

**Virus effects on Sauvignon Blanc wine
quality:
Progress report 2006**

Mundy DC

July 2006

Report to New Zealand Winegrowers
HortResearch Client Report No. 19624
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Mundy DC
HortResearch Marlborough
Marlborough Wine Research Centre
85 Budge Street
PO Box 845, Blenheim, New Zealand
Tel: +64 3 577 2373
Fax: +64 3 578 0153

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This report has been prepared by The Horticulture and Food Research Institute of New Zealand Ltd (HortResearch), which has its Head Office at 120 Mt Albert Rd, Mt Albert, AUCKLAND. This report has been approved by:

Research Scientist

Date: 31 July 2006

Group Leader, Bioprotection

Date: 31 July 2006

CONTENTS

	Page
EXECUTIVE SUMMARY	1
INTRODUCTION	2
MATERIAL AND METHODS.....	3
Yield and ripening progress 2005/06 season	3
RESULTS	5
DISCUSSION.....	9
CONCLUSIONS	11
RECOMMENDATIONS.....	11
REFERENCES	11
ACKNOWLEDGEMENTS.....	12
APPENDIX 1. Experimental block design.....	13

EXECUTIVE SUMMARY

Virus effects on Sauvignon blanc wine quality progress report 2006

Report to New Zealand Winegrowers

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Little has been reported on the impacts of Grapevine Leaf Roll type 3 Virus (GLRaV-3) on white grapes. Marlborough Sauvignon blanc is the flagship white wine of the New Zealand industry. We are not aware of any published studies of that have measured the impact of GLRaV-3 on Marlborough Sauvignon blanc. A recent survey of the wine industry has shown that growers in Marlborough perceive the risk of GLRaV-3 spread in Marlborough is low and that the impact on Marlborough Sauvignon blanc will be low, as it does not show red leaf symptoms as red grapes do (Bonfiglioli & Stewart 2005).

The investigation conducted in the 2005/2006 season measured the impact of GLRaV-3 on the leaves and fruit of 20 vines with and 20 vines without this virus. Significant differences in leaf colour, berry weights and juice nitrogen content were observed. The possible implications of these observations are compared and contrasted with the results of four published papers dealing with the impacts of GLRaV-3 on three white varieties.

The main recommendation from the report is the continued monitoring of these vines for another two seasons. With the continuation of this project, a number of additional activities are recommended that were outside of the scope of the project in the first season.

For further information please contact:

Dion Mundy
HortResearch Marlborough
Marlborough Wine Research Centre
85 Budge Street
PO Box 845, Blenheim, New Zealand
Telephone +64 3 577 2373
Email dmundy@hortresearch.co.nz

INTRODUCTION

The symptoms of Grapevine Leafroll associated Virus type 3 (GLRaV-3) in white wine-grape varieties are often far less obvious than in their red counterparts. While red wine-grape varieties may express leaves showing early “autumn” colorations of red between the green veins (Anon 2005a), white varieties often do not express the same easily detected visual symptoms. In fact infected white varieties may show no symptoms at all: if there are symptoms they are likely to include yellowing of leaves between leaf veins, leaves with the margins rolled backwards or reduced fruit set (Anon 2005b).

In many fruit crops, virus infections typically reduce both yield and quality of fruit, but there is little published information in the scientific literature that quantifies the effect of virus infection on white wine quality and yield. The New Zealand wine industry and Marlborough in particular relies on the production of high quality Sauvignon blanc wines as a flagship for New Zealand wine exports. Because of the lack of strong visual symptoms of virus infection and the lack of information about the effects of virus infection on white wine quality, many growers in Marlborough are unaware or unconcerned about the virus status of their Sauvignon blanc vines (Bonfiglioli & Stewart 2005).

However, a recent report on the economic effects and financial impact of GLRaV3 (Nimmo-Bell 2006) has highlighted the need for robust scientific data on Sauvignon blanc grapes, to allow the economic effects and financial impact of GLRaV3 to be calculated for Marlborough. This project was therefore designed to determine if and how the presence of virus infected Sauvignon blanc vines in the vineyard might affect fruit development and wine quality.

The project was designed as a three year project with phenology studies in year 1, followed by phenology and microvinification studies in year 2 and 3. The project starts to address some of the research themes outlined in Section 2: impact of GLRaV-3 on vine growth and productivity, in the recent review by HortResearch for New Zealand Winegrowers (Charles et al. 2006).

