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Grapevine leafroll disease and Shiraz disease: interim recommendations for management in Australian vineyards



FINAL REPORT TO WINE AUSTRALIA

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Contents

Preface **1**

The purpose of this document **2**

Introduction **3**

Virus disease cycle **5**

Distribution of target viruses **7**

GLD and SD: influence and varietal susceptibility **8**

Economic impacts **10**

A proposed starting point for red and white varieties **11**

A decision support tree for grapevine virus management **12**

Visual symptom identification **14**

White varieties 14

Red varieties 14

Visual assessor training 19

Symptom development in leaves 19

The timing of symptom expression in leaves 21

Frequency and timing of visual inspections 22

Record keeping 22

Distinguishing GLD and SD from other leaf-reddening conditions 24

Roguing **26**

What is roguing and why is it important? 26

Roguing virus-infected vines 26

Remnant grapevine roots 27

Whole-block removal 27

Replanting 28

Top-grafting (red and white varieties) 29

Mealybugs and soft scale insects: the vectors of virus **30**

Vector life cycle and biology **32**

Emergence of vectors and spring activity 32

Feeding and honeydew 32

Egg laying 33

Crawlers 34

Juveniles and adults 34

Numbers of generations 35

Vector monitoring **36**
Time of monitoring (red and white variety vines) 36
Late-vintage inspections (red and white variety vines) 36
Visual vine inspections (red and white variety vines) 37
Collecting vine material (red and white variety vines) 37
Interpreting the results 38
Record keeping 38
Monitoring mealybugs with pheromones 39
Vector management **40**
Biological control (red and white variety vines) 40
Insecticides (red and white variety vines) 40
Vineyard hygiene (red and white variety vines) 42
Laboratory testing for viruses **43**
Diagnostic laboratories 43
Why test for grapevine viruses? **44**
Red varieties 44
White varieties 44
Questions for the laboratory (red and white variety vines) 45
When and where to collect grapevine samples (red and white variety vines) 45
Shopping list 46
How to collect single samples (red and white variety vines) 46
Composite test plan (red and white variety vines) 48
How to collect composite samples (red and white variety vines) 48
Available laboratory tests (red and white variety vines) **49**
Enzyme-linked immunosorbent assay (ELISA) 49
Molecular-based assays 49
Interpreting diagnostic results **50**
Red varieties – single sample testing 50
Composite sample testing 53
Composites back to single tests 53
Future prospects for virus identification: remote sensing **55**
Appendix 1: Key references **56**
Appendix 2: Timeline of management activities **58**
Appendix 3: Figure and table captions detailed in full **59**
Acknowledgements **61**

Preface

The negative influence of viruses on grapevine productivity and grape quality is increasingly recognised in Australian vineyards. Detrimental changes due to virus infection include reduced vine vigour as well as reduced grape quality and yield. Approximately 80 grapevine viruses have been identified globally, with twelve known to be present in Australia. Minimising the influence and spread of endemic viruses, and preventing the establishment of new exotic viruses, is a priority to protect Australian grape and wine production.

The notable viruses of economic importance to grape-growing in Australia are Grapevine leafroll-associated virus 1 and 3 (GLRaV-1 and -3), and Grapevine virus A (GVA), which is often co-infected with one or more leafroll viruses and causes Shiraz disease. Management of viruses is challenging, and growers require standard protocols for virus and vector control that are tried and tested.

This document draws upon the experience of the New Zealand wine industry's *Virus Elimination* project (2009–2015), which used an integrated (multi-tactic) response focusing on preventative measures and early intervention to reduce and contain virus spread. While New Zealand's integrated response is probably compatible in many ways with grape-growing in Australia, differences between countries justify some caution. For example, the New Zealand response targeted only GLRaV-3, owing to it being the sector's most problematic virus.

GLRaV-1, -3, and Shiraz disease cause similar foliar changes and are spread between grapevines by mealybugs and soft scale insects, but Australia's diversity of grape varieties and insects transmitting these viruses exceeds that of New Zealand. Climate is another distinction between the countries. An integrated response to grapevine viruses under Australian conditions has not yet been tested to determine its effectiveness.

Given these differences, this document offers definitive advice only where it is reasonable to do so. Where data are either absent or scarce (a 'knowledge gap'), this is highlighted in the text. A separate document provided to Wine Australia focuses on these knowledge gaps to enable the consideration and prioritisation of future research.

Finally, it is important to recognise that grapevine viruses will continue to spread if those affected by them choose to do nothing. This document acts a starting point for awareness and a call to action for the Australian wine and grape sector to minimise the negative influence of viruses on vineyards.

The purpose of this document

In August 2020, Wine Australia issued a Request for Proposal with the goal of developing a plan to manage Grapevine leafroll disease and Shiraz disease. The project had four objectives. This document addresses one of those objectives:

- **To develop a Best Practice Management guide for virus management in vineyards and nurseries for the Australian wine sector**

As a starting point, this document will inform future communication, training, extension and change of practice activities. It aims to help the Australian grape and wine sector adopt robust virus management practices for implementation in the vineyard and nursery.

A second objective seeks to consolidate vine improvement accreditation.

- **To review the existing vine improvement accreditation schemes to draft the scope, structure, and content of a single, national vine propagation and supply standard**

This objective, which was beyond the scope of this document, is being addressed by other parties who will be reporting to Wine Australia separately. Despite this objective not being addressed in this document, the importance of a robust vine improvement accreditation scheme cannot be overstated. Developing such a scheme that has the confidence of the entire sector is the platform upon which an in-vineyard virus management response is built.

The third and fourth objectives were to:

- **Identify gaps in knowledge relating to the surveillance, sampling, testing and extent and impact of grapevine virus infection in Australia and any other aspect affecting the selection of management responses to grapevine viruses at the sector and vineyard level**
- **Scope options, including detail of research priorities and possible future research questions, to address these knowledge gaps.**

These objectives were addressed alongside the development of this document and are detailed in a separate report provided to Wine Australia to inform future research, development and adoption activities.

Introduction[1]

Key Points

- Multiple viruses cause Grapevine leafroll disease (GLD) and Shiraz disease (SD). In Australia, the key viruses are GLRaV-1, GLRaV-3, and GVA.
- Viruses can be spread by using diseased planting material and sap-sucking mealybugs and soft scale insects.
- GLD and SD harm grapevine physiology, fruit yield, fruit composition, and wine quality.
- Disease control requires a coordinated and integrated management response:
    - Monitoring and managing mealybugs and soft scale insects.
    - Identifying and removing diseased grapevines.
    - Using vines certified as virus-free when planting.

Grapevine leafroll disease (GLD) is a significant grapevine disease worldwide, whereas reports of Shiraz disease (SD) have to date been confined to South Africa and Australia. GLD and SD are caused by viruses in the *Closteroviridae* and *Vitivirus* families, respectively. GLD can be caused by five different viruses (Grapevine leafroll-associated virus-1, -2, -3, -4, and -7). In Australia, those of most concern are Grapevine leafroll-associated virus 1 (GLRaV-1) and Grapevine leafroll-associated virus 3 (GLRaV-3). The other grapevine leafroll viruses (GLRaV-2, -4, and -7) appear at this time to be of little economic importance and are out of scope for this document. However, their presence may still influence management decisions, including determining budwood collection for future propagation.

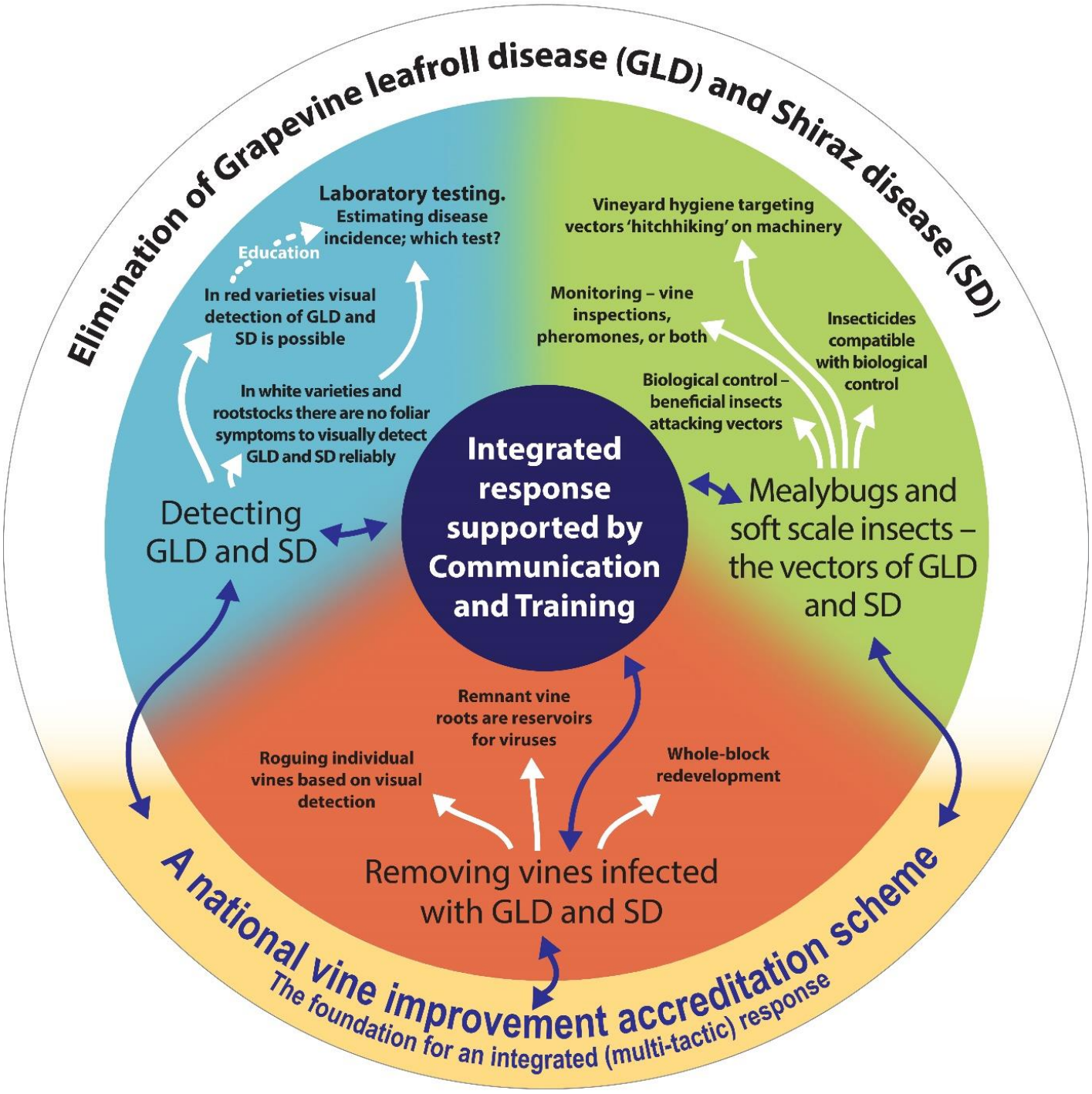
The leading viral causal agent for SD is Grapevine virus A (GVA), although SD-infected grapevines are often co-infected with one or more grapevine leafroll viruses (GLRaV-1, -2, -3, -4, and -7). As the name suggests, the variety Shiraz is particularly sensitive to GVA infection, but it also occurs in other red varieties such as Malbec and Merlot.

Grapevine viruses are spread by grafting and by sap-sucking mealybugs and soft scale insects. GLD and SD can cause obvious visual foliar symptoms, particularly in red varieties, where GLD causes distinctive reddening of leaves while the primary veins remain green. For SD, leaves turn completely red and may remain on the vine during winter. These foliar symptoms provide visual cues that can be used to identify diseased grapevines.

Both diseases are economically important because they negatively influence grapevine physiology, fruit yield, and quality. Ultimately, wine quality may be lowered. To reduce the influence of these viral diseases, vineyard and nursery managers need to control the insects that spread the virus, identify and remove diseased grapevines from the vineyard, and use only certified virus-free grapevines when planting. Communication and information access are also key components for a successful co-ordinated and integrated management response.

[1] Despite the authors having referred to a substantial body of research originating from Australia and elsewhere, for ease of reading this literature is not cited in the document. Instead, Appendix 1 lists the key research papers and other published material used in the development and writing of the document.

Embracing an integrated (multi-tactic) response, focusing on preventative measures and early intervention to reduce and contain virus spread, as depicted below and outlined in the remainder of this document, is key to nullifying the economic influences of GLD and SD in Australian vineyards.



Virus disease cycle

There are two mechanisms by which GLD and SD can infect grapevines (as described in Figure 1):

- Vegetative propagation and grafting: planting diseased grapevines, and by way of top-grafting when using diseased vine material (scion and/or rootstock)
- Insects: up to 14 sap-sucking insect species – mealybugs and soft scale insects – transmitting one or more viruses from infected to healthy grapevines.

Material from diseased mother vines in source blocks (infected with one or more viruses) remains infected when used for propagation on own-roots, grafted plants, and when top-grafting. Top-grafting is of particular concern when the virus status of the grapevines to be worked is unknown and vines have not shown symptoms previously. If healthy plant material is top-grafted onto diseased trunks, the virus will spread to the healthy scion material and cause disease.

When mealybugs and soft scale insects feed on a diseased vine, they acquire virus. GLRaV-3 has been detected in mealybugs up to 16 days after they were denied access to a virus-infected grapevine. If the vectors move onto a neighbouring healthy grapevine and resume feeding, the transfer of virus particles will result in a new infection. This secondary spread of virus in the vineyard occurs through mobile insects walking along canes and wires to neighbouring grapevines. Juvenile life stages are also passively dispersed by wind to new areas in a vineyard; vectors may also ‘hitchhike’ on unclean machinery. Although not widely studied, another possible virus transmission pathway is through vector feeding on the remnant roots of a recently removed diseased grapevine. Vector movement from remnant roots, a known GLRaV-3 reservoir, to the roots of a newly planted grapevine may result in virus transmission following the resumption of vector feeding.

Grapevine leafroll-associated viruses and GVA are not known to be mechanically transmitted on pruning equipment or harvesters, nor are they known to be transmitted by touch. Transmission via seeds has not been reported.

Once a grapevine is infected with one or more viruses, either by propagation of infected material or insect-mediated transmission, the virus will use the same cells and organs that the vine uses to move sugars and nutrients to grow and produce fruit. By using a vine’s vascular system, a replicating virus is transported to the leaves, canes, trunk, and roots. Eventually, plant function is damaged, which may result in a range of symptoms:

- Visible reddening in the leaves of red varieties
- Reduced plant growth
- Poor lignification of canes (for SD)
- Delayed berry ripening.

In red varieties, the interval between infection and visible symptom expression is likely to be at least 12 months, meaning a grapevine infected in one vintage may not show foliar symptoms until the following vintage. As we note later, the absence of foliar symptoms for GLD and SD in white varieties and rootstocks limits the usefulness of visual symptom identification to identify infected vines reliably.

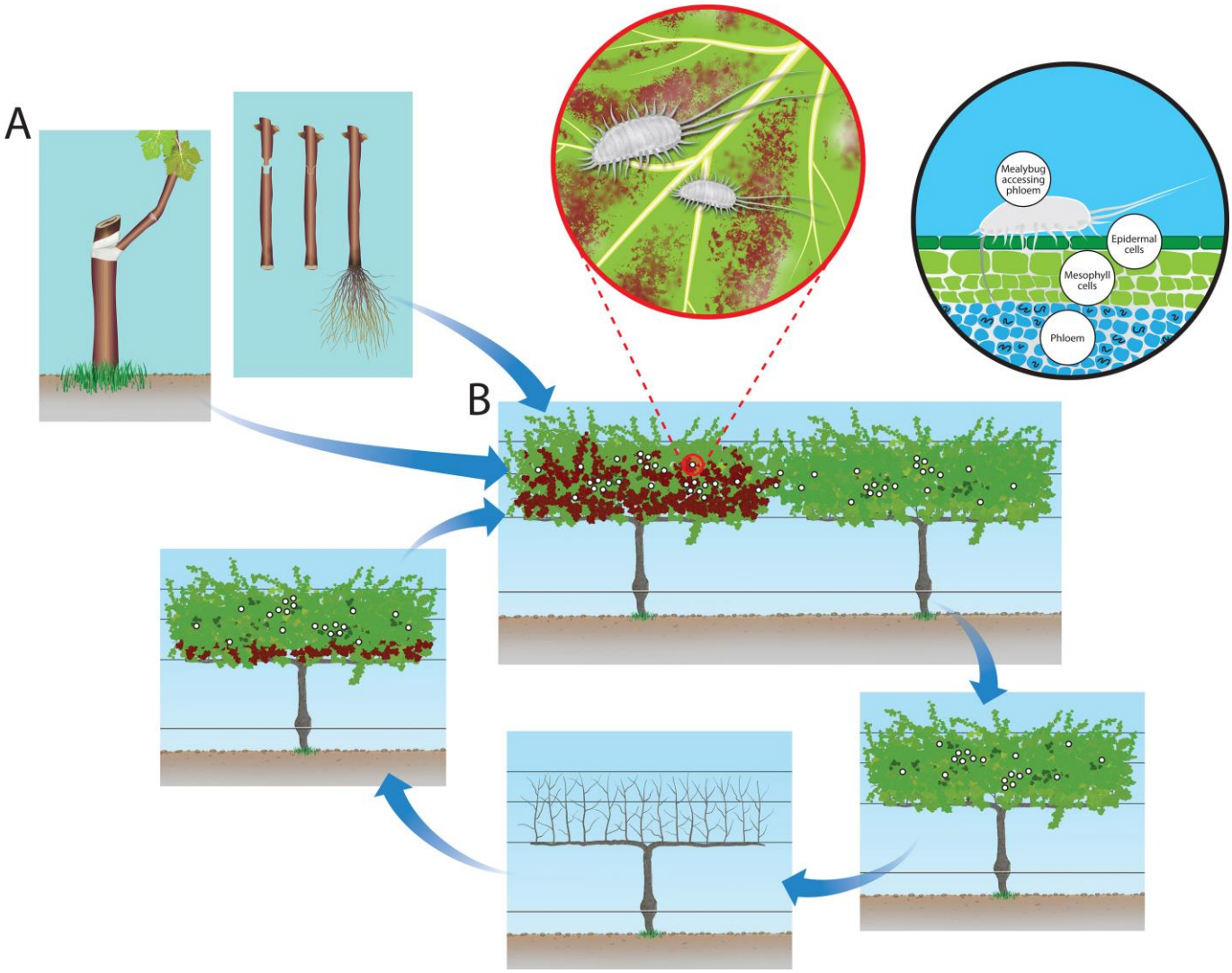


Figure 1. Virus disease cycle showing how Grapevine leafroll disease (GLD) and Shiraz disease (SD) can enter the vineyard and be spread by mealybugs or soft scale insects. Starting from the top left, the propagation of plant material from diseased mother vines for use in top-grafting and grafted grapevines can introduce virus into vineyards (A). Disease will spread from a diseased grapevine to a healthy grapevine by mealybugs or soft scale insects (B). As depicted in the large red and black circles, mealybugs feed on a diseased vine and acquire the virus. When the mealybugs (small white circles) move to a neighbouring (healthy) grapevine and begin feeding again, the virus particles will move from the insect into the plant tissue. The vine is now infected. In red varieties, visible symptom expression of disease takes at least 12 months to become apparent, meaning a grapevine infected in one vintage may not show foliar symptoms until the following vintage.

Distribution of target viruses

Grapevine leafroll disease (GLD), and the causative viruses, GLRaV-1 and GLRaV-3, are found worldwide. GVA is also detected throughout the grape-growing world, but Shiraz disease (SD[2]) has been reported only from Australia and South Africa. Reports suggest SD is caused by a particular strain of GVA that is nominally referred to as 'GVA group II'. Grapevines infected with SD are often co-infected with GLD-causing viruses, particularly GLRaV-3.

Virus strains are often referred to as a variant of a given virus. Where the virus strains possess different genetic makeup, they are often referred to as 'genetic variants' and are grouped based on their similarity. Each grapevine leafroll virus (GLRaV-1, GLRaV-3 and so on) is known to have several different genetic variants. Research is required to understand (1) the distribution of these genetic variants in Australia, (2) the influence they might have on the grapevine in the Australian environment, and (3) whether this genetic variation affects the accuracy of diagnostic testing in the laboratory (red and white varieties) or visual symptom identification in the vineyard (red varieties).

