Wine Australia for Australian Wine

Department of Primary Industries and Regional Development
GOVERNMENT OF WESTERN AUSTRALIA

Do viral infection(s) in the Gingin clone of Chardonnay influence vine phenotype, performance and consequent wine quality?



FINAL REPORT FOR INCUBATOR PROJECT

Project number: **DPR 1801**

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1. Abstract

The most widely planted clone of Chardonnay in Western Australia (WA) is anecdotally thought to be the Gingin clone, which produces some of WA's highest quality Chardonnay, despite poor fruit set, millerandage and low yields. It is thought by many growers that these attributes are due to an interaction with one or more grapevine viruses, in particular Grapevine leafroll virus 1 (GLRV1). This project did not conclusively show a detectable difference between small batch wines made with and without virus when assessed by a panel of experts. One expert was able to reliably detect the difference across two sites, but further research is required.

2. Executive Summary

The interesting question here is whether viral infection of the Gingin Chardonnay clone can influence the vine phenotype, performance and wine quality. Virus infection is not usually thought to be of benefit to berry characteristics, but in this instance, it may be possible that it is contributing to the sought-after unique nature of some high quality wines, particularly Western Australian Chardonnay.

Understanding whether GLRV1 and/or other viruses are consistently present in the Gingin clone, and how they affect vines, berry characteristics and resulting wines will begin to answer many questions and provide a basis for further research into this phenomenon. The research outcomes from this project align to the Wine Australia Strategic Plan 2015-2020 Priority 2: Increasing competitiveness, in particular Strategy 4: Improving resource management and sustainability (Biosecurity, pest and disease management).

In this project, small batch wine was produced from vines with and without Grapevine leafroll virus 3 (GLRV3), as it was difficult to find enough vines with GLRV1 only in the short timeframe of 12 months. The main question being asked of the experts on the sensory evaluation panel was whether there was a difference in the wines, not whether it was a "good" or "bad" difference. Overall, the experts weren't able to reliably pick the difference between a wine made with or without virus, although one particular expert was able to pick the difference. This one individual result shows that the difference may in fact be noticeable to an expert and that further research is likely worthwhile. Future research should also include further chemical analysis of wines with and without virus, and include other varietals.

3. Aims and Background

The Chardonnay Gingin clone grown in WA is known for producing high quality Chardonnay, despite the characteristic poor fruit set, millerandage and low yields. It is thought that these attributes are due to an interaction with one or more grapevine viruses, in particular Grapevine leafrollviruses 1 and 3 (GLRV1&3).

There has been little research on the effect of virus infection on the quality of wine produced from virus infected vines and those published focus on red varieties. These red varieties often show obvious symptoms of infection such as leaf reddening and red blotches. In 2016, a study by Alabi et al.* looked at a virus affected merlot cultivar and determined that the affected vines produced wine with significantly lower alcohol, polymeric pigments and anthocyanins compared with wine made from non-symptomatic vines. Most other studies examine the impact virus infection has

on yields in grapevines. These viruses are known to cause reduced fruit quality and yield (10-70% reported), and in cases where symptomless infection occurs these infections can go unnoticed, but remain in the vineyard as a long-term source of virus for transmission to other vines.

There are no published studies on the effect virus infection has on wine quality produced from white varieties. The aim of this research is to identify the viruses present in vines of the Chardonnay Gingin clone and the effect they have on wine characteristics. GLRV1&3 are the primary virus concern for grape growers in Australia.

This project was developed in consultation with Richard Fennessy, DPIRD Wine Researcher located at the Bunbury office, and with Dr Brenda Coutts, DPIRD Plant Virologist located at the South Perth Office. This project is relevant to the Australian grape and wine community in that it will be the first real effort to determine the difference between wine made from virus-free vines and from virus affected vines, and identify the viruses present. It will be useful to the wine industry by providing some much needed data regarding the anectodal evidence of virus affected vines producing good quality wine. The results will have implications for future management strategies for vineyards all over Australia, not just Western Australia.

4. Materials and Methods

4.1 Project Design

The main aim of this research is to accurately identify the virus composition of a number of individual Gingin Chardonnay clone vines and then make wine from vines with different virus status (single and mixed infection). The combinations of viruses assessed will depend on the range of viruses that are found in the vines tested.