The review by HortResearch (Charles et al. 2006), the report by Nimmo-Bell (2006) and the article by Rod Bonfiglioli & Diane Stewart (2005) all reinforce the statement made in the 2005 MAF viticulture report (Ministry of Agriculture and Forestry 2005) that, “Vineyard decline is another limitation to vineyard performance that needs to be addressed, particularly leaf-roll virus and wood disease in older vineyards.” The first step in addressing the limitation to vineyard performance due to GLRaV-3 in Marlborough is collecting scientific data, for a number of parameters, from virus-infected Sauvignon blanc vines and comparing to non-infected vines over a number of seasons. This report contains detailed information on the performance of vines at one Marlborough site over a single season.

MATERIAL AND METHODS

Virus status of 80 vines was determined to enable the phenology of 20 vines with and 20 vines without detectable levels of GLRaV-3 to be monitored from pre veraison to harvest. The experiment was centred on an area of a block that was virus tested in 2003 and shown to have a 58% level of GLRaV-3 infection within the sampled vines. When the same vines were tested again in 2006, the 13 vines that had not had detectable levels of GLRaV-3 in the experimental area still did not have detectable levels of GLRaV-3. Of the 47 vines that were tested in 2006 but not in 2003, 22 vines had GLRaV-3. Therefore of the 80 vines that have been virus tested in 2006 52.5% of the vines had GLRaV-3. The 20 “clean” vines used in the experiment, which did not have detectable levels of GLRaV-3, were selected so that they had either other clean vines adjacent to them or a post adjacent (Appendix 1). This approach was taken to reduce the risk of virus spread to the clean vines during the planned 3 years’ length of the experiment. Vines were pruned by the vineyard staff on a Scott Henny training system.

YIELD AND RIPENING PROGRESS 2005/06 SEASON

Relative measurements of chlorophyll contents of leaves were made using a Minolta SPAD-502 Chlorophyll Meter. The SPAD leaf chlorophyll meter (SPAD-502, Minolta Corp., Jersey, NJ, USA) meters are calibrated to measure the chlorophyll content in the leaves rapidly and non-destructively. Three field assessments of chlorophyll content of leaves were made in February and March 2006 (22 February, 9 March and 15 March) for each of the individual vines tested for the presence of virus.

The maturity and quality characteristics (average berry weight, soluble solids (°Brix), pH, and titratable acidity) of the berries for the 40 selected vines were assessed seven times (Table 1) during development using standard wine industry analytical techniques. Bunch samples collected during the season were processed within 24 h of sampling. At harvest bunch numbers were recorded to provide average bunch weight data. Total yield was recorded at harvest on an individual vine basis.

Table 1. Dates of sample collection and testing conducted on samples of grapevines in the GLRaV-3 trial, Marlborough, 2006.

Date (2006)	Brix	pH	YAN ¹	TA ²	Yield ³ / vine	
01/02	Yes	Yes	No	Yes		Pre-veraison
14/02	Yes	Yes	Yes	Yes		
20/02	Yes	Yes	Yes	Yes		
27/02	Yes	Yes	Yes	Yes		
06/03	Yes	Yes	Yes	Yes		
13/03	Yes	Yes	Yes	Yes		
20/03	Yes	Yes	Yes	Yes	Yes	Harvest

¹ YAN - Yeast available nitrogen

² TA - Titratable acidity

³ Yield included the recording of bunch number, 30-berry sample weight, total weight of fruit per vine and calculating the average bunch weight per vine

Nitrogen content was assessed six times (Table 1) vines with and without type 3 Leaf Roll Virus using standard wine YAN kits (Unitech Scientific, UNITABTM Reagent, PAN_{bl}-500 and

AMM-500) supplied by Global science. Analysis consisted of determining ammonium and amino acid content in parts per million and adding these two values to obtain yeast available nitrogen (YAN).

Because of the delayed start of the project, flowering dates were not recorded as originally planned. Extra pre-harvest berry development measurements were made using the project time that would have been used to record flowering date if the project had started earlier. The table of experimental activities (Table 2) therefore differs slightly from the proposed timing and activities in the original proposal.

Table 2. Timing of project activities in the GLRaV-3 trial, Marlborough, 2006.

