VISUAL SYMPTOM IDENTIFICATION OF GRAPEVINE LEAFROLL-ASSOCIATED VIRUS 3 IN RED BERRY CULTIVARS SUPPORTS VIRUS MANAGEMENT BY ROGUING

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SUMMARY

Grapevine leafroll-associated virus 3 (GLRaV-3) is the most serious virus in New Zealand and South African vineyards. Its negative influence on berry development is reflected on wine quality, thus making GLRaV-3 control a priority. In red berry cultivars, changes in leaf colour could be useful for the visual identification of GLRaV-3-infected vines with a view to roguing (removing) such vines. We tested the efficacy of visual diagnosis as a potentially cost-effective alternative option to the enzyme-linked immunosorbent assay (ELISA) that is usually used for this purpose. All the vines, or a subsection of vines, in multiple vineyards in New Zealand or South Africa where annual roguing was being performed, were evaluated with the two methods. Of the 114,782 vines assessed visually for symptoms and tested by ELISA, the two methods were in agreement for 114,701 (99.9%) vines, with only 81 vines showing differing results. In 11 of the 44 annual vineyard analyses, visual detection of symptoms was perfectly correlated with ELISA results (sensitivity 100%). The specificity of visual symptom identification compared with ELISA was higher than 99.7% in 43 of the 44 annual vineyard analyses. Symptoms as a predictor of negative ELISA proved to be above 97.5% in all 44 annual vineyard analyses but as a positive predictor, was 100% in 10 of 19 annual vineyard analyses where this could be determined. We conclude that for the red-berried cultivars in this study, visual assessment of foliar symptoms should be adopted as a cost-effective alternative to ELISA during implementation of roguing for GLRaV-3 control.

Keywords: Vitis vinifera, GLRaV-3, plant disease visual diagnostics, integrated virus management, Pseudococcidae.

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INTRODUCTION

Grapevine leafroll-associated virus 3 (GLRaV-3) is one of the most important viral diseases of *Vitis vinifera* L. (Vitaceae). GLRaV-3 occurs in all major winegrowing regions of the world (Maree *et al*., 2013) and adversely influences the quantitative and qualitative parameters of grape and wine production (Cabaleiro *et al*., 1999; Montero *et al*., 2016). Being graft-transmissible (Sheu, 1936), GLRaV-3 is also transmitted (vectored) from vine to vine by dispersing mealybugs (Hemiptera: Pseudococcidae) and soft scale insects (Hemiptera: Coccidae) (Daane *et al*., 2012; Almeida *et al*., 2013).

In New Zealand and South Africa, GLRaV-3 is the most widespread and destructive virus of *Vitis* and its control is a priority (Charles *et al*., 2006; Pietersen *et al*., 2013). Therefore, as well as striving for better vector management, increasing numbers of vineyard owners in both countries are roguing (removing) infected vines as a means of controlling GLRaV-3 (Pietersen *et al*., 2013; Bell, 2015). However, the natural variability of symptom expression in vine foliage is known to hinder roguing efficiency (Maree *et al*., 2013). For example, in rootstocks and many white berry cultivars, foliar symptoms are absent, making visual diagnosis of GLRaV-3 unreliable. At Vergelegen Wine Estate in South Africa, GLRaV-3 is controlled in white cultivars by testing all vines annually by enzyme-linked immunosorbent assay (ELISA) to identify infected vines for roguing (Pietersen *et al*., 2013). Similarly in New Zealand, estimates of virus incidence in Sauvignon blanc, the most commonly grown white berry cultivar, are achieved by ELISA testing (Cohen *et al*., 2012). However, with effective GLRaV-3 control reliant upon annual ELISA testing, often encompassing multiple planted sites, most owners are unable or unwilling to justify this expense. Thus, with no means of being able to identify individual infected vines cost-effectively, management of GLRaV-3 in white berry cultivars remains problematic, with best practice being to plant clean, virus-tested vines and to control the insect vectors (Pietersen *et al*., 2013).