Grapevine leafroll disease and Shiraz disease have been observed in many grape growing regions of Australia. A wide-ranging survey estimating GLD and SD incidence and distribution has not been undertaken, but in 2008 and 2009, 218 grapevine samples from vineyards in New South Wales, Queensland, South Australia, Victoria, and Western Australia showed the presence of GLRaV-1, GLRaV-3, and GVA in all five states. In a recent study, SD has been identified in South Australia vineyards.

[2] SD should not be confused with 'Syrah decline', which has been described in the United States and Europe. Syrah decline, which may be due to genetics, leads to grapevine basal trunk swelling, reduced shoot vigour in spring, red leaves in early summer, and delayed fruit ripening.

GLD and SD: influence and varietal susceptibility

GLRaV-1, GLRaV-3, and GVA can infect all grape varieties. However, the severity of symptom expression can be influenced by the variety, the scion-rootstock combination, environmental factors, virus strain, possible co-infection with other viruses/pathogens, and the duration of the infection.

Typically, GLD and SD can lead to reddening of leaves in red varieties, with GLD leaves retaining green primary veins and SD leaves turning completely red. Rootstock and most white varieties infected with GLD and SD do not show foliar symptoms reliably (described later).

Grapevines with GLD, particularly those infected with GLRaV-1 and GLRaV-3, lose vigour and productivity, and fruit quality is reduced. Fruit yield losses of between 14 and 50% have been reported and are probably caused by the viruses negatively influencing berry size and weight, cluster size, and cluster form (such as loose clusters). The fruit from diseased grapevines, particularly in red varieties, generally ripen more slowly and unevenly than healthy grapevines (Figure 2). Harvested fruit have lower soluble solids content (Brix), higher titratable acidity (particularly malic and tartaric acids), and reduced compounds such as anthocyanins, phenolics, and tannins. Declining wine quality will affect financial sustainability.

Despite its name, Shiraz disease can cause symptoms in red varieties like Malbec and Merlot. Grapevines with SD are often co-infected with GVA and a grapevine leafroll virus, particularly GLRaV-3, and they will also generally display loss of vigour, productivity, fruit quality, and wine quality. Additional symptoms caused by SD include an increased likelihood of primary bud necrosis, retarded canopy growth in spring, uneven lignification, particularly at the buds, and potential longitudinal cracking on non-lignified one-year-old canes. GVA has also been associated with causing stem-grooving of canes.

Despite no reports of SD-related grapevine deaths in Australia due to SD infection on its own, that is apparently not the case in South Africa, where reports confirm vine deaths occurring within five years of planting. In Australia, entire blocks of SD-affected vines have been removed due to lack of productivity, typically within six years of the vineyard becoming infected. The cost of removing virus infected vines and replanting is estimated at around AU$70,000 per hectare.

Some GLD-infected varieties may show no detrimental symptoms and are in fact sought after by winemakers. For example, the entirety of Mendoza Chardonnay clonal material in New Zealand is known to be infected with GLRaV-1 and is nonetheless regarded as a premium clone for Chardonnay wines. Similarly, the Gingin clone of Chardonnay is greatly valued by producers in the Margaret River region of Western Australia and known to to be widely infected with GLRaV1.The extent to which the variety, clone, scion-rootstock combination, and environmental conditions influence symptom expression and severity is likely to be important and require further research in Australia. Symptoms caused by GLD and SD may also vary in different Australian wine regions.

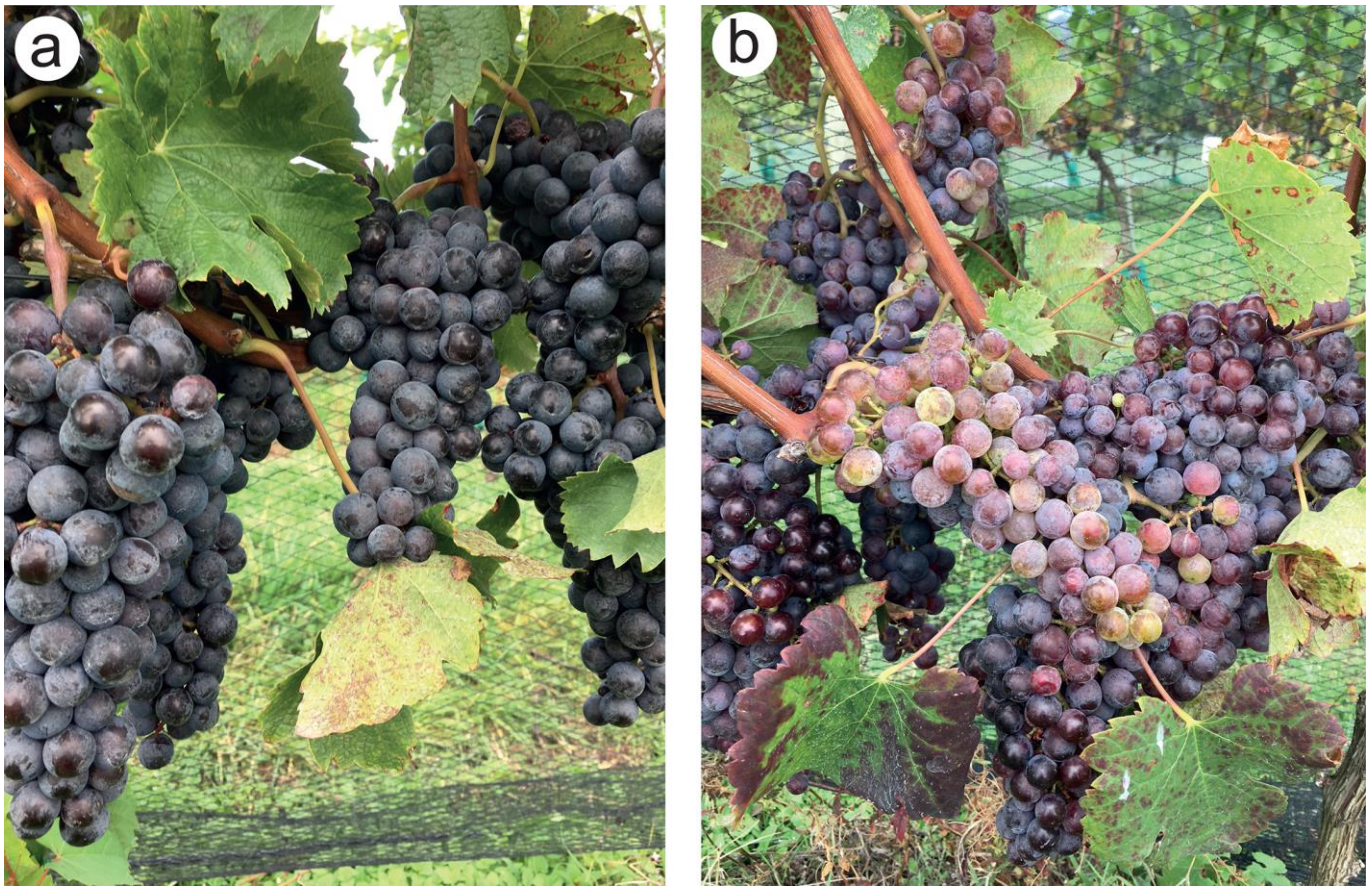


Figure 2. One of the symptoms of Grapevine leafroll-associated virus 3 (GLRaV-3) in red varieties is uneven ripening of fruit. Compare the good colour development among clusters on a GLRaV-3-free Merlot vine (a) with the uneven colour development seen in another Merlot vine infected with GLRaV-3 (b).

Economic impacts

In the absence of disease control, the financial costs of GLD in the USA have been estimated to range from US$25,000 to $226,500 per hectare over the lifetime of a vineyard. These cost estimates depend on the variety, geographic region, disease prevalence in the vineyard, the abundance of mealybug and soft scale insects (i.e. the virus 'vectors'), estimated yield losses, penalties associated with unsatisfactory grape quality, and the extent of disease control measures employed.

Such estimates underscore the importance of maintaining low disease incidence and low vector abundance in reducing the costs of GLD. For example, data modelling undertaken in New Zealand in 2021 suggested that over a period of 20 years, the average annual cost plus loss of income was an estimated NZ$131 per hectare when actively maintaining low GLRaV-3 incidence (<1%) and low mealybug abundance (6 per 100 vine leaves inspected). By contrast, at an initial GLRaV-3 incidence of 10%, but no active management responses, estimated average annual costs plus loss of income increased to NZ$8,236 per hectare.

Responding to GLRaV-3 in New Zealand sees growers identify and remove infected vines when initial incidence is less than 20% in a single variety block. However, in blocks where the widely agreed 20% upper threshold is exceeded, annually removing infected vines can be unsustainable, both practically and economically. Under these conditions, it is suggested that planning begin to schedule a whole-of-block vine replacement program when conditions allow. In Australia, evaluation is needed to assess if 20% is a financially acceptable and biologically viable upper threshold to manage GLRaV-3, and whether this threshold can be applied to vineyards with other viral infections such as GLRaV-1 and GVA.

Based on current literature, the cost-benefit analysis of undertaking active disease and vector management is favourable. However, the economic influence of SD and GLD in Australia and the benefits to be gained from implementing particular management responses will require more evaluation.

A proposed starting point for red and white varieties

This document suggests implementing a range of management tactics when responding to GLD and SD. It offers growers the opportunity to consider and then develop a plan to begin the process of managing the targeted viruses.

Some of the concepts proposed in this document will be new to many growers, so implementing all aspects of the proposed management plan will be challenging. It may mean there are some growers that cannot or will not be able to adopt all the tactics proposed in the timely manner suggested.

If, for whatever reason, it is impractical to adopt all the management tactics suggested, it is important to at least begin assessing virus incidence across the vineyard. A good understanding of virus incidence in different single variety blocks in a vineyard is the basis for other decisions.

For example, if a particularly high-yielding block or one producing award winning wines is shown to have a relatively low virus incidence (say, less than 5%), this information is the basis for decisions related to vector monitoring and vector management. Further, if the block of concern was planted in a red variety (with obvious foliar symptoms), it might also inform decisions related to removing individual virus-infected vines or for sourcing budwood for future vine propagation.

Assessments of GLD and SD in other blocks might highlight moderate to high virus incidence (say 15 – 20%). Again, this information is critical to future decisions related to vector monitoring and management, and if the block was planted in a red variety, it might also determine decisions related to the feasibility of initiating vine removal.

Elsewhere in the vineyard, assessments of GLD and SD could highlight one or more blocks where virus incidence is very high (say 30 – 50% or more). Decisions on what to do in these blocks will be complex and likely to be influenced by other factors. For example, in a block planted with highly valued old or heritage vines aged at 50 or more years, decisions linked to vine removal are not straightforward, particularly if the wine produced continues to be of high quality. The default position under these circumstances may be to begin the process of vector monitoring and/or the adoption of an insecticide program.

It is worth remembering that blocks with high (or even moderate) virus incidence that also support mealybugs and soft scale insects are a risk to vines in surrounding blocks due to human-induced or airborne dispersal of the insect vectors. In addition, such blocks should not be used to source material for vine propagation.

Whatever the virus incidence in different blocks, the knowledge gained from understanding virus incidence is fundamental to informing a host of other decisions with regard to management of GLD and SD.

A decision support tree for grapevine virus management

With this in mind, a ‘Decision Support Tree’ is proposed for those wanting to proactively manage GLD and SD in vineyards (Figure 3).

There are distinct pipelines for white and red varieties, which recognise that visible symptoms are present in most red varieties but not whites (see below). Once virus incidence in a vineyard planted to a white variety is known (by laboratory testing), the decision tree can follow the same pathway as for a red variety.

Whole-block redevelopment is proposed when surpassing an initial 20% virus incidence upper threshold. As noted already, the acceptance and applicability of this upper threshold to grape-growing in Australia requires evaluation, and will depend on the particular situation (see Whole-block removal).

Concepts relating to the decision support tree are explained in detail in the following sections.

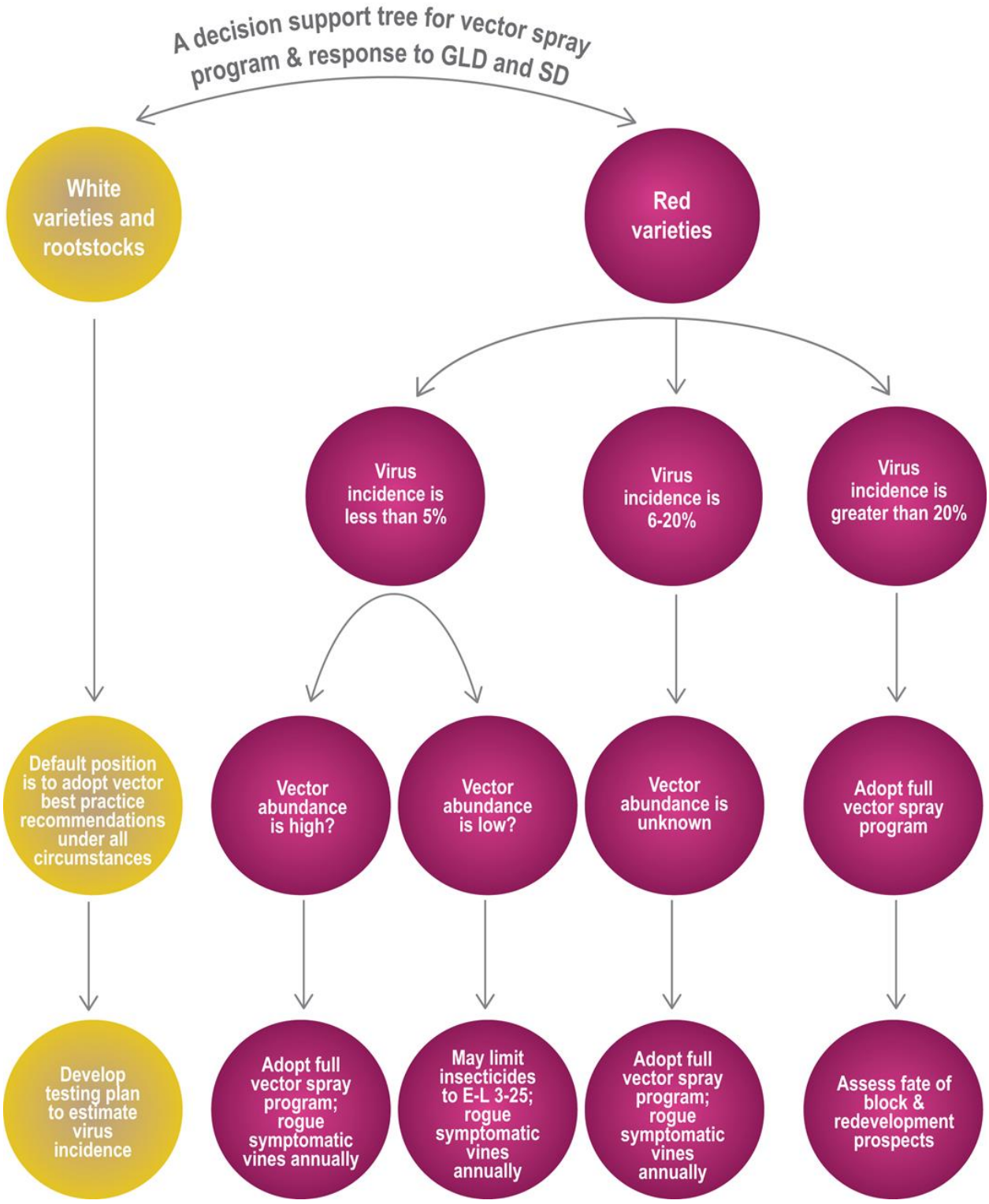


Figure 3. A generalised decision support tree to help vineyard staff begin determining a response to Grapevine leafroll disease (GLD) and Shiraz disease (SD) in white and red varieties. Note: Insecticides targeting mealybug and soft scale insects can be applied from dormancy (Eichhorn Lorenz or E-L 3) up to no later than 80% cap-fall (E-L 25), depending on the insects being targeted and the active constituent(s) being applied (as noted in the *Agrochemicals registered for use in Australian viticulture*, 22/23, The Australian Wine Research Institute, 31 May 2022).

Visual symptom identification[3]

Key Points

- Visual detection of GLD and SD is limited to red varieties only, not white varieties or rootstocks.
- The foliar changes in red varieties are distinctive: inter-vein reddening, green veins, and leafroll on the margins.
- When using trained assessors, these foliar changes offer wineries a reliable, cost-effective diagnostic tool for GLD and SD.
- Symptoms typically develop from mid-vintage among leaves close to the cordon.
- By late-vintage, symptom expression affects progressively more leaves and those higher in the vine canopy.
- The optimal time to start visual monitoring is likely to be late-vintage in all grape-growing regions.

White varieties

Among white varieties and rootstocks infected with GLD or SD, there are either no obvious visual changes to the foliage or, if changes are observed, they may not provide conclusive evidence of infection.

White varieties, rootstocks and a number of red varieties are not sensitive to GVA and do not display symptoms of SD when infected. However, they can still act as a reservoir of virus which can spread to neighbouring vineyards in the presence of insect vectors. In addition, sensitive varieties grafted onto tolerant GVA-affected varieties will develop SD. In New Zealand, the appearance of curling on leaf margins in a variety like Chardonnay is periodically cited as an indication of GLD infection. Although these foliar changes are real, they are likely to have developed over a period of years – too long a timeframe to enable early identification and prompt removal of infected vines. Instead, by remaining *in situ*, the vines are a source of virus inoculum, from which feeding vectors can acquire and then transmit GLD or SD to healthy vines.

An inability to visually detect GLD and SD in white varieties and rootstocks means they continue to pose a risk to other grapevine plantings nearby. Under these circumstances, vector monitoring and management by insecticides may become the default virus management response (discussed later).

The only reliable way to determine the virus status in white varieties and rootstocks is to collect leaves or budwood so they can be tested in an approved laboratory (described later).

Red varieties

In red varieties, vines infected with GLD undergo distinctive foliar changes post-véraison (Figures 4, 5, and 6). Changes include progressive reddening of leaf tissue between the primary veins. The primary veins remain green. There may also be some curling of the leaf margins, hence reference to the term ‘leafroll’.

Where SD infection is limited to GVA only (not co-infected with GLD), the entire leaf turns red, including the primary veins (Figure 7). Evidence suggests foliar changes occur early in the vintage and

[3] Using a modified Eichhorn-Lorenz grapevine phenology model, we propose start dates to undertake different elements of the integrated response to GLD and SD (see Appendix 2).

they can persist well into winter. Eventually, the leaf blade drops, but the petiole remains connected to the cane. (Note: when an SD-infected vine is co-infected with GLD, the foliar changes align more closely to those described for GLD.)

New Zealand's experience of GLD has shown that trained staff can diagnose foliar symptoms in Cabernet Sauvignon, Cabernet Franc, Malbec, Merlot, and Pinot Noir to a high degree of reliability. They can also distinguish between vines that are virus infected and those with benign conditions like mineral deficiency (discussed below). The ease and reliability of detection improves markedly when visual inspections are undertaken late-vintage.

GLRaV-1, -2, -4, and -7 can all cause GLD foliar symptoms which are likely to be readily identifiable, but any subtle differences between these and GLRaV-3 may require further evaluation in the vineyard and/or laboratory testing for confirmation.

In New Zealand, Syrah (Shiraz in Australia) is sometimes noted as being a difficult variety in which to diagnose GLD visually. In Hawke's Bay, where Syrah is widely grown, it is quite common to see multiple vines showing some degree of foliar reddening, and assessors have reported difficulty distinguishing between vines with GLD and those that are otherwise healthy but have some red leaves. Whether factors like the clone, virus variant, or season are influencing the foliar changes is unclear, but assessors have found visual detection to be more reliable when inspections are scheduled post-harvest, when GLD symptoms in Syrah are generally well advanced.