Stage 1:
Identify the location of plantings of the Gingin Chardonnay clone, liaise with growers to participate in the monitoring and provide grapes for small batch wine making. Complete initial virus testing of vines. As we were unsure as to the possibility of finding Gingin Chardonnay with no virus infection, clone 95, and Mendoza Chardonnay were included in the sampling (2 blocks and 1 block respectively).

Stage 2:
Sample Gingin clone vines to test for the presence of GLRV1 and GLRV3. In each vineyard, 20 symptomatic and 20 non-symptomatic vines will be identified, sampled and tested. Following identification of virus-free and virus-infected plantings, further testing to monitor the individual vines at each location was completed during another site visit and again at harvest time. Each vine was identified and sampled, then returned to the DPIRD Diagnostic and Laboratory Services (DDLS) laboratories for testing.

Virus identification and testing was done in the DDLS labs by qPCR with the HSP70-149F/HSP70-293R/HSP70/225P set for GLRV1 and the 56F/285R/181P set for GLRV3 from Osmana et al. 2007. LAMP-PCR in the field for subsequent visits was done using the GLRV1 and GLRV3 primer sets developed in the 2018 Science and

Innovation Awards for Young People in Agriculture, Fisheries and Forestry Wine Australia sponsored award for Dr Monica Kehoe (manuscript in preparation).

For the final confirmation of viral load at harvest time, qPCR as described above and Next generation sequencing by the Oxford Nanopore Technologies MinIon portable sequencer were used for the individual vines identified for wine production. Briefly, the RNA from each sample collected for site 1 without detection (WD) site 1 GLRV3 infected, site 2 WD and site 2 GLRV3 infected were pooled into 4 respective pools of RNA. They then underwent ribosomal depletion using the RiboMinus kit (ThermoFisher) according to manufacturer's instructions, followed by first strand cDNA synthesis and reverse transcription with SuperScript IV First-strand synthesis kit (ThermoFisher) with the use of NGS1random primer (Ng *et al.,* 2015). The Nanopore LSK-109 sequencing kit along with the PBC-001 barcoding kit was used to prepare 4 barcoded libraries which were then sequenced on a MinIon R9.4 Flow Cell, and run for 24 hours.

Stage 3:
Small batch wines were made from grapes harvested from vines with a known viral composition. They were transported to the DPIRD wine laboratory in Bunbury where the fruit was chilled overnight to 4°C. The following day the parcels were separated into treatments (i.e. GLRV3 and WD), weighed, 10 bunches per treatment weighed and photographed to calculate bunch compaction. Bunch compaction was measured by utilising a modified formula from Tello and Ibanez (2014) which consists of (bunch weight/((length + width)/2)2).

The day post-harvest, treatments were destemmed and crushed before being pressed at approximately 600L/t equivalent extraction rate. Juice additions consisted of 60-80ppm potassium metabisulphite (PMS) and 40ppm pectinase enzyme before settling for 5 days at 4°C.

The settled juice was racked and inoculated with Maurivin PDM yeast, *Saccharomyces cerevisiae* (var. *bayanus*) and fermented at 16°C until residual sugar <3g/L. During fermentation an addition of 200ppm Diammonium phosphate (DAP) was added after the first 1°Be drop for the site 1 wines but due to hydrogen sulphide characters, 200ppm DAP was added at the first 1°Be and 5°Be drop for the site 2 wines.

Post fermentation the wines were racked into glass demijohns (4.5-5L) with 120ppm PMS added. Wines were then stored at 4°C for twelve weeks before being bottled under screw cap via a 0.45µm filtration. The wines were held at 18°C for 5 weeks prior to the sensory assessment.

Stage 4:
Assessment of wine quality as a sensory evaluation with a panel of experts. The panel was made up with four male Margaret River winemakers.

Stage 5:
Communication of results to industry, provision of a final report to Wine Australia.

5. Results and Discussion

Stage 1
In 2018-2019, four locations were identified as potentially having at least one planting of Gingin Chardonnay, a willingness to participate in the study and agreeable to allowing the hand harvesting of approximately 25kg of fruit from their vines. In total 7 blocks were virus tested thoroughly, with leaves from 50 individual vines collected. These were the first vine in each panel from a designated (and recorded) row within the block. The leaf samples were transported to the laboratory in Perth where they were tested by qPCR for GLRV1 and GLRV3.

