Journal of Plant Pathology (2018) 100:399–408
https://doi.org/10.1007/s42161-018-0085-z

ORIGINAL ARTICLE

Vineyard-wide control of grapevine leafroll-associated virus 3 requires an integrated response

Vaughn A. Bell[1] · Duncan I. Hedderley[2] · Gerhard Pietersen[3] · Philip J. Lester[4]

Received: 14 February 2018 / Accepted: 18 May 2018 / Published online: 12 June 2018

Abstract

Grapevine leafroll-associated virus 3 (GLRaV-3) negatively alters grape yield and wine quality but adopting practical control actions could avert an epidemic. In 13 New Zealand commercial vineyards that were planted with one of five red berry cultivars ($n = 29{,}943$ vines), we assessed if roguing (removing) GLRaV-3-infected vines could reduce and maintain incidence at <1%. In 2009, baseline GLRaV-3 incidence ranged from 4 to 24%. Annually until 2015, we visually diagnosed and rogued vines with foliar symptoms of GLRaV-3, and monitored vine populations of the virus vector, the mealybug *Pseudococcus calceolariae*. In 2009, 2544 symptomatic vines (12%) were rogued but with incidence declining year-on-year, just 408 vines (1.4%) were rogued in 2015. Mapping virus spread annually showed within-row vines immediately either side of an infected vine ('first' vines) were most at risk of vector mediated transmission, but a temporal decline in these infections was observed. In 2010, 26% of 'first' vines had foliar symptoms, reducing to 6% by 2015. Overall, GLRaV-3 management outcomes were variable. In six vineyards, symptomatic vine incidence reduced to <1% within 3 years of roguing commencing. By contrast, roguing did not contain virus spread in another two vineyards, where cumulative vine losses of 37 and 46% to 2011 and 2013, respectively, was deemed economically unsustainable by the owners who removed all remaining vines. In the remaining five vineyards, annual incidence was consistently >1%. In demonstrating the importance of low vector pressure to successful virus control, we emphasise the need to adopt a multi-tactic response targeting virus and vector populations annually.

Keywords *Vitis vinifera* · GLRaV-3 · Roguing · Mealybug vector *Pseudococcus calceolariae*

Introduction

One of the most economically important and best studied viral diseases of *Vitis vinifera* L. (Vitaceae) is grapevine leafroll disease, with which grapevine leafroll-associated virus 3 (GLRaV-3) is primarily associated (Maree et al. 2013). A type member of the genus *Ampelovirus* (family *Closteroviridae*), GLRaV-3 occurs in all major winegrowing regions of the world (Martelli 2014). Of the viruses affecting *Vitis* in New Zealand, GLRaV-3 is the most widespread and most destructive (Charles et al. 2006), with the potential to adversely alter quantitative and qualitative parameters of grape and wine production (Endeshaw et al. 2014).

GLRaV-3 is a phloem-limited virus initially believed to be transmitted solely through the use of infected propagating material (Sheu 1936). Critical to mitigating this risk was the development of grapevine certification schemes in New Zealand and elsewhere in the world (Almeida et al. 2013). Certification has improved the health of the planting material supplied to owners, thus significantly reducing the risk of primary spread of virus and virus-like diseases such as GLRaV-3 (Walter and Martelli 1997). However, the advances achieved by certification can be quickly negated by vine to vine transmission of GLRaV-3 by insect vectors, as shown by Engelbrecht and Kasdorf (1990) with the mealybug *Planococcus ficus* (Hemiptera:

✉ Vaughn A. Bell
Vaughn.Bell@plantandfood.co.nz

[1] The New Zealand Institute for Plant & Food Research Limited, Private Bag 1401, Havelock North 4157, New Zealand

[2] The New Zealand Institute for Plant & Food Research Limited, Private Bag 11 030, Palmerston North 4442, New Zealand

[3] Agricultural Research Council-Plant Protection Research Institute, c/o Department of Microbiology and Plant Pathology and Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa

[4] Victoria University of Wellington, P O Box 600, Wellington 6140, New Zealand

Pseudococcidae). Multiple species of phloem-feeding mealybugs and soft scale insects (Coccidae) have since been identified as vectors of GLRaV-3 (Daane et al. 2012; Herrbach et al. 2017). In New Zealand, the mealybugs *Pseudococcus calceolariae* and *P. longispinus* are commonly found in vineyards (Charles et al. 2010). Both species are competent vectors of GLRaV-3 (Petersen and Charles 1997).

With no known cure for GLRaV-3 in the vineyard, roguing (removing) infected vines has become an important management option around the world (Almeida et al. 2013; Pietersen et al. 2013, 2017). In red berry cultivars, the identification of vines for roguing is aided by foliar changes characterised by dark red downward curling leaves with green veins (Golino et al. 2002). These changes late in the growing season reliably predicted GLRaV-3 infection, with the results of visual symptom identification shown to be comparable with enzyme linked immunosorbent assay testing (Bell et al. 2017). Conversely, many white berry cultivars remain symptomless when infected with GLRaV-3 and are thus difficult to visually detect (Maree et al. 2013). For this reason, our research was limited to red berry cultivars.

The New Zealand wine sector has long recognised grapevine leafroll disease as a serious threat to production (McKissock 1964) but only recently has research started to quantify the economic costs associated with mitigation. Data from New Zealand vineyards affected by GLRaV-3 together with simulation studies from the USA, concluded that when virus incidence was low, roguing symptomatic vines early improved income and profitability relative to the other management scenarios tested (Atallah et al. 2012; Nimmo-Bell 2006). However, when virus incidence exceeded 20%, roguing was considered uneconomic in New Zealand (Hoskins et al. 2011), while a 25% threshold was proposed in the USA (Atallah et al. 2012).

The implementation of practical measures in South Africa were the first to demonstrate vineyard-wide control of GLRaV-3 (Pietersen et al. 2013). After planting certified virus-free vines, any new symptomatic vines that appeared were quickly rogued, with insecticides applied to control the mealybug vectors. Between 2002 and 2012, this integrated (multi-tactic) response culminated in vineyard-wide virus incidence of <0.03% (Pietersen et al. 2013).