Whether this experience will be repeated in Australia with Shiraz is unclear. Other red varieties commonly grown in Australia (but not New Zealand) may require similar caution when visual inspections are undertaken.

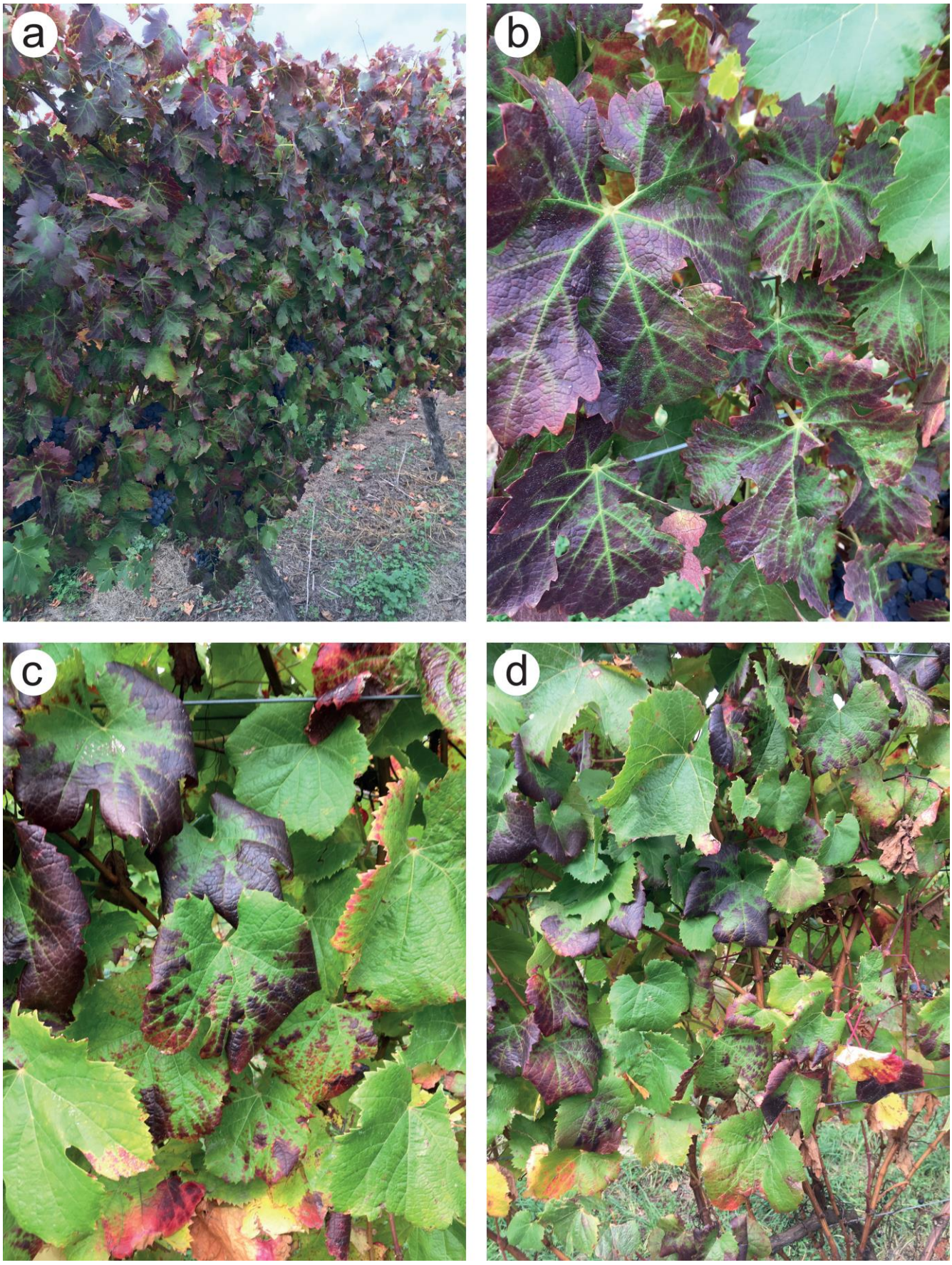


Figure 4. Foliar symptoms of Grapevine leafroll-associated virus 3 in Cabernet Sauvignon (a, b) and Malbec (c, d) vines.

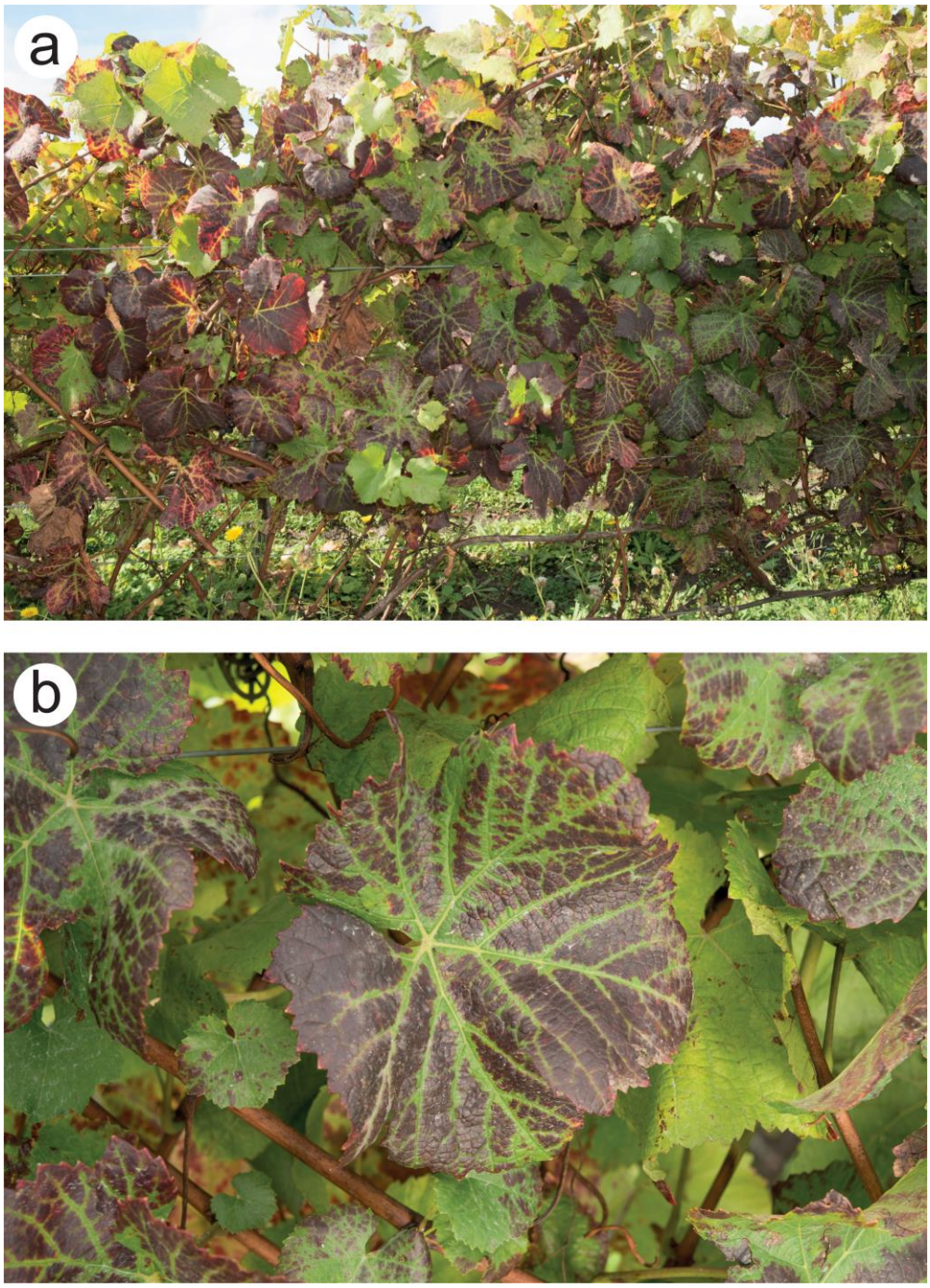


Figure 5. The foliar symptoms of Grapevine leafroll-associated virus 3 in a Pinot Noir vine. The extent of foliar changes are seen throughout the vine canopy late in the vintage (a) and are characterised by inter-vein reddening and green veins (b).



Figure 6. The foliar symptoms of Grapevine leafroll-associated virus 3 in a Merlot vine.

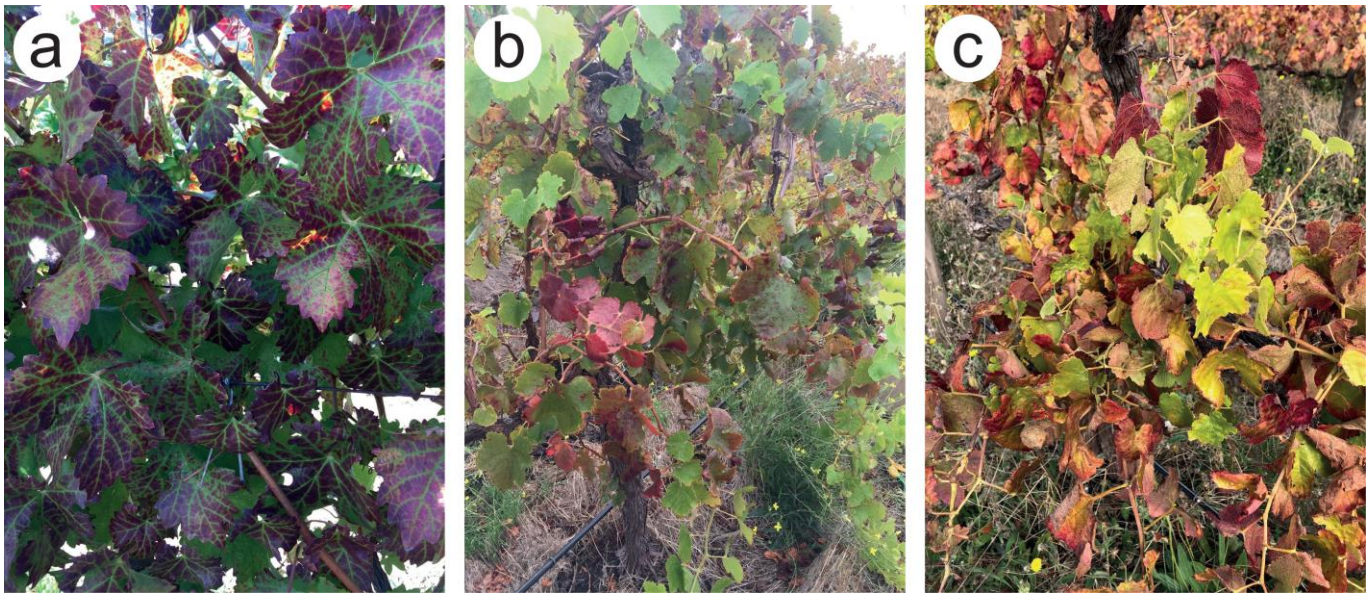


Figure 7. Foliar symptoms of Grapevine leafroll-associated virus 1 (a) and Shiraz disease (b, c).

Visual assessor training

Visual symptom identification (VSI) is the process of looking for the foliar symptoms of GLD and SD in the vineyard. In red varieties, the classic foliar symptoms described earlier mean infected vines can be visually detected with a high degree of reliability. Given that VSI serves as the foundation for most vine removal decisions, training is a necessity.

Experienced VSI assessors prefer to use the vineyard as their ‘classroom’ for training people to recognise the foliar symptoms (and variability) of GLD and SD.

Trainees are also encouraged to calibrate the results of their visual assessments with those of laboratory testing. In this regard, the cost-effectiveness of the enzyme-linked immunosorbent assay (ELISA) (relative to molecular tests) has been widely applied in New Zealand. Typically, a trainee compares a photograph of the foliar symptoms in an individual vine with the laboratory ELISA test results, or they may choose to revisit specific vines where laboratory results do not agree with an initial visual assessment. Comparisons like these help refine the skills needed for VSI.

Symptom development in leaves

In red varieties, the interval between a vine being infected with GLD and SD and the initial expression of foliar symptoms is thought to be about 12 months. During this interval, an infected vine showing no symptoms of virus is often described as being ‘asymptomatic’. Despite the lack of visual symptoms, an asymptomatic vine is a possible source of virus inoculum for feeding mealybugs and soft scale insects, hence the importance of good vector management (described later).

Beyond the asymptomatic period, foliar symptoms begin to develop in the canopy (Figure 8).

- Symptoms develop relatively slowly, with early- or mid-vintage virus expression often first observed among leaves close to the cordon.
- At this time, leaf reddening is often rudimentary, with symptom development on individual leaves sometimes described as ‘patchy’ or ‘spotty’ (Figure 9).

- In the following weeks, reddening progressively develops to entirely fill all (or most) inter-vein areas on affected leaves.
- The primary veins of these leaves remain green.
- As curling on leaf margins may also become evident, there is an increase in the number of symptomatic leaves, with symptom expression becoming more apparent among leaves higher in the vine canopy.
- By late-vintage, large areas of the canopy are dominated by red leaves.
- Improving the outcomes of VSI will benefit from grower workshops. Asking those attending to bring with them a selection of vine leaves with symptoms of GLD and SD, as well as leaves from vines with other leaf-reddening conditions, helped raise awareness and understanding among New Zealand growers.

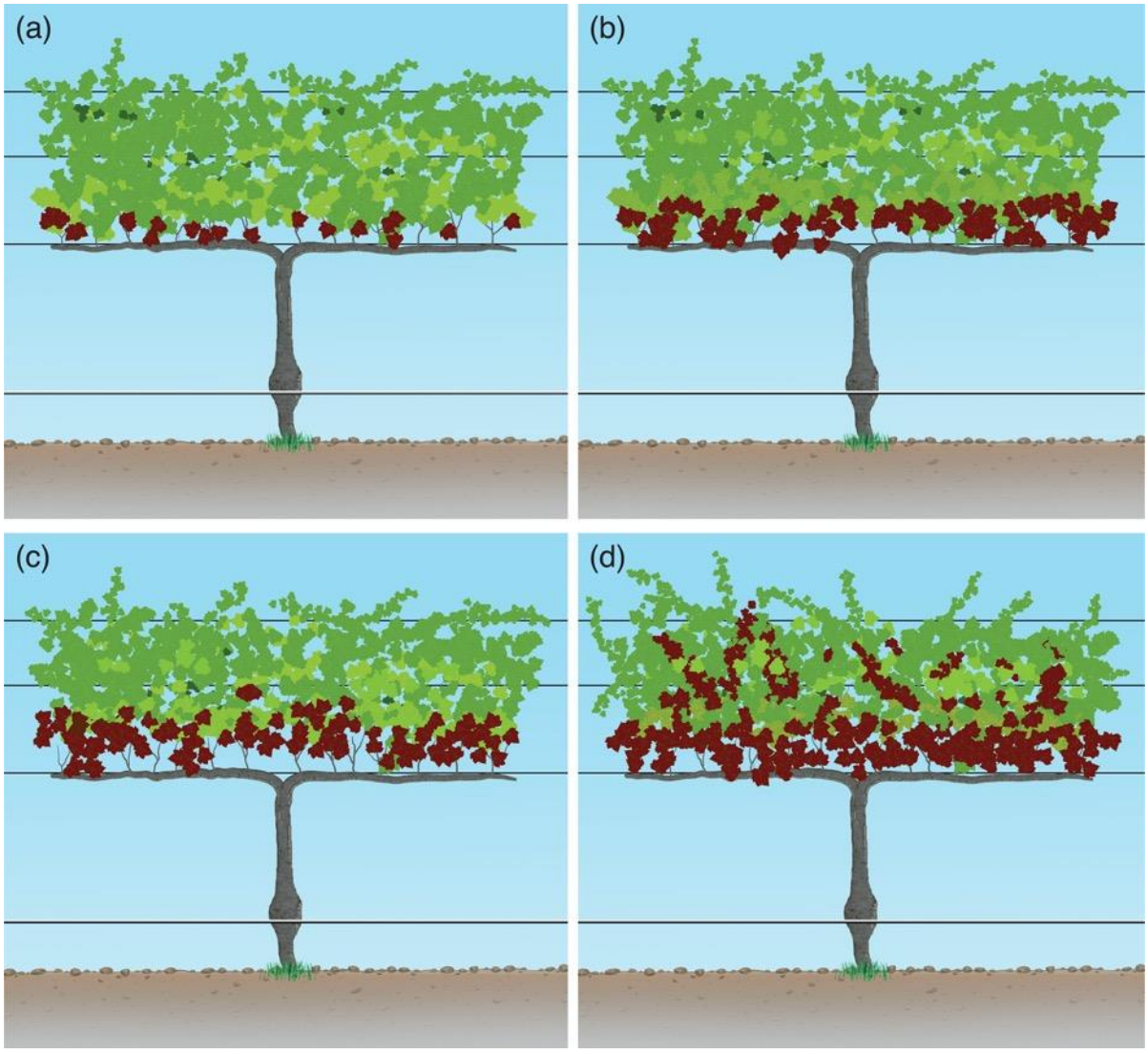


Figure 8. A stylised view of the foliar symptoms of Grapevine leafroll disease and Shiraz disease in red varieties developing over time. Mid-vintage, a small number of leaves close to the vine cordon start to turn red (a); in the following weeks, the numbers of red leaves progressively increases (b and c), and by late-vintage, red leaves are evident higher in the vine canopy (d).

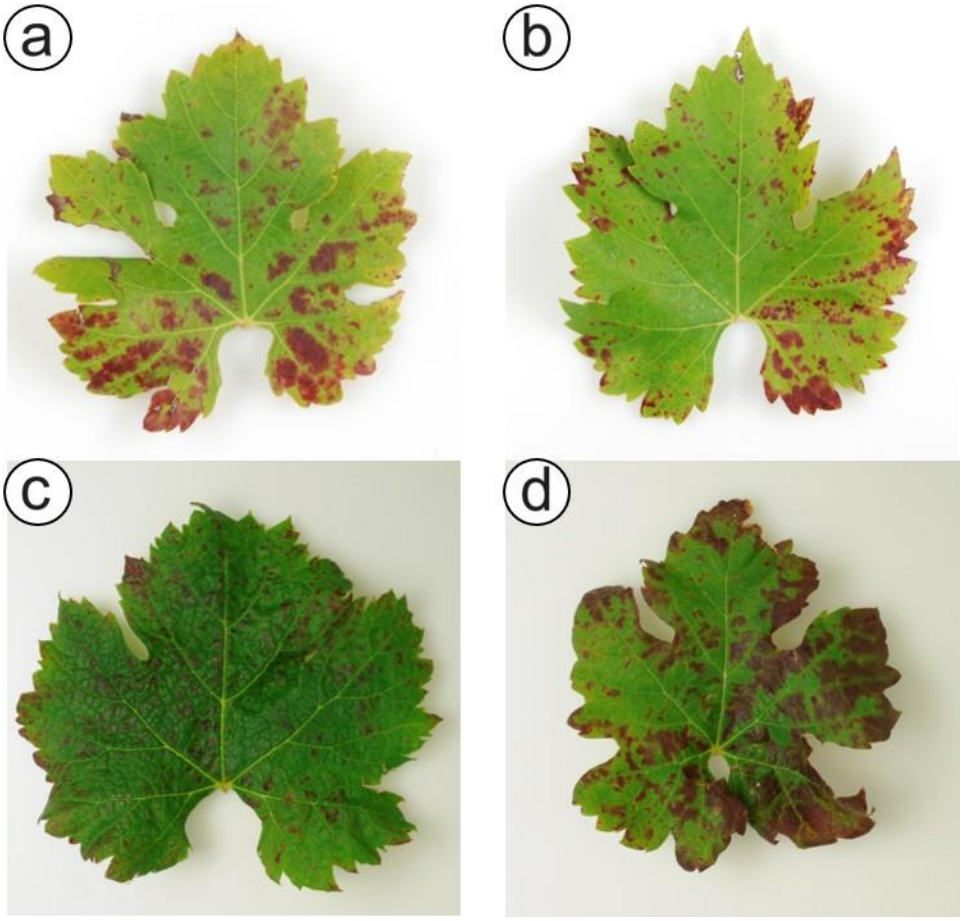


Figure 9. Grapevine leaves showing rudimentary foliar symptoms of Grapevine leafroll-associated virus 3 in Cabernet Sauvignon (a, b) and Merlot (c, d) vines. Early- to mid-vintage symptom development is often characterised by ‘spotty’ or ‘patchy’ reddening on individual vine leaves.