No.	Activity	Timing	Number of samples
1	Virus testing, marking of vines and experimental layout	February	
2	Leaf chlorophyll measurements	February / March	3 x visits to record leaf chlorophyll content and leaf health
3	Berry development	February / March	5 x pre-harvest samplings of berries for YAN ¹ , Brix, TA ² and pH
4	Harvest	March	Yield ³ , Juice Brix, TA, pH and YAN determined
5	Report	July	

¹ YAN - Yeast available nitrogen

² TA - Titratable acidity

³ Yield included the recording of bunch number, 30-berry sample weight, total weight of fruit per vine and calculating the average bunch weight per vine

RESULTS

During the season differences in leaf colour were detected between the vines with virus and those without virus using the SPAD leaf chlorophyll meter (SPAD-502, Minolta Corp., Jersey, NJ, USA). Consistently over time the vines with virus had a lower average SPAD value, which indicated that the leaves on these vines were more yellow than leaves of vines without virus. SPAD values did decrease during the season on both sets of vines but virus-infected vines always had lower values (Figure 1). At the pre-harvest measurement on 15 March 2006, vines with virus had a lower (23.1) SPAD value than vines without the virus (28.3); this difference was highly significant statistically (Table 3).

Table 3. Summary of physiological measurements of vines at harvest 2006 with and without detectable Grapevine Leaf Roll type 3 Virus on Sauvignon blanc grapevines in Marlborough.

	Positive for GLRaV-3	Negative for GLRaV-3	p
Leaf colour measurements			
SPAD ¹	23.1	28.3	<0.001
Yield at harvest			
30-berry sample weight (g)	69.9	59.5	<0.001
Bunch number	53.1	58	0.313
Mean bunch weight (kg)	0.10	0.11	0.272
Total Yield per vine (kg)	5.59	6.49	0.117
Harvest Juice analysis			
Brix °	21.67	22.07	0.360
pH	3.09	3.06	0.124
TA (g/L)	10.0	10.3	0.265
Harvest juice nitrogen concentration			
Ammonium (mg/L)	64.2	80.8	0.006
Amino acid (mg/L)	152.4	172.9	0.123
Total Yeast assailable nitrogen (mg/L)	216.6	253.7	0.030

¹ SPAD values of 20 or lower indicates a yellowing leaf that has started to senesce

The weights of 30-berry samples were significantly different at harvest with vines with no virus detected having lower mean berry weights, 59.5 g compared with 69.9 g (Table 3). Bunch numbers and total yield per vine were not significantly different. Hence the calculated mean bunch weights also were not significantly different (Table 3).

Standard juice analysis at harvest of °Brix, pH and titratable acidity (TA) showed no significant difference between the vines with and the vines without detectable levels of GLRaV-3 (Table 3). °Brix over time did show a trend for the vines with no detectable GLRaV-3 to have slightly higher values (Figure 2).

The juice nitrogen concentrations at harvest were significantly higher for ammonium (80.8 mg/L) and total yeast assailable nitrogen (253.7 mg/L) in the fruit from vines without detectable levels of GLRaV-3, than the ammonium (64.2 mg/L) and total yeast assailable nitrogen (216.6 mg/L) of fruit from vines with GLRaV-3 (Table 3). Differences in ammonium concentrations were observed at all sampling points during berry maturity (Figure 3). Primary amino acid concentrations were not significantly different and a clear trend during the season was not observed (Figure 4). The seasonal trend for Total YAN was for vines without GLRaV-3 to have higher values (Figure 5).