In the present study, we sought to build upon these virus management protocols. However, unlike the very low (<2.5%) and very high virus incidence (100%) reported by Pietersen et al. (2013), the vineyards in this study were characterised by GLRaV-3 incidence at discovery ranging from low (4%) to moderately high (24%). Based on this critical distinction between studies, we sought to determine if integrated virus management using roguing was compatible with winegrowing conditions and vineyard practices in New Zealand. We also monitored mealybug vectors on the vines, with the varying management approaches adopted by different owners providing insights into the relationship between vector abundance and virus control outcomes. Hence, in view of this variable, our objective was to determine if roguing could reduce and maintain annual GLRaV-3 incidence at <1%.

Methods and materials

Study vineyard selection In 2009, we asked the owners of New Zealand commercial vineyards to participate in this study. The 13 owners that volunteered were based in Hawke's Bay (39°39′S 176°52′E), a horticultural region on the east coast of the North Island. Each study vineyard (hereinafter identified as A to O) was planted with one of five red berry cultivars (Table 1).

Table 1 Summary of grapevine cultivar, planting date, the number of vines and baseline incidence of grapevine leafroll-associated virus 3 (GLRaV-3) in the study vineyards

Vineyard ID	Grapevine cultivar	Clone (rootstock)	Year planted	No. of vines	GLRaV-3 incidence (%)[a]	GLRaV-3 monitoring
A	Merlot	6 (SO4)	1997	1536	4.0	2009–2015
B	Cabernet Sauvignon	LC10 & 15 (3309)	2003	3262	24.1	2009–2015
C	Merlot	481 (3309)	2000	1040	10.6	2009–2015
D	Cabernet Sauvignon	LC10 (3309)	2006/7	4204	9.3	2009–2015
E	Cabernet Sauvignon	Erindale & 7 (101–14; 3309)	1999	2251	16.0	2009–2015
F	Malbec	1056 & 595 (101–14; RG)	2002	3072	8.6	2009–2015
I	Cabernet Sauvignon	420A (101–14)	1993/4	1584	15.1	2009–2011
J	Merlot	481 (3309)	2000	2410	8.7	2009–2015
K	Cabernet Sauvignon	420A (RG)	2000	1243	9.9	2009–2013
L	Syrah	MS4012 (RG)	1999	2354	7.1	2010–2015
M	Syrah	MS (101–14)	2001	3118	7.9	2011–2015
N	Syrah	383 (101–14)	2002	1625	22.2	2011–2015
O	Pinot noir	667 (3309)	2004	2221	9.5	2012–2015

GLRaV-3 incidence data from the outset of our research will have included vines that acquired the disease during the interval between initial planting and the start of this research

Vineyards G & H were lost to the study in 2011 for reasons unrelated to GLRaV-3

[a] As determined by visual symptom identification from the number of vines present in 2009

In 2009, GLRaV-3 affected all the vineyards: incidence ranged from 4 to 24%. The vines were grown on a vertical shoot positioned trellis on either one or two cordons.

In screening for GLRaV-3, the New Zealand grafted grapevine standard aims to minimise the risk of infected propagating material being supplied to the sector (Anonymous 2006). However, other than vineyard D, the vines in all the vineyards in 2009 were planted before the strict implementation of the New Zealand certification system, meaning that in most study vineyards GLRaV-3 may have been introduced during initial planting. Replacement vines planted during this study were sourced from nurseries accredited to the New Zealand grafted grapevine standard (V. Bell unpublished data).

With the exception of two certified organic vineyards (C, E), where mealybug insecticides were not used, the vineyards were conventionally managed, with the use of registered pesticides legally permitted by New Zealand Winegrowers, the national industry body. However, other than recommending the adoption of mealybug insecticide best practice, we had no direct influence on this aspect of the study.

During this study, the owners of vineyards I and K decided the negative economic influence of GLRaV-3 necessitated removing all the remaining 'healthy' vines in 2011 and 2013, respectively. We report on the virus incidence and mealybug abundance data collected up to these dates. Also in 2011, vineyards G and H were lost to the study for commercial reasons unrelated to GLRaV-3. Those data are not reported.

To counter these losses, additional vineyards were added to the study. Three vineyards were planted with Syrah vines, with GLRaV-3 data added from 2010 (Vineyard L) and 2011 (M, N). In the fourth vineyard planted in Pinot noir vines (O), GLRaV-3 data were added from 2012. Mealybug data were collected from 2012 from these vineyards.

GLRaV-3 identification and mapping Among red berry cultivars, visual diagnostics based on late-season changes to leaf colour was used to detect GLRaV-3 infection, which is a method shown to be reliable (Bell et al. 2017). The process was standardised by the same experienced assessor (V. Bell) walking the vineyard rows in April each year (Southern Hemisphere autumn). The symptomatic vines were rogued annually by vineyard personnel.

We monitored the spatio-temporal spread of the virus throughout each study vineyard. We refer to those vines immediately surrounding a GLRaV-3-symptomatic vine as 'nearest neighbours', of which there was a maximum of 10 per infected vine (Fig. 1). The 'first' and 'second' vines were within-row positions; 'opposite' and 'diagonal' vines were across-row positions. A fifth position, the 'random' infection, represented a symptomatic vine spatially distinct from the 'nearest neighbours'.

In recording the precise location of vines with GLRaV-3 symptoms, we also recorded the numbers and position of each

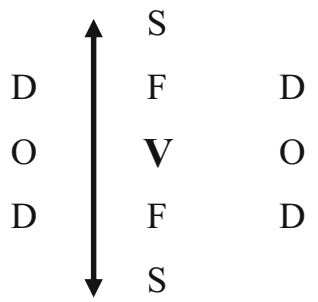

Fig. 1 A diagrammatic view of three vine rows showing the position of the 10 'nearest neighbour' vines relative to a grapevine leafroll-associated virus 3 (GLRaV-3)-symptomatic vine (V). There were four 'nearest neighbour' positions: within-row 'first' (F) and 'second' (S) vines; across-row 'opposite' (O) and 'diagonal' (D) vines. Vine trunks within rows were 1.8 m apart; row width ranged from 2.0–3.0 m, depending on the study vineyard. Black arrow denotes the direction of the vine rows

'nearest neighbour' vine with potential to be infected but which had no virus symptoms at the time of monitoring. The incidence of 'random' infections was a percentage of all other symptomatic, non-'nearest neighbour' vines.