Red wine production in both countries is currently dominated by only a few cultivars, including Merlot, Syrah (known as Shiraz in South Africa) and Cabernet Sauvignon, together with Pinot noir and Malbec in New Zealand and Pinotage in South Africa. In these cultivars post-véraison, GLRaV-3-infected vines are broadly characterised by distinctive dark red downward curling leaves with green veins (Golino *et al*., 2002). By implication, these foliar symptoms could be used for visual diagnosis of GLRaV-3-infected vines, thus offering a potentially cost-effective and reliable means of identifying vines for roguing.

The value of visual assessments to GLRaV-3 control was recently demonstrated in Vergelegen, where annual roguing of symptomatic red berry vines culminated in year-on-year reductions to virus incidence of less than 0.05% (Pietersen *et al*., 2013). Although clearly inferred from the positive outcomes, no empirical data were presented to support the relative accuracy of visual diagnostics in Vergelegen. Thus, for roguing to become an accepted management practice, vineyard owners have to be confident that visual diagnostics are effective and that the results are comparable with ELISA (Sturz *et al*., 1997). Moreover, there can be little tolerance for over-estimating virus incidence (false positives) leading to the removal of healthy vines, or indeed for under-estimating it by way of false negatives (Fox, 1997).

The objective of this research was to assess the accuracy of visual symptom identification compared with the use of ELISA in both countries and to determine if this method was reliable and sufficient for GLRaV-3 control by roguing in vineyards established in red berry cultivars.

MATERIALS AND METHODS

Site selection. The New Zealand study vineyards were located in Hawke's Bay (S39°39′, E176°52′), a horticultural region on the east coast of the North Island. The collection of vine material for testing was undertaken across five spatially distinct commercial vineyards (hereinafter identified as A, B, C, D and E), with each planted in a single red berry variety (Table 1). As part of a larger GLRaV-3 research programme, vines in the five vineyards were visually inspected for symptoms of GLRaV-3 from 2009 (2011 in block D), with this practice continuing annually thereafter. Any symptomatic vines visually identified were rogued later in the same year (Bell, 2015). In this paper, we present the results of visual inspections for each vineyard for a single year only.

In South Africa, assessments were conducted on the historic Vergelegen Wine Estate situated in Somerset West (S34°04′, E18°53′), in which roguing of GLRaV-3-infected vines had been undertaken annually since 2002. The vineyards selected were Foundation blocks within the South African Wine Grape Certification Scheme (SAWGCS) where by regulation they had to be tested by ELISA in seasons where plant material from source vineyards was to be released. In the New Zealand vineyards and Vergelegen, the vines were grown on a vertical shoot positioned trellis with two cordons.

GLRaV-3 visual symptom identification. Changes to leaf colour and morphology were used to visually identify GLRaV-3-symptomatic vines each year. In New Zealand and South Africa, this annual task was undertaken late in the growing season when symptoms are most pronounced (Bell *et al*., 2015) by the same experienced assessors (V. Bell and G. Pietersen, respectively). Using a site-specific spreadsheet, the precise position and number of symptomatic vines visually identified with GLRaV-3 were recorded as the assessor walked the length of the inter-row separating two adjacent vine rows. This process was repeated in all rows in every vineyard. From 2006, however, the assessor in South Africa drove a quad bike along the inter-row and recorded infections in both rows from one side, with only alternating inter-rows being used. Each site was visited on at least two occasions per season in New Zealand, with visits timed for the pre- and post-harvest period (March and April). In South Africa, a single site visit was conducted as late as possible in the post-harvest season but before leaf-fall (usually during April to early-May). This period coincides with the Southern Hemisphere autumn.