Table 1. Results of initial survey for GLRV1 and GLRV3

Location	GLRV1	GLRV3	GLRV1&3	negative	Total tested
Site 1					
B6 - clone 95	0	36	1	13	50
B5 - Gingin	48	0	2	0	50
Site 2					
Clone 95	2	3	1	44	50
"young" Gingin	2	34	3	11	50
Site 3					
mendoza	0	0	50	0	50
mendoza	0	49	0	1	50
Site 4					
Gingin	49	0	0	1	50

Following the initial survey, Site 1 - clone 95 and Site 2 - Gingin clone were chosen for close monitoring through to harvest. These sites were selected as there were enough vines with and without virus infection present which would ensure sufficient grapes would be available for small batch wine making,

Stage 2
On the 4th and 5th February 2019, sites 1 and 2 were revisited and LAMP PCR was used to assess the same vines again for their virus status, in addition, more vines were tested to further identify more vines with viru and vines without detection (WD).

Following the first round testing with qPCR and follow up with LAMP PCR in the field, at Site 1, 14 WD vines, and 20 vines with GLRV3 only were identified; at site 2, 16 WD vines, and 40 vines with GLRV3 only were identified. Neither site had enough vines with GLRV1 only identified to harvest for small batch winemaking despite testing a number of vines either side of the previously identified GLRV1 positive vines.

Stage 3
Site 1 was harvested on 28 February 2019 and site 2 on 11 March 2019. Selectively harvesting vines of either GLRV3 or those without detection (WD) site 1 yielded 39kg and 30kg respectively with the fruit quality deemed sound. Site 2 yielded 22kg (GLRV3) and 15kg (WD). This was a first crop for the block and consequently had small canopies and no bird netting, resulting in sun damaged fruit with moderate disease and as such the fruit quality was deemed poor. The data from the harvest for each site is available in Table 2.

At the time of harvest, leaf samples from each vine were taken, transported to the lab at south perth and tested for GLRV1 and GLRV3 by qPCR, with the results of this testing in Table 3.

Table 2. Harvest data

Site	**1**	**1**	**2**	**2**
Chardonnay clone	Clone 95	Clone 95	Gingin	Gingin
Virus Status	WD	GLRV3	WD	GLRV3
Harvest date	29-Feb	29-Feb	11-Mar	11-Mar
Total quantity	~30kg	~39Kg	~15Kg	~22Kg
Fruit quality	Sound	Sound	Poor	Poor
Av berry weight (g)	1.4	1.3	1.5	1.4
Av bunches/vine (std)	15.8 (2.9)	17.8 (4.6)	7.5 (4.5)	7.4 (6.1)
Av kg's/vine (std)	2.29 (0.76)	2.51 (0.74)	1.22 (1.96)	0.90 (0.82)
Av bunch weight (std)	142.3g (29.43)	141.1g (18.7)	127.8g (86.5)	110.5g (38.8)
Bunch compaction	1.33	1.36	1.14	1.25

Table 3. qPCR result at harvest

Site	**1**	**2**	**3**	**4**
Virus status	WD	GLRV3	WD	GLRV3
WD	14	1	11	2
GLRV3	1	19	2	22
GLRV1	0	0	0	0
Total	15	20	13	24

In addition, to see if any other viruses were present, and as a secondary confirmation of the qPCR results, next generation sequencing was done using the Oxford Nanopore Technologies MinIon sequencer. A total of 9,111,237 reads were produced by this run, with a breakdown of the results presented in table 4.

Table 4. MinIon sequencing

Site	**1**	**2**	**3**	**4**
Virus status	WD	GLRV3	WD	GLRV3
Total reads	1,348,511	3,486,284	2,189,875	2,086567
GLRV3 reads	190	21,123	1,063	2,210
Total	**9,111,237**			

The results showed that GLRV3 was readily detected in pools 2 and 4 which corresponds to site 1 and 2 vines that were harvested with GLRV3. In total 9,111,756 reads were sequenced across the four pools. The detection of some reads in pools 1 and 3 reflects the detection of GLRV3 in 1 vine for pool 1 and 2 vines for pool 3 which correspond to the WD vines from both site 1 and 2. Unfortunately due to the need to harvest at short notice, according to the vineyard schedule there was not enough time in the project to identify or harvest any additional vines following the qPCR results from samples taken at harvest.

