

Grapevine leafroll-associated virus 3 and its management strategies in vineyards

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Grapevine leafroll-associated virus 3 (GLRaV-3) is the most severe grapevine virus pathogen worldwide. It reduces berry yield by up to 40% and significantly hampers wine quality, especially in red varieties. This article summarises the latest local and international studies on how to stop GLRaV-3 from spreading in new and established vineyards and highlights current gaps in management knowledge from an Australian perspective. The best management strategy should involve testing, identifying and roguing infected vines and effective vector management. New cuttings should be sourced from material tested negative for GLRaV-3 from sources that undertake regular testing to ensure a high health status is maintained.

INTRODUCTION

Grapevine leafroll disease is one of the most widespread and economically damaging viral diseases of grapevines globally. The disease is associated with up to 12 different virus species and strains known collectively as grapevine leafroll-associated viruses (GLRaV). This article focuses on grapevine leafroll-associated virus 3 (GLRaV-3), one of the most severe of the leafroll viruses. Once established in a vineyard, GLRaV-3 may spread rapidly and it may take years to eradicate. Many grapevine varieties with GLRaV-3 experience reduced yields, delayed or variable ripening time, and lower quality wine (Naidu *et al.* 2014). GLRaV-3 infection can also reduce the quality of wood for propagation including grafting. Some GLRaV-3 infected vines may remain symptomless, acting as reservoirs for virus spread. Up to 10 genomic groups of GLRaV-3 with symptom intensity from mild to severe have been identified (Wu *et al.* 2020).

CLASSIFICATION OF LEAFROLL-ASSOCIATED VIRUS SPECIES

GLRaV-3 is one of the 12 different virus species and strains belonging to the genus *Ampelovirus*, family *Closteroviridae*, which are associated with grapevine leafroll disease. The *Ampelovirus* genus consists of two subgroups. Subgroup I has three species: GLRaV-1 and GLRaV-3, and GLRaV-13 (Ito and Nakaune 2016), which are associated with severe leafroll disease. Subgroup II has one species, GLRaV-4, with up to 10 strains that produce mild symptoms and may not affect vine yield and quality. However, they

may exhibit synergistic effects in the presence of other viruses. For example, grapevine virus A when mixed with the Subgroup II of ampeloviruses can cause Shiraz Disease (Wu *et al.* 2020). Two other leafroll viruses, GLRaV-2 and GLRaV-7, have their own genus with unknown vectors. To date, GLRaV-7, GLRaV-13 and a few strains of GLRaV-4 have not been detected in Australia.

SYMPTOMS

Vine growth and foliage

GLRaV-3 infection can reduce plant vigour, canopy size, longevity and wood quality for propagation. These conditions are more pronounced in grafted vines. Expression of GLRaV-3 symptoms is influenced by grapevine cultivar, virus variant, rootstock-scion combinations, mixed virus infections and environmental factors. European grapevine cultivars (*Vitis vinifera*) and an Asian *Vitis* species (*V. coignetiae*) exhibit symptoms, while rootstocks including American *Vitis* spp. and most white varieties are asymptomatic.

In red varieties, symptomatic vines develop interveinal red or reddish-purple patches on mature leaves around veraison. Symptoms become more evident as the season progresses. By late autumn, the entire interveinal area of infected leaves turns reddish-purple and the veins turn bright green. In severely infected vines, the entire canopy can turn red by the end of the season (Figures 1 and 2, see page 36).

In red varieties, other stresses such as magnesium, phosphorus or potassium deficiency, or damage from machinery, pests, wind or girdling, can cause coloration that may



Figure 1. Typical symptoms of GLRaV-3 on the leaves of red grapevine varieties.

be confused with leafroll symptoms (Figure 3, see page 36). Molecular testing is required to confirm if symptoms are associated with leafroll disease.

In white varieties, GLRaV-3 can be present without symptoms, or may present as mild interveinal yellowing in leaves with green veins (Figure 4, see page 38). As with red varieties, foliar symptoms become more evident as the season progresses.

Fruit

Compared to healthy vines, GLRaV-3 infected vines have smaller bunches and



Figure 2. Symptoms of grapevine leafroll disease on leaves of a red grapevine variety. This vine tested positive for GLRaV-3. Typical vein greening and backward rolling of the leaves are clear.

berries, with yield losses between 14% and 40% reported (Naidu *et al.* 2014). The magnitude of yield losses depends on cultivar-rootstock combinations, vine age, virus strain and environmental conditions. Berries from infected vines have uneven or delayed ripening, reduced sugars, reduced aroma and less anthocyanin. Compared to berries from healthy vines, the fruit from infected vines is pale with increased titratable acidity. Symptom severity increases over time leading to an increased delay in ripening each year, along with lower yields. Asynchronous ripening of healthy and infected bunches reduces fruit quality over time.