Comparing the results of visual diagnostics with those from ELISA. In New Zealand, the accuracy of visual diagnostics with those from ELISA was compared between 2011 and 2013 by undertaking late-season collections of vine leaves or budwood as specified by Cohen *et al*. (2012). Collections were from vineyards as per Table 1. Within 24 hours of collection, vine samples were sent by overnight courier to the Plant Virus Testing Laboratory, an ISO 17025 ELISA-accredited laboratory at The New Zealand Institute for Plant & Food Research Limited in Auckland. ELISA testing was limited to GLRaV-3 detection only, with the testing protocols using both monoclonal and polyclonal antibodies as described by Cohen *et al*. (2012). Pending the tests, vine samples were stored at 4°C.

In vineyard A in May 2011, budwood was cut from 190 Cabernet Sauvignon vines that had been visually diagnosed with leafroll virus one month earlier. An additional 10 non-symptomatic vines served as negative controls. Budwood was cut from the basal section of a single cane on each of the two cordons per vine and cut to a length of *ca*. 250 mm.

In late March 2012, a single leaf was collected from each of 768 Merlot and 1,125 Cabernet Sauvignon vines in vineyards B and C, respectively. In addition, we sought to assess the presence of GLRaV-3 in asymptomatic vines (or 'false negatives' based on visual diagnostics) by ELISA testing leaves from non-symptomatic vines. A single leaf was collected from close to the cordon of every vine in consecutive rows across 50% of each vineyard. In

Table 1. Summary of the results to validate visual assessment of Grapevine leafroll-associated virus 3 (GLRaV-3) in red berry grapevines against enzyme-linked immunosorbent assay (ELISA) testing of vine petioles, leaves or budwood collected from vineyards in Hawke's Bay, New Zealand and Vergelegen Wine Estate, Somerset West, South Africa. CS=Cabernet Sauvignon; MB=Malbec; MO=Merlot; SH=Syrah (New Zealand), Shiraz (South Africa).