The timing of symptom expression in leaves

Multiple factors influence symptom expression.

- The grapevine variety, clone, virus variant (either as single or dual infections), and the viral load, among other factors, will influence the timing and severity of symptom expression in the foliage.
- For vines infected for multiple years, the high viral load means symptom expression in the foliage is likely to occur well before mid-vintage.
- By contrast, symptom expression is likely to occur much later in the vintage for relatively newly infected vines (say, less than two years).
- The timing of symptom expression in cooler, coastal regions could vary from inland areas. Temperature and other climatic variables may influence the timing and severity of foliar symptom expression, potentially by several weeks.
- The extent to which these and other variables influence symptom expression in different grape-growing regions has been identified as a subject requiring more research.

Frequency and timing of visual inspections

In New Zealand, VSI is recommended for every block planted in a red variety on at least one occasion per vintage. If possible, two visits per block are advised, owing to the variability in the timing of symptom expression among different vines (possibly because of the duration of the infection).

Assessments start late-vintage (mid-March to mid-April in New Zealand), although a post-harvest timeline may be preferred once the pressure of vintage is over. This approach is also used in South Africa.

Post-harvest inspections should consider possible foliar damage to vines due to mechanical harvesters and senescence that can lead to leaf reddening. In combination, both factors may further complicate virus identification.

The same recommendations hold for Australian grape-growers, although the timing of symptom expression may differ from vintage to vintage and between wine regions varying in climate.

Record keeping

For a VSI task to be effective (as with roguing and vector monitoring – discussed below), good record keeping is crucial because you cannot manage effectively what is not measured. Moreover, good records are a repository of historical information linked to operational tactics (e.g. what vines were removed from where and when) and an operational strategy (e.g. review effectiveness and if necessary, modifying future responses to virus and/or vector).

A VSI assessor will follow a previously agreed plan to mark or tag individual virus-infected vines so they can be readily identified after leaf fall. This means of identification is used to inform future vine removal decisions.

- A common method of identification is to tie flagging tape of an agreed colour around the trunk midpoint of each symptomatic vine.
- The easiest method is to manually record every virus-infected vine seen, either on paper or on a portable electronic device. The assessor will record the number of vines visually diagnosed on a per-row basis.
- The left-hand side of an A4-sized document lists in ascending order the row numbers per block.
- An assessor walking (or riding) along the inter-row, notes the appearance of foliar symptoms of GLD or SD and records it against the row number on which the infection was observed.
- Before moving on from this vine, the assessor ties flagging tape of the agreed colour around the vine trunk.
- Where new symptomatic vines are observed, the process is repeated along the entire length of both rows and in the remaining rows of the block.
- The goal is to record and count the number of virus-infected vines visually identified per row across every block that was visited.
- The data sheet will subsequently become the reference directing the vineyard team to particular rows to remove virus-infected (flagged) vines during winter.

The use of GPS technology is an alternative method to electronically record the numbers and position of vines visually diagnosed with GLD or SD.

- Every GPS tag will have a margin of error. If the margin of error in the unit is just a few centimetres, it may not be necessary to tie flagging tape around the trunk of each visually diagnosed virus-infected vine.
- Instead, once the data point is entered, personnel can return later in the year with the GPS unit to precisely locate an individual vine that is to be removed.
- For other GPS units, however, accuracy may be much less precise (potentially with a margin of error several metres in any direction). In this situation, use flagging tape to identify virus-infected vines.

A map of each vineyard block drawn in Microsoft® Excel® (or similar software) is another method to record virus incidence every year (Figure 10).

- Like GPS mapping, a paper or electronic 'map' of a vineyard block is used to record the precise location of every infected vine.
- One cell on the spreadsheet represents a single vine (denoted by an 'x'). In the example map (Figure 10), there are four vines per panel.
- By marking an individual virus-infected vine, an assessor can precisely locate its position in a panel and on a vine row. Presenting information in this way offers a powerful visual representation of GLD and SD.
- Over time, the power of block-specific maps greatly increases, enabling a winery team to visualise the influence of virus in different blocks and even different areas within a block.
- In Figure 10, a single virus-infected vine was initially visually identified in row 3. Over the next two years, additional virus-infected vines were visually detected, all of which were closely associated with the initial infection. This information highlights the influence of the virus and the vector-mediated spread probably responsible for what could be described as a virus 'hot-spot'.
- Record-keeping helps to determine the success (or otherwise) of a virus management response. If the outcomes of annual vine removal are less than expected or predicted, this information should be the catalyst to review the virus and vector management response targeting particular areas of the vineyard.
- Mapping the virus incidence in a vineyard may also inform the mode of transmission, particularly in young vines. Patterns may vary depending whether the virus was present in planting material or is vector-mediated, but assessment will be required on a case-by-case basis.

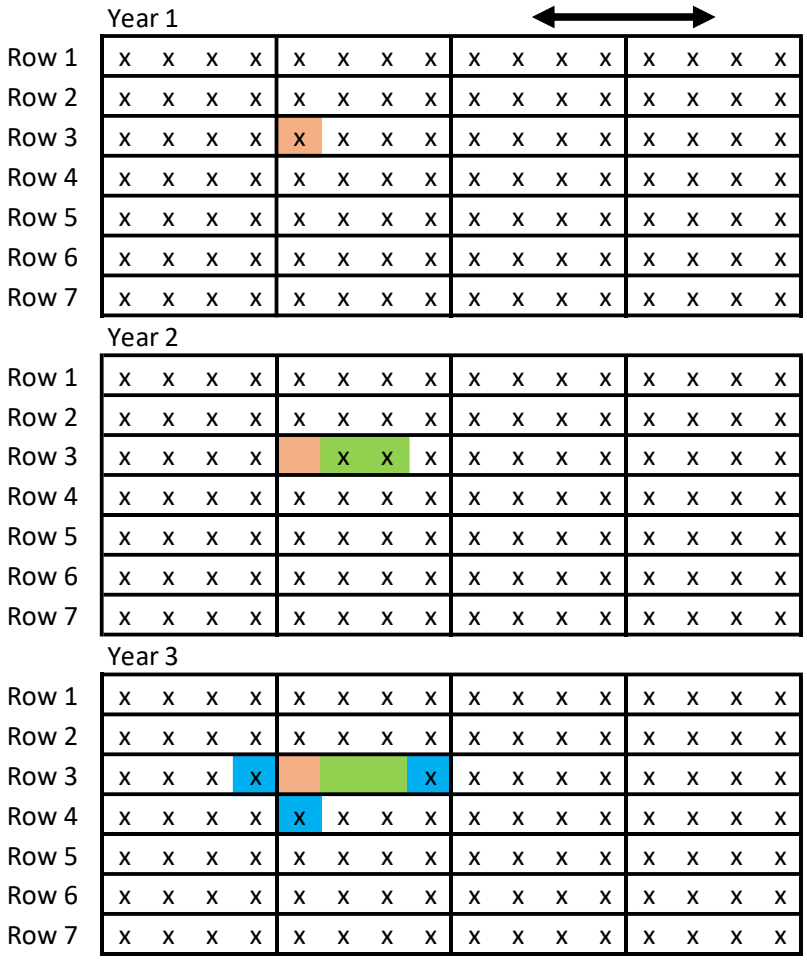


Figure 10. Part of a vineyard depicted on a Microsoft® Excel® spreadsheet over the course of three years. In each of seven rows there were four panels and four vines per panel. Those Grapevine leafroll disease or Shiraz disease infections that were visually identified were colour-coded by year. In year 1, a single vine was visually diagnosed as virus-infected. Its position was recorded on the Excel 'map' (row 3). Its removal during winter of year 1 was denoted by the loss of the 'x'. In year 2, two further vines were visually diagnosed as virus-infected in row 3. Both were removed in winter. By year 3, another three vines were visually diagnosed as virus-infected and the positions were recorded on the map. Note: dispersing vectors often spread virus to neighbouring within-row vines. The black arrow denotes row direction.

Distinguishing GLD and SD from other leaf-reddening conditions

In addition to the foliar symptoms caused by GLD and SD, grapevine leaves turn red for other reasons that assessors should bear in mind. A vine may be deficient in a mineral like magnesium (Figure 11a); it may have one or more canes damaged by the wind or partially girdled by a trellis wire (Figure 11b, c); or the entire canopy may simply turn red for reasons that are not always immediately understood (Figure 11d).

Failure to adequately distinguish between these relatively benign symptoms and those caused by GLD and SD increases the risk of virus-infected vines being retained while otherwise healthy vines are removed.

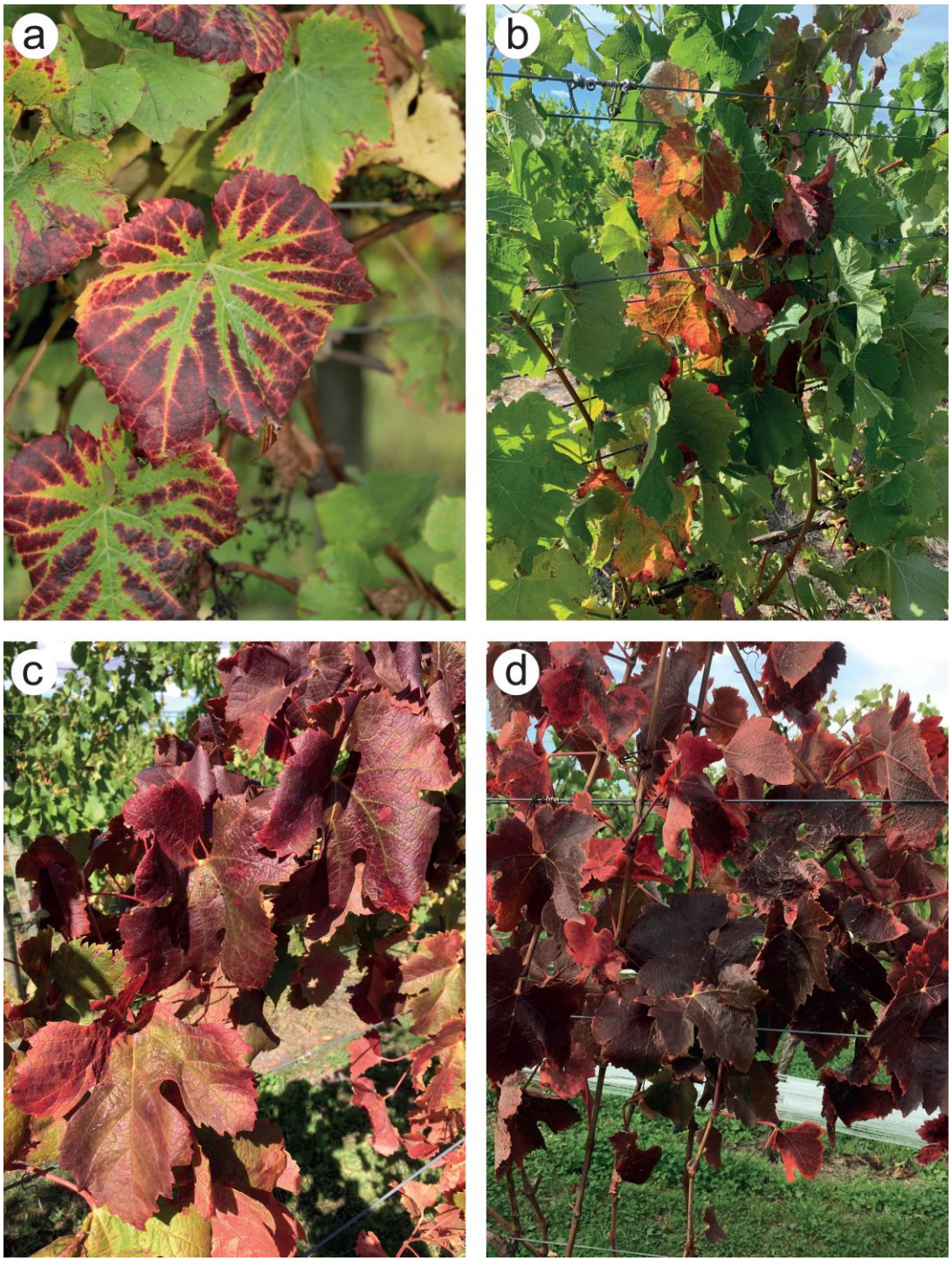


Figure 11. Grapevines can undergo foliar reddening during vintage. Inadequate training can reduce the ability of an assessor to distinguish between vines infected with Grapevine leafroll disease (GLD) and Shiraz disease (SD), and those with magnesium deficiency (a), cane girdling (b, c), and symptoms of unknown status (d).

Roguing

Key Points

- Roguing is the process of physically removing vines infected with GLD and SD.
- Undertaken annually, the timing of roguing is ideally suited for winter months.
- Rogue only those vines expressing foliar symptoms and/or that have tested positive to GLD or SD.
- Vine extraction targets all above ground material, including the trunk, cordons, and canes. Remove and dispose of.
- Remnant roots are a virus reservoir and support vectors. Where practicable, remove them to a depth of about 25 cm.
- Planting replacement vines that have been virus tested can begin as soon as the following spring.

What is roguing and why is it important?

The practice of removing vines infected with one or more viruses is commonly referred to as ‘roguing’. The goal of roguing is to reduce the risk of insect vectors feeding on an infected vine, acquiring the virus, and, after moving to a nearby vine, transmitting virus when they resume feeding. This transmission pathway applies to GLD and SD.

As previously noted, roguing is feasible in red varieties because of the distinctive changes to the colour and form of foliage in infected vines. In New Zealand and South Africa, foliar changes have been shown to be a reliable indicator of GLRaV-3 infection. It is expected that VSI can also be applied to GLRaV-1 and SD, although this may require evaluation.

In commercial vineyards in New Zealand and South Africa, roguing has been used to reduce and sustain GLRaV-3 incidence to less than 1% per annum. Reliable VSI plus effective vector monitoring and, if needed, vector management, are critical to successful outcomes. Underpinning this entire process is the health of the planting material supplied to growers by commercial nurseries.

Compromising any part of this integrated response limits its potential to prevent (or even reasonably contain) vector-mediated spread of GLD and SD to nearby healthy vines.

Roguing virus-infected vines

- Rogue only those vines with foliar symptoms of GLD or SD or where laboratory testing confirms individual vines have tested positive for one or more of the targeted viruses. Roguing is often undertaken during winter months.
- At present, there is no evidence that virus spread is contained more effectively when neighbouring vines with no foliar symptoms are rogued at the same time as one with symptoms.
- Rogue infected vines in the same year they are visually diagnosed, using VSI records to locate each infected vine in the vineyard.
- Where circumstances permit, remove virus-infected vines using a spade and grubber, but note that the ease of roguing is influenced by vine age, rootstock, and soil type. Removing vines by

hand in heavier or stony soils is difficult. Under these circumstances, roguing probably requires that machinery be used, particularly where more vines are being removed.

- Effective roguing comprises the removal of an entire vine, including the roots, trunk, cordon, and canes.
- All rogued vines should be moved to bare ground separate to the areas planted in grapevines. When and where it is feasible to do so, the vines are disposed of by mulching, composting or fire. Converting grapevine wood to biochar may be another consideration. The important goal is to remove from the vineyard virus-infected material and any resident vectors.
- Some New Zealand and South African growers have left ground fallow after roguing individual vines or removing entire blocks. The duration of a fallow period is less important than the attempt to remove remnant roots during this timeframe. However, this is a topic requiring further research.

Remnant grapevine roots

Research in New Zealand showed that remnant roots left after removal of a GLRaV-3-infected vine remained a virus reservoir for a period of at least 12 months. While the tiny, fibrous roots in the soil break down quite rapidly, roots with a diameter of 1 cm or more may persist underground for years, depending on the conditions for decomposition.

An association between larger remnant roots and subterranean mealybugs could represent another virus transmission pathway, one that could jeopardise a future vine replant program.

- Despite the absence of published data confirming a link between remnant roots, subterranean mealybugs, and virus spread to newly planted vines, this scenario remains a plausible virus transmission pathway. Consequently, when roguing, growers should remove as much of a vine's root system as possible.
- Vine age, rootstock and soil type all influence the success of whole-vine extraction.
- Removing every root fragment from every rogued vine is impossible, but aim to remove larger roots to a depth of about 25 cm. This is the area of the root zone where subterranean mealybug populations are most likely to be found.

Whole-block removal

The decision as to when a winery should redevelop an entire area affected by GLD or SD is complex. Tolerance for disease pressure and the financial wherewithal to redevelop part or all of a vineyard will be among a range of factors influencing such decisions. In addition, vine age will also be an important consideration when determining vineyard redevelopment. The idea of removing very old vines (say, 80 to 100 years) is likely to be contentious and far from straightforward. It requires careful consideration of multiple factors, including the costs and benefits of retaining virus-infected vines and the feasibility of collecting budwood for propagation.

For the New Zealand wine sector, it is generally accepted that 20% GLRaV-3 incidence is the threshold beyond which roguing is unlikely to be a viable response, either logistically or economically. Beyond 20%, decisions should instead turn to redeveloping affected areas.

Planning for redevelopment should consider the additional advantages of removing irrigation lines and vine trellising to enable heavy machinery to remove all vines above and below ground without being impeded by the presence of infrastructure.

Removing all the infrastructure also offers an opportunity to review vine orientation and the planting density of replacement vines.

Replanting

After roguing an infected vine (or redeveloping an entire block), growers may delay planting replacement vines. There is no need to maintain a fallow period as long as remnant roots have been removed (see above).

Their decision to do so may be based upon the availability of suitable replacement high-health vines of the variety (or clone) required, or a determination to first ascertain if the roguing response is containing the spread of GLD and/or SD successfully before committing to a replant program. The decision to begin a replant program might follow assessments in subsequent years confirming no (or very few) new infections.

Alternatively, the decision may be made to quickly plant replacement vines in the following spring, an interval of three to four months.

In New Zealand, some vineyard managers have noted difficulties establishing young vines amid adjacent mature vines. Although replanting can be influenced by soil type, other measures can help promote vine establishment. These include:

- At the time of planting, add vine guards so that any herbicide targeting the groundcover does not affect the young vine.
- Maintain good soil moisture during vine establishment. To achieve this, an extra dripper may need to be added to the irrigation line.
- The periodic addition of fertiliser or compost is also helpful.
- As the vine grows, it will need to be trained onto the trellis system.
- Some ongoing remedial weeding may be necessary to maximise soil moisture and nutrients for the young vine.
- Manage insect pest and disease pressure on replacement vines through the usual vineyard spray program.
- Young vines may require additional protection where vertebrate pests are present (e.g. rabbits).
- For blocks planted in red varieties, assessors should continue to visually monitor for GLD or SD so that any replacement vines developing foliar symptoms can be promptly identified and if necessary, rogued.
- Note: an aspect of replanting requiring further evaluation is the age of the replacement vines. Relative to small bare-rooted one-year-old vines, planting potted, two-year-old vines that are already at cordon height could be advantageous to initial vine establishment.

Top-grafting (red and white varieties)

Top-grafting allows varieties to be changed relatively quickly and efficiently. Successful top-grafting can be cheaper than replacing vines, and the timeframe for the resumption of crop production may be as short as one year, given that the existing root structure remains intact.

However, for top grafting to be contemplated, there must first be a comprehensive assessment and a good understanding of the risks from GLD and SD. Accordingly, assessments of virus incidence must encompass the area to be reworked as well as the area from which scion wood is sourced.

- If either area is planted in red varieties, VSI can screen for GLD and SD (assuming suitable assessor training). Whether or not top-grafting is feasible depends on the results of that virus screening.
- If a virus is not visually detected in both the block to be reworked and the scion source block, top-grafting can proceed with some confidence.
- Where GLD or SD is visually detected in either area, even at a relatively low incidence (such as 5%), the decision to act should be considered carefully and proceed only on the basis that VSI and roguing symptomatic vines are undertaken annually in the reworked block. This must be supported by ongoing vector monitoring and, where necessary, with vector management.