Three assumptions underpinned virus transmission from an infected vine to the 'nearest neighbour' vines: (1) where a new symptomatic vine was a 'nearest neighbour' of vines rogued over multiple years, the probable GLRaV-3 infection pathway was attributed to the vine, or vines, rogued most recently and then in the following order: 'first', 'second', 'opposite', 'diagonal'; (2) where a new symptomatic vine had two or more 'nearest neighbours' rogued in the same year, the most probable GLRaV-3 infection pathway was attributed in the following order: 'first', 'second', 'opposite', 'diagonal'; and (3) any symptomatic vine or aggregation of two or more symptomatic vines not identified as a 'nearest neighbour' vine(s), was classified as a 'random' infection(s).

Mealybug monitoring Annually between 2010 and 2015, vine leaves were collected from the study vineyards to assess mealybug numbers. Collections were undertaken just before harvest in the austral autumn period of mid- to late-March, which generally coincides with the emergence of the third and final generation of *P. calceolariae* and *P. longispinus* (Charles 1981). Leaf collections were from vines widely dispersed in each vineyard. One leaf per vine was randomly collected from within a 10–15 cm band around the cordon. A total of 400 leaves were collected per vineyard per year (except for 2010, when 300 were collected). Each leaf was inspected under a binocular microscope in the laboratory. An absolute count of all life stages enabled a measure of the numbers of mealybugs per 100 vine leaves inspected. In most cases, mealybugs were visually identified to species level.

Statistics The GLRaV-3 incidence was expressed as the number of symptomatic vines in a particular position ('first', 'second', 'opposite', 'diagonal', 'random') compared with the total

number of vines assigned to that position, and analysed using generalised linear models with binomial distribution. Data on changes to GLRaV-3 incidence over time were analysed for each position (using data from all vineyards), with vineyard and year as categorical variables. The variation in trends between vineyards (the vineyard * year interaction) was used to check for over-dispersion. Data on the difference between the position within each vineyard and year were analysed with position as a categorical variable. After 6 years of continuous GLRaV-3 management, the study vineyards were divided on the basis of virus management outcomes: among six group 1 vineyards (A, C, E, J, M, and O), GLRaV-3 control was effective and was sustained at <1% for a minimum of two consecutive years; in the seven group 2 vineyards, virus control by roguing was either ineffective (I and K) or annual virus incidence remained >1% when data collection ceased in 2015 (B, D, F, L, and N). Comparisons between mealybug numbers (average numbers per 100 vine leaves) as a subset of group 1 and group 2 vineyards were analysed using generalised linear models with Poisson distribution and estimating the dispersion from vineyard to vineyard variation within both groups. All analyses used GenStat (version 14, 2011, VSNi Limited).

Results

Among the nine study vineyards in 2009, a total of 2544 vines were visually identified with GLRaV-3 (11.7%) (Fig. 2). After correcting for the removal of all residual 'healthy' vines from vineyards I (2011) and K (2013), the addition of data from vineyards L (2010), M, N (2011), and O (2012), and the periodic planting of replacement vines (A, B, D, E, F, J, and M), GLRaV-3 incidence progressively declined: of the 11 vineyards in 2015, 408 symptomatic vines were visually identified (1.4%).

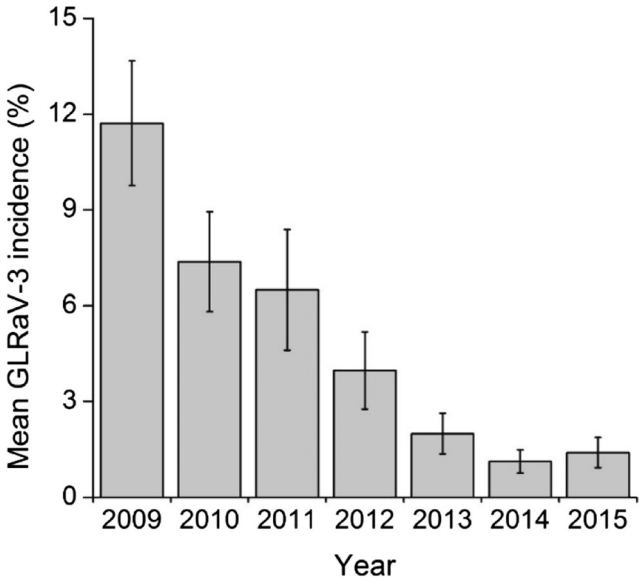

Fig. 2 Mean (±SEM) grapevine leafroll-associated virus 3 (GLRaV-3) incidence recorded in the study vineyards, 2009 to 2015 ($n = 9$ vineyards in 2009, 10 in 2010, 12 in 2011, 2012 and 2013, and 11 in 2014 and 2015). Data reflect the loss of two study vineyards, the addition of data from newly added study vineyards, and the planting of replacement vines

After seven years of data collection, it was clear that some study vineyards had successfully managed GLRaV-3 while in others, the outcomes were less successful. To identify the aspect (or aspects) of the integrated management response that might best explain these differing outcomes, the study vineyards were divided into two groups. In group 1 vineyards (A, C, E, J, M, and O), annual GLRaV-3 incidence reduced to <1% within 3 years of roguing commencing, where it was sustained until monitoring concluded in 2015 (Fig. 3a). Cumulative vine loss slowed from 2012, as represented by the slope of the lines being zero or close to it (Fig. 3c).

Among the group 2 vineyards (B, D, F, L, and N), the effectiveness of roguing was inconclusive, with annual virus incidence >1% through until the conclusion of data collection in 2015 (Fig. 3b). Cumulative vine losses increased year-on-year (Fig. 3d). That no symptomatic vines were removed from vineyard F in 2013 and 2014 represented the only departure from the roguing protocol in this study.