Country	Vineyard ID	Cultivar	Year planted	No. of vines	Date of assessment	Tissue used for ELISA test	Total number of vines tested/assessed	ELISA positive/ visually positive (a)	ELISA negative/ visually positive (b)	ELISA positive/ visually negative (c)	ELISA negative/ visually negative (d)	Sensitivity (%)[1]	95% confidence Interval	Specificity (%)[2]	95% confidence interval	Positive Predictive value (%)[3]	Negative Predicative value (%)[4]
New Zealand																	
	A	CS	2006	4204	02-05-2011	Budwood	200	184	6	0	10	100.0	98.2-100.0	62.5	35.4-84.8	96.8	100.0
	B	MO	1997	1536	31-03-2012	Leaves	768	5	0	0	763	100.0	47.8-100	100.0	99.5-100.0	100.0	100.0
	C	CS	1999	2251	31-03-2012	Leaves	1125	3	0	2	1120	60.0	14.7-94.7	100.0	99.7-100.0	100.0	99.8
	D	SH	2002	1625	05-04-2013	Leaves	1005	15	0	5	985	75.0	50.9-91.3	100.0	99.6-100.0	100.0	99.5
	E	MB	2002	3072	01-05-2013	Budwood	130	50	0	2	78	96.2	86.8-99.5	100.0	95.4-100.0	100.0	97.5
South Africa																	
	Rooiland 6	SH	2002	2739	22-03-2005	Petioles	2739	4	4	2	2729	66.7	22.3-95.7	99.9	99.6-99.9	50.0	99.9
				2723	05-04-2006	Petioles	2723	22	3	0	2698	100.0	84.6-100.0	99.9	99.7-100.0	88.0	100.0
				2659	29-04-2010	Petioles	2659	0	2	0	2657	n/a	n/a	99.9	99.7-100.0	n/a	100.0
				2657	01-05-2011	Petioles	2657	2	0	0	2655	100.0	15.8-100.0	100.0	99.9-100.0	100.0	100.0
	Rooiland 7	CS	2002	4495	26-04-2004	Petioles	4495	13	12	0	4470	100.0	75.3-100.0	99.7	99.5-99.9	52.0	100.0
				4470	04-05-2005	Petioles	4470	6	3	1	4460	85.7	42.1-99.6	99.9	99.8-100.0	66.7	99.9
				4460	05-04-2006	Petioles	4460	7	8	2	4443	77.8	39.9-97.2	99.8	99.7-99.9	46.7	99.9
				4435	01-05-2011	Petioles	4435	0	0	0	4435	n/a	n/a	100.0	99.9-100.0	n/a	100.0
	Rooiland 8.1	CS	2002	2681	22-03-2005	Petioles	2681	1	1	0	2679	100.0	2.5-100.0	100.0	99.8-100.0	50.0	100.0
				2679	05-04-2006	Petioles	2679	3	0	5	2671	37.5	8.5-75.5	100.0	99.9-100.0	100.0	99.8
	Rooiland 8.2	CS	2002	2665	04-05-2005	Petioles	2665	0	0	0	2665	n/a	n/a	100.0	99.9-100.0	n/a	100.0
				2665	04-04-2006	Petioles	2665	0	0	0	2665	n/a	n/a	100.0	99.9-100.0	n/a	100.0
				2656	08-04-2008	Petioles	2656	0	0	0	2656	n/a	n/a	100.0	99.9-100.0	n/a	100.0
	Rooiland 9.1	CS	2002	2810	05-05-2005	Petioles	2810	0	0	0	2810	n/a	n/a	100.0	99.9-100.0	n/a	100.0
				2810	06-04-2006	Petioles	2810	1	0	1	2808	50.0	1.3-98.7	100.0	99.9-100.0	100.0	99.9
				2799	09-04-2008	Petioles	2799	1	0	0	2798	100.0	2.5-100.0	100.0	99.9-100.0	100.0	100.0
				2796	01-05-2011	Petioles	2796	0	0	0	2796	n/a	n/a	100.0	99.9-100.0	n/a	100.0
	Rooiland 9.2	SH	2002	3020	05-05-2005	Petioles	3020	0	0	0	3020	n/a	n/a	100.0	99.9-100.0	n/a	100.0
				3020	04-04-2006	Petioles	3020	0	0	4	3016	0.0	0.0-60.2	100.0	99.9-100.0	n/a	99.9
				3011	01-05-2011	Petioles	3011	0	0	1	3010	0.0	0.0-97.5	100.0	99.9-100.0	n/a	99.9
	Rooiland 10.1	CS	2002	3020	05-05-2005	Petioles	3020	0	0	2	3018	0.0	0.0-84.2	100.0	99.9-100.0	n/a	99.9
				3018	04-04-2006	Petioles	3018	0	0	2	3016	0.0	0.0-84.2	100.0	99.9-100.0	n/a	99.9
				3000	08-04-2008	Petioles	3000	2	1	0	2997	100.0	15.8-100.0	99.9	99.8-100.0	66.7	100.0
	Rooiland 10.2a	CS	2002	1353	05-05-2005	Petioles	1353	0	0	1	1352	0.0	0.0-97.5	100.0	99.7-100.0	n/a	99.9
				1352	04-04-2006	Petioles	1352	0	0	1	1351	0.0	0.0-97.5	100.0	99.7-100.0	n/a	99.9
				1339	29-04-2010	Petioles	1339	1	0	0	1338	100.0	2.5-100.0	100.0	99.7-100.0	100.0	100.0
				1338	01-05-2011	Petioles	1338	0	0	0	1338	n/a	n/a	100.0	99.7-100.0	n/a	100.0
	Rooiland 10.2b	CS	2002	665	05-05-2005	Petioles	655	0	0	0	655	n/a	n/a	100.0	99.4-100.0	n/a	100.0
				665	04-04-2006	Petioles	655	0	0	1	654	0.0	0.0-97.5	100.0	99.4-100.0	n/a	99.8
				654	29-04-2010	Petioles	654	0	0	0	654	n/a	n/a	100.0	99.4-100.0	n/a	100.0
				654	01-05-2011	Petioles	654	0	0	0	654	n/a	n/a	100.0	99.4-100.0	n/a	100.0
	Rooiland 11.1	CS	2002	3719	06-05-2005	Petioles	3719	0	0	0	3719	n/a	n/a	100.0	99.9-100.0	n/a	100.0
				3719	04-04-2006	Petioles	3719	0	0	1	3718	0.0	0.0-97.5	100.0	99.9-100.0	n/a	99.9
	Rooiland 11.2	SH	2002	4263	06-05-2005	Petioles	4263	0	0	0	4263	n/a	n/a	100.0	99.9-100.0	n/a	100.0
				4263	04-04-2006	Petioles	4263	2	0	0	4261	100.0	15.8-100.0	100.0	99.9-100.0	100.0	100.0
				4261	17-04-2007	Petioles	4261	3	6	0	4252	100.0	29.2-100.0	99.9	99.7-99.9	33.3	100.0
				4240	01-05-2011	Petioles	4240	0	0	0	4240	n/a	n/a	100.0	99.9-100.0	n/a	100.0
	Rooiland 12.1	MO	2002	3901	06-05-2005	Petioles	3901	0	0	1	3900	0.0	0.0-97.5	100.0	99.9-100.0	n/a	99.9
				3900	05-04-2006	Petioles	3900	0	0	1	3899	0.0	0.0-97.5	100.0	99.9-100.0	n/a	99.9
Totals							114782	325	46	35	114376						