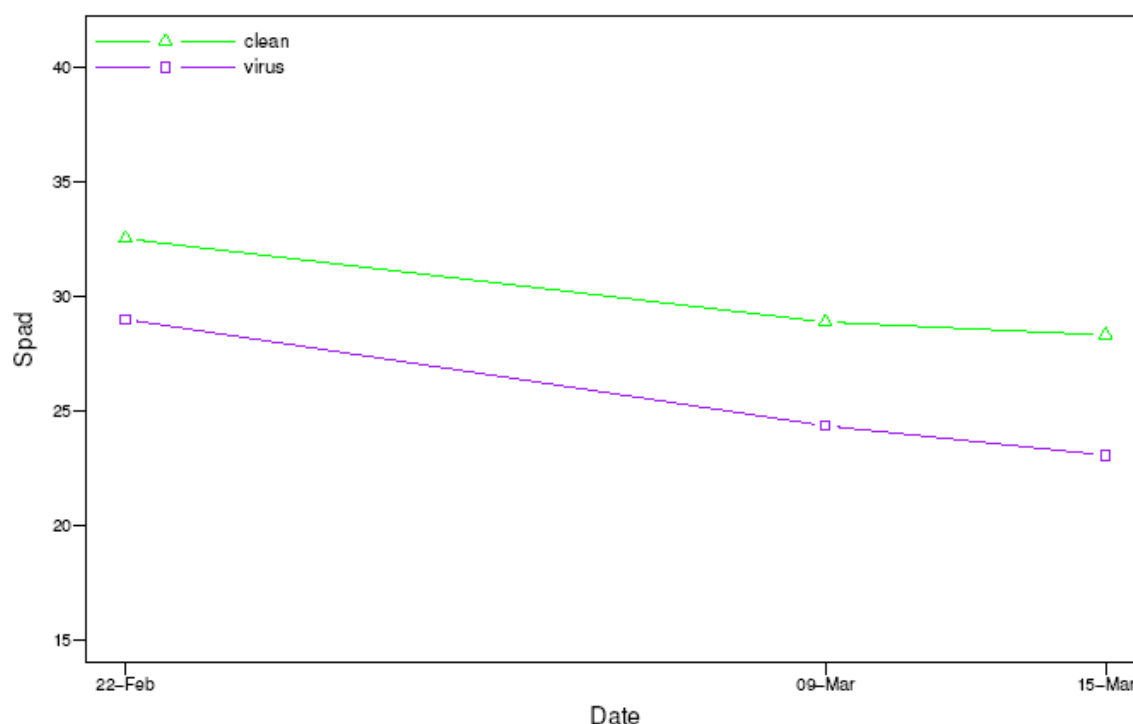


Figure 1. Leaf colour values as measured with a SPAD leaf chlorophyll meter (SPAD-502, Minolta Corp., Jersey, NJ, USA) which are calibrated to measure the chlorophyll content in the leaves rapidly and non-destructively. The SPAD value for vines with and without Grapevine Leaf Roll Virus type 3 in Marlborough Sauvignon blanc grapes before harvest in 2006 were recorded. SPAD values of 20 or lower indicates a yellowing leaf that has started to senesce. Measurements plotted are the means from 80 vines sampled.

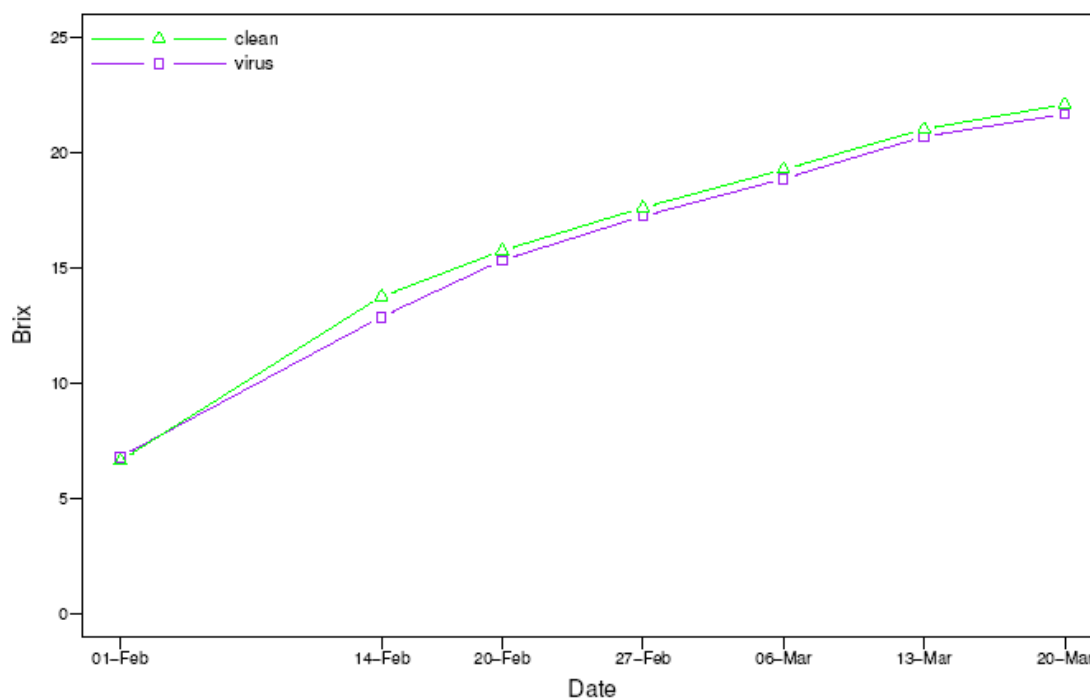


Figure 2. °Brix values for vines with and without Grapevine Leaf Roll Virus type 3 in Marlborough Sauvignon blanc grapes before harvest in 2006. Measurements are the means from 20 vines with and 20 vines without virus.