The position is more complex for white varieties and rootstocks because of the lack of visual virus symptoms.

- Under these circumstances, the feasibility of top-grafting relies solely on vine samples being collected and sent to a suitable laboratory, where they can be tested for GLD and SD.
- For white varieties, laboratory testing for GLRaV-1, GLRav-3, and/or GVA is undertaken to better understand disease incidence in a block, to estimate risk to neighbouring blocks, and whether vine material should be used as budwood for the purpose of nursery propagation and top-grafting.
- Without the depth of understanding and risk assessment that virus testing confers, top grafting in white varieties should be approached with caution because of the risk of virus exchange between rootstock and scion.

Mealybugs and soft scale insects: the vectors of virus

Key Points

- As well as being transmitted through infected planting material, GLD and SD is spread vine to vine by mealybug and soft scale insects.
- These multiple species of sap-sucking insects are collectively referred to as 'vectors' of GLD and SD.
- Vectors feed on all grapevine cultivars, but some cultivars may be more susceptible to vectors than others.
- Vector species diversity almost certainly varies between and even within different grape-growing regions.

As well as being transmitted via infected planting material, GLD and SD are spread by mealybugs (four species) and soft scale insects (up to 10 species), all of which have been recorded in Australian vineyards at various times (Table 1).

Table 1. Species (and common names) of mealybug and scale insects that have been recorded in Australian vineyards. Most of the species listed can acquire Grapevine leafroll-associated virus 1 (GLRaV-1), GLRaV-3, and/or Shiraz disease when feeding on infected vines. Having dispersed and resumed feeding on a healthy grapevine host, they can then transmit one or more of these pathogens. (Species list of soft scale insects from Rakimov et al. 2013)

Mealybugs (Hemiptera: Pseudococcidae)	Scale insects (Hemiptera: Coccidae)
Planococcus citri (citrus mealybug)	*Coccus hesperidum* (soft brown scale)
Pseudococcus calceolariae (citrophilus mealybug)	*Co. elongatus*
P. longispinus (longtailed mealybug)	*Co. longulus* (long soft scale)
P. viburni (obscure mealybug)	*Coccus* sp.
	Parasaissetia nigra (nigra scale)
	Parthenolecanium sp.
	Part. persicae (grapevine scale)
	Part. pruinosum (frosted scale)
	Saissetia oleae
	Saissetia sp. (soft scale)

The insects spreading GLD and SD from infected to healthy vines are collectively referred to as the 'vectors', of which the longtailed mealybug and the grapevine scale are just two example species (Figure 12).

The presence of different vector species will vary within and between grape-growing regions, and possibly between states. Mixed populations are likely, meaning there could be one or more species of mealybug within a vineyard, and these could co-exist with one or more species of soft scale insects.

The mix of species may periodically change owing to the influence of vineyard practices like insecticide use.

Mealybugs and soft scale insects settle and feed on multiple plant species, including grapevines. These are referred to as ‘host’ plants.

All grapevine varieties can host the vectors, but some varieties (and possibly some clones) may be more susceptible to vectors than others.

Grapevine susceptibility to vectors is likely to vary by region, and this could be influenced by temperature, humidity, canopy density, soils, site topography, and other variables.

The severity and persistence of vector infestations are strongly influenced by factors like sprayer maintenance and the effectiveness with which contact insecticides cover and penetrate the vine canopy.

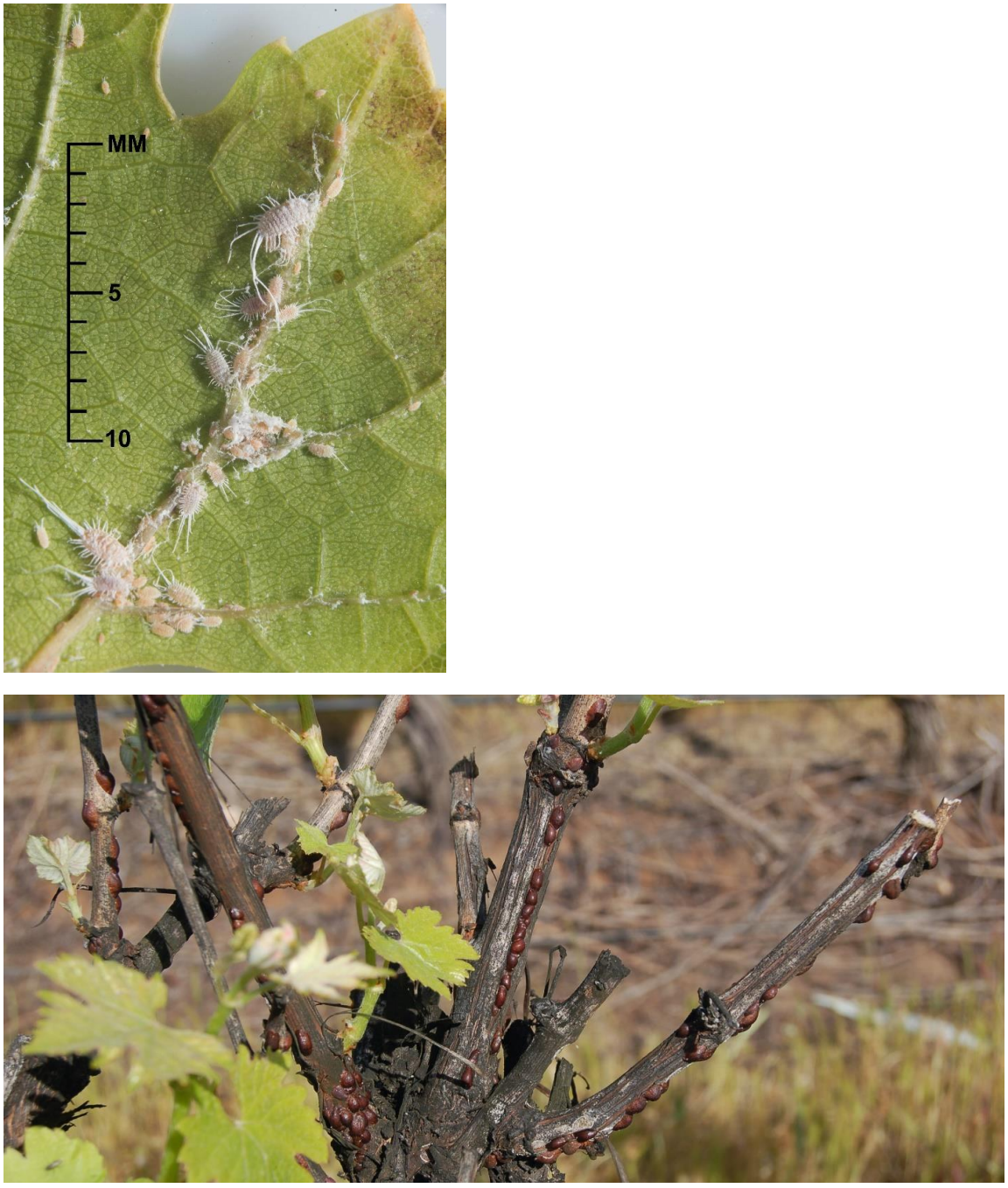


Figure 12. The longtailed mealybug, *Pseudococcus longispinus* (top); the grapevine scale, *Parthenolecanium persicae* (bottom).

Vector life cycle and biology

Key Points

- Mealybug and soft scale insects have different life histories, but they share some biological traits.
- They overwinter on grapevines, typically in the cracks and crevices on old wood, and under flaking bark.
- Their feeding generates waste honeydew, which when disposed of supports the growth of black sooty mould.
- Mature females of all vector species lay hundreds of eggs from which emerge mobile crawlers.
- A crawler can only acquire GLD and SD by feeding on an infected vine - reinforcing the value of roguing.
- Crawlers on one vine passively wind-blown onto another vine may spread GLD or SD when they resume feeding.
- The numbers of generations per species varies, but some mealybugs likely have four generations per vintage.

Different mealybug species have different life histories and biological traits. Some of this variability will be subtle, while other aspects will be markedly different. Similarly, life history and biological variability will also characterise different species of soft scale insects.

The following is a brief, generalised overview of the life cycle and biology of the vectors.

Emergence of vectors and spring activity

Vectors become active in spring, emerging from overwintering sites under grapevine bark or within cracks and crevices on old vine wood. Unlike the longtailed mealybug, the citrophilus and obscure mealybugs can colonise the roots of host plants, including grapevines.

As temperatures increase in spring and vine phenology advances, the vectors progressively move onto the developing vine canopy. They typically settle on the underside of leaves, where they are often closely associated with the primary veins. Their mouth parts are adapted to access phloem sap.

Feeding and honeydew

Older juveniles and adult female vectors are immobile or largely immobile (species dependent). These life stages settle for relatively long periods in a tightly defined location (on foliage, canes, old wood, or fruit). Where population densities are low, vectors tend to reside lower in the vine canopy, close to the cordon from which they emerged after winter.

Where population densities are high, vectors are often distributed across the entire height and depth of the canopy.

Vectors can be found on leaves for an entire vintage, but véraison will trigger movement by some individuals into the fruit clusters, where they remain feeding/reproducing for the remainder of the vintage.

Feeding vectors produce a droplet of honeydew from their posterior end. This may result in greater ant activity as they seek to ‘farm’ the vectors for the honeydew. Where ant activity increases in the vine canopy it can be useful to assessors looking for the presence of mealybugs and soft scale insects.

When honeydew is not farmed by ants and is instead flicked away by the vector, this waste by-product may land on leaves, clusters, canes, and cordons. As a nutrient-rich substrate, honeydew supports the growth of black sooty mould (Figure 13). Fruit may be rejected at harvest because of the risk of wine taint caused by black sooty mould. Outbreaks of soft scale insects and sooty mould were observed across a number of Australian wine regions in the 2017 vintage and again in 2022.

Honeydew-soaked leaves and fruit clusters often co-occur with black sooty mould, although we note that rather than an industry-wide issue, the appearance of either is possibly region-dependent. Of relevance here is that both are visually distinctive and indicate that vectors are present in the vine canopy and are abundant and potentially widely distributed across the vineyard. Such insights should be used as part of a vector monitoring program, and should inform a future vector management program that potentially involves insecticides.

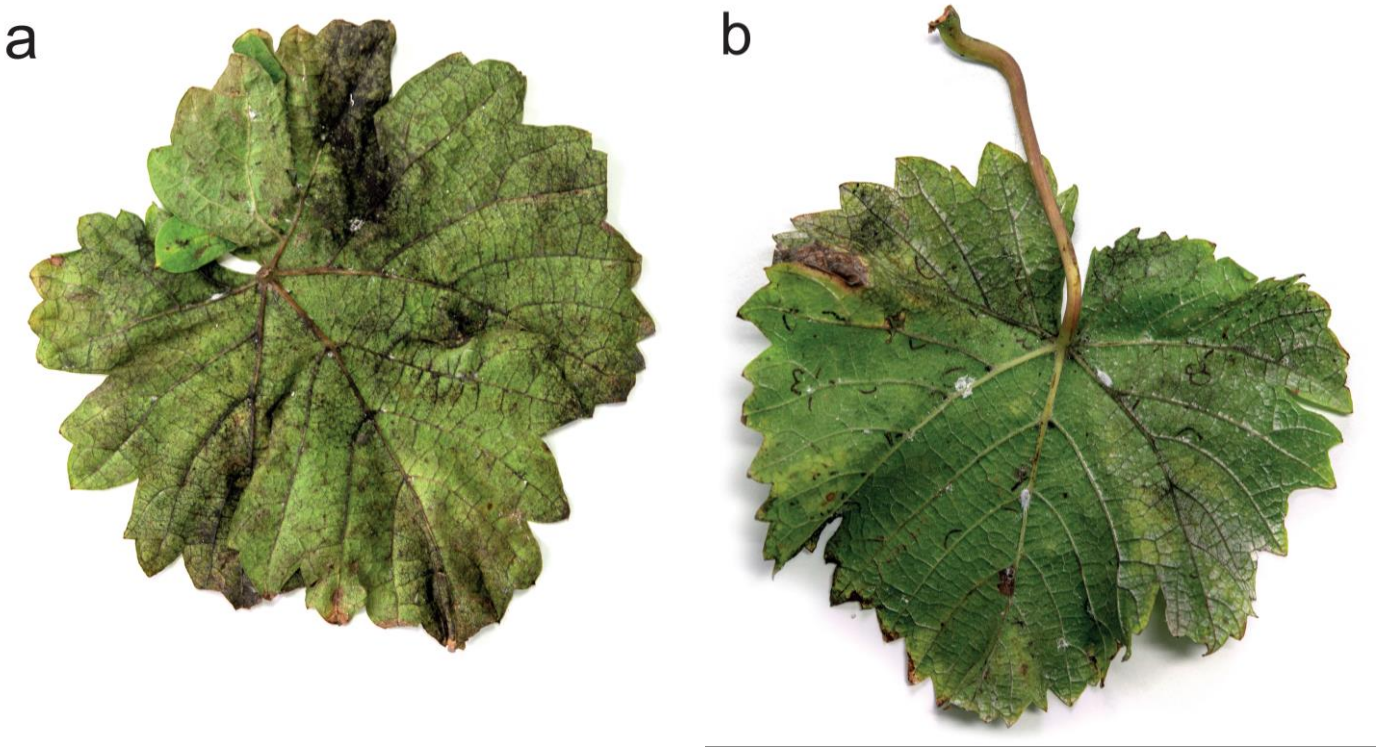


Figure 13. Grapevine leaves affected by black sooty mould. Both the upper (a) and lower (b) surface may be affected, with mealybugs accessing phloem sap from within the primary veins (b).

Egg laying

For many species of soft scale insects, males are either absent from the population (females only) or are only rarely detected (species and environment dependent). Consequently, asexual reproduction dominates for these species and is independent of males.

The numbers of eggs produced by a female varies between species: a female grapevine scale lays an estimated 1000 eggs; a female frosted scale can lay up to 450 eggs.

Among the mealybug species, populations increase by way of sexual reproduction only. After mating, a female mealybug produces around 200 eggs. With approximately 50% of the eggs produced eventually becoming sexually mature females, the population increase in the vines can be exponential, and this is particularly evident when control measures are inadequate. The descendants

of a first-generation egg-laying female in early spring can theoretically reach 200 million by the time the fourth generation begins to emerge in autumn (Table 2).

Table 2. An example of the potential for mealybug population increase in an Australian vineyard where there are up to four generations per year, and where the measures adopted to contain population growth are inadequate.

Generation	Mealybug population increase (50% of which are male)
G1	One female produces **200** eggs (less 100 males)
G2	100 females each lay 200 eggs or **20,000** eggs in total (less 10,000 males)
G3	10,000 females each lay 200 eggs or **2,000,000** eggs in total (less 1,000,000 males)
G4	1,000,000 females each lay 200 eggs or **200,000,000** eggs in total (less 100,000,000 males)

Crawlers

After emerging from the egg, the first life stage of both vector groups are known as crawlers. Their small size (about 0.4 mm in length) means crawlers are difficult to monitor by eye in the vineyard. Instead, a 10x hand lens is recommended to be used when inspecting vine leaves and clusters for these insects, particularly crawlers.

As the name suggests, crawlers are mobile. They walk with relative ease throughout the vine canopy. Although they are wingless, crawlers can be passively dispersed by the wind to areas well beyond the vine from which they emerged from the egg. Of all the life stages, mealybug crawlers are the most efficient at acquiring GLD and transmitting it to healthy grapevines. Indeed, research in New Zealand showed that a single mealybug (citrophilus and longtailed) crawler could infect a vine with GLD.

Among the soft scale insects, the crawler life stage is also likely to be the most competent at transmitting GLD and SD.

When a virus-infected vector moults, it is unlikely to continue to carry virus infection. It can only re-acquire these viruses when it resumes phloem feeding on an infected vine. Assuming virus-infected vines are numerous and widely distributed in the vineyard, a pattern of virus acquisition when feeding and virus loss when moulting is probably repeated throughout an individual's lifespan. This semi-persistent virus infection status can be permanently disrupted by roguing those vines infected with GLD and SD (described earlier).

Juveniles and adults

Mealybugs and soft scale insects typically develop through two or three juvenile life stages, depending on the species. During this time they are either actively feeding or seeking feeding sites, and they are mobile, walking easily within a grapevine and between them.

Juvenile and mature female mealybugs are segmented, soft bodied, and covered in a waxy secretion that is at least partially water repellent.

For soft scale insects, the juveniles of many species lack a waxy secretion, meaning they are likely to be more susceptible to desiccation and the influence of contact insecticides.

After several moults, both vector groups develop into mature adults. For mealybugs, a sexually mature adult male is quite different from juveniles and adult females (Figure 14). As juveniles, male mealybugs cannot be visually distinguished from the female form because they all look similar. They

all feed and in so doing, they are all able to acquire and transmit GLD and SD. However, during their final moult, they undergo a pupation. The newly emerged male has no functional mouth parts, so cannot feed and cannot acquire (or transmit) virus. Instead, the sole focus of the mature male is to locate one or more sexually mature females with which to mate, to begin the cycle again through another generation.



Figure 14. A sexually mature male citrophilus mealybug (*Pseudococcus calceolariae*). Receptors in the male's antennae detect the airborne sex pheromone of a 'calling' female of the same species. Note the prominent waxy filaments from the posterior end.

Numbers of generations

In Australia, vineyard mealybugs potentially have up to four generations per year. A single generation is often reported for soft scale insects, but this aspect of their biology remains unclear.

Vector monitoring

Key Points

- Knowing if the vectors are present on grapevines is an important step to reducing the influence of GLD and SD.
- Inspections for vectors are ideally timed for late-vintage, when they are often in the vine canopy and are easier to find.
- The objective of searching is to estimate the scope and distribution of vector populations in vines across the vineyard.
- Assessors look for vectors on vine leaves, in clusters, ants actively foraging for vector honeydew, or black sooty mould.
- Over time, good record keeping provides invaluable information on persistent vector 'hot-spots' requiring attention.

Managing GLD and SD effectively relies on knowing if the insect vectors are present and where, and what response is needed to reduce the risk of vector-mediated virus transmission to healthy red and white variety vines.

As with visual symptom identification, effective vector monitoring requires well-trained staff who know what they are looking for, where in the vines they can find them, and the optimal time to begin looking.

Time of monitoring (red and white variety vines)

In New Zealand, the optimal time to start vector monitoring is late-vintage. This timeline is likely to also apply to Australia, but may vary slightly between different grape-growing regions.

If present, vector populations generally peak late in the vintage and are therefore more likely to be seen on vine leaves and in clusters.

Some personnel may want to start vector monitoring in spring but this is not recommended. Early in a vintage, vector population densities are naturally low in many vineyards, meaning opportunities to detect them are also low. If the spring monitoring results substantially underestimate the vector risk, vineyard managers may mistakenly decide not to apply insecticides that spring.

Late-vintage inspections (red and white variety vines)

Late-vintage inspections of the vines for vectors are recommended, starting immediately before, during, or after harvest.

Late-vintage inspections aim to estimate the scope of a vector problem in the vineyard. This will include some measure of vector abundance (percentage of infested leaves or clusters). In addition, monitoring should provide insights into vector distribution throughout the vineyard to highlight areas where vector presence is deemed high (so-called 'hot-spots'), or in areas where they are not detected or rarely detected ('cold-spots').

This information forms the basis for planning a vector insecticide program for the following spring.

Visual vine inspections (red and white variety vines)

Vector monitoring should be undertaken every vintage in all vineyards.

Vector monitoring can be as simple as just looking at the vines as you walk (or ride slowly) down randomly selected vine rows. Assessors are looking for mealybugs and soft scale insects on vine leaves or in the grape clusters, or for signs of vector presence, including black sooty mould and ants actively foraging for honeydew.

The number of rows visited when monitoring will be determined by the number of people available to undertake the task, their experience, and the time available for this activity.

Other determinants of monitoring may include the recent history of vectors in the vineyard (including the possibility of block to block variability), the incidence of GLD and/or SD, and therefore the risk of their being spread by the vectors to healthy vines.