VECTORS

Grapevine leafroll associated viruses can be transmitted through vegetative propagation of grapevines, including grafting, or by the phloem sap-sucking insect vectors mealybugs and soft scales. These insect vectors generally move along the rows from one vine to the next, spreading the virus in a clustered pattern. Leafroll viruses are not transmitted by pruning.

Several species of mealybugs (*Pseudococcidae*) and soft scale (*Coccidae*) have been found in vineyards infected with GLRaV-1, -3, and -4. In Australia, three mealybug species are frequently found and all

are effective GLRaV-3 vectors: *Pseudococcus calceolariae* (citrophilus mealybug), *Ps. longispinus* (long-tailed mealybug) and *Ps. viburni* (obscure mealybug). Mealybug nymphs overwinter under vine bark and in crevices (Figure 5a, see page 40) and, in the case of the citrophilus and obscure mealybugs, in the rootzone below ground. In spring, they move out onto vines. Female mealybugs lay a large number of eggs which quickly hatch into crawlers (first instar nymphs). The nymphs are highly mobile and effective GLRaV-3 vectors. Mealybugs can have three to four generations per year. When conditions are favourable (mild/warm conditions around 25°C and humid) mealybug populations increase rapidly. Mealybugs can survive on remnant vine roots and spread GLRaV-3 to newly-established vineyards, sometimes even after a fallow period.

Parthenolecanium persicae is the most common species of soft scales found in Australian vineyards (Figure 5b, see page 40) and can spread GLRaV-3 and grapevine virus A simultaneously. Scale insects have one generation per year. Second or third instar nymphs overwinter under vine bark and in crevices. They emerge in spring and begin to grow rapidly. The female lays between 100 and 2000 eggs depending on the species. The crawlers (first instar nymphs) emerge in late October (mid-spring) and are highly mobile and effective GLRaV-3 vectors.

VINEYARD SPREAD OF GRAPEVINE LEAFROLL-ASSOCIATED VIRUS 3

The spread of GLRaV-3 virus in vineyards can occur in two ways: primary spread and secondary spread. Primary spread of

GLRaV-3 occurs when it is introduced into a newly-planted vineyard via virus-infected planting material or from vectors carrying the virus in from another vineyard. Vectors can be introduced into a vineyard via wind, ants, birds, machinery or vineyard workers' clothing and equipment. Secondary spread occurs when vectors carry the virus from the primary source of infection to other vines. Typically, it is vines on either side of an infected vine in a row that become infected. If symptoms do show on these vines, this may not occur for one or two years after infection (Constable 2020, Bell 2020). These vines can test positive by polymerase chain reaction (PCR) one year before symptoms appear. The rate of secondary spread is correlated with vector abundance. In South Africa, 100% of vines became infected 15 years after first symptoms of GLRaV-3 appeared (Pietersen *et al.* 2013). This rapid rate of spread does not seem to occur in Australia; one reason might be the absence of the aggressive mealybug vector *Planococcus ficus*.

MANAGEMENT STRATEGIES FOR LEAFROLL DISEASE

Management of the spread of GLRaV-3 in vineyards requires monitoring and testing of vineyards, roguing of GLRaV-3 infected vines, control of insect vectors and planting virus-tested propagating material. This integrated approach has been used effectively in vineyards in South Africa, New Zealand and California (Bell *et al.* 2021). In New Zealand, roguing vines and controlling mealybugs dramatically decreased the number of infected vines over six years (Bell *et al.* 2018). In South Africa, practices including herbicide spraying

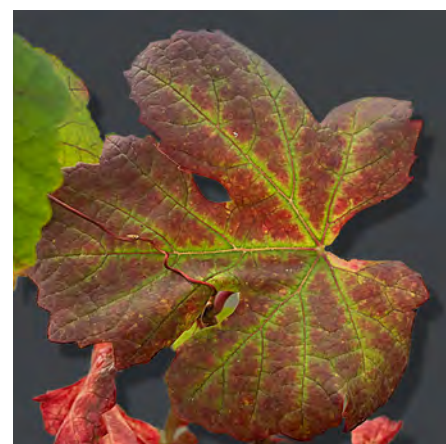


Figure 3. An unknown disorder in Cabernet Sauvignon vines. These vines tested negative for all known leafroll viruses by polymerase chain reaction (PCR).