Of the remaining group 2 vineyards (I and K), annual virus incidence was persistently high (Fig. 3b), with cumulative vine losses of 37% by 2011 and 46% by 2013, respectively (Fig. 3d). The inability of roguing to adequately contain virus spread resulted in reduced economic viability of both vineyards. Hence, the owner of vineyard I decided to remove all the remaining 'healthy' vines in July 2011 (Southern Hemisphere winter); the same decision was taken by the owner of vineyard K in July 2013.

Of the 2544 symptomatic vines rogued in 2009, 742 vines were rogued from the group 1 vineyards (10.3% of vines in those vineyards), and 1802 from the group 2 vineyards (13.5% of vines in those vineyards). By 2015, 408 vines were rogued, with 40 from group 1 (0.3% of vines) and 368 from group 2 vineyards (2.5% of vines). Early in the study, the percentages between the two groups were not significantly different, but from 2013 the percentage of rogued vines in group 1 vineyards was significantly lower than for the group 2 vineyards (binomial generalised linear model: 2009 $p = 0.483$; 2010 $p = 0.968$; 2011 $p = 0.071$; 2012 $p = 0.069$; 2013 $p = 0.001$, 2014 $p = 0.001$; 2015 $p = 0.001$).

'Nearest neighbour' Vineyard-specific analyses showed that nearest neighbour vines were significantly more likely to be GLRaV-3-infected compared with the 'random' position (binomial generalised linear models $p < 0.001$; Table 2). When excluding 'random' infections, significant differences in virus incidence were detected among the 'nearest neighbours', with 'first' vines at higher risk of GLRaV-3 infection (Table 2). However, among the group 1 vineyards in particular, this

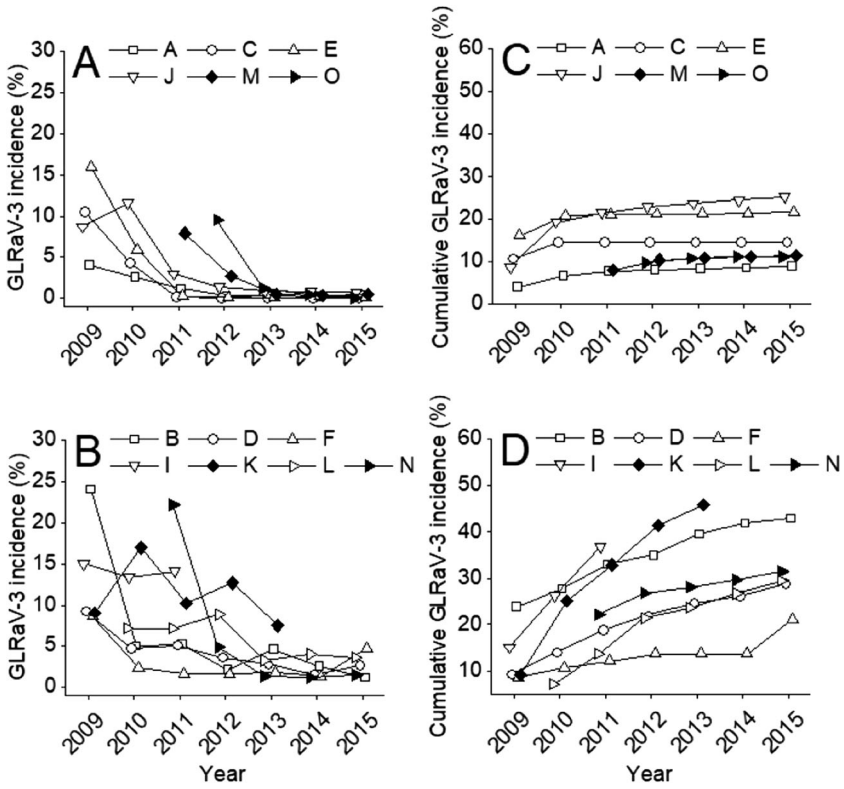

Fig. 3 Percent annual grapevine leafroll-associated virus 3 (GLRaV-3) incidence (graphs **a** and **b**) and cumulative GLRaV-3 incidence (graphs **c** and **d**) in the 13 study vineyards, 2009 to 2015. In group 1 vineyards (graphs **a** and **c**), GLRaV-3 incidence reduced to <1% where it was sustained for at least two consecutive years up until 2015. In group 2 vineyards (graphs **b** and **d**), GLRaV-3 control was either ineffective (I and K) or annual virus incidence was >1% (B, D, F, L, and N). Data included the planting of replacement vines and the addition of vineyards L, M, N, and O after 2009. Data from each vineyard in 2009 included cumulative numbers of vines that will have acquired GLRaV-3 in the interval between initial planting and the commencement of this study

influence became less pronounced as annual roguing progressively reduced the numbers of infected vines.

In pooling the data from all vineyards, between year analyses within a nearest neighbour position showed significant differences in GLRaV-3 incidence (Fig. 4). 'First' vines were most at risk of GLRaV-3 in all years: in 2010, mean incidence was 26.5% but after 5 years of roguing, an average of 6.1% of 'first' vines was visually identified with virus in 2015, a between year reduction that was highly significant (binomial generalised linear model $p < 0.001$, after fitting a vineyard effect).

For all other 'nearest neighbour' positions, a similar between-year pattern of decline in GLRaV-3 incidence was observed (binomial generalised linear models; 'second' $p < 0.001$; 'opposite' $p < 0.001$; 'diagonal' $p = 0.08$, after fitting a vineyard effect).

There was also a year-on-year reduction in new 'random' outbreaks of GLRaV-3. In 2010, an average of 3.6% of all the non-'nearest neighbour' vines were visually identified with GLRaV-3 symptoms; by 2015, 'random' infections averaged 0.2%, a between year reduction that was highly significant (binomial generalised linear models $p < 0.001$, after fitting a vineyard effect).