Collecting vine material (red and white variety vines)

From simple visual inspection of vines, vector monitoring can be expanded to include collecting a specific number of vine leaves from a target area (Figure 15). For example, a sampling protocol might include collecting 100 vine leaves per hectare or per block (irrespective of area). Leaves may be substituted for 50 to 100 clusters per unit of area.

- Each sample collected should be individually inspected for vectors with the aid of magnification in the office or laboratory. Mealybugs in particular can be seen close to the primary veins as they access phloem sap.
- In New Zealand, leaves are the preferred means of monitoring vectors because, unlike clusters, they are clean, easy to handle, and two-dimensional.
- A vector-counting protocol could be based on a simple presence/absence score. For example, the result of searching in a particular area of the vineyard might read, ‘at least one mealybug was observed on 10 leaves (or 10 clusters) from the 100 samples inspected.’
- The result could be further described as mealybugs were detected on 10% of leaves (or clusters) from the particular area visited.
- An alternative is to count all the vectors seen per leaf (or cluster) to include all life stages (crawlers, juveniles, adults). This is a ‘gold standard’ for vector monitoring. When applied in New Zealand by scientists, this method increased the understanding of vectors and improved the quality of the information shared with growers about monitoring and management recommendations.
- This method might note that, from 100 leaves inspected, a total of 100 mealybugs (across all life stages) were found, giving an average of one mealybug for every leaf inspected.
- Understanding the mealybug population can be further enhanced by also recording the proportion of the 100 leaves (or clusters) found with vectors.
- In the example above, the 100 mealybugs might have been found on just five of the 100 leaves inspected. Therefore, the data interpretation is expanded to note that just 5% of leaves were found with at least one mealybug. With so few leaves found with mealybugs, the result infers that mealybugs were patchily (rather than evenly) distributed among the vines in the area sampled.

- Conversely, if the 100 mealybugs found were recorded on 75 of the 100 leaves inspected, the 75% leaf infestation implies that rather than a patchy distribution, the mealybugs were more evenly distributed across much of the entire area sampled.

- Whether these data are used to decide on an insecticide response is context dependent. Relevant questions should include: What is the prevalence of GLD and SD? Is there an overlap in populations of vectors and virus-infected vines?

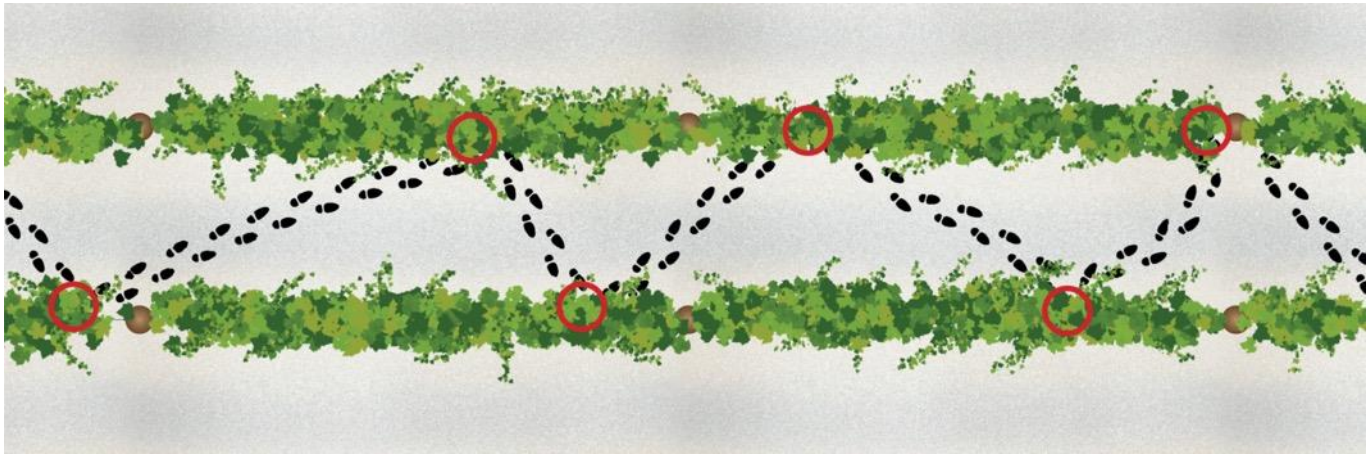


Figure 15. Aerial view of two vine rows. The red circles denote sampling points from which a single vine leaf (or a berry cluster) was collected before walking to the next vine in either row. Back in the laboratory or another facility, the vine material should be inspected using at least a 10x hand lens to assess presence and/or estimate the abundance of virus vectors: mealybugs and soft scale insects.

Interpreting the results

By repeating a monitoring protocol across multiple areas, independent of the grapevine variety, the resulting vector data set increases in value, particularly in the context of year-on-year comparisons within and between vineyards.

It might, for example, highlight areas that tend to remain free, or relatively free, of vectors. Suppose these areas overlap with areas that are also free (or relatively free) of GLD and SD. In that case, there may be a much-reduced reliance on insecticides, or they may be omitted altogether or possibly applied in alternate vintages.

Conversely, data over multiple years might highlight areas where vines continue to be susceptible to vector infestations. Their presence in areas overlapping with areas also affected by GLD and/or SD should inform future decisions about the need to start an insecticide response or to maintain a reliance on it (described later).

Indeed, the data might also highlight areas where insecticides have historically been used, but where monitoring has shown vector populations remain persistently high. Such insights should be used to trigger a review of the insecticide program to ascertain if, where, and how improvements to it can be implemented.

Record keeping

A consistent monitoring method repeated year on year with good record keeping will be a valuable resource to guide future management decisions. It enables comparisons of vector numbers on vine leaves or clusters from one year to the next.

Monitoring mealybugs with pheromones

Four mealybug species have been recorded in Australian vineyards: the longtailed, obscure, citrophilus, and citrus mealybugs. All four species reproduce sexually. Hence, an adult female ‘calls’ for an adult male by releasing a sex pheromone. Males of the same species respond to that pheromone.

In recent years, scientists have successfully developed synthetic ‘mimics’ of these naturally occurring compounds found in these four mealybug species. As a result, the availability of synthetic sex pheromones has potential to offer growers a passive, sensitive, and efficient method for monitoring mealybugs in vineyards.

However, with this technology still in its early stages of development and understanding in Australian vineyards, further research is required to determine best practice application.

Among soft scale insects, asexual reproduction dominates. For many species, this means males are absent all or most of the time. Hence, with no need to ‘call’ for a mate, there are no known synthetic sex pheromones available for soft scale insects.

Vector management

Key Points

- Overseas evidence highlights the importance of ongoing vector management in the grapevines.
- Two vector management tools are contemplated: biological control and insecticides.
- Biological control is an important natural ecosystem service whereby beneficial insects attack vector species.
- Use of insecticides should be limited to products that complement biological control rather than disrupting it.
- Cleaning equipment and machinery can prevent vineyard-wide movement of vector 'hitchhikers'.

Evidence from virus management programs in New Zealand has consistently highlighted the importance of biological control or the use of insecticides to manage vector populations in the vines.

Biological control (red and white variety vines)

Biological control is a natural ecosystem service that is an important component of pest and disease management in horticulture. Insect predators and parasitoids – collectively known as beneficial insects or natural enemies – attack mealybugs and soft scale insects (and other insect pests) in and around the vineyard environment.

Natural enemies of scale and mealybugs found in Australian vineyards include parasitic wasps, beetles, predatory moth larvae, lacewings and a predatory mite. Actions that favour a healthy natural enemy population can help to control vector populations. Such actions include providing a habitat for their food and shelter and minimising the use of pesticides known to be toxic to beneficial insects.

There is potential for some vineyard management practices to disrupt biological control. For example, the use of broad-spectrum insecticides targeting vectors is likely to reduce the number of beneficial insects in the vineyard. Under these circumstances, the ability of beneficial insects like parasitoids to attack the vectors and reduce pest pressure is likely to be diminished.

To compensate for disrupted biological control, vineyard and nursery managers may have no option but to increase the number of applications to achieve reasonable vector control, thereby increasing their reliance on insecticides and perpetuating the cycle.

Insecticides (red and white variety vines)

Scale insects and mealybugs are difficult to control, mainly due to their habit of overwintering under grapevine bark. There are also relatively few chemical options currently available. Insecticides approved by Australian regulators for use in vineyards against mealybugs and soft scale insects may vary from those approved for use by individual wineries. Such variability is not unexpected, but along with the winery view of particular products and their use, product label recommendations must always be observed.

Table 3 outlines those insecticide active constituents targeting the insect vectors affecting wine grapes. For the active constituents listed, the tabulated information was sourced from the 'Dog Book'

compiled by The Australian Wine Research Institute, 31 May 2022. Whenever and wherever possible, the insecticide program adopted should be compatible with biological control, such is its importance to vector management. Hence, omitted from this list is reference to active constituents in Activity Group 1A (carbamates) and 1B (organophosphates).

Before a new vintage begins, it is critical that all spray machinery is properly serviced and any damage is repaired.

To achieve an effective insecticide program, chemical and water rates must be correctly calculated to reflect the target. For example, adjustments to the sprayer setup are necessary in vineyards with variable vine planting densities (e.g. 2- and 3-m wide rows). Similarly, sprayer adjustments may also need to reflect different phenological stage of the vines and, therefore, differing canopy densities. Contain tractor speed to within an acceptable limit.

For contact insecticides, in particular, coverage is king, so it is critical that sprayers are calibrated to wet the entire vine and that as much product as possible remains on the vine (as opposed to running off onto the ground).

These and other insecticide best practice messages, which includes following all label recommendations around aspects that include but are not limited to withholding periods, should be the subject of future grower workshops.

In the absence of reliable changes to the foliage among white varieties, the default position for many growers will be to apply insecticides to the vines to minimise the risk of mealybugs and soft scale insects spreading virus to healthy vines.

Table 3. Insecticide active constituents targeting mealybugs and/or soft scale insects that are currently registered for use in Australia as recorded in *Agrochemicals registered for use in Australian viticulture* (The Dog Book), Australian Wine Research Institute (updated 31 May 2022).

Active constituents (product names)	Activity Group	Target insect group(s)	Brief description of Mode of action	Recommended application †
Acetamiprid + Pyriproxyfen (Trivor®).	4A + 7C	Mealybugs and scale insects	Contact insecticide. Nerve action, growth regulator. Targets immature life stages.	Foliar applied in spring. Apply no more than once per growing season and no later than E-L 19 (c. 16 leaves separated; flowering begins).
Buprofezin (Applaud®, Buprofezin 440, Scale & Bug, Uptown®).	16	Mealybugs	Contact insecticide. Insect growth regulator. Interferes with the moulting of immature life stages.	Foliar applications in spring. Two applications separated by 14 days, with the first being 2 weeks prior to 80% flowering. The second application is to be no later than E-L 25 (80% cap fall).
Paraffinic oil (Bioclear®, BioPest™, D-C-Maxx® nC24, Heavy Paraffinic Dormancy Spray Oil, isoCLEAR® HPO).	n/a	Mealybugs and scale insects		Dormancy spray.
Petroleum oil (All Seasons White Oil, D-C-Tron® Plus Spray Oil, Sacoa Summer Spray Oil™, Stifle™).	n/a	Scale insects		Dormancy spray.
Spirotetramat (Movento® 240 SC; Viento 240 SC).	23	Mealybugs and scale insects (suppression only for the latter group).	Systemic. A lipid biosynthesis inhibitor. Reduces fecundity and fertility, primarily against immature life stages.	Foliar application in spring. Use no later than E-L 18 (14 leaves separated). For mealybugs, consult product label as registration may apply to specific species.

† Manufacturer's label recommendations in respect of timing of application, product rates, and other factors, including health and safety guidelines, must be adopted at all times.

Vineyard hygiene (red and white variety vines)

Vectors ‘hitchhiking’ on machinery and people may be carrying virus infection.

Thoroughly cleaning vineyard equipment and machinery is an important step to minimising the potential movement of vectors within and between vineyards. Potential sources of vector contamination include tractors, mechanical harvesters, leaf trimmers, pre-pruning machines, and cultivators.

To achieve the hygiene standards required, implementing one or more of the following actions is proposed:

- Develop a vineyard-wide plan to be adopted annually that takes account of recent virus incidence and vector abundance assessments.
- Clearly communicate the expectations of the plan to all relevant vineyard personnel.
- The plan should also include communicating hygiene expectations to external contractors undertaking any work, whether they are using their own machinery or gear belonging to the vineyard.
- After exiting one block, all machinery should be cleaned of all vine material before entering another block. This can be achieved by hand initially, but ideally the task will be completed through the use of high pressure water.
- In cleaning all visible parts of all equipment, ensure hard-to-reach places like crevices and shelves on the equipment are also targeted. Shredded leaves are capable of supporting live vectors.
- The use of soft detergent or steam cleaning (above 100ºC) will aid sanitation efforts.
- In blocks where virus-infection is known to co-occur with vectors, extra care should be taken to clean all machinery thoroughly before exiting the site and entering another block (or another vineyard).
- Where and when it is feasible to do so, plan to access blocks of young vines and those where virus incidence is low or non-existent before accessing older blocks where vector populations or virus incidence might be higher.
- In blocks (or vineyards) where there are no data related to virus incidence and/or vector abundance, assume a worst-case scenario, incorporate all reasonable precautions linked to machine hygiene, and start work in young, ‘clean’ blocks before entering older blocks (or vineyards).
- Avoid disposal or composting of grape marc near established vineyard blocks because of its potential to support vectors.

Laboratory testing for viruses

Key Points

- Laboratory testing supports:
  - Training for visual symptom identification in red varieties.
  - Estimating disease incidence within a vineyard.
- Disease incidence estimates can indicate whether a block is of high risk to neighbouring blocks and guide vector management decisions.
- Mature leaves are collected in late summer or autumn.
- Mature canes are collected in autumn through to late winter.

Diagnostic laboratories

There are three diagnostic laboratories in Australia that are accredited to test for GLRaV-1, GLRaV-3, and GVA as well as a suite of other grapevine viruses including GLRaV-2, GLRaV-4/9, Grapevine virus B (GVB), Grapevine rupestris stem pitting-associated virus (GRSPaV), Grapevine fleck virus (GFkV) and Grapevine Pinot gris virus (GPGV).

- **Australian Wine Research Institute (AWRI)**

  Phone: +61 (08) 8313 6600

  Affinity Labs
  Level 2 Reception
  The Australian Wine Research Institute
  Cnr. Hartley Grove and Paratoo Road
  Urrbrae SA 5064

- **Crop Health Services**

  Phone: +61 (03) 9032 7515

  AgriBio Specimen Reception
  Main Loading Dock
  5 Ring Road
  La Trobe University
  Bundoora VIC 3083

- **DPIRD Diagnostic Laboratory Services**

  Phone: +61 (08) 9368 3351

  Specimen Reception C Block
  Department of Primary Industries and Regional Development
  3 Baron-Hay Court
  South Perth WA 6151

Why test for grapevine viruses?

Red varieties

For red varieties, laboratory testing for GLRaV-1, GLRaV-3, and/or GVA is often performed on individual grapevines. It may be undertaken to support training for visual symptom identification (VSI), roguing decisions, and to screen source plant material for propagation.

In the early stages of VSI training, a subset of grapevines within different blocks should be selected for testing, particularly for blocks planted with different scion/rootstock combinations. (For the purposes of this document, a block is defined as an area planted in the same grapevine variety and clone, sourced from the same nursery, and planted at the same time.)

Testing should target grapevines presenting foliar symptoms as well as those with no apparent symptoms, along with vines where the virus status based on visual symptoms is uncertain.

Training helps assessors to become more confident with their visual assessments for GLD and SD, and importantly, to become confident with identifying non-symptomatic grapevines, particularly grapevines with mineral deficiencies that are often confused as diseased. As assessors become more experienced, the need for laboratory tests on individual grapevines is likely to reduce or possibly stop altogether. However, if visual assessments cannot confirm a grapevine's infection status conclusively, laboratory testing should continue.

In circumstances where a high proportion of grapevines in a block display inconclusive foliar symptoms, plant material can be collected for 'composite testing', similar to the white varieties testing strategy (described in the following section). Composite testing can reduce the overall number of laboratory tests required and can provide useful insights into disease incidence.

Be mindful that GLRaV-2, -4, and -7 can also cause GLD foliar symptoms. However, these viruses are out of scope for this document, and their influence on grapevines and the specific management decisions associated with each virus requires further evaluation.

White varieties

For white varieties, laboratory testing for GLRaV-1, GLRaV-3, and/or GVA is undertaken to better understand disease incidence in a block, to estimate risk to neighbouring blocks, roguing decisions, and to screen source plant material for propagation. It may also guide decisions linked to vector tolerance and management responses.

In situations where a grower considers VSI is feasible in specific white varieties, laboratory tests must be used to confirm the virus status. Testing must include a sub-set of grapevines presenting foliar symptoms believed to be those of GLD or SD as well as those vines without any apparent symptoms of virus.

In New Zealand vineyards, incidence of GLD caused by GLRaV-3 in a block can be determined statistically by collecting and testing samples from 200 grapevines evenly distributed through a block. These are generally tested as 40 composite samples, whereby one composite sample includes leaf or cane material from five separate grapevines.

The scope and intensity of the sampling needed to provide robust, reliable information under Australian conditions may require further research, but it is generally accepted that sampling in a regular and even distribution within a block allows for:

- Better coverage of the entire block, ensuring results of disease incidence are representative
- Greater ease for tracing positive tests results to an individual grapevine
- Systematically generate maps to record disease presence, potential hotspots, and changes occurring over time.

Questions for the laboratory (red and white variety vines)

Prior to collecting vine samples for testing, contact the diagnostic laboratory for price lists and instructions. A laboratory should be able to answer the following questions:

- What are the biosecurity requirements for transporting plant material inter-state?
- What grapevine viruses can you test for?
- Do you perform composite sample testing?
- Can you follow up with single sample testing within composite samples that test positive?
- Do you re-test samples that generate inconclusive results?
- What is the cost difference for testing leaf samples compared with mature cane samples?
- What is the expected turnaround time for results?
- How long do you hold the vine material in the laboratory?
- Is there a preferred method of labelling samples to help track and trace?
- Do you have a preferred packaging and courier method (including courier day) for vine material?

When and where to collect grapevine samples (red and white variety vines)

Before starting this process, talk with the laboratory about any biosecurity regulations that need to be followed whenever there is potential for plant-based samples to be transported interstate.

Also, check with the laboratory on their recommendations for sampling procedure. The following contains general information and is a guide to use in the absence of any other information.

During vintage, leaf tissue is the preferred material to collect, especially late in the season before leaf fall (Table 4). Target older leaves from mature lignified canes at the basal section of the canopy close to the fruiting zone. For red varieties, prioritise the collection of leaves showing visible disease symptoms.

If collections occur after leaf fall, collect mature lignified canes. Canes should be taken from the canopy base, close to the fruiting zone, and include at least two to three buds (or nodes).

When collecting material from one grapevine for one laboratory test, collect a minimum of two leaves or two canes from different areas of the vine canopy.

Table 4: Grape plant sample collection for single and composite tests for grapevine viruses.

	Single samples	Composite samples
Leaf	Collect **up to 10** leaves per vine. Minimum of 2 leaves.	Up to **50** leaves from **5** vines can be combined as a composite sample.
	Choose leaves from the base–middle of mature canes from each selected vine. Choose leaves from different positions of the canopy, and either side of the trunk.	
	For each sample, use unique identifiers (e.g. Block J, row #235, panel #5, vine #2).	
	Leaf samples can be collected in late summer or autumn (from mid-February until leaf senescence).	
Cane	Collect **up to 4** canes per vine. Minimum of 2 canes.	Up to **20** canes from up to **5** vines can be combined as a composite sample.
	Cane samples should be collected from mature wood about **10–20** buds from the base of the cane. Then re-cut for a length of **2–3** buds (approximately 20 cm). Make sure the wood is healthy and **alive**. Choose canes from different positions on the grapevine, and either side of the trunk.	
	For each sample, use unique identifiers (e.g., Block J, row #235, panel #5, vine #2).	
	Cane samples can be collected in autumn or winter: 1. From March till late winter for scion samples 2. Not to be collected before late March for rootstock samples.	