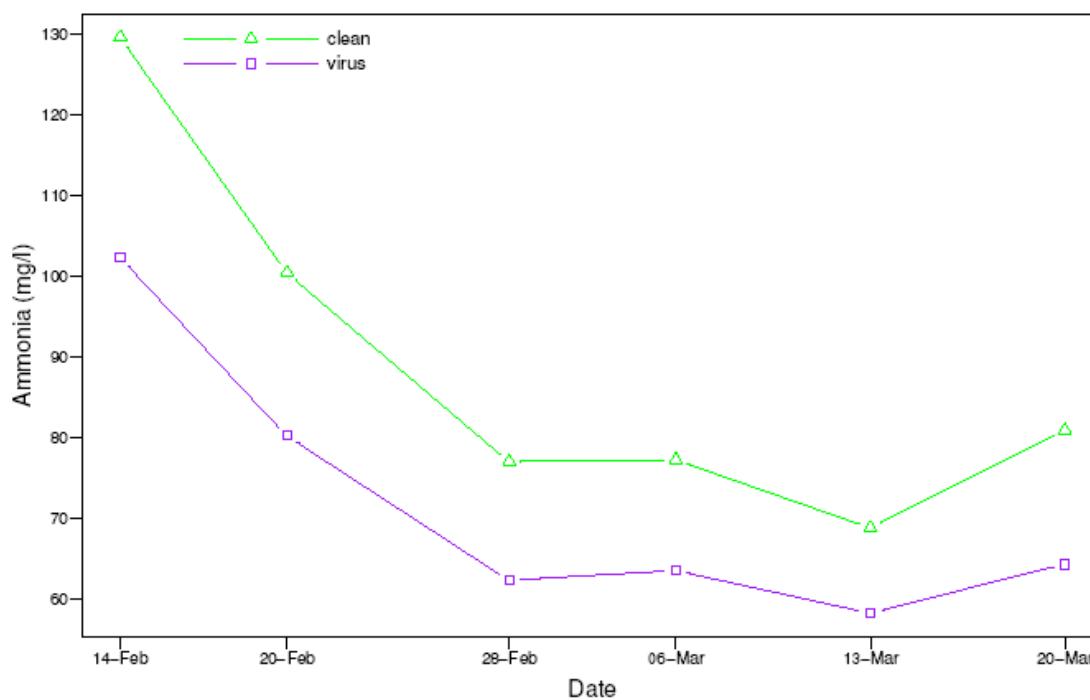


Figure 3. Ammonium values for vines with and without Grapevine Leaf Roll Virus type 3 in Marlborough Sauvignon blanc grapes before harvest in 2006. Measurements are the means from 20 vines with and 20 vines without virus.