Mealybug monitoring *Pseudococcus calceolariae* was the most abundant species found on vine leaves in all vineyards, representing c. 98% of the mealybugs visually identified to species level between 2010 and 2015. *Pseudococcus longispinus* was occasionally detected.

Among group 1 vineyards, an average of <20 mealybugs per 100 vine leaves inspected was found between 2010 and 2014 (range: 7 to 18) (Fig. 5). In 2015, the increased average to 25 mealybugs was influenced by uncharacteristic changes in vineyards M and O, where there were 92 and 43 mealybugs per 100 leaves, respectively. However, with virus incidence of <0.5% in both vineyards, the risk of vector-mediated virus spread was low.

Among the group 2 vineyards, an average of ≥39 mealybugs per 100 vine leaves (range: 39 to 93) was recorded during this study (Fig. 5). Thus, in group 2 vineyards, mealybug numbers per year were between two- and six-fold higher than group 1 vineyards but the differences were not always statistically significant due to high levels of variation (Fig. 6: generalised linear models using a Poisson distribution: 2010 $p = 0.115$; 2011 $p = 0.111$; 2012 $p = 0.010$; 2013 $p = 0.005$, 2014 $p = 0.005$; 2015 $p = 0.279$).

Discussion

This study demonstrated that roguing symptomatic vines was an effective method for controlling GLRaV-3 in red berry cultivars, particularly when supported by low numbers of mealybugs on vine leaves. In the group 1 vineyards, integrating these and other factors successfully controlled GLRaV-3 within 3 years of roguing commencing, with annual incidence sustained below 1% through until the conclusion of data

Table 2 Percent grapevine leafroll-associated virus 3 (GLRaV-3) incidence among the nearest neighbour and random positions in the 13 study vineyards. Results of fitting binomial generalised linear models in 2010 to 2015

Vineyard ID	*P* values for differences		Percent GLRaV-3 incidence by year					Approximate LSD[a]
	Between positions NN&R[b]	Between positions NN	First	Second	Opposite	Diagonal	Random	
2010								
A	<0.001	<0.001	20.7a[c]	0.0c	5.6b	1.9bc	1.3c	6.0
B	<0.001	0.006	11.1a	4.0bc	7.3ab	4.9bc	2.7c	3.6
C	0.081	0.489	7.5a	5.4ab	3.4ab	4.2ab	3.1b	5.1
D	<0.001	<0.001	10.6a	6.5b	6.4b	4.3bc	2.6c	2.9
E	<0.001	<0.001	15.3a	5.7b	3.5bc	5.3b	2.6c	3.8
F	<0.001	0.006	14.1a	6.3ab	3.8bc	1.3bc	1.8c	6.5
I	<0.001	<0.001	57.4a	26.0b	23.3b	17.1b	3.2c	10.4
J	<0.001	<0.001	42.4a	15.8b	8.8c	6.5c	8.4c	6.6
K	<0.001	<0.001	59.8a	35.0b	35.6bc	22.1c	6.8d	12.4
2011								
A	<0.001	0.285	6.3a	2.0ab	2.2ab	2.1ab	0.5b	4.2
B	<0.001	0.013	13.1a	7.4b	6.9b	7.7b	4.9b	3.9
C	0.340	0.486	0.6a	0.0a	0.0a	0.0a	0.0a	0.5
D	<0.001	<0.001	13.9a	5.7b	1.9 cd	3.8bc	1.5d	2.4
E	0.048	0.279	0.9a	0.4ab	0.0ab	0.3ab	0.0b	0.8
F	<0.001	<0.001	19.8a	5.7b	1.6b	1.7b	0.3c	5.1
I	<0.001	<0.001	47.9a	32.5b	15.5c	10.3c	2.1d	9.4
J	<0.001	<0.001	10.6a	3.4b	1.7bc	2.2b	0.6c	3.0
K	<0.001	<0.001	32.8a	13.2b	7.7bc	4.1c	2.7c	7.2
L	<0.001	<0.001	56.1a	19.3b	5.4c	2.4 cd	1.9d	6.7
2012								
A	0.675	0.397	0.8a	0.0a	0.0a	0.0a	0.3a	0.8
B	<0.001	<0.001	6.1a	1.8b	1.8b	1.0bc	0.6c	1.8
C	0.998	1.000	0.0a	0.0a	0.0a	0.0a	0.0a	0.0
D	<0.001	0.002	6.5a	3.0b	3.0b	3.2b	0.9c	1.9
E	0.107	0.234	0.5a	0.3a	0.0a	0.0a	0.0a	0.5
F	<0.001	<0.001	18.0a	2.8bc	5.5b	1.4 cd	0.3d	4.8
J	<0.001	<0.001	4.9a	0.4b	0.8b	2.9a	0.1b	1.9
K	<0.001	<0.001	29.8a	16.3b	5.9 cd	13.3bc	3.2d	7.9
L	<0.001	<0.001	38.7a	13.9bc	10.5c	18.5b	1.6d	7.0
M	<0.001	<0.001	20.5a	3.4b	2.7b	1.5bc	0.7c	3.8
N	<0.001	<0.001	25.9a	9.0b	5.4bc	2.5 cd	0.8d	5.7
2013								
A	0.846	0.112	1.6a	0.0a	0.0a	0.0a	0.3a	1.0
B	<0.001	<0.001	7.4a	2.4b	3.1b	2.6b	1.0c	2.1
C	0.998	1.000	0.0a	0.0a	0.0a	0.0a	0.0a	0.0
D	<0.001	0.166	4.4a	2.8ab	2.3b	3.2ab	1.0c	1.7
E	0.188	0.411	0.3a	0.3a	0.0a	0.0a	0.0a	0.4
F	<0.001	<0.001	19.0a	5.0b	0.5c	2.7b	0.2c	4.3
J	<0.001	0.008	2.4a	0.0bc	0.8ab	0.8ab	0.0c	1.2
K	<0.001	<0.001	20.9a	4.7b	2.0bc	5.2b	0.9c	5.6
L	<0.001	<0.001	15.1a	5.9b	2.3b	3.3b	0.6c	4.2
M	<0.001	0.002	3.8a	0.0b	0.6b	0.6b	0.1b	1.5
N	<0.001	<0.001	7.8a	0.6bc	0.9b	0.5bc	0.0c	2.3
O	<0.001	0.002	6.3a	1.9b	1.0bc	0.6bc	0.3c	2.6
2014								
A	0.332	1.000	0.0a	0.0a	0.0a	0.0a	0.1a	0.1
B	<0.001	0.007	4.0a	2.5a	2.1a	1.0b	0.7b	1.7
C	0.998	1.000	0.0a	0.0a	0.0a	0.0a	0.0a	0.0
D	<0.001	0.007	3.0a	1.7ab	1.5ab	0.8b	0.2c	1.2
E	0.051	0.413	0.3a	0.7a	0.0a	0.2a	0.0a	0.7
F	<0.001	<0.001	12.3a	2.3b	1.3b	1.3b	0.3b	3.4
J	0.192	0.042	1.4a	0.7ab	0.0b	0.2ab	0.2b	1.0
L	<0.001	<0.001	14.6a	7.0b	3.7b	5.1b	0.7c	4.4
M	0.098	0.090	1.2a	0.9a	0.3a	0.0a	0.2a	1.1
N	<0.001	<0.001	4.2a	3.0ab	0.0c	0.9bc	0.1c	2.3
O	0.183	0.037	1.9a	0.0a	0.5a	0.0a	0.2a	1.2