Table 5. Wine making

Site	**1**	**1**	**2**	**2**
Virus Status	WD	GLRV3	WD	GLRV3
Juice Baume	11.8	11.2	11.2	11.4
Juice pH	3.17	3.20	3.22	3.28
Juice TA (g/L)	7.5	7.5	7.87	6.78
Duration of fermentation (days)	25	14	17	17
Wine pH	3.17	3.17	3.22	3.26
Wine TA (g/L)	7.5	7.1	6.8	6.3
Glucose/fructose (g/L)	2.7	0.3	0.3	0.3
Alc (%)	12.6	11.5	12.0	12.3

Stage 4

The objective of the sensory assessment was to identify if a panel of experienced winemakers could distinguish clonal wines made from vines with and without GLRV3. The backgrounds of the panelists are summarised in table 6 below.

A triangle test was used as described by Meilgaard, Civille and Carr (2007).

Ideally the panel should have been larger than four for statistical rigour but were only able to secure the time of these panelists on the day of the assessment.

Table 6. Sensory panel makeup

Panellist	Age	Years of winemaking
1	31-39	17
2	31-39	15
3	40-49	29
4	31-39	15

Each panellist was presented with six brackets per site, within the brackets were six possible combinations of the two treatments (ABB, BAA, AAB, BBA, ABA and BAB). The wines were identified by randomised three digit numbers and on a scoring sheet the panellists were asked to select the wine which was different and note why it was different.

Initially the panellists were not informed of the nature of the trial other than it was involving 2019 Margaret River Chardonnay to avoid potential biases. As a group, the results (Tables 7.1 and 7.2) showed they were unable to distinguish between the treatments (with and without GLRV3) as collectively they could only correctly identify the different wine 50% of the time. However, panelist 1 showed an ability to pick the treatments with a degree of confidence, p value of 0.001 when the combined results are analysed.

This may indicate that a highly skilled assessor may in fact be able to separate wines made from virus infected vines and those that are not. Further work is required to have greater confidence in the results.

Table 7.1 Sensory evaluation – site 1

Site 1			***Clone 95***		
Treatment A			No GLRV3 (WD)		
Treatment B			GLRV3		
Bracket	Panelist 1	Panelist 2	Panelist 3	Panelist 4	Correct Result
ABB	1	1	1	0	**3**
BAA	0	0	0	1	**1**
AAB	1	0	0	1	**2**
BBA	1	0	1	0	**2**
ABA	0	0	1	0	**1**
BAB	1	1	1	0	**3**
Correct Answer	**4**	**0**	**4**	**2**	
Total correct picks: 12					
From: 24					

Table 7.2 Sensory evaluation – site 2

Site 2			***Gingin***		
Treatment A			No GLRV3 (WD)		
Treatment B			GLRV3		
Bracket	Panelist 1	Panelist 2	Panelist 3	Panelist 4	Correct Result
BAB	1	1	1	0	**3**
BAA	1	1	0	0	**2**
AAB	1	0	0	0	**1**
ABA	1	0	1	1	**3**
BBA	1	0	1	0	**2**
ABB	1	0	0	0	**1**
Correct Answer	**6**	**2**	**3**	**1**	
Total correct picks: 12					
From: 24					

6. Conclusion and Recommendations

Overall, this work has provided some preliminary data towards the question of whether or not infection with Grapevine leafroll virus infection affects the end quality of the wine produced.

The results from the limited sensory evaluation show that the experts were unable to distinguish between the WD and GLRV3 wines, with collectively only 50% of the results given being correct. However one single panelist was successful in picking the difference for 4 out of 6 brackets at site 1 and 6 out of 6 for site 2. If not for the project being limited by time it would have been interesting to follow this up with further panelists. Unfortunately the timing of our sensory evaluation clashed with the timings of other industry events, but was unavoidable in the short time frame to the end of this project.

There did not appear to be any major differences in the harvest and wine making data between the 4 treatments, however due to time and budgetary constraints we were unable to perform repetitions within the experiment. Other limiting factors to this

project included the short time frame of 12 months, and the limited budget which meant only a small number of sites could be selected for the small batch wine making. While the project was meant to focus solely on Gingin Chardonnay, the decision to include a clone 95 block was fortuitous given the difficulties in obtaining sound fruit from the chosen Gingin block at site 2.