[1] Sensitivity = a/(a+c); [2] Specificity = d/(b+d); [3] Positive Predictive Value = a/(a+b); [4] Negative Predictive Value = d/(c+d); n/a = not applicable.

early April 2013, this protocol was repeated in vineyard D, which was planted in Syrah vines. A single leaf was taken from each of 1,005 vines (62% of all vines).

In vineyards B, C and D, the sampled leaves from every GLRaV-3-symptomatic vine and the non-symptomatic within-row vines either side of it, were individually tested by ELISA. The remaining non-symptomatic leaves from each bag of twenty leaves were then tested as a composite sample using protocols modified by Cohen *et al.* (2012). Composite samples that tested positive were re-tested as composites of five leaves and finally, single-vine extracts were prepared to identify the individual infected vine(s).

In vineyard E in May 2013, budwood from 50 Malbec vines visually identified with GLRaV-3 one month earlier was collected in the same manner as described for vineyard A. Previous research indicated that GLRaV-3 spread was generally clustered around earlier infections, with adjacent within-row vines the most likely to become infected (Habili and Nutter, 1997; Pietersen *et al*., 2013; Bell, 2015). Therefore, to assess the prevalence of asymptomatic infections within a row, budwood was taken from a further 80 vines immediately adjacent to those vines rogued either in 2012 or from those yet to be rogued in 2013. At the time of sampling, none of the 80 vines had visible symptoms of GLRaV-3.

In South Africa, all vineyards that were being ELISA-tested by Vititec in accordance with SAWGCS requirements for foundation vineyards, were also visually assessed. The petioles of three basal leaves were collected from each vine in early-April to early-May. The petioles of ten such vines were pooled as a single composite sample for testing by ELISA. The ELISA conducted was based on the antisera of Goszczynski *et al.* (1995) and tests were done for GLRaV-1, –2 and –3 in a single test. In instances where viruses were tested separately, results referred only to GLRaV-3 infection, with no instances of GLRaV-1 and –2 found on Vergelegen (G. Pietersen, unpublished results). When the composite preparation yielded a positive result, all the individual vines making up the composite sample were tested separately to identify the specific infected vine(s). Where GLRaV-3 was detected, the vine was rogued during the subsequent winter (usually within one month).