Figure 4. Symptoms of GLRaV-3 on a white grapevine variety. While the canopy is pale, a close inspection of the leaves (inset on the right) shows green veins in a yellow background.

of infected vines, controlling mealybugs with insecticide and roguing vines resulted in a reduction of the infection rate of GLRaV-3 from 100% in a 41ha vineyard in 2002 to 0.027% in 2012 (Pietersen *et al.* 2013).

Monitoring and testing

The process of identifying GLRaV-3 infected vines differs for red and white varieties. In white varieties, visual identification of the infected vines is difficult and suspected vineyards should be screened before winter using composite vine sampling and molecular testing. In these varieties molecular diagnostics should be conducted each year since the virus symptoms are difficult to identify visually. In red varieties, visual symptoms in late autumn are often sufficient to identify GLRaV-3-infected vines. Mapping the pattern of spread of GLRaV-3 can help identify the source of the virus and the direction and speed of the spread. Remote sensing technology, for example, the use of drones equipped with RGB cameras, can speed up the mapping process; however, 'ground-truthing' the data by molecular testing in a laboratory is important.

Roguing of infected vines

Once identified, GLRaV-3 positive vines should be removed, including all remnant roots to a depth of 30cm as these can act as reservoirs for vectors that can re-infect new plantings. The vines on either side of the rogued vine should be monitored closely before harvest each year to detect and remove new infections.

In Lodi, California, this mechanism was successfully deployed on an 18ha block of Cabernet Sauvignon planted in 2013 where the first virus symptoms were observed in

2015. In 2016, 8% of the vines ($n = 2468$) showed leafroll symptoms and molecular testing confirmed the presence of GLRaV-3. In 2016, all infected vines were rogued and in 2017 only 1.2% of vines showed symptoms. The roguing exercise reduced the infection rate dramatically (Bolton 2020).

Studies in New Zealand, South Africa and the USA showed that roguing of infected vines is economical when the infection rate is below 20%. Beyond 20% infection, it becomes economically unfeasible to rogue symptomatic vines. Depending on the region, 20% infection can provide an economical return in Australian vineyards (Constable 2020). In the Riverland region of South Australia, a Shiraz vineyard on Ramsey rootstock established in 1994 shows more than 50% GLRaV-3 infection but it is still producing 11 tonnes per hectare, a satisfactory yield for the grower. However, virus-free vines could potentially yield much more. The capacity to operate with 50% infection might either be due to the presence of a milder strain of GLRaV-3 or to the vigorous growth of Shiraz on Ramsey in a warm region like the Riverland with sufficient irrigation. This highlights the need for region-specific research on the physiological effects of GLRaV-3 strains on vine performance and grape quality to develop Australian-specific management recommendations.

Vector control

Essential to any vector management program are strategies that aim to reduce the movement of live vectors within and between vineyard blocks. This should include an understanding of the viral loads within each block so that movement of personnel and equipment can be ordered from uninfected first to the most highly infected last. Appropriate equipment sanitation practices to

remove soil and plant debris should also be included between the appropriate blocks.

The natural enemies of scale and mealybug insects such as ladybirds, parasitic wasps and lacewings typically keep populations under control, but if the pests are protected by ants or for some other reason get established, their control might require chemical application. This is difficult because the nature of scale and mealybug insects is to shelter under bark, making good chemical contact difficult. Systemic insecticides can overcome this, but any chemical intervention needs to be considered carefully. Examples of insecticides used to control insect pests but later having negative secondary impacts are common.

Ideally, pest and beneficial insect populations should be monitored and, where possible, sprays should be applied only when and where pest thresholds are reached. This allows beneficial insects to repopulate from areas that were not sprayed. In addition, the agrochemicals that have the least off-target impact should be chosen from the options available. The University of California integrated pest management guidelines for mealybug control recommend in early spring using the active constituent buprofezin, in late spring using spirotetramat (first choice) or clothianidin (soil application) and in summer using buprofezin (only agrochemicals registered in Australia have been included from these guidelines). Due to maximum residue limit regulatory constraints imposed by some export markets, the nature of these active constituents or the timing of their use might prevent them from being used. Agrochemical recommendations for export wine can be found in the 'dog book', available from the AWRI website and as a smartphone app.

