titre would assist in understanding when GPGV concentrations are highest to assist with accurate and sensitive testing.

Comparative testing

AWRI Commercial Services detected GPGV in 16/16 samples that had previously tested positive. CHS detected GPGV in 13/16 samples using the endpoint RT-PCR assay of Beuve *et al.,* (2015), 15/16 samples using the endpoint RT-PCR assays of Glasa *et al.,* (2014) and 16/16 samples using the real time PCR assay of Bianchi *et al.,* (2015). These results and the results of the Italian validation work indicate that several robust testing procedures are available for detection of GPGV and that differences in extraction procedures may not make a difference to GPGV detection if the method is optimised. However, the results also show that reliable GPGV detection is dependent upon the RT-PCR assay that is used.

Conclusions

Based on this preliminary study, GPGV can be reliably detected using the test methods of the CHS and AWRI Commercial Services diagnostic laboratories. Similar proficiency tests could be done in future for GPGV and other grapevine viruses to provide confidence in the test methods used by different laboratories, importantly including that used at the Australian border.

GPGV was detected in autumn, winter and spring at the cool and warm climate sites. Further work is required to assess test reliability in summer at both sites. There is also a need to conduct a similar trial over several seasons to confirm the most reliable time point to conduct GPGV testing.

GPGV may be unevenly distributed in leaves and shoots which could impact detection, but there was no apparent difference in the reliability of different tissues sample types used for GPGV detection. A broader trial should be done to provide a statistical basis for sampling to improve detection of GPGV.

This study used one table grape variety and one wine grape variety and further work is required to determine the reliability of GPGV detection using the methods in this study in other wine and table grape varieties and rootstocks. There is also a requirement to examine the impact of GPGV strain on diagnostic test reliability.

The association between GPGV and disease in Australia is uncertain and its contribution to disease needs to be examined. GPGV was not associated with disease in the variety grown at the cool climate study site.

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Table 1. The seasonal detection, in autumn, winter and spring 2018, of *Grapevine Pinot gris virus* in samples of two shoots (a), four shoots (ab) and 10 leaves with petioles (c) of 15 grapevines from the cool climate vineyard.

Vine number	Vine material sampled	Sample timing GPGV result[1]		
		Autumn	Winter	Spring
1	a	POS	POS	POS
	ab	NEG	POS	POS
	c	POS	NT	POS
2	a	POS	POS	POS
	ab	POS	POS	POS
	c	POS	NT	POS
3	a	POS	POS	POS
	ab	POS	POS	POS
	c	POS	NT	POS
4	a	POS	POS	POS
	ab	POS	POS	POS
	c	POS	NT	POS
5	a	POS	POS	POS
	ab	POS	POS	POS
	c	POS	NT	POS
6	a	POS	POS	POS
	ab	POS	POS	POS
	c	POS	NT	POS
7	a	POS	POS	POS
	ab	POS	POS	POS
	c	POS	NT	POS
8	a	POS	POS	POS
	ab	POS	POS	POS
	c	POS	NT	POS
9	a	POS	POS	POS
	ab	POS	POS	POS
	c	POS	NT	POS
10	a	POS	POS	POS
	ab	POS	POS	POS
	c	POS	NT	POS
11	a	POS	POS	POS
	ab	POS	POS	POS
	c	POS	NT	POS
12	a	POS	POS	POS
	ab	POS	POS	POS
	c	NEG	NT	POS
13	a	POS	POS	POS
	ab	POS	POS	POS
	c	POS	NT	POS
14	a	POS	POS	NEG
	ab	POS	POS	POS
	c	POS	NT	NEG
15	a	POS	POS	NT
	ab	POS	POS	NT
	c	POS	NT	NT
16	a	NEG	NEG	NEG
	ab	NEG	NEG	NEG
	c	NEG	NT	NEG

[1]POS = GPGV detected; NEG = GPGV not detected; NT = not tested.

Table 2. The seasonal detection, in autumn, winter and spring, of *Grapevine Pinot gris virus* in samples of two shoots (a), four shoots (ab) and 10 leaves with petioles (c) of 15 grapevines from the warm climate vineyard.

Vine number	Vine material sampled	Sample timing GPGV result[1 2]		
		Autumn BLOCK 1	Winter BLOCK 2	Spring BLOCK 2
1	a	POS	POS	POS
	ab	POS	POS	POS
	c	POS	NT	POS
2	a	POS	POS	POS
	ab	POS	POS	POS
	c	POS	NT	POS
3	a	POS	NEG	NEG
	ab	POS	NEG	NEG
	c	POS	NT	POS
4	a	POS	NEG	NEG
	ab	POS	NEG	NEG
	c	POS	NT	POS
5	a	POS	POS	POS
	ab	POS	POS	POS
	c	POS	NT	POS
6	a	POS	POS	POS
	ab	POS	NEG	NEG
	c	POS	NT	NEG
7	a	POS	POS	POS
	ab	POS	POS	POS
	c	POS	NT	POS
8	a	POS	POS	POS
	ab	POS	POS	POS
	c	POS	NT	POS
9	a	POS	POS	POS
	ab	POS	POS	POS
	c	POS	NT	POS
10	a	POS	POS	POS
	ab	NEG	POS	POS
	c	NEG	NT	POS
11	a	POS	POS	POS
	ab	POS	POS	POS
	c	POS	NT	POS
12	a	POS	POS	POS
	ab	POS	POS	POS
	c	POS	NT	POS
13	a	POS	POS	POS
	ab	POS	POS	POS
	c	POS	NT	POS
14	a	NEG	POS	POS
	ab	POS	POS	POS
	c	POS	NT	POS
15	a	POS	NEG	NEG
	ab	NEG	NEG	NEG
	c	NEG	NT	NEG

[1]POS = GPGV detected; NEG = GPGV not detected; NT = not tested.