Analysis. The sensitivity (probability of a positive assessment when GLRaV-3 is present) and specificity (probability of a negative assessment when GLRaV-3 is absent) (Lalkhen and McCluskey, 2008) were calculated by comparing visual assessments to detect GLRaV-3 with the results of ELISA, the "gold standard" method specified in the New Zealand Winegrowers Grafted Grapevine Standard (Anonymous, 2006) and in the SAWGCS (Almeida *et al*., 2013). Percentages were calculated separately for each vineyard. GenStat (version 16, VSN International Ltd, Hemel Hempstead, UK) and MedCalc® Statistical Software (www.medcalc.org/calc/diagnostic_test.php) were used for the following calculations (see Table 1): Sensitivity = a/(a+c); Specificity = d/(b+d); Positive Predictive Value = a/(a+b); Negative Predictive Value = d/(c+d); where a) ELISA-positive, visually positive samples, b) ELISA-negative, visually positive samples, c) ELISA-positive, visually negative samples and d) ELISA-negative, visually negative samples. All values are expressed as percentages. Confidence Intervals are 'exact' Clopper-Pearson confidence intervals.

RESULTS

The number of vines which a) tested positive by ELISA and displayed symptoms, b) tested negative by ELISA but had displayed symptoms for each evaluation season and vineyard, c) tested positive by ELISA but had not displayed symptoms and d) tested negative by ELISA and did not display symptoms, are presented in Table 1. The sensitivity, specificity, positive predictive value and negative predictive value are provided in the same table.

Of the 114,782 vines assessed visually and tested by ELISA, the two methods were in agreement for 114,701 (99.9%) vines, with only 81 vines yielding differing results. Of these, 35 were vines that were positive in ELISA but visually they were non-symptomatic. In the other 46 vines, symptoms were observed but the vines tested negative in ELISA. Because of a low annual incidence of infection in the majority of vineyards analysed, the sensitivity of visual detection compared with ELISA could be determined in only 29 instances. In 11 of these, visual detection of symptoms was perfectly correlated with ELISA results (sensitivity 100%). In 10 annual vineyard analyses, a total of 15 infected vines (considered newly infected as they had not tested positive the previous year) were detected by ELISA. Foliar symptoms were not observed in any of these vines in the season of analysis. In the last eight annual vineyard analyses where sensitivity could be determined, it ranged from 37.5 to 96.2% because of the presence of a number of vines without symptoms being detected by ELISA. The specificity of visual symptom identification compared with ELISA was higher than 99.7% in 43 of the 44 annual vineyard analyses, while the remaining annual vineyard analysis had a specificity of 62.5%. Presence of symptoms as a predictor of negative ELISA was above 97.5% in all 44 annual vineyard analyses but as a positive predictor, it was 100% in 10 of 19 annual vineyard analyses where this could be determined. In the remaining nine annual vineyard analyses, positive predictor values ranged from 37.5 to 96.8%.

DISCUSSION

For the red berry cultivars affected by GLRaV-3, visual symptom identification was an accurate method for

identifying infected vines, both in terms of its sensitivity and specificity. Agreement between visual symptom identification and ELISA of 99.93% was observed amongst 114,782 vines analysed, with only 81 vines (0.07%) having conflicting results.

By undertaking visual symptom identification, owners aim to identify GLRaV-3-infected vines reliably, cost-effectively and in a timely manner allowing them to remove sources of virus inoculum quickly. If achieved, owners can greatly reduce the incidence of virus foci and lower the risk of vector-mediated virus transmission to healthy vines (Pietersen *et al*., 2013; Bell, 2015). Our results suggest factors like poorly defined and delayed symptom development, or latent and asymptomatic infections, do not adversely influence GLRaV-3 control.