Table 2 (continued)

Vineyard ID	*P* values for differences		Percent GLRaV-3 incidence by year					Approximate LSD[a]
	Between positions NN&R[b]	Between positions NN	First	Second	Opposite	Diagonal	Random	
2015								
A	0.155	0.052	0.7a	0.0a	2.5a	0.0a	0.2a	1.6
B	0.024	0.076	2.3a	1.2ab	0.7b	0.8b	0.3b	1.2
C	0.998	1.000	0.0a	0.0a	0.0a	0.0a	0.0a	0.0
D	<0.001	<0.001	5.6a	2.9b	1.7b	1.9b	0.3c	1.6
E	0.885	0.534	0.3a	0.0a	0.0a	0.2a	0.1a	0.4
F	<0.001	<0.001	33.1a	13.2b	5.6c	5.9c	1.0d	5.8
J	0.011	0.612	1.4a	0.6ab	0.6ab	0.7ab	0.1b	1.2
L	<0.001	<0.001	18.9a	5.4b	2.1bc	1.7bc	0.3c	4.0
M	<0.001	0.003	2.7a	0.4b	0.8ab	0.0b	0.1b	1.4
N	<0.001	0.758	1.4ab	1.9ab	1.3ab	2.5a	0.0b	2.3
O	0.178	0.441	0.5a	0.0a	0.0a	0.0a	0.0a	0.4

Vineyards G and H were excluded from all analyses post-2011 following the removal of the residual vines for commercial reasons unrelated to GLRaV-3

Vineyard I was excluded from all analyses post-2011 following the loss of all residual vines

Vineyard K was excluded from all analyses post-2013 following the loss of all remaining vines

[a] *LSD* least significant difference

[b] *NN* nearest neighbours, *R* random

[c] Within a row, percentages with a common letter beside them are not significantly different ($\alpha = 0.05$; pairwise likelihood ratio test)

collection in 2015. The results were consistent with studies undertaken in South Africa (Pietersen et al. 2013) and California, USA (Ricketts et al. 2015) where effective GLRaV-3 control relied on integrating the use of certified virus-free vines with a protocol that included good vector management and the annual roguing of symptomatic vines.

Before committing to roguing, owners need to be confident that it can reduce GLRaV-3 incidence and contain spread so as to minimise the loss of healthy, productive vines. Thus, a crucial economic consideration becomes one of differentiating between vines that must be rogued in order to achieve effective virus control from those that can be safely retained.

We found that of the 'nearest neighbours', 'first' vines were most at risk of GLRaV-3 infection, which supported the findings of Habili and Nutter (1997), and Pietersen et al. (2013). Although this was an important result, we regard the overall risk to 'first' vines as being relatively low. In 2010, an average of one quarter of 'first' vines had foliar symptoms of GLRaV-3, meaning that three-quarters of 'first' vines were non-symptomatic. By 2015, an average of 94% of 'first' vines were non-symptomatic, but when looking at group 1 vineyards only, this increased to 99%. Consequently, with most 'first' vines unlikely to be infected with GLRaV-3, the New Zealand wine sector rogue symptomatic vines only (Andrew et al. 2015). Any unseen asymptomatic 'first' red berry vines from one year should have foliar symptoms the following year thereby enabling prompt removal (Bell et al. 2015).

The relative lack of effective virus control among group 2 vineyards suggests that under certain conditions removing symptomatic vines only is sub-optimal, as was found for all but the smallest outbreaks of cocoa swollen shoot disease in western Africa (Thresh and Owusu 1986). There, the more effective response for controlling the spread of this mealybug-vectored pathogen was to rogue the symptomatic tree plus all of the apparently healthy immediate neighbours (Thresh and Owusu 1986). Indeed, in modelling spatial-dynamic diffusion of grapevine leafroll disease, Atallah et al. (2015) showed roguing infected vines and their equivalent of 'first' vines that laboratory tests confirmed were positive for the virus, improved vineyard net present value by up to 19% relative to a 'no-control' option. In