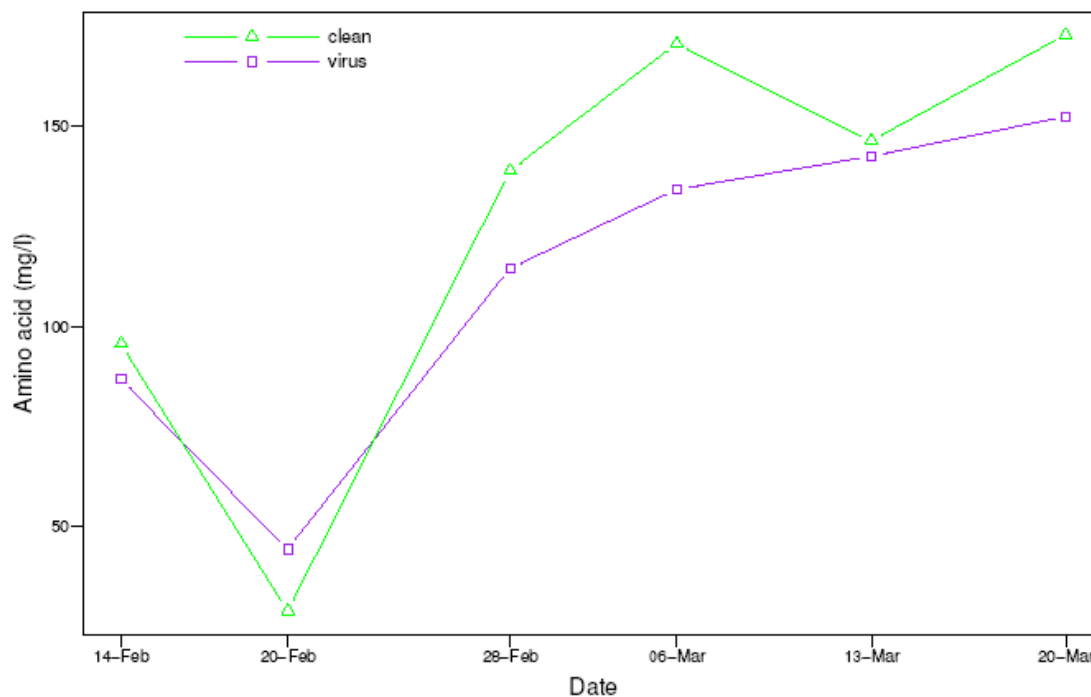


Figure 4. Primary amino acid values for vines with and without Grapevine Leaf Roll Virus type 3 in Marlborough Sauvignon blanc grapes before harvest in 2006. Measurements are the means from 20 vines with and 20 vines without virus.

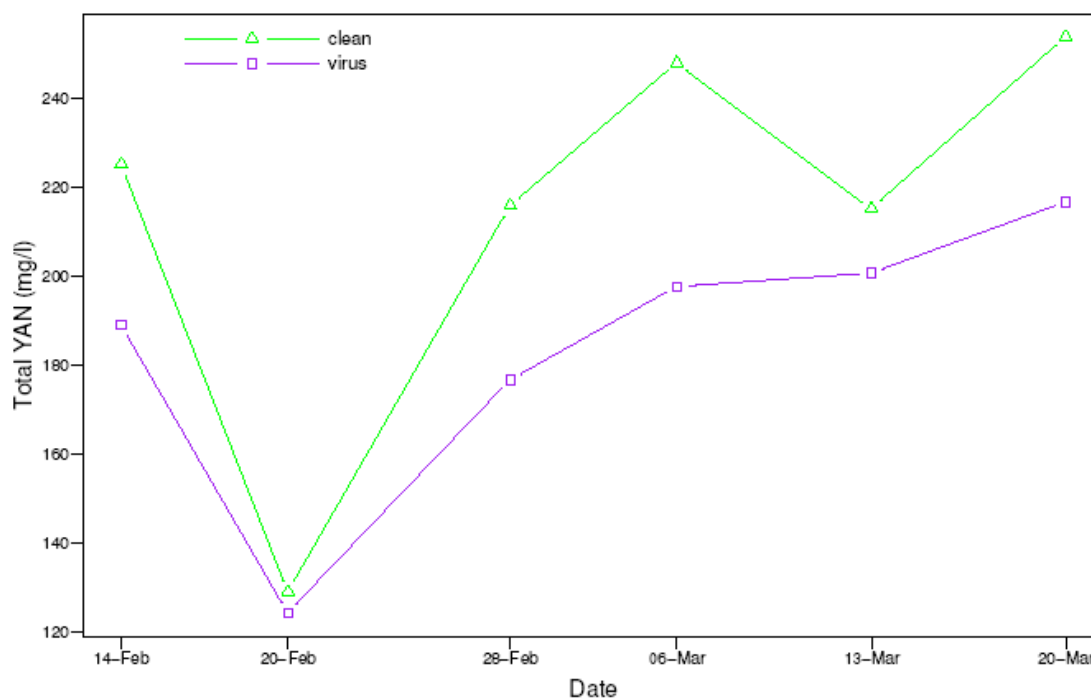


Figure 5. Total Yeast available nitrogen values for vines with and without Grapevine Leaf Roll Virus type 3 in Marlborough Sauvignon blanc grapes before harvest in 2006. Measurements are the means from 20 vines with and 20 vines without virus.

DISCUSSION

A key question for the New Zealand wine industry and in particular the Marlborough wine industry is, “What impact does the Grapevine Leaf Roll type 3 Virus have on Sauvignon blanc grapes grown under Marlborough conditions?” In order to understand this question we must first look at the reported information available on the impacts of GLRaV-3 in other countries and other grape varieties.