[2]The autumn samples were collected from a different group of grapevines to the winter and spring samples.

Table 3. Autumn 2018 detection of *Grapevine Pinot gris virus* from individual shoots and leaves sampled from vines 14 and 15 from the cool climate vineyard.

Vine number	Vine material sampled	GPGV result[1]
14	Shoot – 1	POS
	Shoot – 2	POS
	Shoot – 3	POS
	Shoot – 4	POS
	Leaf – 1	POS
	Leaf – 2	POS
	Leaf – 3	NEG
	Leaf – 4	POS
	Leaf – 5	POS
	Leaf – 6	NEG
	Leaf – 7	POS
	Leaf – 8	POS
	Leaf – 9	POS
	Leaf – 10	POS
15	Shoot – 1	POS
	Shoot – 2	NEG
	Shoot – 3	POS
	Shoot – 4	POS
	Leaf – 1	POS
	Leaf – 2	POS
	Leaf – 3	NEG
	Leaf – 4	POS
	Leaf – 5	NEG
	Leaf – 6	NEG
	Leaf – 7	POS
	Leaf – 8	POS
	Leaf – 9	POS
	Leaf – 10	POS

[1]POS = GPGV detected; NEG = GPGV not detected; NT = not tested.

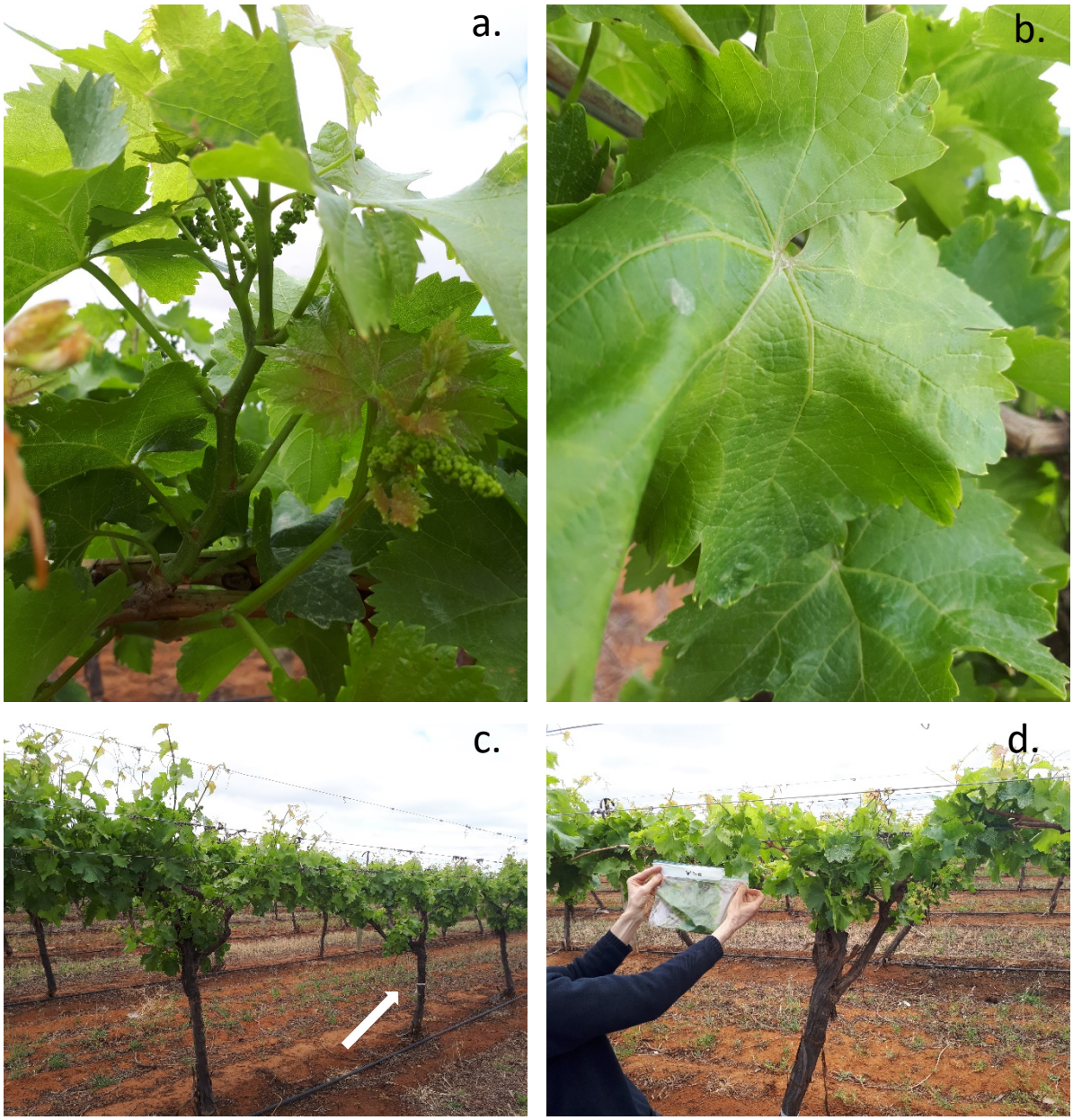


Figure 1. Symptoms observed on grapevines (table grape cultivar Crimson seedless) infected with *Grapevine pinot gris virus* from the warm climate study site in spring 2018: examples include a. zig zag shoots, b. mild leaf mottling; and c. stunted growth of grapevine 1 (arrow) and d. stunted growth of grapevine 8.