Where ELISA detected GLRaV-3 in asymptomatic vines, this may be due to the relatively recent infection of such vines, with ELISA capable of detecting GLRaV-3 before foliar symptoms are expressed, i.e. earlier in the so-called latency period. Such a scenario can be expected where roguing is being applied efficiently and hence only relatively new infections exist. Additionally, late infection in the season is the most difficult to detect as there are few or no symptoms and little time prior to senescence. However, these vines are likely to express symptoms in the following year (Bell *et al*., 2015). With just 35 latently infected vines detected in this study, it seemed there was minimal under-estimation of GLRaV-3 incidence. In vineyards C and D in New Zealand for example, 2130 vines were assessed but just seven ELISA-positive, visually negative vines were detected (0.3%). It is unclear if these vines completely lacked foliar symptoms at the time of monitoring or if symptom development was so rudimentary and limited to a small number of leaves per vine, that they were simply overlooked. Irrespective of cause, the long term effect of a relatively small number of asymptomatic infected vines is unlikely to influence GLRaV-3 control adversely when effective vector management and sustained annual roguing are implemented, as was demonstrated by Pietersen *et al*. (2013) and Bell (2015).

We also found that foliar symptoms predicted GLRaV-3 infection reliably, with just 46 vines observed with symptoms that subsequently tested negative in ELISA. In this regard, the notable exception was vineyard A in New Zealand where specificity of 62.5% was low compared with that found in other study vineyards. In 2011, GLRaV-3 was visually identified in 190 Cabernet Sauvignon vines but only 184 of them were positive by ELISA. However, upon re-inspection of the six vines 12 months later, the characteristic foliar symptoms of GLRaV-3 were observed in five of them (vineyard personnel removed the vines before ELISA testing could be undertaken). It is possible that the ELISA tests in 2011 generated false negatives caused by an uneven distribution of GLRaV-3 (Rowhani *et al*., 1997; Cohen *et al*., 2004) and if so, the virus may have been present in canes not sampled for ELISA. Other explanations include the possibility of a GLRaV-3 serotype with low titres; or antibody affinity resulting in poor ELISA detection (Cohen *et al*., 2012); or foliar symptoms caused by other leafroll associated viruses; or the symptoms were misidentified and were caused by other factors producing foliar changes similar to those of GLRaV-3 (e.g. some mineral deficiencies) (Bell, 2015).

Under the conditions tested in New Zealand and South Africa, we now have evidence that vineyard owners do not need to validate field assessments of GLRaV-3 with supporting ELISA tests (though ELISA is required when used to corroborate visual assessments during training). Instead, in the red berry cultivars described here, owners can be confident that visual diagnostics usefully underpin integrated virus management when undertaken by trained personnel. In terms of the SAWGCS at least, we are now recommending that assessment for GLRaV-3 of red-berried foundation vineyards no longer be done with ELISA, but should instead be based upon foliar inspections for symptoms only, using trained personnel. By adopting this method, resources, funds and the finite testing period in autumn can be directed towards ELISA testing larger numbers of vines among green-berried cultivars.

In conclusion, this study supports the use of visual symptom identification as a reliable indicator of GLRaV-3 in several red berried grapevine cultivars. Visual assessments by trained personnel timed for late in the growing season can greatly improve prospects of identifying most sources of virus inoculum so that they can be removed quickly. Importantly, we found that in two winegrowing countries with contrasting environments, this outcome was achievable without incurring ELISA or other laboratory test-related costs and delays. Under the conditions tested in New Zealand and South Africa, the finding of a low incidence of asymptomatic vines indicated they pose a low risk to overall virus identification and virus control, particularly when supported by effective vector management. Although our data support the efficacy of visual diagnostics, it remains to be seen if outcomes are influenced by grapevine cultivars not part of this study, by GLRaV-3 genetic variants, or different cultivar/variant combinations. Hence, research is needed to assess the extent to which these factors might influence the efficacy of visual diagnostics for GLRaV-3 control.

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