Shopping list

Before collecting vine samples, the following items are needed:

- Plastic snap-lock bags (ideally with an area to write identifying labels)
- Permanent black marker pen
- Secateurs (if collecting cane material)
- Cooler bag or polystyrene container for storing collected material
- Frozen blocks to keep plant material chilled
- Plant tags/ties (when collecting composite test samples).

How to collect single samples (red and white variety vines)

When collecting mature vine leaves:

- Samples should include an intact leaf blade plus its petiole (Figure 16).
- Choose leaves from different positions throughout the grapevine canopy, including both sides of the trunk (for bi-lateral grapevines).
- Stack the leaves on top of each other in the same orientation, and lay them flat inside a plastic snap-lock bag.
- Seal the bag, leaving a gap to provide airflow and evacuate residual air from inside the plastic bag.
- Using the marker pen, label the bag with a unique identifier (e.g., Block J, row #235, panel #5, vine #2).

- Place grapevine samples in a chilled container with the frozen blocks. Keep out of direct sunlight.

When collecting mature canes:

- Prune mature wood at the node position closest to the fruiting wire. These are typically 10–20 buds in length (Figure 16).
- Choose canes from different positions throughout the canopy, including both sides of the trunk (for bi-lateral grapevines).
- Trim the canes to lengths of 15–20 cm (approximately 2–3 buds).
- Ensure the wood collected is alive and healthy.
- Place the canes in the plastic snap-lock bag.
- Follow the same steps as described for leaf collection: seal the bag leaving a gap, evacuate the residual air, label bags with a unique identifier, and keep plant samples cool and out of direct sunlight.

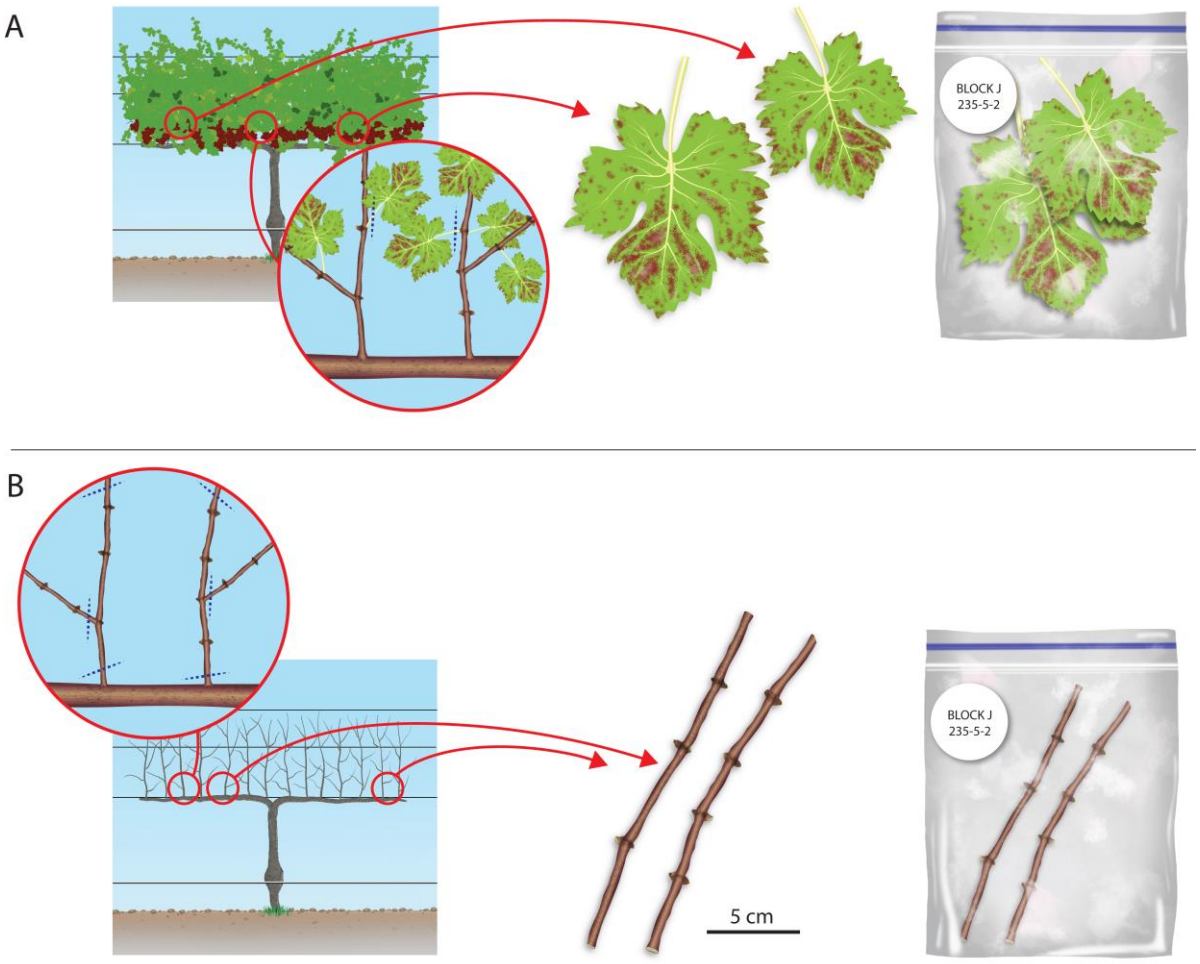


Figure 16. Leaf (A) and mature grapevine cane (B) sample collection for laboratory virus testing. Ideally, collect a minimum of two mature leaves from the base of lignified canes closest to the fruit zone late in the vintage, but prior to leaf fall Leaves are stacked on top of each other in the same direction and placed in snap-lock plastic bags (A). Post-leaf fall, collect a minimum of two mature, lignified cane samples. They should be approximately 20 cm in length and they should ideally include nodes closest to the fruiting zone. Ensure the wood is alive. Place the cane samples in the plastic snap-lock bag before despatch to an accredited testing laboratory (B).

Composite test plan (red and white variety vines)

A plan is essential to ensure grapevine samples are systematically collected to represent an entire target area.

For example, to composite test a block of 100 rows each planted with 200 grapevines, a vineyard manager might develop the following plan:

- One composite test sample will consist of five different grapevines.
- Vine material will be collected from every 10th row.
    - Year 1: collections are undertaken in Rows 1, 10, 20, 30, 40, 50, 60, 70, 80, and 90.
    - Year 2: collections are undertaken in Rows 5, 15, 25, 35, 45, 55, 65, 75, 85, and 95.
- For each row, plant material will be collected from every 20th grapevine.
    - Samples will be collected from a total of 10 grapevines that make up two composite test samples.
    - The first five grapevines in the row will make up one composite test sample, and the last five grapevines in the row will make up the second composite test sample.
- For the block, a total of 100 grapevines will be sampled, generating 20 composite test samples.

How to collect composite samples (red and white variety vines)

- Mature leaves and canes are collected from grapevines as described for single test samples.
- The key difference will be the need for larger sample bags able to contain up to 50 mature leaves or 20 mature canes.
- If single grapevine test information will subsequently be required from the composite test samples, an additional step is required.
    - The leaves or canes collected from a single vine, including different positions of the canopy from both sides of the trunk (for bi-lateral grapevines), must be bundled together by wrapping a plant tag around the leaf petiole or around the canes.
    - These are placed in a plastic snap-lock bag that is then sealed.
    - If a composite test sample consists of five grapevines, a complete composite test sample bag will contain five bundles of vine material.
    - Each bundle should have a unique identifier. For example, the number ‘1’ can be used for the first grapevine sampled, number ‘2’ for the second grapevine sampled, and so on up to number ‘5’ to represent the fifth and last grapevine sampled for a five-grapevine composite test.
- Each composite test sample should also have a unique identifier (block name, row number and composite number).
- For both leaf and cane collection, appropriately label the snap-lock bags with a traceable identification code, slightly seal the bag, leaving a gap to provide airflow and squeeze out any remaining air inside the plastic bag before completing the seal. Keep the plant samples cool and out of direct sunlight.

Available laboratory tests (red and white variety vines)

Two main tests are used to detect GLRaV-1, GLRaV-3, and GVA. These are the serological test called enzyme-linked immunosorbent assay (ELISA), and variations on the molecular test such as conventional end-point reverse transcription polymerase chain reaction (RT-PCR) and qualitative real-time reverse transcription polymerase chain reaction (RT-qPCR).

Enzyme-linked immunosorbent assay (ELISA)

ELISA is an antibody-based assay that detects the protein that protects the virus' genome-forming virus particles, virions. This assay is the gold-standard laboratory test method used in New Zealand when testing for GLRaV-3. It is robust and cost-effective. ELISA has demonstrated wide specificity, with antibody providers happy to work with researchers to ensure that the antibody mix detects new-to-science GLRaV-3 genetic variants. However, it can be less sensitive than RT-qPCR, so low quantities of virus in plant samples might generate a so-called 'false negative' (when a plant sample has the virus in question, but the test is unable to detect its presence because the virus amount is too low or unevenly distributed in the vine).

The suitability of ELISA testing for key grapevine viruses under Australian conditions requires more research. At present, ELISA tests are only offered for GLRaV-3 (not GLRaV-1 and GVA) and at some of the laboratories listed in this document. The unavailability of ELISA testing more widely is a potential barrier to growers accessing what is a reliable testing option, particularly in situations where the main disease issue is GLRaV-3. Moreover, the relative cost efficiency of ELISA compared with molecular tests, will be an important tool to aid VSI assessor training.

Molecular-based assays

Molecular-based assays detect the virus genome sequence that encodes the virus proteins needed to replicate and move within a vine's vascular system. Whilst this assay is specific and sensitive, it is more expensive than ELISA. The price of this process may alter owing to the worldwide ramp-up of the similar method used for detecting SARS-CoV-2 (the causative agent of COVID-19). It may also be too specific, in that such assays can potentially miss virus strains with slight differences in the virus genome sequence, thereby causing 'false negatives'. As for ELISA, the specificity of the molecular assays can be iteratively improved based on knowledge of the genetic variation of each virus species present in the country. Molecular-based assays can be beneficial for growers wanting to screen vineyards for a panel of viruses. This panel testing can assist with the identification of the virus(es) of importance for specific vineyards.

The timeline between laboratory receipt of samples and availability of results can be up to 15 working days.

Interpreting diagnostic results

The recommended interpretation of laboratory results is based on current knowledge of each virus.

The recommendations in this section are for wine-producing blocks, rather than source blocks (in which there is a lower tolerance for viruses like GLRaV-2, GLRaV-4, and GLRaV-7).

Red varieties – single sample testing

Single-sample tests from red variety vines are used to confirm the presence or absence of one or more viruses to aid VSI training. This confirmation testing should include grapevines visually assessed for disease, those appearing **healthy (no foliar symptoms)**, and grapevines where visual symptoms are unclear.

Figure 17 shows possible scenarios and subsequent actions after receiving laboratory test results for plant samples from red varieties **with visual symptoms of GLD and/or SD**.

- A grapevine sample showing visual symptoms of GLD or SD returns a positive laboratory test. The VSI assessment is confirmed. Proceed with roguing and the multi-tactic disease management steps described earlier.
- A grapevine sample showing visual symptoms for GLD or SD returns a negative laboratory test. A sampling error might have occurred, or the foliar symptoms might have been caused by another grapevine leafroll virus (GLRaV-2, -4, or -7) or other leaf-reddening condition (see Distinguishing GLD and SD from other leaf-reddening conditions).
    - If another vine sample is collected and submitted for testing to confirm GLD or SD status, and the test is again negative, the grapevine might have been misidentified. Under these circumstances, the grapevine in question should be retained and revisited in the following vintage.
    - If re-testing is not an option, continue to monitor in the following vintage.
    - If laboratory testing for other grapevine leafroll viruses such as GLRaV-2, -4, or -7 results in a negative test, the grapevine might have been misidentified. Continue to monitor this grapevine in the following vintage.
    - If laboratory testing for other grapevine leafroll virus such as GLRaV-2, -4, or -7 results in a positive test, the next management step will depend on the virus detected. For example, if GLRaV-2 is detected, the risk of it spreading to another vine is low because there are no known insect vectors, unlike GLRaV-1 and GLRaV-3. However, GLRaV-2 is graft transmissible, and vines testing positive should be rogued if there is a chance of budwood being collected for future propagation.

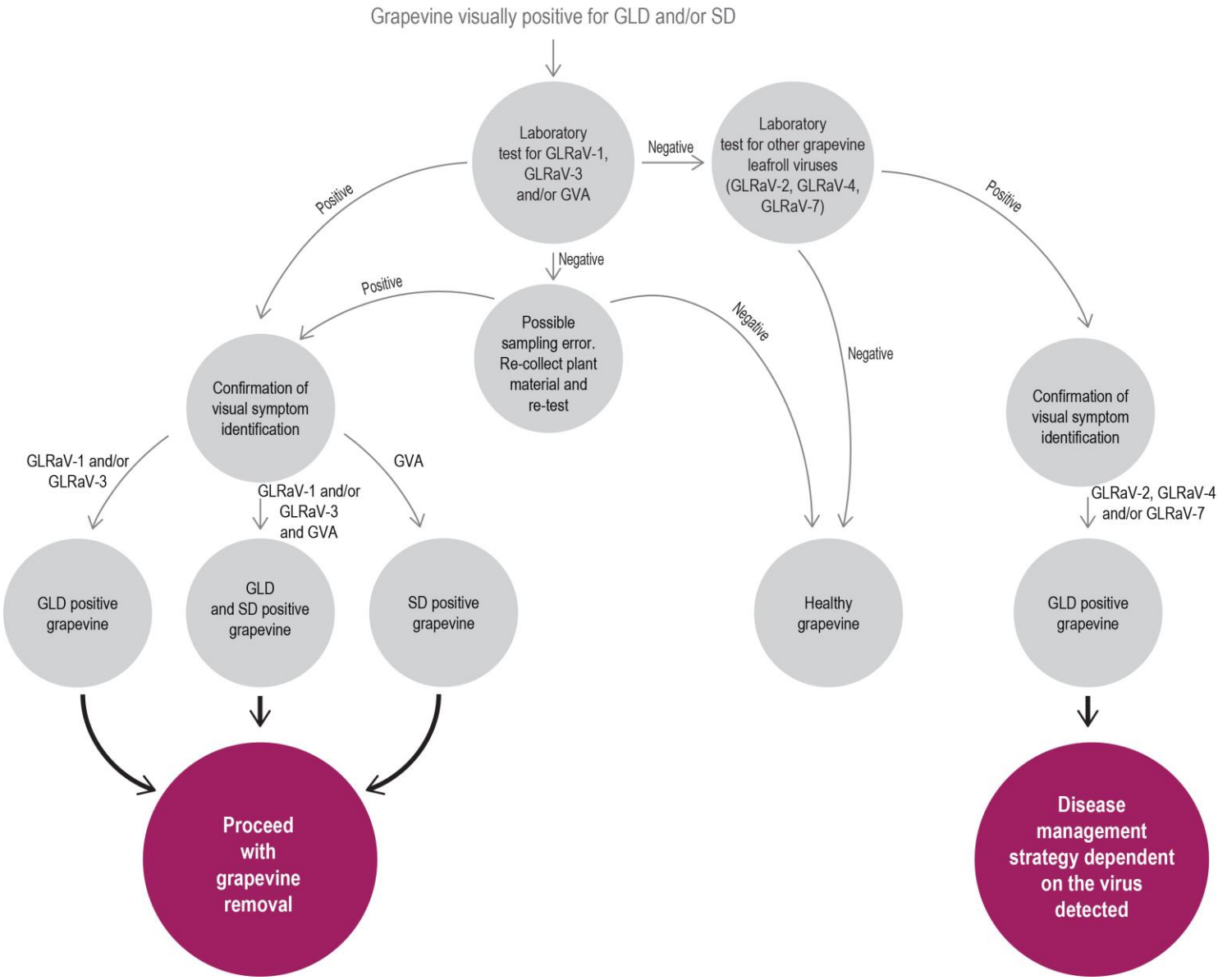


Figure 17. Decision support tree for interpreting laboratory results of grapevine samples taken from red varieties with visual symptoms of Grapevine leafroll disease (GLD) and Shiraz disease (SD).

Figure 18 shows possible scenarios and subsequent actions after receiving laboratory test results for samples from red varieties **with no visual symptoms of GLD and/or SD**.

- A sample from a grapevine with no visual symptoms tests negative for a grapevine leafroll virus or GVA. The VSI assessment has been confirmed. No further action is required.
- A sample from a grapevine with no visual symptoms tests positive for a grapevine leafroll virus or GVA. The grapevine might have a new infection, or a mistake with sampling might have occurred. Another plant sample should be collected and submitted for testing.
    - If the re-test also leads to a positive laboratory result for GLRaV-1 or GLRaV-3 or GVA, the grapevine probably has a new infection. Proceed with disease management steps, including roguing.

- If the re-test also leads to a positive laboratory result for GVA only, the grapevine probably has a new infection. Monitor the grapevine for visual symptoms in the next vintage.
- It is possible that a positive virus infection associated with no symptoms will be of interest to researchers and in any case, it should be monitored next vintage.

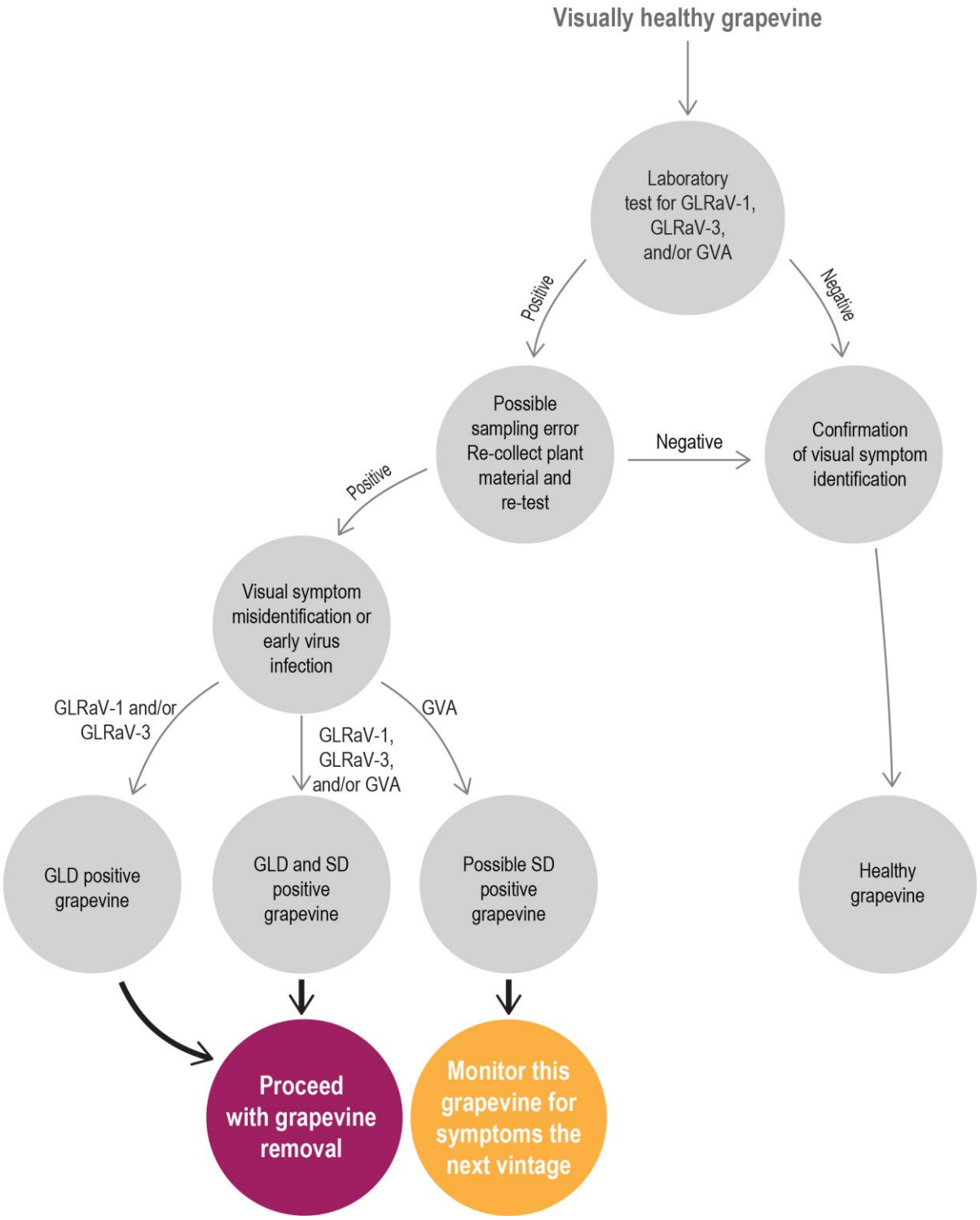


Figure 18. Decision support tree for interpreting laboratory results of grapevine samples from red varieties with no visual symptoms of Grapevine leafroll disease (GLD) or Shiraz disease (SD).