Other vector control strategies include biological control through natural predators such as lacewings, lady beetles, mites and predaceous midges. The use of pheromones for mating disruption has also proved to be effective (Ballesteros *et al.* 2021), although research supporting this in an Australian context is lacking.

Use of clean planting material

The use of high health planting material that is free from viruses is one of the most important elements of virus management. ▶

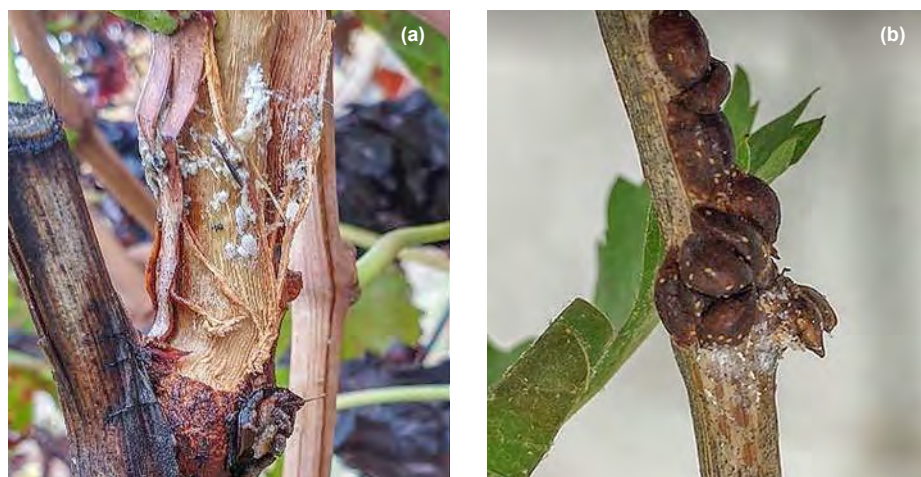


Figure 5 (a) Cluster of obscure mealybugs (*Pseudococcus viburni*) under the bark of a Shiraz cane; (b) Grapevine scale (*Parthenolecanium persicae*) on a Shiraz cane. Crawlers are visible on the top of the shell as small white dots.

There are a number of virus testing facilities in Australia that can be used to confirm this (AWRI Commercial Services in SA, AgriBio in Victoria, DPIRD Diagnostics and Laboratory Services (DDLS) in WA). If a block is being replanted as a virus remediation strategy, then a fallow period may be required. Molecular testing of volunteer vines after a fallow period is recommended to ensure they are free of GLRaV-3. In 2018, in a block of Shiraz at McLaren Vale, virus positive volunteer vines emerged after roguing. It was recommended to kill the infected vines with herbicides and leave the block fallow for another year before establishing a new vineyard using clean planting material.

Virus elimination is also an option for varieties infected with virus. Natural heat therapy has been used to eliminate viruses by growing vines in a hot climate. In the Sahara Desert of Tunisia, a domestic grapevine variety called Sakasly, 100% infected with GLRaV-3, was grown and the virus was monitored by ELISA and PCR over two years. In year one, 80% of the originally-infected vines tested negative and two years later, 93-95% of vines tested negative (Ben Salem Fnayou *et al.* 2006). It might be possible to apply this type of natural thermotherapy for virus elimination in Australia as there are many remotely segregated desert-like areas that could be used for vine propagation and to establish wood as nuclear stock. In these areas, the vines are protected against mealybug invasions as their eggs do not hatch at temperatures above 37°C (Braybrook 2012). These slow-growing, hot conditions can also be mimicked in controlled-temperature growth rooms and glasshouses,

a technique already being used for virus elimination in Australia by some laboratories and nurseries. This method, while very effective, can take up to five years. Tissue culture coupled with thermotherapy and chemotherapy is another virus elimination procedure that can reduce the time to produce virus-free plantlets to approximately two years depending on the variety. This virus elimination service is currently offered by AWRI Commercial Services.

CONCLUDING REMARKS

Grapevine leafroll disease is caused by up to 12 different virus species and strains all belonging to the same family, *Closteroviridae*. One of these viruses, grapevine leafroll-associated virus 3 (GLRaV-3), is highly pathogenic to the grapevine. Unlike some fungal grapevine pathogens, viruses cannot be controlled through agrochemical application, but their incidence can be alleviated by planting certified high health material sourced from vineyards that undergo robust virus testing regimes to ensure they are free from GLRaV-3. Further management strategies should include monitoring of vineyards at least annually, testing for GLRaV-3, roguing infected vines and robust vector management programs. Research in Australia is still required to further tailor these management strategies to our unique conditions.

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