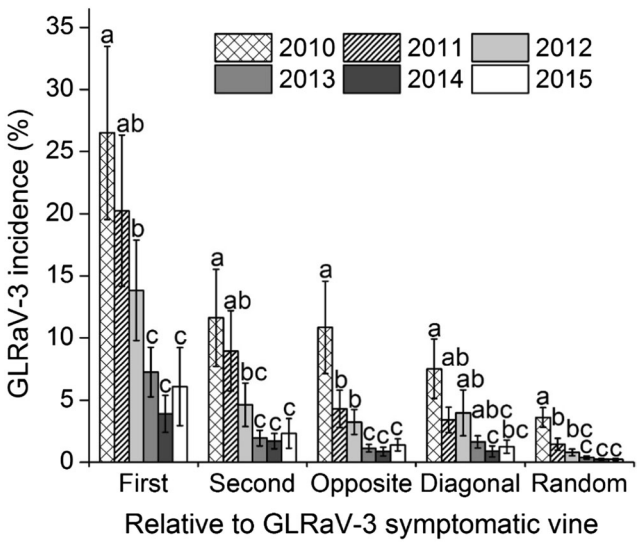

Fig. 4 Mean (±SEM) grapevine leafroll-associated virus 3 (GLRaV-3) incidence among the 'nearest neighbour' and 'random' vine positions in the study vineyards, 2010 to 2015 ($n = 9$ study vineyards in 2010, 10, 11 and 12 in 2011, 2012, and 2013, respectively, and 11 in 2014 and 2015). Statistically significant differences between years within a position are denoted by different letters ($\alpha = 0.05$)

some New Zealand vineyards that were not part of the present study, symptomatic plus 'first' vines were removed concurrently without any laboratory testing of the non-symptomatic vines. While we have not yet evaluated the efficacy of this so-called 1 + 2 response, we suggest the prospect of effective virus control by this method is low when vector pressure is high. For instance, in the group 2 vineyards I and K, all symptomatic vines were rogued in 2009, resulting in losses of 15 and 10%, respectively. A 1 + 2 response would have immediately increased vine losses to 25 and 15%, respectively, but with mealybugs abundant for the duration of monitoring, we suggest the effectiveness of this response would have been confounded by vector mobility and their viruliferous status. Instead, effective GLRaV-3 management occurred only when there was a relatively low probability of *P. calceolariae* encountering virus foci.

These insights are important because in New Zealand a 1 + 2 strategy cost NZ$1800 per ha more than roguing symptomatic vines only (Nimmo-Bell 2006). Therefore, to maximise vineyard profitably and longevity, the conditions under which roguing in New Zealand could or should be modified requires further evaluation based on differing virus/vector scenarios.

The presence of 'random' infections seems likely to be another troubling aspect of GLRaV-3 epidemiology. With no predictability as to where 'random' infections might occur, they can confound control efforts by increasing the spatial distribution of virus foci and thus widening the areas requiring intervention. One explanation for 'random' infections may be the passive aerial dispersal of viruliferous vectors (Charles et al. 2009). In this study though, 'random' outbreaks were relatively rare events when compared with infections amongst 'nearest neighbours'. We conclude that the result of finding so few 'random' infections from 2013 was likely to have been strongly influenced by the combination of annual roguing and relatively low numbers of mealybugs.

During this study, we ensured that the protocols for each variable monitored – the visual virus diagnostics, the application of roguing, and mealybug assessments – was standardised across vineyards. Thus, of these variables, mealybug abundance in the vine canopy provided the most plausible explanation for the contrasting virus control outcomes between vineyards. Indeed, links between the speed of pathogen spread and vector abundance was found during a multi-year study of GLRaV-1 in Burgundy (Le Maguet et al. 2013). In one vineyard, a significant correlation was found between temporal changes to incidence (from 5 to 86%) and the detection of

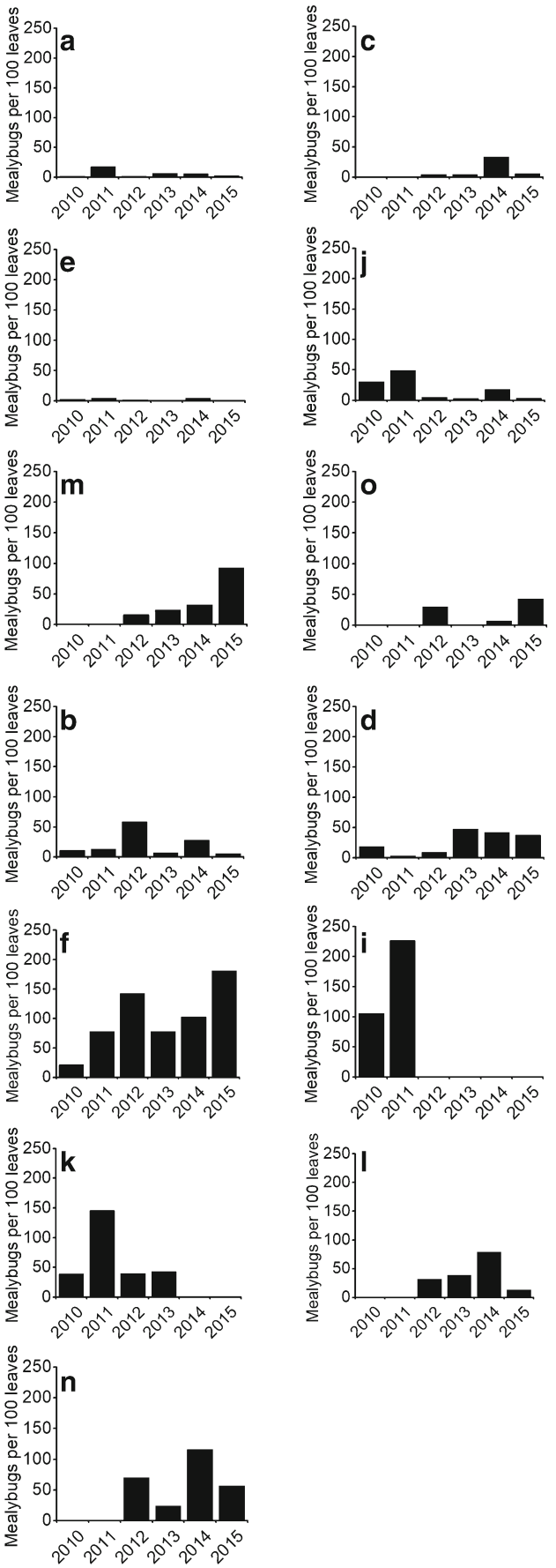

Fig. 5 Numbers of mealybugs found per 100 vine leaves inspected in the group 1 (**a**, **c**, **e**, **j**, **m**, and **o**; six upper graphs) and group 2 vineyards (**b**, **d**, **f**, **i**, **k**, **l**, and **n**; seven lower graphs), 2010 to 2015. Each of 400 vine leaves was inspected for mealybugs per vineyard per year; 300 in 2010. In vineyards I and K, the residual vines were removed in July 2011 and July 2013, respectively. In vineyards **l**, **m**, **n**, and **o**, leaf assessments for mealybugs started in 2012