In addition, given what is typically known about existing mature blocks of Gingin Chardonnay it is surprising that it was so difficult to find enough GLRV1 infected vines to perform the experimental work for this project. As GLRV1 and GLRV3 have similar effects on the vine we elected to use GLRV3 vs WD vines to ensure we had enough grapes to make the required small batch wines.

Interestingly, we were informed that site 2 was a young planting of the Gingin clone of Chardonnay, however unlike the other Gingin planting tested GLRV1 was not readily detected by either qPCR or LAMP PCR in very many of these vines. Initially it was thought given the young age of the vines it may be possible the virus titre had not yet reached a detectable level .

The results do show that a single skilled assessor was able to detect some difference between the wines. If the opportunity arose this would be an ideal experiment to repeat, at both sites 1 and 2, particularly as site 2 matures. In addition, broadening the experiment to more than 2 sites might help to elucidate the effects more clearly if they are really there. One of the questions that was most commonly asked when speaking with growers when visiting their properties or at presentations to industry was whether or not we would expand this to other varietals, with a particular interest in Shiraz and Cabernet. Including one of the red varietals, alongside Chardonnay would be of great interest to us, and it seems to industry in Western Australia too.

This interest in this project has highlighted the importance of continuing efforts to understand the effect of virus infection on the end quality of wine. The idea that virus infection is good for the wine quality is a potentially harmful one, and does not fit with current best management practice for the control of viruses in a vineyard – that is, that virus presence should be monitored for in the vines and vectors and where possible infected vines should be rogued out. When infection levels are very high, some growers will remove the block and look to replant with healthy vines such is the gains in yield and quality of fruit from doing this. If growers wish to maintain virus in some blocks, but not others this poses many new questions for on-farm hygiene management.

It is still worth investigating this question further, in particular:

1) Whether or not the effect on end wine quality is an adverse one? This research has indicated that highly skilled panelists can detect a difference. This research did not ask if the difference was good or bad.

2) Having the remaining small batch wines from this experiment, or from future experiments sent for chemical analysis as well as sensory evaluation to really dig down in to the changes in end wine that is produced from vines with or without virus.

3) Determining whether there is a threshold of number of vines infected in a block before the effect becomes noticeable. Similar to experiments with

smoke tainted grapes – blending wine made from virus or WD vines in varying proportions to see if there is an acceptable % of infected vines before the difference in taste and/or quality becomes detectable.

7. Extension

The progress and results of this research were communicated to industry in a number of ways:

1) Through visits to growers properties as part of the initial survey for this project and in return visits to the two chosen sites. In all cases the growers were kept informed of the results from qPCR and LAMP PCR of samples from their properties.
2) Presentation to members of the Margaret River Wine Association as part of their "Kick the dirt viti" meeting on July 3 2019. There were approximately 30 people present at this meeting.
3) Presentation to growers in the McLaren Vale region via Zoom, organized through Joe Siebert of DJs Growers. There were approximately 15 people present at this meeting.
4) Presentation to Wine Australia delegation and regional representatives at the Margaret River Education Campus on 20 August 2019
5) Invitation to present at the South Australian and also the West Australian Virus symposium being organized for May 2020.
6) Through the posting of regular updates and photos to twitter via my own account - @monica_kehoe (532 followers), including the tagging of @wine_australia and @DPIRDmedia.
7) Articles written for the December 2018 and both the March and June 2019 WA wine industry newsletters (https://www.agric.wa.gov.au/newsletters/win) (470 subscribers).

8. Researcher benefit and feedback

The regional incubator program has given me the opportunity to build my skills in leading a project, delivering milestones, collaboration and most importantly to continue to build the network of researchers, growers, agronomists and other people in the wine industry that I began with the Science Award project and have continued here. I have forged working relationships with a number of vineyards, who have expressed their support for this project, and any future projects that I may be involved in to continue this work. Their response was very positive and the interactions have helped to further develop my extension and field capabilities. The opportunity to begin to explore the effects of grapevine virus effect on end wine quality has been fantastic, and has given me the drive to pursue further funding and opportunities to continue with research on grapevine virus diseases.

If there was any feedback regarding the incubator program, it is only that it would be great to have the opportunity to extend beyond a 12 month program. Two years would enable researchers to repeat experiments that rely on harvest or sensory evaluation for results.

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