Composite sample testing

Laboratory testing on composite samples from red or white varieties helps to estimate disease incidence at the block level. Disease incidence information is then used to determine whether to plan for future redevelopment of the block (high disease incidence above), or management of the block (low incidence) as outlined in the previous sections of this document.

If any of the composite samples test positive for one or more viruses, some disease is probably present in the block. Consider the following scenarios for the next steps.

- If a high number of composite samples test positive for (1) GLRaV-1 or GLRaV-3 or (2) GLRaV-1 or GLRaV-3 and GVA, and samples are from a red variety showing some visual symptoms, this block should be rogued and actively managed as described in this document, including for insect vectors.
- If a high number of composite samples test positive for (1) GLRaV-1 or GLRaV-3 or (2) GLRaV-1 or GLRaV-3 and GVA, and samples are from a white or red variety showing no visual symptoms, next steps might include a continuation of vector management, and, in following vintages, active monitoring for virus symptoms (in red variety blocks). Further composite testing should be conducted in subsequent years.
- If a high number of composite samples test positive for GVA only and samples are from a red variety showing some visual symptoms, this block should continue to be actively managed as described in this document.
- If a high number of composite samples test positive for GVA only and samples are from a white or red variety showing no visual symptoms, next steps are likely to include a continuation of vector management. In following vintages, monitoring for virus symptoms (in red variety blocks) and further composite testing should be conducted.
- If a low number of composite samples test positive for one or more viruses, the vineyard manager might simply record incidence. In following vintages, monitoring, vector management and testing should be continued.
- If all the composite samples test negative for one or more viruses, virus infection in the block is either absent or virus infection is very low (<1%).

Composites back to single tests

Vineyard and nursery managers might want to ‘drill down’ for further details on individual vines after receiving results from composite tests. In general, this single vine information might be for the purpose of identifying individual vines that might be rogued or which remain in the ground. Such decisions require additional laboratory testing.

If samples from the different grapevines are appropriately bundled and labelled (as described in the section “How to collect composite samples”), the laboratory should be instructed to test each as a single sample. For unbundled and unlabelled samples, new samples from each grapevine will need to be collected and sent to the laboratory.

Figure 19 shows possible scenarios and subsequent actions following this additional single sample testing.

- If a sample tests positive for GLRaV-1 or GLRaV-3, or GLRaV-1 or GLRaV-3 and GVA, the grapevine should be removed (rogued). The block should be actively managed as described

in this document. If a sample tests positive for GVA only and the grapevine is a red variety showing visual symptoms, it should be removed.

- If a plant sample (either white or red variety) tests positive for GVA only and the grapevine shows no visual or detrimental symptoms, the vineyard manager might choose to monitor for symptoms in the next vintage. If there is a chance budwood will be collected for future propagation, budwood should not be used from this grapevine and the grapevine should be removed.
- If a sample tests negative in the laboratory for grapevine leafroll virus or GVA, the grapevine is assumed to be healthy.

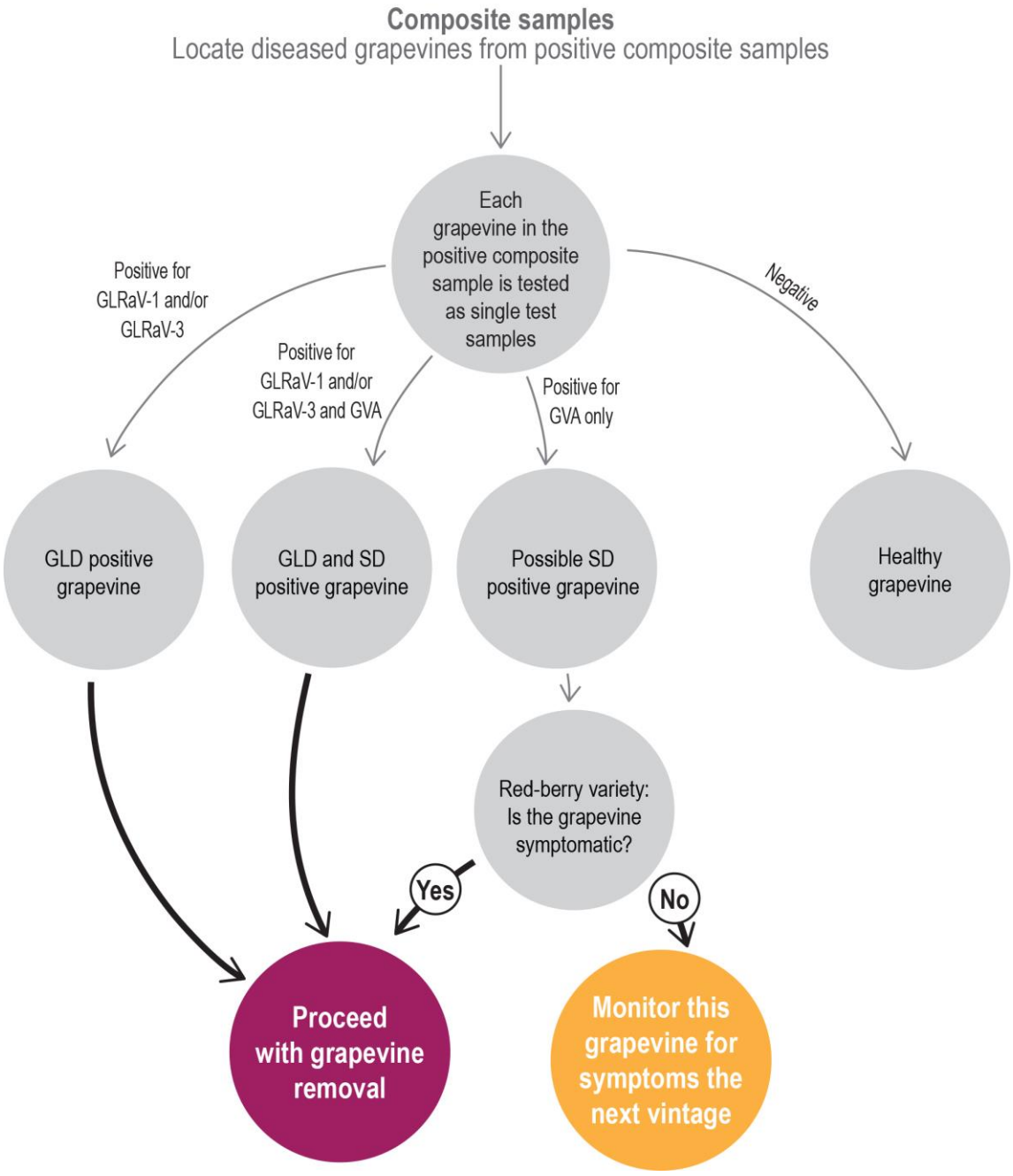


Figure 19. Decision support tree to aid the interpretation of laboratory results of grapevine samples subjected to composite testing for Grapevine leafroll disease (GLD) and Shiraz disease (SD).

Future prospects for virus identification: remote sensing

Remote sensing, including hyperspectral imaging, may aid the detection of virus-infected vines in future, especially in white varieties where visible symptoms are not readily apparent to the human eye. Importantly, this technology may allow surveillance on a vineyard-wide scale to inform growers about blocks requiring follow-up laboratory testing of vine samples to estimate virus incidence.

In acknowledging the possibilities of this technology, we note it remains in a development phase. Therefore, its application in the vineyard in future is dependent upon the outcomes of ongoing research and co-development with growers and potential industry providers.

Appendix 1: Key references

A selection of the published papers and other reference material commonly referred to by the authors during the writing of this document. Note: for the sake of brevity, this list does not include all the literature reviewed during the development and writing of the document.

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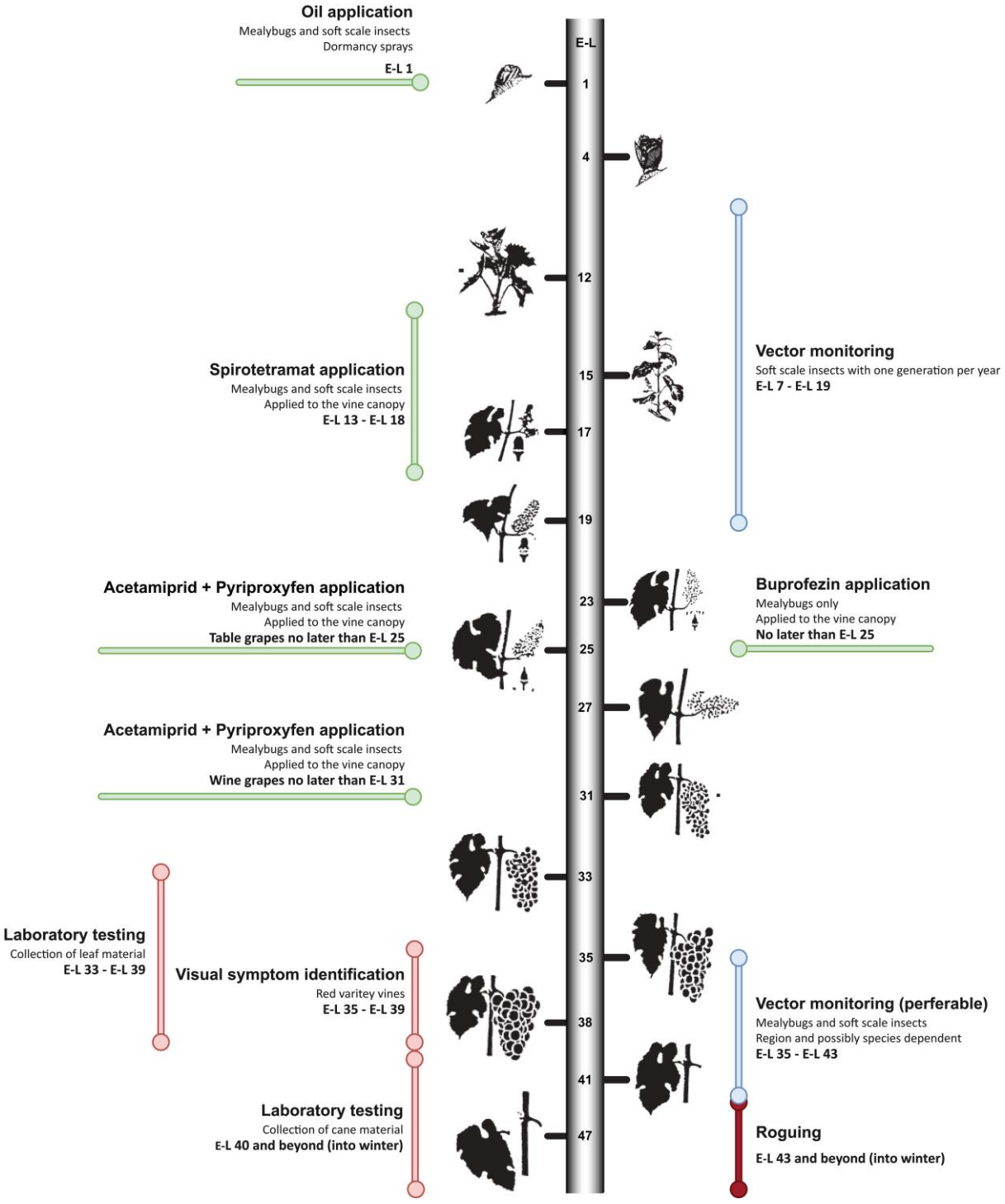
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Appendix 2: Timeline of management activities

Summary of the different elements of the integrated response to Grapevine leafroll disease and Shiraz disease, and proposed start and end dates based on the modified Eichhorn-Lorenz (E-L) grapevine phenology model.
(Tony Corbett, The New Zealand Institute for Plant and Food Research Limited)



Appendix 3: Figure and table captions detailed in full

Figure 1. Virus disease cycle showing how Grapevine leafroll disease (GLD) and Shiraz disease (SD) can enter the vineyard and be spread by mealybugs or soft scale insects. Starting from the top left, the propagation of plant material from diseased mother vines for use in top-grafting and grafted grapevines can introduce virus into vineyards (A). Disease will spread from a diseased grapevine to a healthy grapevine by mealybugs or soft scale insects (B). As depicted in the large red and black circles, mealybugs feed on a diseased vine and acquire the virus. When the mealybugs (small white circles) move to a neighbouring (healthy) grapevine and begin feeding again, the virus particles will move from the insect into the plant tissue. The vine is now infected. In red varieties, visible symptom expression of disease takes at least 12 months to become apparent, meaning a grapevine infected in one vintage may not show foliar symptoms until the following vintage. Tony Corbett, The New Zealand Institute for Plant and Food Research Limited.

Figure 2. One of the symptoms of Grapevine leafroll-associated virus 3 (GLRaV-3) in red varieties is uneven ripening of fruit. Compare the good colour development among clusters on a GLRaV-3-free Merlot vine (a) with the uneven colour development seen in another Merlot vine infected with GLRaV-3 (b), Hawke's Bay, New Zealand, 25 March 2020.

Figure 3. A generalised decision support tree to help vineyard staff begin determining a response to Grapevine leafroll disease (GLD) and Shiraz disease (SD) in white and red varieties. Note: Insecticides targeting mealybug and soft scale insects can be applied from dormancy (Eichhorn Lorenz or E-L 3) up to no later than 80% cap-fall (E-L 25), depending on the insects being targeted and the active constituent(s) being applied (as noted in the *Agrochemicals registered for use in Australian viticulture*, 20/21, The Australian Wine Research Institute, December 2020). Tony Corbett, The New Zealand Institute for Plant and Food Research Limited.

Figure 4. Foliar symptoms of Grapevine leafroll-associated virus 3 in Cabernet Sauvignon (a, b) and Malbec (c, d) vines growing in Hawke's Bay, New Zealand, April 2019.

Figure 5. The foliar symptoms of Grapevine leafroll-associated virus 3 in a Pinot Noir vine, Hawke's Bay, New Zealand, April 2019. The extent of foliar changes are seen throughout the vine canopy late in the vintage (a) and are characterised by inter-vein reddening and green veins (b).

Figure 6. The foliar symptoms of Grapevine leafroll-associated virus 3 in a Merlot vine, Hawke's Bay, New Zealand, April 2020.

Figure 7. Foliar symptoms of Grapevine leafroll-associated virus 1 (a) and Shiraz disease (b, c). The images were kindly supplied by Qi Wu, The Australian Wine Research Institute.

Figure 8. A stylised view of the foliar symptoms of Grapevine leafroll disease and Shiraz disease in red varieties developing over time. Mid-vintage, a small number of leaves close to the vine cordon start to turn red (a); in the following weeks, the numbers of red leaves progressively increases (b and c), and by late-vintage, red leaves are evident higher in the vine canopy (d). Tony Corbett, The New Zealand Institute for Plant and Food Research Limited.

Figure 9. Grapevine leaves showing rudimentary foliar symptoms of Grapevine leafroll-associated virus 3 in Cabernet Sauvignon (a, b) and Merlot (c, d) vines growing in Hawke's Bay, New Zealand. Early- to mid-vintage symptom development is often characterised by 'spotty' or 'patchy' reddening on individual vine leaves.

Figure 10. Part of a vineyard depicted on a Microsoft® Excel® spreadsheet over the course of three years. In each of seven rows there were four panels and four vines per panel. Those Grapevine leafroll disease or Shiraz disease infections that were visually identified were colour-coded by year. In year 1, a single vine was visually diagnosed as virus-infected. Its position was recorded on the Excel 'map' (row 3). Its removal during winter of year 1 was denoted by the loss of the 'x'. In year 2, two further vines were visually diagnosed as virus-infected in row 3. Both were removed in winter. By year 3, another three vines were visually diagnosed as virus-infected and the positions were recorded on the map. Note: dispersing vectors often spread virus to neighbouring within-row vines. The black arrow denotes row direction.

Figure 11. Grapevines can undergo foliar reddening during vintage. Inadequate training can reduce the ability of an assessor to distinguish between vines infected with Grapevine leafroll disease (GLD) and Shiraz disease (SD), and those with magnesium deficiency (a), cane girdling (b, c), and symptoms of unknown status (d).

Figure 12. The longtailed mealybug, *Pseudococcus longispinus* (top); the grapevine scale, *Parthenolecanium persicae* (bottom; image kindly supplied by Jenny Venus).

Figure 13. Grapevine leaves affected by black sooty mould. Both the upper (a) and lower (b) surface may be affected, with mealybugs accessing phloem sap from within the primary veins (b).

Figure 14. A sexually mature male citrophilus mealybug (*Pseudococcus calceolariae*). Receptors in the male's antennae detect the airborne sex pheromone of a 'calling' female of the same species. Note the prominent waxy filaments from the posterior end. Robert Lamberts, The New Zealand Institute for Plant and Food Research Limited.

Figure 15. Aerial view of two vine rows. The red circles denote sampling points from which a single vine leaf (or a berry cluster) was collected before walking to the next vine in either row. Back in the laboratory or another facility, the vine material should be inspected using at least a 10x hand lens to assess presence and/or estimate the abundance of virus vectors: mealybugs and soft scale insects. Tony Corbett, The New Zealand Institute for Plant and Food Research Limited.

Figure 16. Leaf (A) and mature grapevine cane (B) sample collection for laboratory virus testing. Ideally, collect a minimum of two mature leaves from the base of lignified canes closest to the fruit zone late in the vintage, but prior to leaf fall Leaves are stacked on top of each other in the same direction and placed in snap-lock plastic bags (A). Post-leaf fall, collect a minimum of two mature, lignified cane samples. They should be approximately 20 cm in length and they should ideally include nodes closest to the fruiting zone. Ensure the wood is alive. Place the cane samples in the plastic snap-lock bag before despatch to an accredited testing laboratory (B). Tony Corbett, The New Zealand Institute for Plant and Food Research Limited.

Figure 17. Decision support tree for interpreting laboratory results of grapevine samples taken from red varieties with visual symptoms of Grapevine leafroll disease (GLD) and Shiraz disease (SD). Tony Corbett, The New Zealand Institute for Plant and Food Research Limited.

Figure 18. Decision support tree for interpreting laboratory results of grapevine samples from red varieties with no visual symptoms of Grapevine leafroll disease (GLD) or Shiraz disease (SD). Tony Corbett, The New Zealand Institute for Plant and Food Research Limited.

Figure 19. Decision support tree to aid the interpretation of laboratory results of grapevine samples subjected to composite testing for Grapevine leafroll disease (GLD) and Shiraz disease (SD). Tony Corbett, The New Zealand Institute for Plant and Food Research Limited.

Table 1. Species (and common names) of mealybug and scale insects that have been recorded in Australian vineyards. Most of the species listed can acquire Grapevine leafroll-associated virus 1 (GLRaV-1), GLRaV-3, and/or Shiraz disease when feeding on infected vines. Having dispersed and resumed feeding on a healthy grapevine host, they can then transmit one or more of these pathogens (Species list of soft scale insects from Rakimov et al. 2013).

Table 2. An example of the potential for mealybug population increase in an Australian vineyard where there are up to four generations per year, and where the measures adopted to contain population growth are inadequate.

Table 3. Insecticide active constituents targeting mealybugs and/or soft scale insects that are currently registered for use in Australia as recorded in *Agrochemicals registered for use in Australian viticulture* (The Dog Book), Australian Wine Research Institute (updated 31 May 2022).

Table 4: Grape plant sample collection for single and composite tests for grapevine viruses.

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