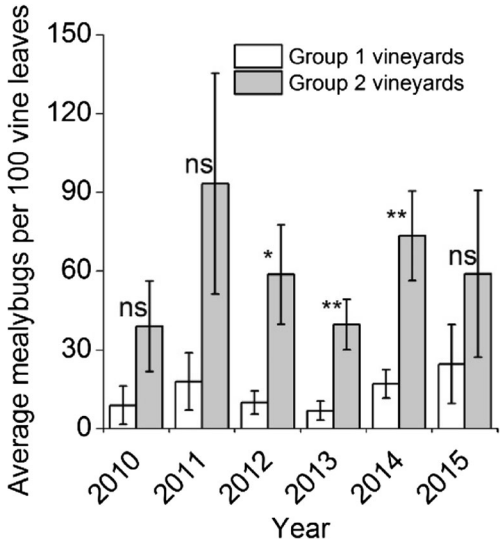

Fig. 6 Comparisons of the average (±SEM) numbers of mealybugs per 100 vine leaves inspected between group 1 and group 2 study vineyards, 2010 to 2015. Among group 1 vineyards (A, C, E, J, M, and O), grapevine leafroll-associated virus 3 (GLRaV-3) incidence reduced to <1% where it was sustained until data collection ceased in 2015. GLRaV-3 control in group 2 vineyards was either ineffective (I, K) or annual incidence was >1% (B, D, F, L, and N). Each of 400 vine leaves was inspected per vineyard per year; 300 in 2010. Significant within-year differences between group 1 and group 2 vineyards are denoted by $*p = 0.01$; $**p = 0.005$; ns = not significant, with $p > 0.05$

the mealybug vector *Phenacoccus aceris* on three-quarters of vines. In a second vineyard over the same period, GLRaV-1 incidence remained largely unchanged at about 5%, with just 6% of vines found with *P. aceris* (Le Maguet et al. 2013). These results supported the present study: in vineyards I and K, we attributed the inability of roguing to control GLRaV-3 to the high numbers of *P. calceolariae*; by contrast, virus control in group 1 vineyards benefited from consistently low numbers of the same vector.

Infestations of mealybugs in the vines were inevitably influenced by owners adopting different management approaches. As noted, the use of registered mealybug insecticides was sanctioned by New Zealand Winegrowers in all but two of the study vineyards. When reviewing the annual spray diary data, we noted that among the group 2 vineyards there was often poor compliance with insecticide label recommendations, particularly in respect of the pre-flowering insect growth regulator, buprofezin (Young 2013). Although the correct quantities of active ingredient was applied to the vines, water volumes of 300–500 L/ha were often used instead of the recommended 1000 L/ha (V. Bell unpublished data). The commensurate reduction in efficacy of this contact insecticide (Lo et al. 2009) meant control of this cryptic pest was inconsistent between years in some group 2 vineyards (B, D, L, and N) and entirely ineffective in the others (F, I and K). By contrast, of those group 1 vineyards where mealybug insecticides were used, good compliance with best practice was evident (A, J, M, and O). Though a plausible explanation, we cannot be certain that the buprofezin applications contributed to these results.

The remaining group one vineyards C and E were certified organic, with insecticides not applied to the vines. While the relative absence of mealybugs from the vines in both vineyards may have been due to practices used, substantial infestations in organic vineyards not part of this study suggest it may not be a general trend (V. Bell personal observations). The effect of mealybug biological control (Charles et al. 2010) and cultural practices require further investigation, along with a range of other factors possibly contributing to the positive outcomes observed in both vineyards.

When this study commenced in 2009, GLRaV-3 incidence ranged from 4 to 24%, which we regarded as low to moderately high. Consequently, in order to achieve effective virus control we considered there would be limited tolerance for overlapping virus/vector populations. However, despite finding mealybugs on vine leaves in all vineyards, the decline in virus incidence among the group 1 vineyards suggested some 'tolerance' for *P. calceolariae*. While we have not yet examined the issue of an economic injury threshold for this vector, the message conveyed in New Zealand was that eradication of *P. calceolariae* was not needed for effective virus control. However, research is required to determine if such a scenario applies to winegrowing regions beyond New Zealand where four or more vector generations per year could alter the dynamics of virus management. Insights like these could be critical to determining the feasibility of roguing and/or the manner of its implementation.

Finally, in emphasising the importance of mealybug vectors to virus management outcomes, we acknowledge other factors may also have influenced the results. Examples include the moderately high virus incidence recorded in vineyard B in 2009, which at 24%, exceeded our roguing threshold of 20%. Furthermore, the complete absence of roguing for two years in vineyard F would have disadvantaged efforts to control GLRaV-3. Thus, the influence of these and other factors on virus management outcomes, either in isolation or in combination with vector management initiatives, needs further evaluation. Despite such gaps in our knowledge, our results support Pietersen et al. (2013) and Ricketts et al. (2015) and their recommendation that effective GLRaV-3 control relies on the adoption of an integrated management response.

Acknowledgements This work formed part of the New Zealand Grape and Wine Research programme, jointly funded by New Zealand Winegrowers Inc. (NZW) and The New Zealand Institute for Plant & Food Research (PFR) Strategic Science Investment Fund. VAB acknowledges the support of Dr. Simon Hooker, Ruby Andrew, Nick Hoskins (NZW), Philippa Stevens, and Dr. Jim Walker (PFR). This research owes much to the generosity of the many vineyard owners and their staff, and to the technical support of Tara Taylor and Terrence Makea (PFR, Hawke's Bay). Constructive comments on earlier iterations of this paper were provided by Drs Karmun Chooi and Arnaud Blouin (PFR, Auckland) and two anonymous reviewers.

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