A recent review of GLRaV-3 research world wide (Charles et al. 2006) provides a summary of papers that report on one or more of the following impacts on vine growth and fruit product in red grapes: reduced photosynthesis (net photosynthesis, transpiration rate, stomatal conductance, and chlorophyll and carotenoid content), phloem degeneration, reduced root growth, reduced cane growth, delayed phenology, reduced yield, reduced fruit quality, delayed sugar accumulation, higher titratable acidity, lower anthocyanin content, lower pH (higher concentrations of hydrogen ions), and lower wine quality.

Reported findings on the impact of GLRaV-3 on white *Vitis vinifera* are more limited. Reports that are available deal with Riesling (Schoeffling 1980; Wolpert & Vilas 1992), Albarino (Cabaleiro et al. 1999) and Sultana grapes (Hale & Woodham 1979). A number of other papers report the presence of GLRaV-3 on white *Vitis vinifera* but do not report the impact of the virus on the vine performance (Cabaleiro & Segura 1997; Borgo et al. 2000; Komínek & Holleínová 2003; Scalabrelli et al. 2003; de Borbon et al. 2004). The results of the four papers that do report impacts of GLRaV-3 on vine performance are therefore used to compare and contrast the results of the first year of experimentation on Marlborough Sauvignon blanc.

One of the early visual signs of GLRaV-3 in red grapes is the change of leaf colour from green to red. In white grapes the change, if observed, is from green to yellow. The significant difference in SPAD values of leaves on vines with GLRaV-3 compared with leaves on vines without virus during this investigation suggests that GLRaV-3 does affect chlorophyll content of leaves of Sauvignon blanc vines grown in Marlborough. While Cabaleiro et al. (1999) reported significant reduction in net photosynthesis of leaves with visual symptoms of GLRaV-3, no measurements of leaf colour were reported. No direct comparisons of the leaf colour or levels of pigmentation can be made, but the grouping of leaves for photosynthesis measurements into leaves with and without symptoms suggests visual differences were observed. Measurements of photosynthesis were outside the scope of this project in season 1, but could be added for the second and third season as the expertise and equipment are available. The cumulative photosynthetic ability of the vine could also be measured by recording trunk diameter, as has been done in other virus studies (Cabaleiro et al. 1999).

The significantly higher berry weight of vines with virus in the Marlborough study was consistent with the findings for Alarino (Cabaleiro et al. 1999) but contrasts with the findings for Sultana grapes (Hale & Woodham 1979). While the berry weights for Riesling (Wolpert & Vilas 1992) were reported not to be significantly different, the trend in all three seasons was for vines with virus to have higher mean berry weights than the vines without virus. In the Marlborough study, bunch number and total yields were not significantly different and hence neither was the mean bunch weight, suggesting that bunches with virus must have had fewer berries per bunch, to have the same bunch weight as the bunches on vines without virus.

In Riesling (Schoeffling 1980; Wolpert & Vilas 1992), Alarino (Cabaleiro et al. 1999) and Sultana (Hale & Woodham 1979), a higher concentration of soluble solids was observed for fruit from vines without virus. However, in Alarino for one of the three seasons, the difference was not significant. While the °Brix values at harvest were not significantly higher on vines without GLRaV-3 in Marlborough in the 2005/2006 season, the trend during the season was for higher °Brix values on these vines (Figure 2). During the 2005/2006 season a number of the experiments conducted in Marlborough showed either reduced or no difference in °Brix acclimation even when that experiment had observed differences in the previous season. Very favourable conditions for photosynthesis in Marlborough during the 2005/2006 season may have masked differences that would be observed in a different season. It is therefore important to collect data for another two seasons, to determine if GLRaV-3 has an impact on the soluble solids content (°Brix) of Sauvignon blanc in Marlborough.

Cabaleiro (1999) and Wolpert & Vilas (1992) report lower pH of juice when virus is present although in both cases this was not significant in all three years of the study. In Marlborough, pH differences were not observed in the 2005/2006 season. More data would be required to determine if this was a seasonal result or if GLRaV-3 does not affect the pH of juice at harvest.

Titrateable acidity was not significantly affected in Marlborough for the 2005/2006 season, which is consistent with the findings for Sultana grapes (Hale & Woodham 1979). However for Riesling and Albarino, higher total acidity has been reported for vines with virus (Cabaleiro et al. 1999); (Schoeffling 1980; Wolpert & Vilas 1992). Another two seasons of observations would be required to determine if the results reported here are the norms or the exception.

Improvements in vegetative characteristics, fruit quality and wine sensory analysis scores were reported when virus was removed from Riesling clones (Schoeffling 1980). Delayed sugar accumulation of 1-1.6 °Brix in 3 seasons for Riesling was observed, but juice pH and acidity were not affected by infection (Wolpert & Vilas 1992)., No differences in pruning weight and trunk perimeter, but increased berry weights, differences in °Brix, pH and titrateable acidity with GLRaV-3 infection of Albarino in some seasons, as well as differences in net photosynthesis were all reported by (Cabaleiro et al. 1999). Higher malate, tartrate and potassium concentrations of Sultana grapes were reported by (Hale & Woodham 1979).

One of the key assumptions when investigating the impact of GLRaV-3 on Sauvignon blanc in Marlborough is that virus infection may affect the volatiles and other quality attributes within the berry. While we did not make wine nor determine if the taste of the final product was altered, we did measure the nitrogen contents in the juice that are precursors for a number of flavour compounds in wine. Vines with GLRaV-3 had significantly lower ammonium and total yeast available nitrogen. The lower concentrations of nitrogen compounds in the fruit during the period from veraison to harvest (Figures 3, 4 and 5), may affect the production of the flavours required to make high quality Marlborough Sauvignon blanc. Continued testing of YAN levels and micro vinification will be required to determine if this is the case.

CONCLUSIONS

The primary assumption in this project is that the presence of some virus infected vines within a vineyard will reduce the quality of the wine produced from that vineyard. In the first year, we have shown that GLRaV-3 can reduce leaf colour, increase berry weight without changing yield, and decrease the nitrogen concentration of the fruit. The next step is to repeat research with fruit harvested to make wines, and to determine if the observed changes affect the quality of the final product.

RECOMMENDATIONS

The main recommendation for this project is that the experiment continues for two more seasons of field observations. In Season 2 and 3 flowering date should be recorded and the number of samples collected pre-harvest will be reduced.

We also recommend that the following additional activities be included in the project for years 2 and 3:

1. Making of micro vine wines at a target Brix of 21.5°
2. Measurements of photosynthesis as an indication of changes from observed colour differences in leaves
3. Measurements of trunk diameter as an indication of cumulative effects on growth potential.

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APPENDIX 1. EXPERIMENTAL BLOCK DESIGN

row	501	502	503	504	505	506	507	508	509	510	511	512
Bay 1	1									0		
										1		
1	X	X	X	X	X	X	X	X	X	X	X	X
		1										
2		0							1			
		0										
2	X	X	X	X	X	X	X	X	X	X	X	X
										0		
3	1									1		
										0		
3	X	X	X	X	X	X	X	X	X	X	X	X
4		1							1			
	X	X	X	X	X	X	X	X	X	X	X	X
4	1									1		
5	X	X	X	X	X	X	X	X	X	X	X	X
		0										
6		0							1			
		1							0			
6	X	X	X	X	X	X	X	X	X	X	X	X
7	1									1		
	1									0		
7	X	X	X	X	X	X	X	X	X	X	X	X
8		1									1	
	X	X	X	X	X	X	X	X	X	X	X	X
8	1											0
9												1
	X	X	X	X	X	X	X	X	X	X	X	X
9			1								1	
10	X	X	X	X	X	X	X	X	X	X	X	X
												0
11			1									0
												0

row	501	502	503	504	505	506	507	508	509	510	511	512
				1								
				1								
				1								
12				0							1	
	X	X	X	X	X	X	X	X	X	X	X	X
			1	1								1
13												
	X	X	X	X	X	X	X	X	X	X	X	X
											0	
				1							0	
											0	
14											0	
	X	X	X	X	X	X	X	X	X	X	X	X
			0									1
			1									1
			0									0
15			0									1
	X	X	X	X	X	X	X	X	X	X	X	X
												1
16				1							1	0
	X	X	X	X	X	X	X	X	X	X	X	X
			1									0
												0
												0
17												0

Following testing February 2006

- 1 Vine positive for GLRaV 3 used in the experiment
- 0 Vine with no detectable GLRaV 3 used in the experiment
- 1 Vine positive for GLRaV 3 not used in the experiment
- 0 Vine with no detectable GLRaV 3 not used in the experiment